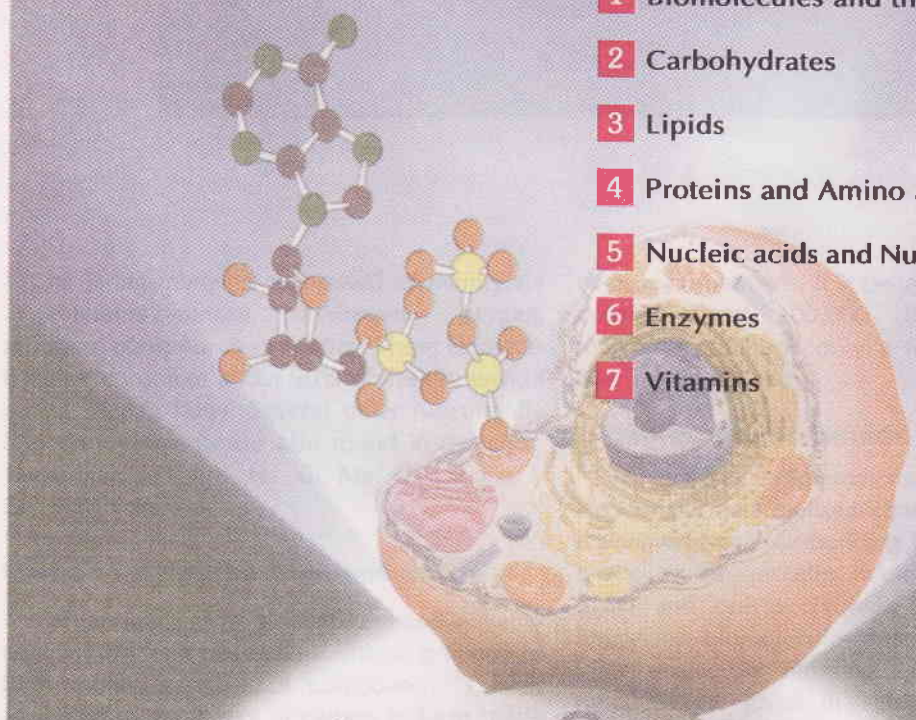
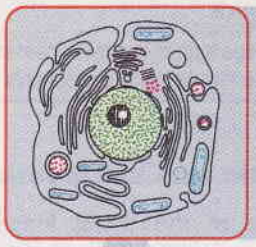
CHEMICAL CONSTITUENTS OF LIFE



Section I

1 Biomolecules and the Cell



The cell speaks :

"I am the unit of biological activity;
Organized into subcellular organelles;
Assigned to each are specific duties;
Thus, I truly represent life!"

The living matter is composed of mainly six elements—***carbon***, ***hydrogen***, ***oxygen***, ***nitrogen***, ***phosphorus*** and ***sulfur***. These elements together constitute about 90% of the dry weight of the human body. Several other functionally important elements are also found in the cells. These include Ca, K, Na, Cl, Mg, Fe, Cu, Co, I, Zn, F, Mo and Se.

Carbon—a unique element of life

Carbon is the most predominant and versatile element of life. It possesses a unique property to form infinite number of compounds. This is attributed to the ability of carbon to form stable covalent bonds and C–C chains of unlimited length. It is estimated that about ***90% of compounds*** found in living system invariably ***contain carbon***.

Chemical molecules of life

Life is composed of lifeless chemical molecules. A single cell of the bacterium, *Escherichia coli* contains about 6,000 different organic compounds. It is believed that man may contain about 100,000 different types of molecules although only a few of them have been characterized.

Complex biomolecules

The organic compounds such as amino acids, nucleotides and monosaccharides serve as the ***monomeric units*** or building blocks of complex biomolecules—proteins, nucleic acids (DNA and RNA) and polysaccharides, respectively. The important biomolecules (macromolecules) with their respective building blocks and major functions are given in ***Table 1.1***. As regards lipids, it may be noted that they are not biopolymers in a strict sense, but majority of them contain fatty acids.

Structural heirarchy of an organism

The macromolecules (proteins, lipids, nucleic acids and polysaccharides) form supramolecular assemblies (e.g. membranes) which in turn organize into organelles, cells, tissues, organs and finally the whole organism.

Table 1.1 The major complex biomolecules of cells

Biomolecule	*Building block (repeating unit)*	*Major functions*
1. Protein	Amino acids	Fundamental basis of structure and function of cell (static and dynamic functions).
2. Deoxyribonucleic acid (DNA)	Deoxyribonucleotides	Repository of hereditary information.
3. Ribonucleic acid (RNA)	Ribonucleotides	Essentially required for protein biosynthesis.
4. Polysaccharide (glycogen)	Monosaccharides (glucose)	Storage form of energy to meet short term demands.
5. Lipid	Fatty acids, glycerol	Storage form of energy to meet long term demands; structural components of membranes.

Chemical composition of man

The chemical composition of a normal man, weighing 65 kg, is given in ***Table 1.2***. Water is the solvent of life and contributes to more than 60% of the weight. This is followed by protein (mostly in muscle) and lipid (mostly in adipose tissue). The carbohydrate content is rather low which is in the form of glycogen.

THE CELL

The cell is the structural and functional unit of life. It may be also regarded as the ***basic unit of biological activity.***

The concept of cell originated from the contributions of Schleiden and Schwann (1838). However, it was only after 1940, the complexities of cell structure were exposed.

Table 1.2 Chemical composition of a normal man (weight 65 kg)

Constituent	*Percent (%)*	*Weight (kg)*
Water	61.6	40
Protein	17.0	11
Lipid	13.8	9
Carbohydrate	1.5	1
Minerals	6.1	4

Prokaryotic and eukaryotic cells

The cells of the living kingdom may be divided into two categories

1. **Prokaryotes** (*Greek* : pro – before; karyon – nucleus) lack a well defined nucleus and possess relatively simple structure. These include the various bacteria.

2. **Eukaryotes** (*Greek* : eu – true; karyon – nucleus) possess a well defined nucleus and are more complex in their structure and function. The higher organisms (animals and plants) are composed of eukaryotic cells.

A comparison of the characteristics between prokaryotes and eukaryotes is listed in ***Table 1.3***.

EUKARYOTIC CELL

The human body is composed of about 10^{14} cells. There are about 250 types of specialized cells in the human body e.g. erythrocytes, nerve cells, muscle cells, β cells of pancreas. An eukaryotic cell is generally 10 to 100 μm in diameter. A diagrammatic representation of a typical rat liver cell is depicted in ***Fig.1.1***.

The plant cell differs from an animal cell by possessing a rigid cell wall (mostly composed of cellulose) and chloroplasts. The latter are the sites of photosynthesis.

Table 1.3 Comparison between prokaryotic and eukaryotic cells

Characteristic	*Prokaryotic cell*	*Eukaryotic cell*
1. Size	Small (generally 1-10 μm)	Large (generally 10-100 μm)
2. Cell membrane	Cell is enveloped by a rigid cell wall	Cell is enveloped by a flexible plasma membrane
3. Sub-cellular organelles	Absent	Distinct organelles are found (e.g. mitochondria, nucleus, lysosomes)
4. Nucleus	Not well defined; DNA is found as nucleoid, histones are absent	Nucleus is well defined, surrounded by a membrane; DNA is associated with histones
5. Energy metabolism	Mitochondria absent, enzymes of energy metabolism bound to membrane	Enzymes of energy metabolism are located in mitochondria
6. Cell division	Usually fission and no mitosis	Mitosis
7. Cytoplasm	Organelles and cytoskeleton absent	Contains organelles and cytoskeleton (a network of tubules and filaments)

The cell consists of well defined subcellular organelles, enveloped by a plasma membrane. By differential centrifugation of tissue homogenate, it is possible to isolate each cellular organelle in a relatively pure form (***Refer Chapter 41***). The distribution of major enzymes and metabolic pathways in different cellular organelles is given in the chapter on enzymes (***Refer Fig.6.6***). The subcellular organelles are briefly described in the following pages.

Nucleus

Nucleus is the largest cellular organelle, surrounded by a double membrane nuclear envelope. The outer membrane is continuous with the membranes of endoplasmic reticulum. At certain intervals, the two nuclear membranes have nuclear pores with a diameter of about 90 nm. These pores permit the free passage of the products synthesized in the nucleus into the surrounding cytoplasm.

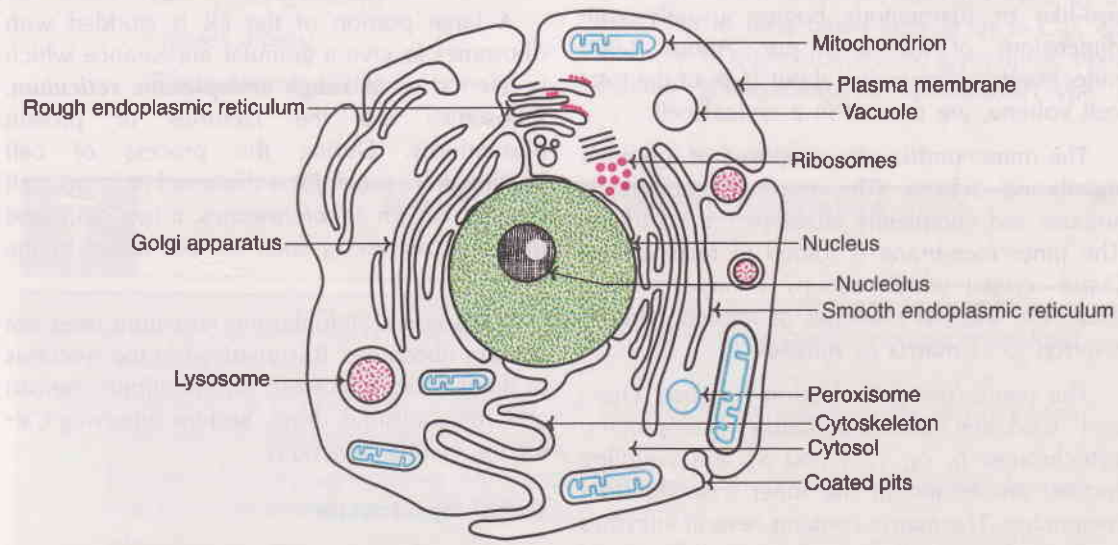


Fig. 1.1 : Diagrammatic representation of a rat liver cell.

Nucleus contains ***DNA***, the repository of genetic information. Eukaryotic DNA is associated with basic protein (histones) in the ratio of 1 : 1, to form ***nucleosomes***. An assembly of nucleosomes constitutes ***chromatin*** fibres of chromosomes (*Greek*: chroma – colour; soma – body). Thus, a single human chromosome is composed of about a million nucleosomes. The number of chromosomes is a characteristic feature of the species. Humans have 46 chromosomes, compactly packed in the nucleus.

The nucleus of the eukaryotic cell contains a dense body known as ***nucleolus***. It is rich in RNA, particularly the ribosomal RNA which enters the cytosol through nuclear pores.

The ground material of the nucleus is often referred to as ***nucleoplasm***. It is rich in enzymes such as DNA polymerases and RNA polymerases. To the surprise of biochemists, the enzymes of glycolysis, citric acid cycle and hexose monophosphate shunt have also been detected in the nucleoplasm.

Mitochondria

The mitochondria (*Greek*: mitos – thread; chondros – granule) are the centres for the cellular respiration and energy metabolism. They are regarded as the ***power houses of the cell*** with variable size and shape. Mitochondria are rod-like or filamentous bodies, usually with dimensions of 1.0 × 3 µm. About 2,000 mitochondria, occupying about $^1/_5$th of the total cell volume, are present in a typical cell.

The mitochondria are composed of a double membrane system. The outer membrane is smooth and completely envelops the organelle. The inner membrane is folded to form ***cristae*** (*Latin* – crests) which occupy a larger surface area. The internal chamber of mitochondria is referred to as ***matrix*** or ***mitosol***.

The components of electron transport chain and oxidative phosphorylation (flavoprotein, cytochromes b, c_1, c, a and a_3 and coupling factors) are buried in the inner mitochondrial membrane. The matrix contains several enzymes concerned with the energy metabolism of carbohydrates, lipids and amino acids (e.g., citric acid cycle, β-oxidation). The matrix enzymes also participate in the synthesis of heme and urea. Mitochondria are the ***principal producers of ATP*** in the aerobic cells. ATP, the energy currency, generated in mitochondria is exported to all parts of the cell to provide energy for the cellular work.

The mitochondrial matrix contains a circular double stranded DNA (***mtDNA***), RNA and ribosomes. Thus, the mitochondria are equipped with an independent protein synthesizing machinery. It is estimated that about 10% of the mitochondrial proteins are produced in the mitochondria.

The structure and functions of mitochondria closely ***resemble prokaryotic cells***. It is hypothesized that mitochondria have evolved from aerobic bacteria. Further, it is believed that during evolution, the aerobic bacteria developed a symbiotic relationship with primordial anaerobic eukaryotic cells that ultimately led to the arrival of aerobic eukaryotes.

Endoplasmic reticulum

The network of membrane enclosed spaces that extends throughout the cytoplasm constitutes endoplasmic reticulum (ER). Some of these thread-like structures extend from the nuclear pores to the plasma membrane.

A large portion of the ER is studded with ribosomes to give a granular appearance which is referred to as ***rough endoplasmic reticulum***. Ribosomes are the factories of protein biosynthesis. During the process of cell fractionation, rough ER is disrupted to form small vesicles known as ***microsomes***. It may be noted that microsomes as such do not occur in the cell.

The smooth endoplasmic reticulum does not contain ribosomes. It is involved in the synthesis of lipids (triacylglycerols, phospholipids, sterols) and metabolism of drugs, besides supplying Ca^{2+} for the cellular functions.

Golgi apparatus

Eukaryotic cells contain a unique cluster of ***membrane vesicles*** known as ***dictyosomes***

which, in turn, constitute Golgi apparatus (or Golgi complex). The newly synthesized proteins are handed over to the Golgi apparatus which catalyse the addition of carbohydrates, lipids or sulfate moieties to the proteins. These chemical modifications are necessary for the transport of proteins across the plasma membrane.

Certain proteins and enzymes are enclosed in membrane vesicles of Golgi apparatus and secreted from the cell after the appropriate signals. The digestive enzymes of pancreas are produced in this fashion.

Golgi apparatus are also involved in the ***membrane synthesis***, particularly for the formation of intracellular organelles (e.g. peroxisomes, lysosomes).

Lysosomes

Lysosomes are spherical vesicles enveloped by a single membrane. Lysosomes are regarded as the digestive tract of the cell, since they are actively involved in digestion of cellular substances—namely proteins, lipids, carbohydrates and nucleic acids. Lysosomal enzymes are categorized as ***hydrolases***. These include the following enzymes (with substrate in brackets)

α-Glucosidase (glycogen)

Cathepsins (proteins)

Lipases (lipids)

Ribonucleases (RNA)

The pH of the lysosomal matrix is more acidic (pH < 5) than the cytosol (pH~7) and this facilitates the degradation of different compounds. The lysosomal enzymes are responsible for ***maintaining the cellular compounds in a dynamic state***, by their degradation and recycling. The degraded products leave the lysosomes, usually by diffusion, for reutilization by the cell. Sometimes, however, certain residual products, rich in lipids and proteins, collectively known as ***lipofuscin*** accumulate in the cell. Lipofuscin is the ***age pigment*** or wear and tear pigment which has been implicated in ageing process.

The digestive enzymes of cellular compounds are confined to the lysosomes in the best interest of the cell. Escape of these enzymes into cytosol will destroy the functional macromolecules of the cell and result in many complications. The occurrence of several diseases (e.g. arthritis, muscle diseases, allergic disorders) has been partly attributed to the release of lysosomal enzymes.

Peroxisomes

Peroxisomes, also known as ***microbodies***, are single membrane cellular organelles. They are spherical or oval in shape and contain the enzyme ***catalase***. Catalase protects the cell from the toxic effects of H_2O_2 by converting it to H_2O and O_2. Peroxisomes are also involved in the oxidation of long chain fatty acids ($> C_{18}$), and synthesis of plasmalogens and glycolipids. Plants contain ***glyoxysomes***, a specialized type of

BIOMEDICAL / CLINICAL CONCEPTS

- *A living cell is a true representative of life with its own organization and specialized functions.*
- *Accumulation of lipofuscin, a pigment rich in lipids and proteins, in the cell has been implicated in ageing process.*
- *Leakage of lysosomal enzymes into the cell degrades several functional macromolecules and this may lead to certain disorders (e.g. arthritis).*
- *Zellweger syndrome is a rare disease characterized by the absence of functional peroxisomes.*

peroxisomes, which are involved in the glyoxylate pathway.

Peroxisome biogenesis disorders (PBDs), are a group of rare diseases involving the enzyme activities of peroxisomes. The biochemical abnormalities associated with PBDs include increased levels of very long chain fatty acids (C_{24} and C_{26}) and decreased concentrations of plasmalogens. The most severe form of PBDs is ***Zellweger syndrome***, a condition characterized by the absence of functional peroxisomes. The victims of this disease may die within one year after birth.

Cytosol and cytoskeleton

The ***cellular matrix*** is collectively referred to as cytosol. Cytosol is basically a compartment containing several enzymes, metabolites and salts in an aqueous gel like medium. More recent studies however, indicate that the cytoplasm actually contains a complex network of protein filaments, spread throughout, that constitutes cytoskeleton. The cytoplasmic filaments are of three types – ***microtubules***, actin filaments and intermediate filaments. The filaments which are polymers of proteins are responsible for the structure, shape and organization of the cell.

INTEGRATION OF CELLULAR FUNCTIONS

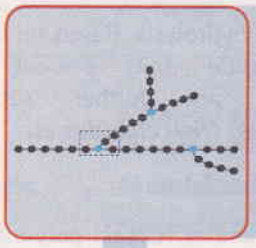
The eukaryotic cells perform a wide range of complex reactions/functions to maintain tissues, and for the ultimate well-being of the whole organism. For this purpose, the various intracellular processes and biochemical reactions are tightly controlled and integrated. Division of a cell into two daughter cells is good example of the orderly occurrence of an integrated series of cellular reactions.

Apoptosis is the programmed cell death or cell suicide. This occurs when the cell has fulfilled its biological functions. Apoptosis may be regarded as a ***natural cell death*** and it differs from the cell death caused by injury due to radiation, anoxia etc. Programmed cell death is a highly regulated process.

SUMMARY

1. *Life is composed of lifeless chemical molecules. The complex biomolecules, proteins, nucleic acids (DNA and RNA), polysaccharides and lipids are formed by the monomeric units amino acids, nucleotides, monosaccharides and fatty acids, respectively.*
2. *The cell is the structural and functional unit of life. The eukaryotic cell consists of well defined subcellular organelles, enveloped in a plasma membrane.*
3. *The nucleus contains DNA, the repository of genetic information. DNA, in association with proteins (histones), forms nucleosomes which, in turn, make up the chromosomes.*
4. *The mitochondria are the centres for energy metabolism. They are the principal producers of ATP which is exported to all parts of the cell to provide energy for cellular work.*
5. *Endoplasmic reticulum (ER) is the network of membrane enclosed spaces that extends throughout the cytoplasm. ER studded with ribosomes, the factories of protein biosynthesis, is referred to as rough ER. Golgi apparatus are a cluster of membrane vesicles to which the newly synthesized proteins are handed over for further processing and export.*
6. *Lysosomes are the digestive bodies of the cell, actively involved in the degradation of cellular compounds. Peroxisomes contain the enzyme catalase that protects the cell from the toxic effects of H_2O_2. The cellular ground matrix is referred to as cytosol which, in fact, is composed of a network of protein filaments, the cytoskeleton.*
7. *The eukaryotic cells perform a wide range of complex functions in a well coordinated and integrated fashion. Apoptosis is the process of programmed cell death or cell suicide.*

2 Carbohydrates



The carbohydrates speak :

"We are polyhydroxyaldehydes or ketones;
Classified into mono-, oligo- and polysaccharides;
Held together by glycosidic bonds;
Supply energy and serve as structural constituents."

Carbohydrates are the most abundant organic molecules in nature. They are primarily composed of the elements ***carbon***, ***hydrogen*** **and** ***oxygen***. The name carbohydrate literally means 'hydrates of carbon'. Some of the carbohydrates possess the empirical formula $(C.H_2O)_n$ where $n \leq 3$, satisfying that these carbohydrates are in fact carbon hydrates. However, there are several non-carbohydrate compounds (e.g. acetic acid, $C_2H_4O_2$; lactic acid, $C_3H_6O_3$) which also appear as hydrates of carbon. Further, some of the genuine carbohydrates (e.g. rhamnohexose, $C_6H_{12}O_5$; deoxyribose, $C_5H_{10}O_4$) do not satisfy the general formula. Hence carbohydrates cannot be always considered as hydrates of carbon.

Carbohydrates may be defined as polyhydroxyaldehydes or ketones or compounds which produce them on hydrolysis. The term 'sugar' is applied to carbohydrates soluble in water and sweet to taste.

Functions of carbohydrates

Carbohydrates participate in a wide range of functions

1. They are the most abundant dietary ***source of energy*** (4 Cal/g) for all organisms.

2. Carbohydrates are precursors for many organic compounds (fats, amino acids).

3. Carbohydrates (as glycoproteins and glycolipids) participate in the structure of cell membrane and cellular functions such as cell growth, adhesion and fertilization.

4. They are structural components of many organisms. These include the fiber (cellulose) of plants, exoskeleton of some insects and the cell wall of microorganisms.

5. Carbohydrates also serve as the storage form of energy (glycogen) to meet the immediate energy demands of the body.

CLASSIFICATION OF CARBOHYDRATES

Carbohydrates are often referred to as saccharides (*Greek*: sakcharon–sugar). They are broadly classified into three major groups—***monosaccharides, oligosaccharides*** **and** ***polysaccharides***. This categorization is based on

Table 2.1 Classification of monosaccharides with selected examples

Monosaccharides (empirical formula)	*Aldose*	*Ketose*
Trioses ($C_3H_6O_3$)	Glyceraldehyde	Dihydroxyacetone
Tetroses ($C_4H_8O_4$)	Erythrose	Erythrulose
Pentoses ($C_5H_{10}O_5$)	Ribose	Ribulose
Hexoses ($C_6H_{12}O_6$)	Glucose	Fructose
Heptoses ($C_7H_{14}O_7$)	Glucoheptose	Sedoheptulose

the number of sugar units. ***Mono-* and *oligo-saccharides*** are sweet to taste, crystalline in character and soluble in water, hence they are commonly known as ***sugars***.

Monosaccharides

Monosaccharides (*Greek* : mono-one) are the simplest group of carbohydrates and are often referred to as simple sugars. They have the general formula $C_n(H_2O)_n$, and they cannot be further hydrolysed. The monosaccharides are divided into different categories, based on the functional group and the number of carbon atoms

Aldoses : When the functional group in monosaccharides is an aldehyde $\left(\begin{matrix}H\\|\\-C=O\end{matrix}\right)$, they are known as aldoses e.g. glyceraldehyde, glucose.

Ketoses : When the functional group is a keto $\left(\begin{matrix}|\\-C=O\end{matrix}\right)$ group, they are referred to as ketoses e.g. dihydroxyacetone, fructose.

Based on the number of carbon atoms, the monosaccharides are regarded as *trioses (3C), tetroses (4C), pentoses (5C), hexoses (6C) and heptoses (7C)*. These terms along with functional groups are used while naming monosaccharides. For instance, ***glucose is an aldohexose while fructose is a ketohexose*** (***Table 2.1***).

The common monosaccharides and disaccharides of biological importance are given in the ***Table 2.2***.

Oligosaccharides

Oligosaccharides (*Greek*: oligo-few) contain 2-10 monosaccharide molecules which are liberated on hydrolysis. Based on the number of monosaccharide units present, the oligosaccharides are further subdivided to ***disaccharides***, ***trisaccharides*** etc.

Polysaccharides

Polysaccharides (*Greek*: poly-many) are polymers of monosaccharide units with high molecular weight (up to a million). They are usually tasteless (non-sugars) and form colloids with water. The polysaccharides are of two types – ***homopolysaccharides*** and ***heteropolysaccharides***.

MONOSACCHARIDES— STRUCTURAL ASPECTS

Stereoisomerism is an important character of monosaccharides. Stereoisomers are the compounds that have the same structural formulae but differ in their spatial configuration.

A carbon is said to be ***asymmetric when*** it is ***attached to four different atoms or groups***. The number of asymmetric carbon atoms (n) determines the possible ***isomers*** of a given compound which is equal to 2^n. Glucose contains 4 asymmetric carbons, and thus has 16 isomers.

Glyceraldehyde —the reference carbohydrate

Glyceraldehyde (triose) is the simplest monosaccharide with one asymmetric carbon atom. It exists as two stereoisomers and has been chosen as the reference carbohydrate to represent the structure of all other carbohydrates.

Table 2.2 Monosaccharides and disaccharides of biological importance

Monosaccharides	*Occurrence*	*Biochemical importance*
Trioses		
Glyceraldehyde	Found in cells as phosphate	Glyceraldehyde 3-phosphate is an intermediate in glycolysis
Dihydroxyacetone	Found in cells as phosphate	Its 1-phosphate is an intermediate in glycolysis
Tetroses		
D-Erythrose	Widespread	Its 4-phosphate is an intermediate in carbohydrate metabolism
Pentoses		
D-Ribose	Widespread as a constituent of RNA and nucleotides	For the structure of RNA and nucleotide coenzymes (ATP, NAD^+, $NADP^+$)
D-Deoxyribose	As a constituent of DNA	For the structure of DNA
D-Ribulose	Produced during metabolism	It is an important metabolite in hexose monophosphate shunt
D-Xylose	As a constituent of glycoproteins and gums	Involved in the function of glycoproteins
L-Xylulose	As an intermediate in uronic acid pathway	Excreted in urine in essential pentosuria
D-Lyxose	Heart muscle	As a constituent of lyxoflavin of heart muscle
Hexoses		
D-Glucose	As a constituent of polysaccharides (starch, glycogen, cellulose) and disaccharides (maltose, lactose, sucrose). Also found in fruits	The 'sugar fuel' of life; excreted in urine in diabetes. Structural unit of cellulose in plants
D-Galactose	As a constituent of lactose (milk sugar)	Converted to glucose, failure leads to galactosemia
D-Mannose	Found in plant polysaccharides and animal glycoproteins	For the structure of polysaccharides
D-Fructose	Fruits and honey, as a constituent of sucrose and inulin	Its phosphates are intermediates of glycolysis
Heptoses		
D-Sedoheptulose	Found in plants	Its 7-phosphate is an intermediate in hexose monophosphate shunt, and in photosynthesis
Disaccharides	***Occurrence***	***Biochemical importance***
Sucrose	As a constituent of cane sugar and beet sugar, pineapple	Most commonly used table sugar supplying calories
Lactose	Milk sugar	Exclusive carbohydrate source to breast fed infants. Lactase deficiency (lactose intolerance) leads to diarrhea and flatulence
Maltose	Product of starch hydrolysis, occurs in germinating seeds	An important intermediate in the digestion of starch

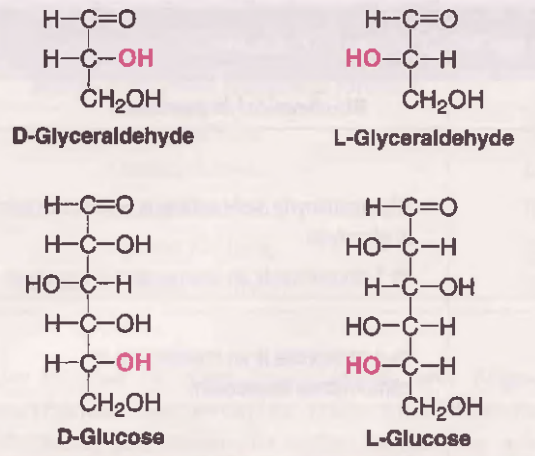


Fig. 2.1 : D-and-L- forms of glucose compared with D- and L- glyceraldehydes (the reference carbohydrate).

D- and L-isomers

The D and L isomers are mirror images of each other. The spatial orientation of −H and −OH groups on the carbon atom (C_5 for glucose) that is adjacent to the terminal primary alcohol carbon determines whether the sugar is D- or L-isomer. If the −OH group is on the right side, the sugar is of D-series, and if on the left side, it belongs to L-series. The structures of D- and L-glucose based on the ***reference monosaccharide, D- and L-glyceraldehyde*** (glycerose) are depicted in ***Fig.2.1***.

It may be noted that the naturally occurring monosaccharides in the mammalian tissues are mostly of D-configuration. The enzyme machinery of cells is specific to metabolise D-series of monosaccharides.

In the medical practice, the term ***dextrose*** is used for ***glucose in solution***. This is because of the dextrorotatory nature of glucose.

Optical activity of sugars

Optical activity is a characteristic feature of compounds with ***asymmetric carbon*** atom. When a beam of polarized light is passed through a solution of an optical isomer, it will be rotated either to the right or left. The term ***dextrorotatory*** **(+)** ***and levorotatory*** **(–)** are used to compounds that respectively rotate the plane of polarized light to the right or to the left.

An optical isomer may be designated as D(+), D(–), L(+) and L(–) based on its structural relation with glyceraldehyde. It may be noted that the D- and L-configurations of sugars are primarily based on the structure of glyceraldehyde, the optical activities however, may be different.

Racemic mixture : If D- and L-isomers are present in equal concentration, it is known as racemic mixture or DL mixture. Racemic mixture ***does not exhibit any optical activity***, since the dextro- and levorotatory activities cancel each other.

Configuration of D-aldoses

The configuration of possible D-aldoses starting from D-glyceraldehyde is depicted in ***Fig.2.2***. This is a representation of ***Killiani-Fischer synthesis***, by increasing the chain length of an aldose, by one carbon at a time. Thus, starting with an aldotriose (3C), aldotetroses (4C), aldopentoses (5C) and aldohexoses (6C) are formed. Of the 8 aldohexoses, glucose, mannose and galactose are the most familiar. Among these, D-glucose is the only aldose monosaccharide that predominantly occurs in nature.

Configuration of D-ketoses

Starting from dihydroxyacetone (triose), there are five keto-sugars which are physiologically important. Their structures are given in ***Fig.2.3***.

Epimers

If two monosaccharides ***differ*** from each other in their ***configuration around a single specific carbon*** (other than anomeric) atom, they are referred to as *epimers* to each other (***Fig.2.4***). For instance, ***glucose and galactose are epimers*** with regard to carbon 4 (C_4-epimers). That is, they differ in the arrangement of −OH group at C_4. Glucose and mannose are epimers with regard to carbon 2 (C_2-epimers).

The interconversion of epimers (e.g. glucose to galactose and vice versa) is known as

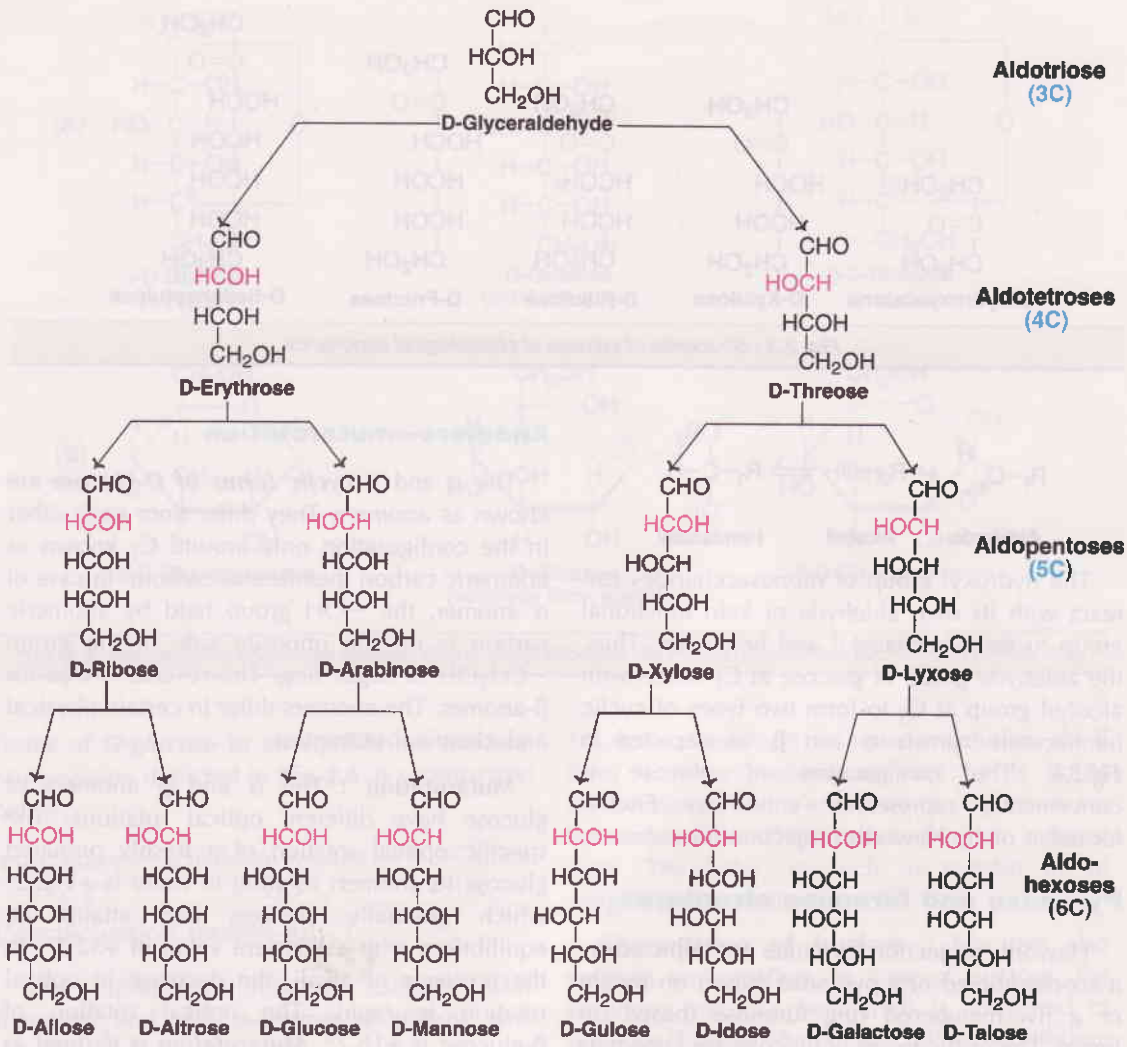


Fig. 2.2 : The structural relationship between D-aldoses shown in Fischer projection. (The configuration around C_2 (red) distinguishes the members of each pair).

epimerization, and a group of enzymes—namely—***epimerases*** catalyse this reaction.

Enantiomers

Enantiomers are a special type of stereoisomers that are ***mirror images of each other***. The two members are designated as D- and L-sugars. Enantiomers of glucose are depicted in ***Fig.2.5***.

Majority of the sugars in the higher animals (including man) are of D-type (***Fig.2.5***).

The term ***diastereomers*** is used to represent the ***stereoisomers*** that are ***not mirror images of one another***.

STRUCTURE OF GLUCOSE

For a better understanding of glucose structure, let us consider the formation of hemiacetals and hemiketals, respectively produced when an aldehyde or a ketone reacts with alcohol.

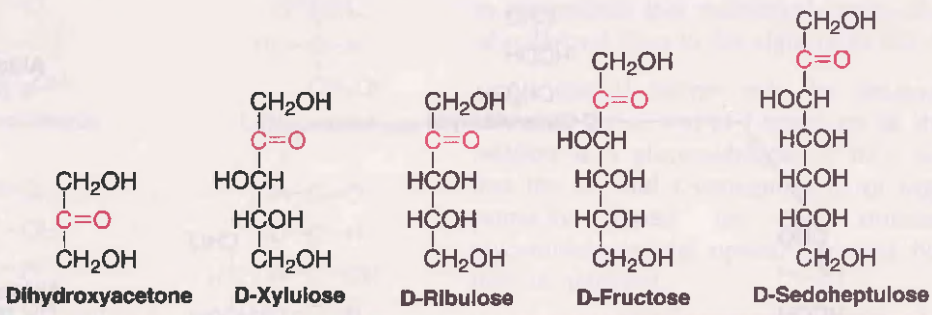
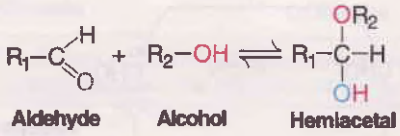


Fig. 2.3 : Structures of ketoses of physiological importance.



The hydroxyl group of monosaccharides can react with its own aldehyde or keto functional group to form hemiacetal and hemiketal. Thus, the aldehyde group of glucose at C_1 reacts with alcohol group at C_5 to form two types of cyclic hemiacetals namely α and β, as depicted in ***Fig.2.6***. The configuration of glucose is conveniently represented either by Fischer formulae or by Haworth projection formulae.

Pyranose and furanose structures

Haworth projection formulae are depicted by a six-membered ring pyranose (based on pyran) or a five-membered ring furanose (based on furan). The cyclic forms of glucose are known as α-D-glucopyranose and α-D-glucofuranose (***Fig.2.7***).

Anomers—mutarotation

The *α* and *β* ***cyclic forms of D-glucose*** are known as *anomers*. They differ from each other in the configuration only around C_1 known as *anomeric* carbon (hemiacetal carbon). In case of α anomer, the −OH group held by anomeric carbon is on the opposite side of the group $-CH_2OH$ of sugar ring. The reverse is true for β-anomer. The anomers differ in certain physical and chemical properties.

Mutarotation : The α and β anomers of glucose have different optical rotations. The specific optical rotation of a freshly prepared glucose (α anomer) solution in water is +112.2° which gradually changes and attains an equilibrium with a constant value of +52.7°. In the presence of alkali, the decrease in optical rotation is rapid. The optical rotation of β-glucose is +18.7°. ***Mutarotation is defined as the change in the specific optical rotation representing the interconversion of α and β***

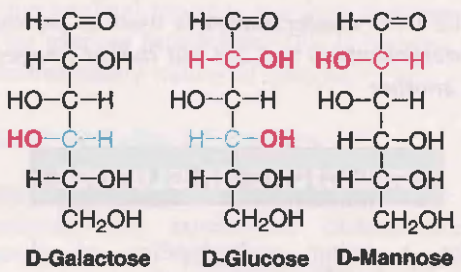


Fig. 2.4 : Structures of epimers (glucose and galactose are C_4-epimers while glucose and mannose are C_2-epimers).

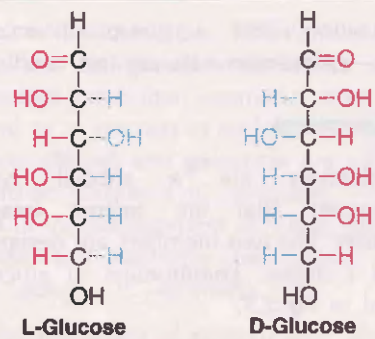


Fig. 2.5 : Enantiomers (mirror images) of glucose.

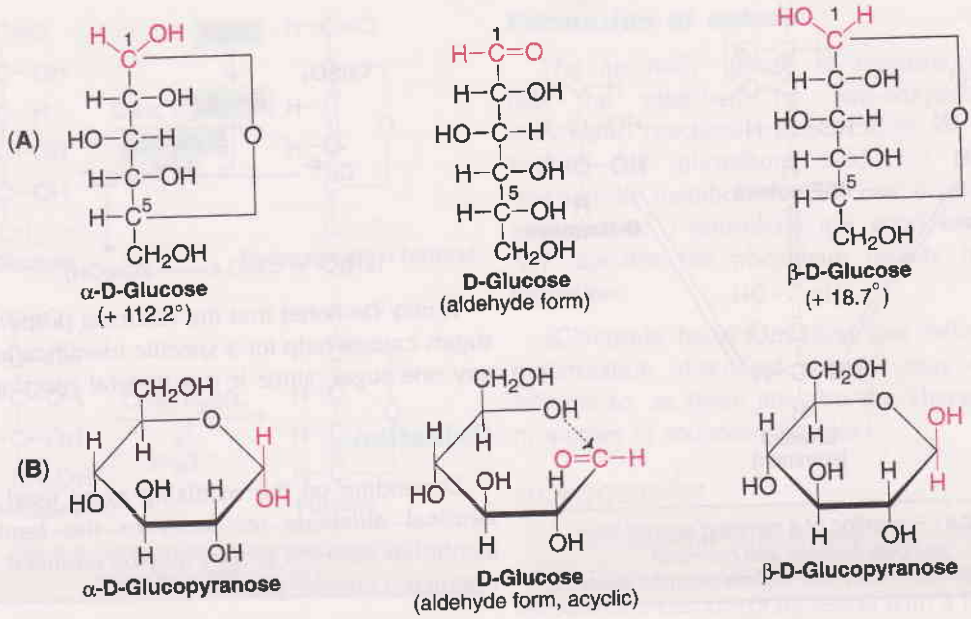


Fig. 2.6 : Mutarotation of glucose representing α and β anomers (A) Fischer projections (B) Haworth projections.

forms of D-glucose to an equilibrium mixture. Mutarotation depicted in ***Fig. 2.6***, is summarized below.

α-D-Glucose ⇌ Equilibrium mixture ⇌ β-D-Glucose
+ 112.2° + 52.7° + 18.7°
(Specific optical rotation $[\alpha]_D^{20}$)

The equilibrium mixture contains 63% β-anomer and 36% α-anomer of glucose with 1% open chain form. In aqueous solution, the β form is more predominant due to its stable conformation. The α and β forms of glucose are interconvertible which occurs through a linear form. The latter, as such, is present in an insignificant quantity.

Mutarotation of fructose : Fruc
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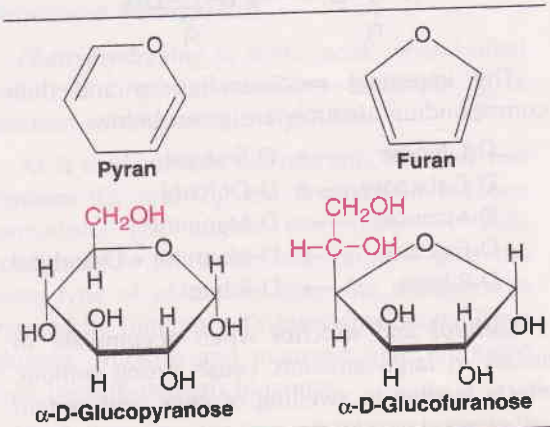

Fig. 2.7 : Structure of glucose-pyranose and furanose forms.

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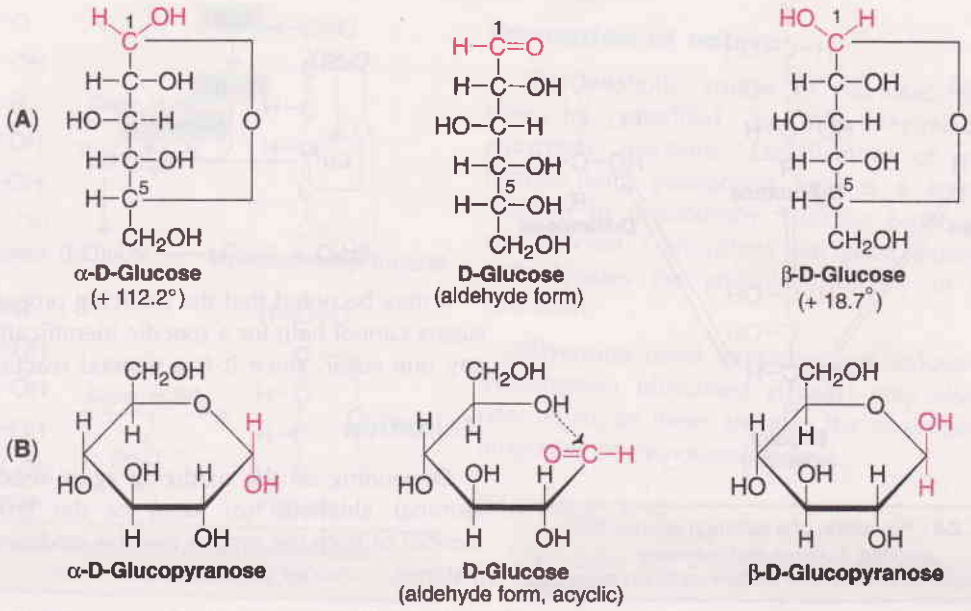

Fig. 2.6 : Mutarotation of glucose representing α and β anomers (A) Fischer projections (B) Haworth projections.

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+ 112.2° + 52.7° + 18.7°

(Specific optical rotation $[\alpha]_D^{20}$)

The equilibrium mixture contains 63% β-anomer and 36% α-anomer of glucose with 1% open chain form. In aqueous solution, the β form is more predominant due to its stable conformation. The α and β forms of glucose are interconvertible which occurs through a linear form. The latter, as such, is present in an insignificant quantity.

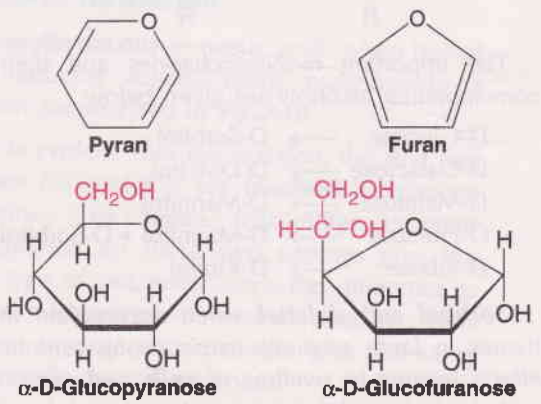


Fig. 2.7 : Structure of glucose-pyranose and furanose forms.

Mutarotation of fructose : Fructose also exhibits mutarotation. In case of fructose, the pyranose ring (six-membered) is converted to furanose (five-membered) ring, till an equilibrium is attained. And fructose has a specific optical rotation of –92° at equilibrium.

The conversion of dextrorotatory (+) sucrose to levorotatory fructose is explained under inversion of sucrose (see later in this chapter).

REACTIONS OF MONOSACCHARIDES

Tautomerization or enolization

The process of shifting a hydrogen atom from one carbon atom to another to produce ***enediols*** is known as ***tautomerization.*** Sugars possessing anomeric carbon atom undergo tautomerization in alkaline solutions.

When glucose is kept in alkaline solution for several hours, it undergoes isomerization to form

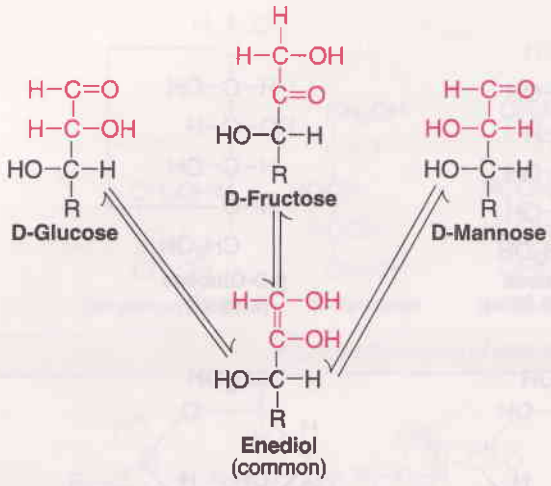


Fig. 2.8 : Formation of a common enediol from glucose, fructose and mannose (R corresponds to the end 3 carbon common structure).

D-fructose and D-mannose. This reaction—known as the ***Lobry de Bruyn-von Ekenstein transformation***—results in the formation of a common intermediate—namely ***enediol***—for all the three sugars, as depicted in ***Fig.2.8.***

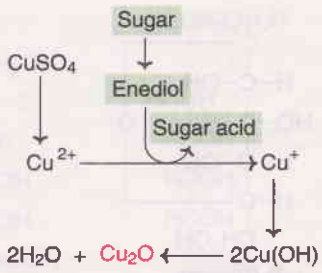
The enediols are highly reactive, hence sugars in alkaline solution are powerful reducing agents.

Reducing properties

The sugars are classified as reducing or non-reducing. The reducing property is attributed to the free aldehyde or keto group of anomeric carbon.

In the laboratory, many tests are employed to identify the reducing action of sugars. These include ***Benedict's test, Fehling's test, Barfoed's test*** etc. The reduction is much more efficient in the alkaline medium than in the acid medium.

The enediol forms (explained above) or sugars reduce cupric ions (Cu^{2+}) of copper sulphate to cuprous ions (Cu^{+}), which form a yellow precipitate of cuprous hydroxide or a red precipitate of cuprous oxide as shown next.



It may be noted that the reducing property of sugars cannot help for a specific identification of any one sugar, since it is a general reaction.

Oxidation

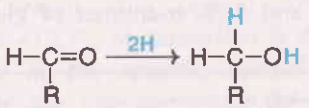
Depending on the oxidizing agent used, the terminal aldehyde (or keto) or the terminal alcohol or both the groups may be oxidized. For instance, consider glucose :

1. Oxidation of aldehyde group (CHO ⟶ COOH) results in the formation of gluconic acid.

2. Oxidation of terminal alcohol group (CH_2OH ⟶ COOH) leads to the production of glucuronic acid.

Reduction

When treated with reducing agents such as sodium amalgam, the aldehyde or keto group of monosaccharide is reduced to corresponding alcohol, as indicated by the general formula :



The important monosaccharides and their corresponding alcohols are given below.

D-Glucose ⟶ D-Sorbitol
D-Galactose ⟶ D-Dulcitol
D-Mannose ⟶ D-Mannitol
D-Fructose ⟶ D-Mannitol + D-Sorbitol
D-Ribose ⟶ D-Ribitol

Sorbitol and ***dulcitol*** when accumulate in tissues in large amounts cause strong osmotic effects leading to swelling of ***cells***, and certain pathological conditions. e.g. ***cataract***, peripheral neuropathy, nephropathy. Mannitol is useful to reduce intracranial tension by forced diuresis.

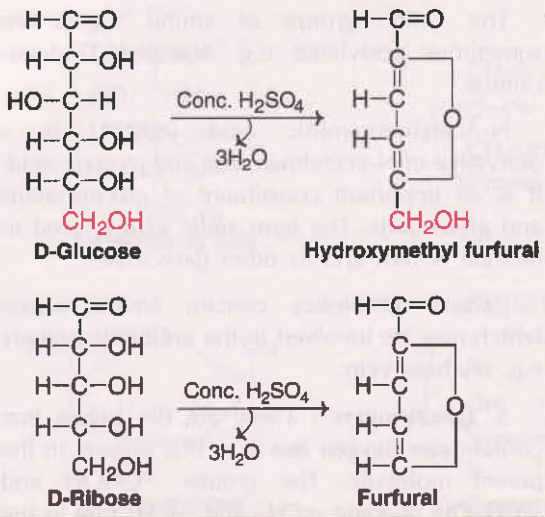


Fig. 2.9 : Dehydration of monosaccharides with concentrated H_2SO_4.

Dehydration

When treated with concentrated sulfuric acid, monosaccharides undergo dehydration with an elimination of 3 water molecules. Thus hexoses give hydroxymethyl furfural while pentoses give furfural on dehydration (***Fig.2.9***). These ***furfurals*** can condense with phenolic compounds (α-naphthol) to form coloured products. This is the chemical basis of the popular ***Molisch test***. In case of oligo- and polysaccharides, they are first hydrolysed to monosaccharides by acid, and this is followed by dehydration.

Osazone formation

Phenylhydrazine in acetic acid, when boiled with reducing sugars, forms osazones in a reaction summarized in ***Fig.2.10***.

As is evident from the reaction, the ***first two carbons*** (C_1 and C_2) are ***involved*** in osazone formation. The sugars that differ in their configuration on these two carbons give the same type of osazones, since the difference is masked by binding with phenylhydrazine. Thus glucose, fructose and mannose give the same type (needle-shaped) osazones.

Reducing disaccharides also give osazones—maltose sunflower-shaped, and lactose powder-puff shaped.

Formation of esters

The alcoholic groups of monosaccharides may be esterified by non-enzymatic or enzymatic reactions. Esterification of carbohydrate with phosphoric acid is a common reaction in metabolism. Glucose 6-phosphate and glucose 1-phosphate are good examples. ATP donates the phosphate moiety in ester formation.

[Glycoside bond formation (see below) and mutarotation (discussed already) may also be referred to, as these are also the characteristic properties of monosaccharides.]

GLYCOSIDES

Glycosides are formed when the hemiacetal or hemiketal hydroxyl group (of anomeric carbon) of a carbohydrate reacts with a hydroxyl group of another carbohydrate or a non-carbohydrate (e.g. methyl alcohol, phenol, glycerol). The bond so formed is known as ***glycosidic bond*** and the non-carbohydrate moiety (when present) is referred to as ***aglycone***.

The monosaccharides are held together by glycosidic bonds to result in di-, oligo- or polysaccharides (see later for structures).

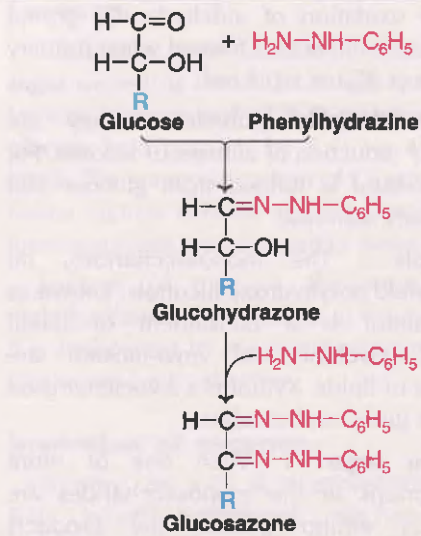


Fig. 2.10 : A summary of osazone formation (R represents C_3 to C_6 of glucose).

Naming of glycosidic bond : The nomenclature of glycosidic bonds is based on the linkages between the carbon atoms and the status of the anomeric carbon (α or β). For instance, lactose—which is formed by a bond between C_1 of β-galactose and C_4 of glucose—is named as β(1 → 4) glycosidic bond. The other glycosidic bonds are described in the structure of di- and polysaccharides.

Physiologically important glycosides

1. **Glucovanillin** (vanillin-D-glucoside) is a natural substance that imparts vanilla flavour.

2. **Cardiac glycosides** (steroidal glycosides) : Digoxin and digitoxin contain the aglycone steroid and they stimulate muscle contraction.

3. **Streptomycin**, an antibiotic used in the treatment of tuberculosis is a glycoside.

4. **Ouabain** inhibits $Na^+ - K^+$ ATPase and blocks the active transport of Na^+.

DERIVATIVES OF MONOSACCHARIDES

There are several derivatives of monosaccharides, some of which are physiologically important

1. **Sugar acids :** Oxidation of aldehyde or primary alcohol group in monosaccharide results in sugar acids. Gluconic acid is produced from glucose by oxidation of aldehyde (C_1 group) whereas glucuronic acid is formed when primary alcohol group (C_6) is oxidized.

2. **Sugar alcohols** (polyols) **:** They are produced by reduction of aldoses or ketoses. For instance, sorbitol is formed from glucose and mannitol from mannose.

3. **Alditols :** The monosaccharides, on reduction, yield polyhydroxy alcohols, known as alditols. Ribitol is a constituent of flavin coenzymes; glycerol and *myo*-inositol are components of lipids. Xylitol is a sweetener used in sugarless gums and candies.

4. **Amino sugars :** When one or more hydroxyl groups of the monosaccharides are replaced by amino groups, the products formed are amino sugars e.g. D-glucosamine, D-galactosamine. They are present as constituents of heteropolysaccharides.

The amino groups of amino sugars are sometimes acetylated e.g. N-acetyl D-glucosamine.

N-Acetylneuraminic acid (NANA) is a derivative of N-acetylmannose and pyruvic acid. It is an important constituent of glycoproteins and glycolipids. The term ***sialic acid*** is used to include NANA and its other derivatives.

Certain antibiotics contain amino sugars which may be involved in the antibiotic activity e.g. erythromycin.

5. **Deoxysugars :** These are the sugars that contain ***one oxygen less*** than that present in the parent molecule. The groups −CHOH and $-CH_2OH$ become $-CH_2$ and $-CH_3$ due to the absence of oxygen. D-2-Deoxyribose is the most important deoxysugar since it is a structural constituent of DNA (in contrast to D-ribose in RNA).

6. **L-Ascorbic acid** (vitamin C) **:** This is a water-soluble vitamin, the structure of which closely resembles that of a monosaccharide.

The structures of selected monosaccharide derivatives are depicted in ***Fig.2.11.***

DISACCHARIDES

Among the oligosaccharides, disaccharides are the most common (***Fig.2.12***). As is evident from the name, a disaccharide consists of two monosaccharide units (similar or dissimilar) held together by a ***glycosidic bond.*** They are crystalline, water-soluble and sweet to taste. The disaccharides are of two types

1. **Reducing** disaccharides with ***free aldehyde or keto*** group e.g. maltose, lactose.

2. **Non-reducing** disaccharides with ***no free aldehyde or keto*** group e.g. sucrose, trehalose.

Maltose

Maltose is composed of ***two α-D-glucose*** units held together by α (1 → 4) glycosidic bond. The free aldehyde group present on C_1 of second glucose answers the reducing reactions, besides

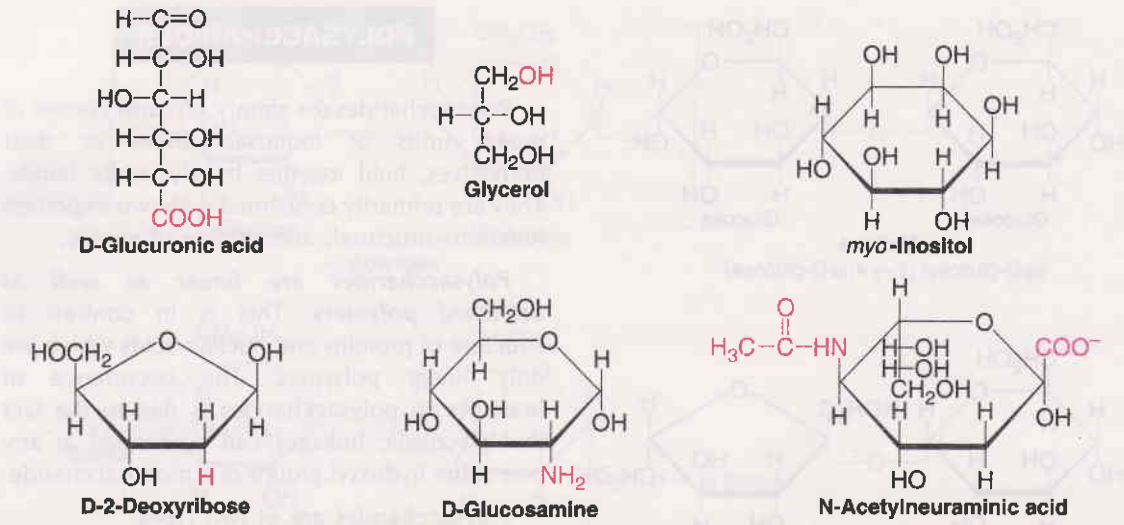


Fig. 2.11 : Structures of monosaccharide derivatives (selected examples).

the osazone formations (sunflower-shaped). Maltose can be hydrolysed by dilute acid or the enzyme maltase to liberate two molecules of α-D-glucose.

In ***isomaltose***, the glucose units are held together by α (1 → 6) glycosidic linkage.

Cellobiose is another disaccharide, identical in structure with maltose, except that the former has β (1 → 4) glycosidic linkage. Cellobiose is formed during the hydrolysis of cellulose.

Sucrose

Sucrose (***cane sugar***) is the sugar of commerce, mostly produced by sugar cane and sugar beets. Sucrose is made up of ***α-D-glucose and β-D-fructose***. The two monosaccharides are held together by a glycosidic bond ($\alpha_1 \rightarrow \beta_2$), between C_1 of α-glucose and C_2 of β-fructose. The reducing groups of glucose and fructose are involved in glycosidic bond, hence sucrose is a ***non-reducing sugar, and it cannot form osazones***.

Sucrose is the major carbohydrate produced in ***photosynthesis***. It is transported into the storage organs of plants (such as roots, tubers and seeds). Sucrose is the most abundant among the naturally occurring sugars. It has distinct advantages over other sugars as a storage and transport form. This is due to the fact that in sucrose, both the functional groups (aldehyde and keto) are held together and protected from oxidative attacks.

Sucrose is an important source of dietary carbohydrate. It is sweeter than most other common sugars (except fructose) namely glucose, lactose and maltose. Sucrose is employed as a sweetening agent in food industry. The intestinal enzyme—sucrase—hydrolyses sucrose to glucose and fructose which are absorbed.

Lactose

Lactose is more commonly known as ***milk sugar*** since it is the disaccharide found in milk. Lactose is composed of ***β-D-galactose and β-D-glucose*** held together by β (1 → 4) glycosidic bond. The anomeric carbon of C_1 glucose is free, hence lactose exhibits reducing properties and forms osazones (powder-puff or hedgehog shape).

Lactose of milk is the most important carbohydrate in the nutrition of young mammals. It is hydrolysed by the intestinal enzyme lactase to glucose and galactose.

Inversion of sucrose

Sucrose, as such is dextrorotatory (+66.5°). But, when hydrolysed, sucrose becomes levorotatory (–28.2°). The process of change in optical rotation from dextrorotatory (+) to levorotatory (–) is referred to as inversion. The

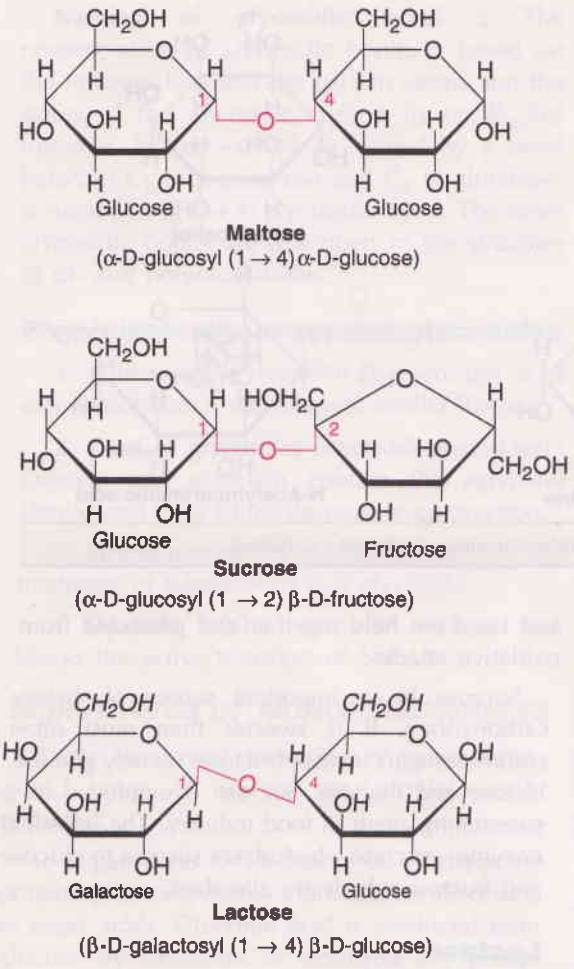


***Fig. 2.12** : Structures of disaccharides —maltose, sucrose and lactose.*

hydrolysed mixture of sucrose, containing glucose and fructose, is known as ***invert sugar.*** The process of inversion is explained below.

Hydrolysis of sucrose by the enzyme ***sucrase*** (***invertase***) or dilute acid liberates one molecule each of glucose and fructose. It is postulated that sucrose (dextro) is first split into α-D-glucopyranose (+52.5°) and β-D-fructofuranose, both being dextrorotatory. However, β-D-fructofuranose is less stable and immediately gets converted to β-D-fructopyranose which is strongly levorotatory (–92°). The overall effect is that dextro sucrose (+66.5°) on inversion is converted to levo form (–28.2°).

POLYSACCHARIDES

Polysaccharides (or simply ***glycans***) consist of repeat units of monosaccharides or their derivatives, held together by glycosidic bonds. They are primarily concerned with two important functions-structural, and storage of energy.

Polysaccharides are linear as well as branched polymers. This is in contrast to structure of proteins and nucleic acids which are only linear polymers. The occurrence of branches in polysaccharides is due to the fact that glycosidic linkages can be formed at any one of the hydroxyl groups of a monosaccharide.

Polysaccharides are of two types

1. **Homopolysaccharides** which on hydrolysis yield only a single type of monosaccharide. They are named based on the nature of the monosaccharide unit. Thus, ***glucans*** are polymers of glucose whereas ***fructosans*** are polymers of fructose.

2. **Heteropolysaccharides** on hydrolysis yield a mixture of a few monosaccharides or their derivatives.

HOMOPOLYSACCHARIDES

Starch

Starch is the carbohydrate reserve of plants which is the most important dietary source for higher animals, including man. High content of starch is found in cereals, roots, tubers, vegetables etc. Starch is a homopolymer composed of D-glucose units held by α-glycosidic bonds. It is known as ***glucosan or glucan.***

Starch consists of two polysaccharide components-water soluble ***amylose*** (15-20%) and a water insoluble ***amylopectin*** (80-85%). Chemically, amylose is a long unbranched chain with 200–1,000 D-glucose units held by α (1 → 4) glycosidic linkages. Amylopectin, on the other hand, is a branched chain with α (1 → 6) glycosidic bonds at the branching points and α (1 → 4) linkages everywhere else (***Fig.2.13***). Amylopectin molecule containing a few

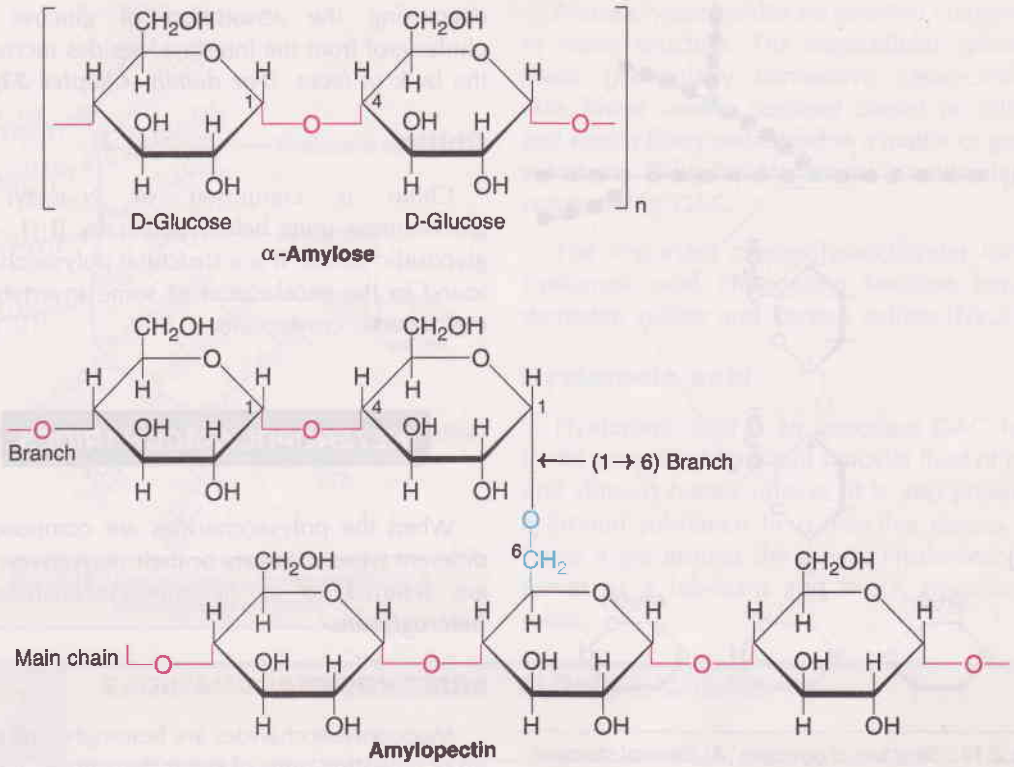


Fig. 2.13 : Structure of starch (α-amylose and amylopectin).

thousand glucose units looks like a branched tree (20–30 glucose units per branch).

Starches are hydrolysed by ***amylase*** (pancreatic or salivary) to liberate dextrins, and finally maltose and glucose units. Amylase acts specifically on α (1 → 4) glycosidic bonds.

Dextrins

Dextrins are the ***breakdown products of starch*** by the enzyme amylase or dilute acids. Starch is sequentially hydrolysed through different dextrins and, finally, to maltose and glucose. The various intermediates (identified by iodine colouration) are soluble starch (blue), ***amylodextrin*** (violet), ***erythrodextrin*** (red) and ***achrodextrin*** (no colour).

Inulin

Inulin is a polymer of fructose i.e., ***fructosan.*** It occurs in dahlia bulbs, garlic, onion etc. It is a low molecular weight (around 5,000) polysaccharide easily soluble in water. Inulin is not utilized by the body. It is used for assessing kidney function through measurement of ***glomerular filtration rate (GFR).***

Glycogen

Glycogen is the carbohydrate reserve in animals, hence often referred to as ***animal starch.*** It is present in high concentration in liver, followed by muscle, brain etc. Glycogen is also found in plants that do not possess chlorophyll (e.g. yeast, fungi).

The structure of glycogen is similar to that of amylopectin with more number of branches. ***Glucose*** is the repeating unit in glycogen joined together by α (1 → 4) glycosidic bonds, and α (1 → 6) glycosidic bonds at branching points (***Fig.2.14***). The molecular weight (up to 1×10^8) and the number of glucose units (up to 25,000) vary in glycogen depending on the source from which glycogen is obtained.

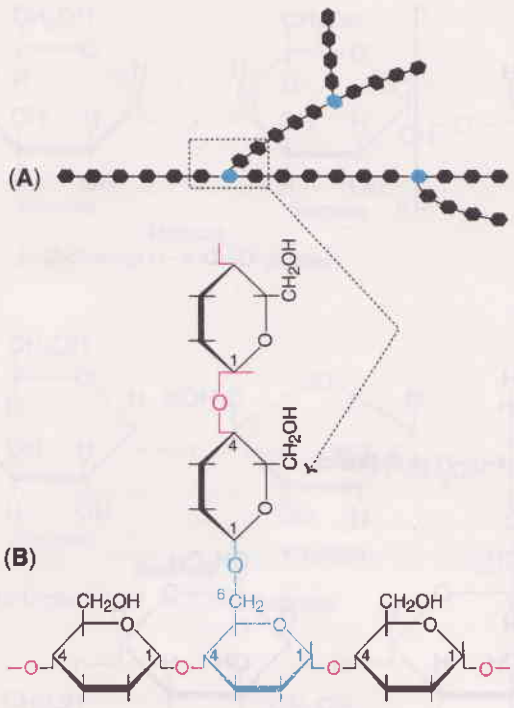


Fig. 2.14 : Structure of glycogen (A) General structure (B) Enlarged at a branch point.

Cellulose

Cellulose occurs exclusively in plants and it is the most abundant organic substance in plant kingdom. It is a predominant constituent of plant cell wall. Cellulose is totally absent in animal body.

Cellulose is composed of ***β-D-glucose*** units linked by β ***(1 → 4) glycosidic bonds*** (***Fig.2.15***). Cellulose cannot be digested by mammals—including man—due to lack of the enzyme that cleaves β-glycosidic bonds (α amylase breaks α bonds only). Certain ruminants and herbivorous animals contain microorganisms in the gut which produce enzymes that can cleave β-glycosidic bonds. Hydrolysis of cellulose yields a disaccharide ***cellobiose,*** followed by ***β-D-glucose.***

Cellulose, though not digested, has great importance in human nutrition. It is a major constituent of ***fiber,*** the non-digestable carbohydrate. The functions of dietary fiber include decreasing the absorption of glucose and cholesterol from the intestine, besides increasing the bulk of feces. (***For details, Chapter 23***)

Chitin

Chitin is composed of N-acetyl D-glucosamine units held together by β (1 → 4) glycosidic bonds. It is a structural polysaccharide found in the exoskeleton of some invertebrates e.g. insects, crustaceans.

HETEROPOLYSACCHARIDES

When the polysaccharides are composed of different types of sugars or their derivatives, they are referred to as heteropolysaccharides or ***heteroglycans.***

MUCOPOLYSACCHARIDES

Mucopolysaccharides are heteroglycans made up of repeating units of sugar derivatives, namely amino sugars and uronic acids. These are more commonly known as ***glycosaminoglycans (GAG)***. Acetylated amino groups, besides sulfate and carboxyl groups are generally present in GAG structure. The presence of sulfate and carboxyl groups contributes to acidity of the molecules, making them acid mucopolysaccharides.

Some of the mucopolysaccharides are found in combination with proteins to form ***mucoproteins*** or ***mucoids*** or ***proteoglycans*** (***Fig.2.16***). Mucoproteins may contain up to 95% carbohydrate and 5% protein.

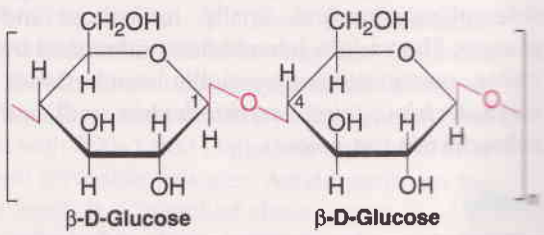


Fig. 2.15 : Structure of cellulose (The repeating unit may be several thousands).

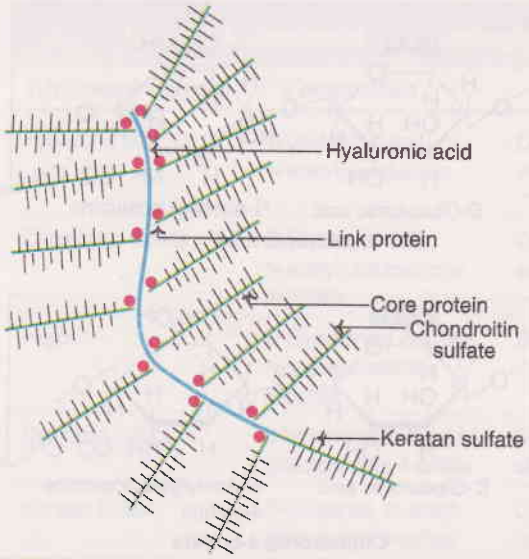


Fig. 2.16 : Diagrammatic representation of a proteoglycan complex.

Mucopolysaccharides are essential components of tissue structure. The extracellular spaces of tissue (particularly connective tissue-cartilage, skin, blood vessels, tendons) consist of collagen and elastin fibers embedded in a matrix or ground substance. The ground substance is predominantly composed of GAG.

The important mucopolysaccharides include hyaluronic acid, chondroitin 4-sulfate, heparin, dermatan sulfate and keratan sulfate (***Fig.2.17***).

Hyaluronic acid

Hyaluronic acid is an important GAG found in the ground substance of synovial fluid of joints and vitreous humor of eyes. It is also present as a ground substance in connective tissues, and forms a gel around the ovum. Hyaluronic acid serves as a lubricant and shock absorbant in joints.

BIOMEDICAL / CLINICAL CONCEPTS

- *Glucose is the most important energy source of carbohydrates to the mammals (except ruminants). The bulk of dietary carbohydrate (starch) is digested and finally absorbed as glucose into the body.*
- *Dextrose (glucose in solution in dextrorotatory form) is frequently used in medical practice.*
- *Fructose is abundantly found in the semen which is utilized by the sperms for energy.*
- *Several diseases are associated with carbohydrates e.g., diabetes mellitus, glycogen storage diseases, galactosemia.*
- *Accumulation of sorbitol and dulcitol in the tissues may cause certain pathological conditions e.g. cataract, nephropathy.*
- *Inulin, a polymer of fructose, is used to assess renal function by measuring glomerular filtration rate (GFR).*
- *The non-digestible carbohydrate cellulose plays a significant role in human nutrition. These include decreasing the intestinal absorption of glucose and cholesterol, and increasing bulk of feces to avoid constipation.*
- *The mucopolysaccharide hyaluronic acid serves as a lubricant and shock absorbant in joints.*
- *The enzyme hyaluronidase of semen degrades the gel (contains hyaluronic acid) around the ovum. This allows effective penetration of sperm into the ovum.*
- *The mucopolysaccharide heparin is an anticoagulant (prevents blood clotting).*
- *The survival of Antarctic fish below –2°C is attributed to the antifreeze glycoproteins.*
- *Streptomycin is a glycoside employed in the treatment of tuberculosis.*

Hyaluronic acid is composed of alternate units of D-glucuronic acid and N-acetyl D-glucosamine. These two molecules form disaccharide units held together by β (1 → 3) glycosidic bond (***Fig.2.16***). Hyaluronic acid contains about 250–25,000 disaccharide units (held by β 1 → 4 bonds) with a molecular weight up to 4 million.

Hyaluronidase is an enzyme that breaks (β 1 → 4 linkages) hyaluronic acid and other GAG. This enzyme is present in high concentration in testes, seminal fluid, and in certain snake and insect venoms. Hyaluronidase of semen is assigned an important role in fertilization as this enzyme clears the gel (hyaluronic acid) around the ovum allowing a better penetration of sperm into the ovum. Hyaluronidase of bacteria helps their invasion into the animal tissues.

Chondroitin sulfates

Chondroitin 4-sulfate (*Greek*: chondros-cartilage) is a major constituent of various mammalian tissues (bone, cartilage, tendons, heart, valves, skin, cornea etc.). Structurally, it is comparable with hyaluronic acid. Chondroitin 4-sulfate consists of repeating disaccharide units composed of D-glucuronic acid and N-acetyl D-galactosamine 4-sulfate (***Fig.2.17***).

Chondroitin 6-sulfate is also present in many tissues. As evident from the name, the sulfate group is found on C_6 instead of C_4.

Heparin

Heparin is an anticoagulant (prevents blood clotting) that occurs in blood, lung, liver, kidney, spleen etc. Heparin helps in the release of the enzyme lipoprotein lipase which helps in clearing the turbidity of lipemic plasma.

Heparin is composed of alternating units of N-sulfo D-glucosamine 6-sulfate and glucuronate 2-sulfate (***Fig.2.17***).

Dermatan sulfate

The name dermatan sulfate is derived from the fact that this compound mostly occurs in the skin. It is structurally related to chondroitin

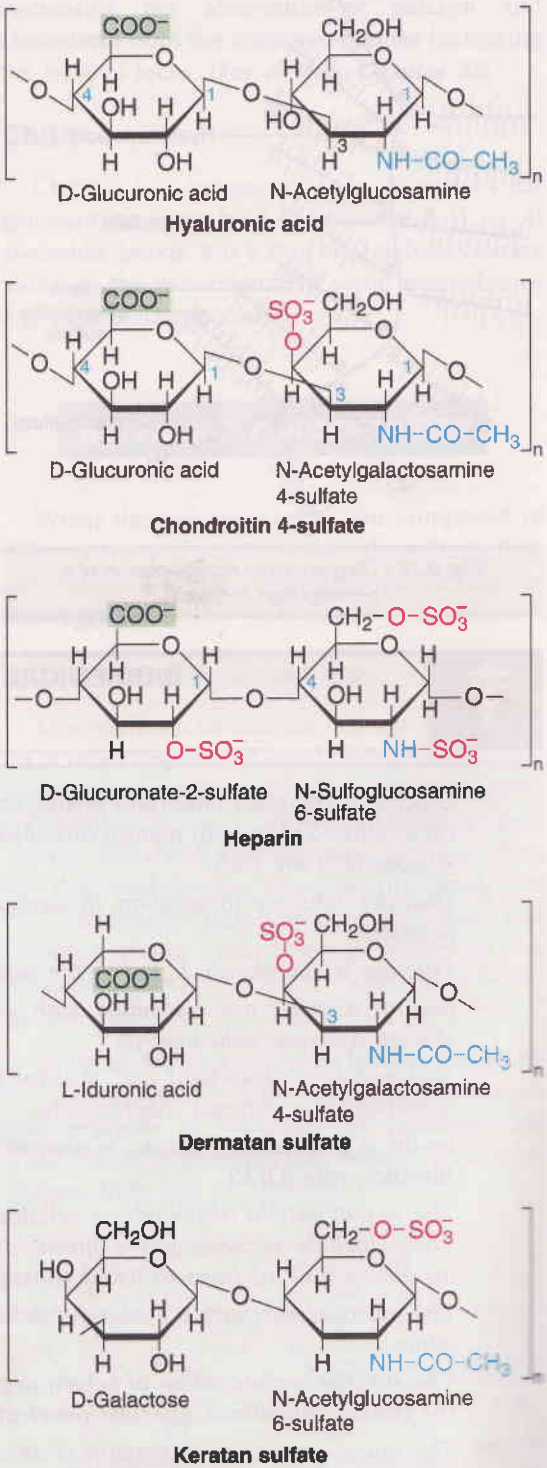


Fig. 2.17 : Structures of common glycosaminoglycans – the disaccharides as repeating units.

Table 2.3 A summary of glycosaminoglycans – composition, distribution and functions

Glycosaminoglycan	*Composition*	*Tissue distribution*	*Function(s)*
Hyaluronic acid	D-Glucuronic acid, N-acetylglucosamine	Connective tissue, synovial fluid, vitrous humor	Serves as a lubricant, and shock absorber. Promotes wound healing
Chondroitin sulfate	D-Glucuronic acid, N-acetylgalactosamine 4-sulfate	Cartilage, bone, skin, blood vessel walls	Helps to maintain the structure and shapes of tissues
Heparin	D-Glucuronate 2-sulfate, N-sulfoglucosamine 6-sulfate	Blood, lung, liver, kidney, spleen	Acts as an anticoagulant
Dermatan sulfate	L-Iduronic acid, N-acetyl-galactosamine 4-sulfate	Blood vessel valves, heart valves, skin	Maintains the shapes of tissues
Keratan sulfate	D-Galactose, N-acetyl-glucosamine 6-sulfate	Cartilage, cornea, connective tissues	Keeps cornea transparent

4-sulfate. The only difference is that there is an inversion in the configuration around C_5 of D-glucuronic acid to form L-iduronic acid (***Fig.2.17***).

Keratan sulfate

It is a heterogeneous GAG with a variable sulfate content, besides small amounts of mannose, fructose, sialic acid etc. Keratan sulfate essentially consists of alternating units of D-galactosamine and N-acetylglucosamine 6-sulfate.

A summary of the glycosaminoglycans with regard to composition, distribution and functions is given in ***Table 2.3***.

GLYCOPROTEINS

Several proteins are covalently bound to carbohydrates which are referred to as glycoproteins. The carbohydrate content of glycoprotein varies from 1% to 90% by weight. Sometimes the term ***mucoprotein*** is used for glycoprotein with carbohydrate concentration more than 4%. Glycoproteins are very widely distributed in the cells and perform variety of functions. These include their role as enzymes, hormones, transport proteins, structural proteins and receptors. A selected list of glycoproteins and their major functions is given in ***Table 2.4***.

The carbohydrates found in glycoproteins include mannose, galactose, N-acetylglucosamine, N-acetylgalactosamine, xylose, L-fucose and N-acetylneuraminic acid (NANA). NANA is an important sialic acid (***See Fig.2.11***).

Antifreeze glycoproteins : The Antarctic fish live below –2°C, a temperature at which the

Table 2.4 A selected list of glycoproteins and their major functions

Glycoprotein(s)	*Major function(s)*
Collagen	Structure
Hydrolases, proteases, glycosidases	Enzymes
Ceruloplasmin	Transport
Immunoglobulins	Defense against infection
Synovial glycoproteins	Lubrication
Thyrotropin, erythropoietin	Hormones
Blood group substances	Antigens
Fibronectin, laminin	Cell-cell recognition and adhesion
Intrinsic factor	Absorption of vitamin B_{12}
Fibrinogen	Blood clotting

blood would freeze. It is now known that these fish contain ***antifreeze glycoprotein*** which lower the freezing point of water and interfere with the crystal formation of ice. Antifreeze glycoproteins consist of 50 repeating units of the tripeptide, ***alanine-alanine-threonine***. Each threonine residue is bound to β-galactosyl (1 → 3) α N-acetylgalactosamine.

Blood group substances

The blood group antigens (of erythrocyte membrane) contain carbohydrates as glycoproteins or glycolipids. N-Acetylgalactosamine, galactose, fucose, sialic acid etc. are found in the blood group substances. The carbohydrate content also plays a determinant role in blood grouping.

SUMMARY

1. *Carbohydrates are the polyhydroxyaldehydes or ketones, or compounds which produce them on hydrolysis. The term sugar is applied to carbohydrates soluble in water and sweet to taste. Carbohydrates are the major dietary energy sources, besides their involvement in cell structure and various other functions.*
2. *Carbohydrates are broadly classified into 3 groups—monosaccharides, oligosaccharides and polysaccharides. The monosaccharides are further divided into different categories based on the presence of functional groups (aldoses or ketoses) and the number of carbon atoms (trioses, tetroses, pentoses, hexoses and heptoses).*
3. *Glyceraldehyde (triose) is the simplest carbohydrate and is chosen as a reference to write the configuration of all other monosaccharides (D- and L- forms). If two monosaccharides differ in their structure around a single carbon atom, they are known as epimers. Glucose and galactose are C_4 – epimers.*
4. *D-Glucose is the most predominant naturally occurring aldose/monosaccharide. Glucose exists as α and β anomers with different optical rotations. The interconversion of α and β anomeric forms with change in the optical rotation is known as mutarotation.*
5. *Monosaccharides participate in several reactions. These include oxidation, reduction, dehydration, osazone formation etc. Formation of esters and glycosides by monosaccharides is of special significance in biochemical reactions.*
6. *Among the oligosaccharides, disaccharides are the most common. These include the reducing disaccharides namely lactose (milk sugar) and maltose (malt sugar) and the non-reducing sucrose (cane sugar).*
7. *Polysaccharides are the polymers of monosaccharides or their derivatives, held together by glycosidic bonds. Homopolysaccharides are composed of a single monosaccharide (e.g., starch, glycogen, cellulose, inulin). Heteropolysaccharides contain a mixture of few monosaccharides or their derivatives (e.g., mucopolysaccharides).*
8. *Starch and glycogen are the carbohydrate reserves of plants and animals respectively. Cellulose, exclusively found in plants, is the structural constituent. Inulin is utilized to assess kidney function by measuring glomerular filtration rate (GFR).*
9. *Mucopolysaccharides (glycosaminoglycans) are the essential components of tissue structure. They provide the matrix or ground substance of extracellular tissue spaces in which collagen and elastin fibers are embedded. Hyaluronic acid, chondroitin 4-sulfate, heparin, are among the important glycosaminoglycans.*
10. *Glycoproteins are a group of biochemically important compounds with a variable composition of carbohydrate (1-90%), covalently bound to protein. Several enzymes, hormones, structural proteins and cellular receptors are in fact glycoproteins.*

SELF-ASSESSMENT EXERCISES

I. Essay questions

1. Define and classify carbohydrates with suitable examples. Add a note on the functions of carbohydrates.
2. Describe the structure and functions of mucopolysaccharides.
3. Give an account of the structural configuration of monosaccharides, with special reference to glucose.
4. Discuss the structure and functions of 3 biochemically important disaccharides.
5. Define polysaccharides and describe the structure of 3 homopolysaccharides.

II. Short notes

(a) Epimers, (b) Mutarotation, (c) Osazone formation, (d) Glycosidic bond, (e) Sugar derivatives, (f) Anomers, (g) Enediol, (h) Amino sugars, (i) Inversion of sucrose, (j) Deoxysugars.

III. Fill in the blanks

1. Name a non-reducing disaccharide _____.
2. The carbohydrate that is taken as a reference for writing the configuration of others _____.
3. If two monosaccharides differ in configuration around a single carbon atom, they are known as _____.
4. The α and β cyclic forms of D-glucose are referred to as _____.
5. The non-carbohydrate moiety found in glycosides is known as _____.
6. Give an example of a glycoside antibiotic _____.
7. The glycosidic bonds at the branching points in the structure of starch are _____.
8. The polysaccharide employed for the assessment of kidney function _____.
9. The glycosaminoglycan that serves as a lubricant and shock absorbant of joints _____.
10. Name the sialic acid, mostly found in the structure of glycoproteins and glycolipids _____.

IV. Multiple choice questions

11. Ribose and deoxyribose differ in structure around a single carbon, namely

    (a) C_1 (b) C_2 (c) C_3 (d) C_4.

12. One of the following is not an aldose

    (a) Glucose (b) Galactose (c) Mannose (d) Fructose.

13. The glycosaminoglycan that serves as an anticoagulant

    (a) Heparin (b) Hyaluronic acid (c) Chondroitin sulfate (d) Dermatan sulfate.

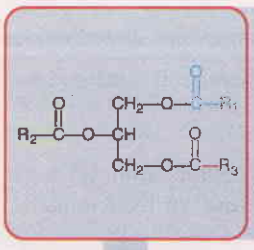
14. The following polysaccharide is composed of β-glycosidic bonds

    (a) Starch (b) Glycogen (c) Dextrin (d) Cellulose.

15. The carbon atoms involved in the osazone formation

    (a) 1 and 2 (b) 2 and 3 (c) 3 and 4 (d) 5 and 6.

3 Lipids



The fat speaks :

"With water, I say, 'Touch me not';
To the tongue, I am tasteful;
Within limits, I am dutiful;
In excess, I am dangerous!"

Lipids (*Greek*: lipos–fat) are of great importance to the body as the chief concentrated storage form of energy, besides their role in cellular structure and various other biochemical functions. As such, lipids are a ***heterogeneous group*** of compounds and, therefore, it is rather difficult to define them precisely.

Lipids may be regarded as organic substances relatively insoluble in water, soluble in organic solvents (alcohol, ether etc.), actually or potentially related to fatty acids and utilized by the living cells.

Unlike the polysaccharides, proteins and nucleic acids, lipids are not polymers. Further, lipids are mostly small molecules.

Classification of lipids

Lipids are broadly classified (modified from Bloor) into simple, complex, derived and miscellaneous lipids, which are further subdivided into different groups

1. **Simple lipids :** Esters of fatty acids with alcohols. These are mainly of two types

   (a) **Fats and oils** (triacylglycerols) **:** These are esters of fatty acids with glycerol. The ***difference*** between fat and oil is only ***physical***. Thus, oil is a liquid while fat is a solid at room temperature.

   (b) **Waxes :** Esters of fatty acids (usually long chain) with alcohols other than glycerol. These alcohols may be aliphatic or alicyclic. Cetyl alcohol is most commonly found in waxes.

2. **Complex (or compound) lipids :** These are esters of fatty acids with alcohols containing additional groups such as phosphate, nitrogenous base, carbohydrate, protein etc. They are further divided as follows

   (a) **Phospholipids :** They contain phosphoric acid and frequently a nitrogenous base. This is in addition to alcohol and fatty acids.

(i) **Glycerophospholipids :** These phospholipids contain glycerol as the alcohol e.g., lecithin, cephalin.

(ii) **Sphingophospholipids :** Sphingosine is the alcohol in this group of phospholipids e.g., sphingomyelin.

(b) **Glycolipids :** These lipids contain a fatty acid, carbohydrate and nitrogenous base. The alcohol is sphingosine, hence they are also called as glycosphingolipids. Glycerol and phosphate are absent e.g., cerebrosides, gangliosides.

(c) **Lipoproteins :** Macromolecular complexes of lipids with proteins.

(d) **Other complex lipids :** Sulfolipids, aminolipids and lipopolysaccharides are among the other complex lipids.

3. **Derived lipids :** These are the derivatives obtained on the hydrolysis of group 1 and group 2 lipids which possess the characteristics of lipids. These include glycerol and other alcohols, fatty acids, mono- and diacylglycerols, lipid (fat) soluble vitamins, steroid hormones, hydrocarbons and ketone bodies.

4. **Miscellaneous lipids :** These include a large number of compounds possessing the characteristics of lipids e.g., carotenoids, squalene, hydrocarbons such as pentacosane (in bees wax), terpenes etc.

NEUTRAL LIPIDS : The lipids which are uncharged are referred to as neutral lipids. These are mono-, di-, and triacylglycerols, cholesterol and cholesteryl esters.

Functions of lipids

Lipids perform several important functions

1. They are the concentrated fuel reserve of the body (triacylglycerols).

2. Lipids are the constituents of membrane structure and regulate the membrane permeability (phospholipids and cholesterol).

3. They serve as a source of fat soluble vitamins (A, D, E and K).

4. Lipids are important as cellular metabolic regulators (steroid hormones and prostaglandins).

5. Lipids protect the internal organs, serve as insulating materials and give shape and smooth appearance to the body.

FATTY ACIDS

Fatty acids are carboxylic acids with hydrocarbon side chain. They are the simplest form of lipids.

Occurrence

Fatty acids mainly occur in the esterified form as major constituents of various lipids. They are also present as free (unesterified) fatty acids. Fatty acids of animal orgin are much simpler in structure in contrast to those of plant origin which often contain groups such as epoxy, keto, hydroxy and cyclopentane rings.

Even and odd carbon fatty acids

Most of the fatty acids that occur in natural lipids are of even carbons (usually 14C – 20C). This is due to the fact that biosynthesis of fatty acids mainly occurs with the sequential addition of 2 carbon units. ***Palmitic acid (16C) and stearic acid (18C) are the most common.*** Among the odd chain fatty acids, propionic acid (3C) and valeric acid (5C) are well known.

Saturated and unsaturated fatty acids

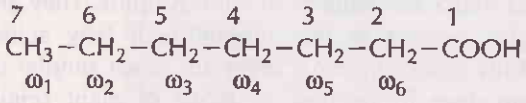
Saturated fatty acids do not contain double bonds, while unsaturated fatty acids contain one or more double bonds. Both saturated and unsaturated fatty acids almost equally occur in the natural lipids. Fatty acids with one double bond are monounsaturated, and those with 2 or more double bonds are collectively known as ***polyunsaturated fatty acids (PUFA).***

Nomenclature of fatty acids

The naming of a fatty acid (systematic name) is based on the hydrocarbon from which it is derived. The saturated fatty acids end with a suffix ***-anoic*** (e.g., octanoic acid) while the unsaturated fatty acids end with a suffix ***-enoic***

(e.g., octadecanoic acid). In addition to systematic names, fatty acids have common names which are more widely used (***Table 3.1***).

Numbering of carbon atoms : It starts from the carboxyl carbon which is taken as number 1. The carbons adjacent to this (carboxyl C) are 2, 3, 4 and so on or alternately α, β, γ and so on. The terminal carbon containing methyl group is known omega (ω) carbon. Starting from the methyl end, the carbon atoms in a fatty acid are numbered as omega 1, 2, 3 etc. The numbering of carbon atoms in two different ways is given below



Length of hydrocarbon chain of fatty acids

Depending on the length of carbon chains, fatty acids are categorized into 3 groups—***short chain*** with less than 6 carbons; ***medium chain*** with 8 to 14 carbons and ***long chain*** with 16 to 24 carbons.

Shorthand representation of fatty acids

Instead of writing the full structures, biochemists employ shorthand notations (by numbers) to represent fatty acids. The general rule is that the total number of carbon atoms are written first, followed by the number of double bonds and finally the (first carbon) position of

Table 3.1 Selected examples of biochemically important fatty acids

Common Name	*Systematic name*	*Abbreviation**	*Structure*
I. Saturated fatty acids			
Acetic acid	Ethanoic acid	2 : 0	CH_3COOH
Propionic acid	n-Propanoic acid	3 : 0	CH_3CH_2COOH
Butyric acid	n-Butanoic acid	4 : 0	$CH_3(CH_2)_2COOH$
Valeric acid	n-Pentanoic acid	5 : 0	$CH_3(CH_2)_3COOH$
Caproic acid	n-Hexanoic acid	6 : 0	$CH_3(CH_2)_4COOH$
Caprylic acid	n-Octanoic acid	8 : 0	$CH_3(CH_2)_6COOH$
Capric acid	n-Decanoic acid	10 : 0	$CH_3(CH_2)_8COOH$
Lauric acid	n-Dodecanoic acid	12 : 0	$CH_3(CH_2)_{10}COOH$
Myristic acid	n-Tetradecanoic acid	14 : 0	$CH_3(CH_2)_{12}COOH$
Palmitic acid	n-Hexadecanoic acid	16 : 0	$CH_3(CH_2)_{14}COOH$
Stearic acid	n-Octadecanoic acid	18 : 0	$CH_3(CH_2)_{16}COOH$
Arachidic acid	n-Eicosanoic acid	20 : 0	$CH_3(CH_2)_{18}COOH$
Behenic acid	n-Docosanoic acid	22 : 0	$CH_3(CH_2)_{20}COOH$
Lignoceric acid	n-Tetracosanoic acid	24 : 0	$CH_3(CH_2)_{22}COOH$
II. Unsaturated fatty acids			
Palmitoleic acid	*cis*-9-Hexadecenoic acid	16 : 1; 9	$CH_3(CH_2)_5CH = CH(CH_2)_7COOH$
Oleic acid	*cis*-9-Octadecenoic acid	18 : 1; 9	$CH_3(CH_2)_7CH = CH(CH_2)_7COOH$
Linoleic acid**	*cis, cis*-9,12–Octadeca-dienoic acid	18 : 2; 9, 12	$CH_3(CH_2)_4CH = CHCH_2CH = CH(CH_2)_7COOH$
Linolenic acid**	All *cis*–9,12,15–Octa-decatrienoic acid	18 : 3; 9, 12, 15	$CH_3CH_2CH = CHCH_2CH = CHCH_2CH = CH(CH_2)_7COOH$
Arachidonic acid	All *cis*–5,8,11,14–Eicosatetraenoic acid	20 : 4; 5, 8, 11, 14	$CH_3(CH_2)_4CH = CHCH_2CH = CHCH_2CH = CHCH_2CH = CH(CH_2)_3COOH$

** Total number of carbon atoms, followed by the number of double bonds and the first carbon position of the double bond(s).*

*** Essential fatty acids.*

double bonds, starting from the carboxyl end. Thus, saturated fatty acid, palmitic acid is written as 16 : 0, oleic acid as 18 : 1; 9, arachidonic acid as 20 : 4; 5, 8, 11, 14.

There are other conventions of representing the double bonds. Δ^9 indicates that the double bond is between 9 and 10 of the fatty acid. ω 9 represents the double bond position (9 and 10) from the ω end. Naturally occurring unsaturated fatty acids belong to ω 9, ω 6 and ω 3 series.

ω 3 series	Linolenic acid (18 : 3; 9, 12, 15)
ω 6 series	Linoleic acid (18 : 2; 9, 12) and arachidonic acid (20 : 4; 5, 8, 11, 14)
ω 9 series	Oleic acid (18 : 1; 9)

The biochemically important saturated and unsaturated fatty acids are given in the ***Table 3.1***.

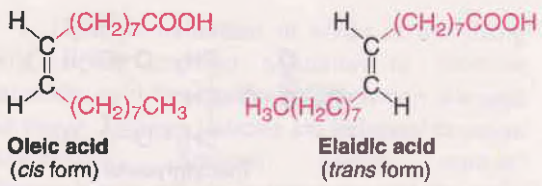
ESSENTIAL FATTY ACIDS

The fatty acids that cannot be synthesized by the body and, therefore, ***should be supplied in the diet*** are known as essential fatty acids (EFA). Chemically, they are ***polyunsaturated fatty acids***, namely ***linoleic acid*** (18 : 2; 9, 12) and ***linolenic acid*** (18 : 3; 9, 12, 15). ***Arachidonic acid*** (20 : 4; 5, 8, 11, 14) becomes essential, if its precursor linoleic acid is not provided in the diet in sufficient amounts. The structures of EFA are given in the ***Table 3.1***.

Biochemical basis for essentiality : Linoleic acid and linolenic acid are essential since humans lack the enzymes that can introduce double bonds beyond carbons 9 to 10.

Functions of EFA : Essential fatty acids are required for the membrane structure and function, transport of cholesterol, formation of lipoproteins, prevention of fatty liver etc. They are also needed for the synthesis of another important group of compounds, namely ***eicosanoids*** (***Chapter 32***).

Deficiency of EFA : The deficiency of EFA results in ***phrynoderma*** or ***toad skin***, characterized by the presence of horny eruptions on the posterior and lateral parts of limbs, on the back and buttocks, loss of hair and poor wound healing.



Oleic acid (*cis* form) **Elaidic acid** (*trans* form)

Fig. 3.1 : Cis-trans isomerism in unsaturated fatty acids.

Isomerism in unsaturated fatty acids

Unsaturated fatty acids exhibit ***geometric isomerism*** depending on the orientation of the groups around the double bond axis.

If the atoms or acyl groups are present on the same side of the double bond, it is a ***cis configuration***. On the other hand, if the groups occur on the opposite side, it is a ***trans configuration***. Thus oleic acid is a *cis* isomer while elaidic acid is a *trans* isomer, as depicted in ***Fig.3.1***. *Cis* isomers are less stable than *trans* isomers. Most of the naturally occurring unsaturated fatty acids exist as *cis* isomers.

In the *cis* isomeric form, there is a molecular binding at the double bond. Thus, oleic acid exists in an L-shape while elaidic acid is a straight chain. Increase in the number of double bonds will cause more bends (kinks) and arachidonic acid with 4 double bonds will have a U-shape. It is believed that *cis* isomers of fatty acids with their characteristic bonds will compactly pack the membrane structure.

Hydroxy fatty acids : Some of the fatty acids are hydroxylated. β-Hydroxybutyric acid, one of the ketone bodies produced in metabolism, is a simple example of hydroxy fatty acids. Cerebronic acid and recinoleic acid are long chain hydroxy fatty acids.

Cyclic fatty acids : Fatty acids with cyclic structures are rather rare e.g., ***chaulmoogric acid*** found in chaulmoogra oil (used in leprosy treatment) contains cyclopentenyl ring.

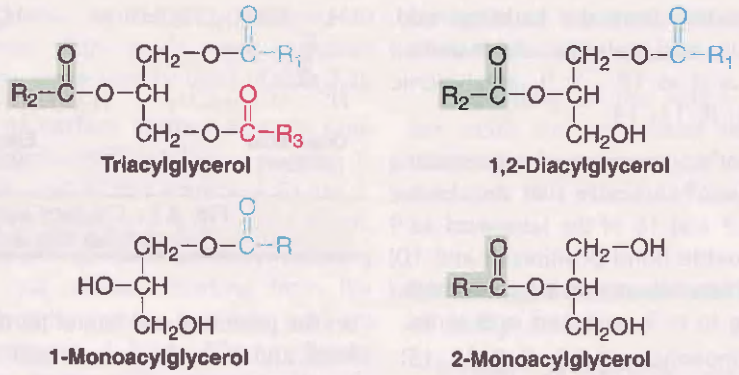


Fig. 3.2 : General structures of acylglycerols
(For palmitoyl R = $C_{15}H_{31}$; for stearoyl R = $C_{17}H_{35}$; For linoleoyl R = $C_{17}H_{31}$)

Eicosanoids : These compounds are related to eicosapolyenoic fatty acids and include prostaglandins, prostacyclins, leukotrienes and thromboxanes. They are discussed together (***Chapter 32***).

TRIACYLGLYCEROLS

Triacylglycerols (***formerly triglycerides***) are the esters of glycerol with fatty acids. The fats and oils that are widely distributed in both plants and animals are chemically triacylglycerols. They are insoluble in water and non-polar in character and commonly known as ***neutral fats***.

Fats as stored fuel : Triacylglycerols are the most abundant group of lipids that primarily function as fuel reserves of animals. The fat reserve of normal humans (men 20%, women 25% by weight) is sufficient to meet the body's caloric requirements for 2-3 months.

Fats primarily occur in adipose tissue : Adipocytes of adipose tissue—predominantly found in the subcutaneous layer and in the abdominal cavity—are specialized for storage of triacylglycerols. The fat is stored in the form of globules dispersed in the entire cytoplasm. And surprisingly, triacylglycerols are not the structural components of biological membranes.

Structures of acylglycerols : Monoacylglycerols, diacylglycerols and triacylglycerols, respectively consisting of one, two and three molecules of fatty acids esterified to a molecule of glycerol, are known (***Fig.3.2***). Among these, triacylglycerols are the most important biochemically.

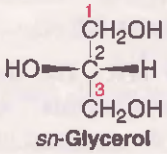
Simple triacylglycerols contain the same type of fatty acid residue at all the three carbons e.g., tristearoyl glycerol or tristearin.

Mixed triacylglycerols are more common. They contain 2 or 3 different types of fatty acid residues. In general, fatty acid attached to C_1 is saturated, that attached to C_2 is unsaturated while that on C_3 can be either. Triacylglycerols are named according to placement of acyl radical on glycerol e.g., 1,3-palmitoyl 2-linoleoyl glycerol.

Triacylglycerols of plants, in general, have higher content of unsaturated fatty acids compared to that of animals.

Stereospecific numbering of glycerol

The structure of glycerol gives an impression that carbons 1 and 3 are identical. This is not true in a 3-dimensional structure. In order to represent the carbon atoms of glycerol in an unambiguous manner, biochemists adopt a ***s***tereospecific ***n***umbering (***sn***) and prefix glycerol with ***sn***.



It should be noted that C_1 and C_3 are different. Cells possess enzymes that can distinguish these two carbons. Thus glycerokinase phosphorylates *sn*-3 (and not *sn*-1) glycerol to give *sn*-glycerol 3-phosphate.

PROPERTIES OF TRIACYLGLYCEROLS

A few important properties of triacylglycerols, which have biochemical relevance, are discussed below

1. **Hydrolysis :** Triacylglycerols undergo stepwise enzymatic hydrolysis to finally liberate free fatty acids and glycerol. The process of hydrolysis, catalysed by ***lipases*** is important for digestion of fat in the gastrointestinal tract and fat mobilization from the adipose tissues.

2. **Saponification :** The hydrolysis of triacylglycerols by alkali to produce glycerol and soaps is known as saponification.

Triacylglycerol + 3 NaOH ⟶
Glycerol + 3 R-COONa (soaps)

3. **Rancidity :** Rancidity is the term used to represent the deterioration of fats and oils resulting in an unpleasant taste. Fats containing unsaturated fatty acids are more susceptible to rancidity.

Rancidity occurs when fats and oils are exposed to air, moisture, light, bacteria etc. ***Hydrolytic rancidity*** occurs due to partial hydrolysis of triacylglycerols by bacterial enzymes. Oxidative rancidity is due to oxidation of unsaturated fatty acids. This results in the formation of unpleasant products such as dicarboxylic acids, aldehydes, ketones etc. Rancid fats and oils are unsuitable for human consumption.

Antioxidants : The substances which can prevent the occurrence of oxidative rancidity are known as antioxidants. Trace amounts of antioxidants such as tocopherols (vitamin E), hydroquinone, gallic acid and α-naphthol are added to the commercial preparations of fats and oils to prevent rancidity. Propyl gallate, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are the antioxidants used in food preservation.

4. **Lipid peroxidation *in vivo* :** In the living cells, lipids undergo oxidation to produce peroxides and free radicals which can damage the tissue. The free radicals are believed to cause inflammatory diseases, ageing, cancer, atherosclerosis etc. It is fortunate that the cells possess antioxidants such as vitamin E, urate and superoxide dismutase to prevent *in vivo* lipid peroxidation (***Chapter 34***).

Tests to check purity of fats and oils

Adulteration of fats and oils is increasing day by day. Several tests are employed in the laboratory to check the purity of fats and oils. Some of them are discussed hereunder

Iodine number : It is defined as the ***grams*** (number) ***of iodine absorbed by 100 g of fat*** or ***oil***. Iodine number is useful to know the relative unsaturation of fats, and is directly proportional to the content of unsaturated fatty acids. Thus lower is the iodine number, less is the degree of unsaturation. The iodine numbers of common oils/fats are given below.

Fat/oil	*Iodine number*
Coconut oil	7 — 10
Butter	25 — 28
Palm oil	45 — 55
Olive oil	80 — 85
Groundnut oil	85 — 100
Cottonseed oil	100 — 110
Sunflower oil	125 — 135
Linseed oil	175 — 200

Determination of iodine number will help to know the degree of adulteration of a given oil.

Saponification number : It is defined as the ***mg*** (number) ***of KOH required to hydrolyse (saponify) one gram of fat or oil***. Saponification number is a measure of the average molecular size of the fatty acids present. The value is higher for fats containing short chain fatty acids. The saponification numbers of a few fats and oils are given below

Human fat : 195–200
Butter : 230–240
Coconut oil : 250–260

Reichert-Meissl (RM) number : It is defined as the number of ml 0.1 N KOH required to completely neutralize the soluble volatile fatty acids distilled from 5 g fat. RM number is useful in testing the purity of butter since it contains a good concentration of volatile fatty acids (butyric acid, caproic acid and caprylic acid). This is in contrast to other fats and oils which have a negligible amount of volatile fatty acids. Butter has a RM number in the range 25-30, while it is less than 1 for most other edible oils. Thus any ***adulteration of butter can be easily tested*** by this sensitive RM number.

Acid number : It is defined as the number of mg of KOH required to completely neutralize free fatty acids present in one gram fat or oil. In normal circumstances, refined oils should be free from any free fatty acids. Oils, on decomposition—due to chemical or bacterial contamination—yield free fatty acids. Therefore, oils with increased acid number are unsafe for human consumption.

PHOSPHOLIPIDS

These are complex or ***compound lipids*** containing phosphoric acid, in addition to fatty acids, nitrogenous base and alcohol (***Fig.3.3***).

There are two classes of phospholipids

1. Glycerophospholipids (or phosphoglycerides) that contain glycerol as the alcohol.

2. Sphingophospholipids (or sphingomyelins) that contain sphingosine as the alcohol.

Glycerophospholipids

Glycerophospholipids are the major lipids that occur in biological membranes. They consist of glycerol 3-phosphate esterified at its C_1 and C_2 with fatty acids. Usually, C_1 contains a saturated fatty acid while C_2 contains an unsaturated fatty acid.

1. **Phosphatidic acid :** This is the simplest phospholipid. It does not occur in good concentration in the tissues. Basically, phosphatidic acid is an intermediate in the synthesis of triacylglycerols and phospholipids.

The other glycerophospholipids containing different nitrogenous bases or other groups may be regarded as the derivatives of phosphatidic acid.

2. **Lecithins (*phosphatidylcholine*):** These are the most abundant group of phospholipids in the cell membranes. Chemically, lecithin (*Greek* : lecithos—egg yolk) is a phosphatidic acid with choline as the base. Phosphatidylcholines represent the ***storage form of body's choline***.

BIOMEDICAL / CLINICAL CONCEPTS

- *Lipids are important to the body as constituents of membranes, source of fat soluble (A, D, E and K) vitamins and metabolic regulators (steroid hormones and prostaglandins).*
- *Triacylglycerols (fats) primarily stored in the adipose tissue are concentrated fuel reserves of the body. Fats found in the subcutaneous tissue and around certain organs serve as thermal insulators.*
- *The unsaturated fatty acids–linoleic and linolenic acid—are essential to humans, the deficiency of which causes phrynoderma or toad skin.*
- *The cyclic fatty acid, namely chaulmoogric acid,is employed in the treatment of leprosy.*
- *Fats and oils on exposure to air, moisture, bacteria etc. undergo rancidity (deterioration). This can be prevented by the addition of certain antioxidants (vitamin E, hydroquinone, gallic acid).*
- *In food preservation, antioxidants—namely propyl gallate, butylated hydroxyanisole and butylated hydroxytoluene—are commonly used.*

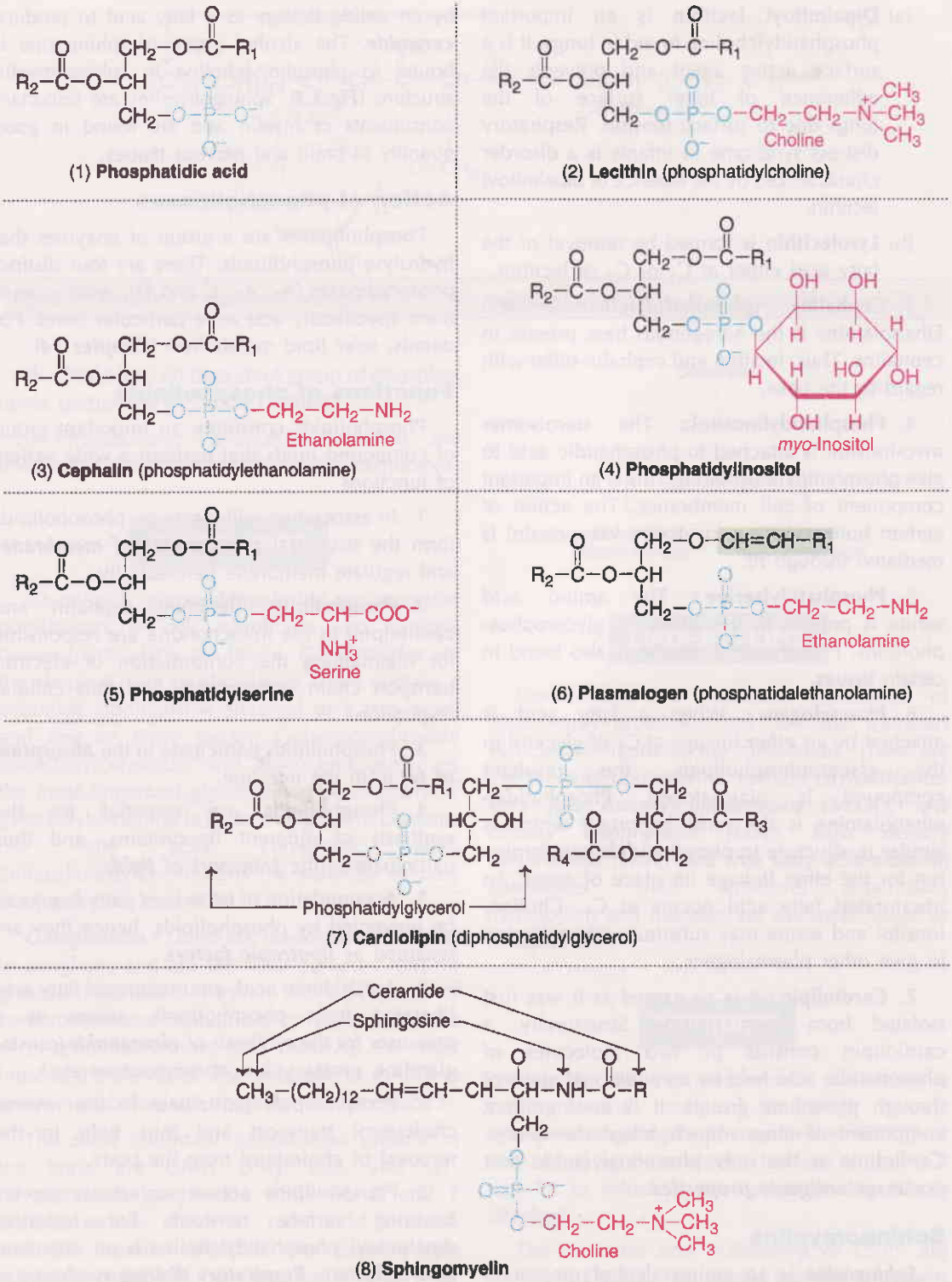


Fig. 3.3 : Structures of phospholipids.

(a) **Dipalmitoyl lecithin** is an important phosphatidylcholine found in lungs. It is a surface active agent and prevents the adherence of inner surface of the lungs due to surface tension. Respiratory distress syndrome in infants is a disorder characterized by the absence of dipalmitoyl lecithin.

(b) **Lysolecithin** is formed by removal of the fatty acid either at C_1 or C_2 of lecithin.

3. **Cephalins** (***phosphatidylethanolamine***) : Ethanolamine is the nitrogenous base present in cephalins. Thus, lecithin and cephalin differ with regard to the base.

4. **Phosphatidylinositol :** The steroisomer *myo*-inositol is attached to phosphatidic acid to give phosphatidylinositol(PI). This is an important component of cell membranes. The action of certain hormones (e.g. oxytocin, vasopressin) is mediated through PI.

5. **Phosphatidylserine :** The amino acid serine is present in this group of glycerophospholipids. Phosphatidylthreonine is also found in certain tissues.

6. **Plasmalogens :** When a fatty acid is attached by an ***ether*** linkage at C_1 of glycerol in the glycerophospholipids, the resultant compound is plasmalogen. Phosphati***dal***-ethanolamine is the most important which is similar in structure to phosphati***dyl***ethanolamine but for the ether linkage (in place of ester). An unsaturated fatty acid occurs at C_1. Choline, inositol and serine may substitute ethanolamine to give other plasmalogens.

7. **Cardiolipin :** It is so named as it was first isolated from heart muscle. Structurally, a cardiolipin consists of two molecules of phosphatidic acid held by an additional glycerol through phosphate groups. It is an important component of inner mitochondrial membrane. Cardiolipin is the only phosphoglyceride that possesses ***antigenic properties***.

Sphingomyelins

Sphingosine is an amino alcohol present in sphingomyelins (sphingophospholipids). They do not contain glycerol at all. Sphingosine is attached by an amide linkage to a fatty acid to produce ***ceramide***. The alcohol group of sphingosine is bound to phosphorylcholine in sphingomyelin structure (***Fig.3.3***). Sphingomyelins are important constituents of myelin and are found in good quantity in brain and nervous tissues.

Action of phospholipases

Phospholipases are a group of enzymes that hydrolyse phospholipids. There are four distinct phospholipases (A_1, A_2, C and D), each one of them specifically acts on a particular bond. For details, refer lipid metabolism (***Chapter 14***).

Functions of phospholipids

Phospholipids constitute an important group of compound lipids that perform a wide variety of functions

1. In association with proteins, phospholipids form the structural ***components of membranes*** and regulate membrane permeability.

2. Phospholipids (lecithin, cephalin and cardiolipin) in the mitochondria are responsible for maintaining the conformation of electron transport chain components, and thus cellular respiration.

3. Phospholipids participate in the ***absorption of fat*** from the intestine.

4. Phospholipids are essential for the synthesis of different lipoproteins, and thus participate in the ***transport of lipids***.

5. Accumulation of fat in liver (fatty liver) can be prevented by phospholipids, hence they are regarded as ***lipotropic factors***.

6. Arachidonic acid, an unsaturated fatty acid liberated from phospholipids, serves as a precursor for the synthesis of ***eicosanoids*** (prostaglandins, prostacyclins, thromboxanes etc.).

7. Phospholipids participate in the reverse cholesterol transport and thus help in the removal of cholesterol from the body.

8. Phospholipids act as surfactants (agents lowering surface tension). For instance, dipalmitoyl phosphatidylcholine is an important lung surfactant. ***Respiratory distress syndrome*** in infants is associated with insufficient production of this surfactant.

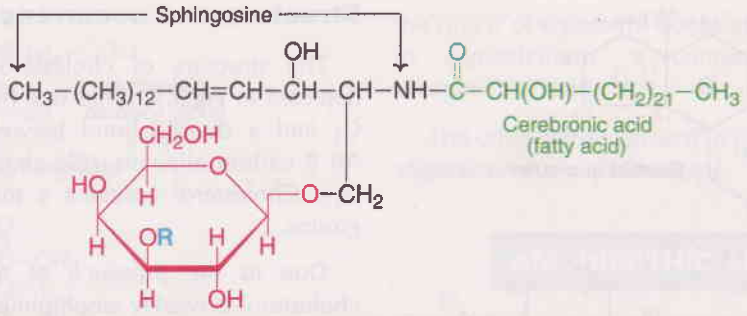


Fig. 3.4 : Structure of galactosylceramide (R = H). For sulfagalactosylceramide R is a sulfatide ($R = SO_4^{2-}$).

9. Cephalins, an important group of phospholipids participate in blood clotting.

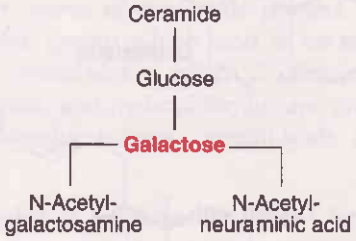
10. Phospholipids (phosphatidylinositol) are involved in signal transmission across membranes.

GLYCOLIPIDS

Glycolipids (***glycosphingolipids***) are important constituents of cell membrane and nervous tissues (particularly the brain). ***Cerebrosides*** are the simplest form of glycolipids. They contain a ceramide (sphingosine attached to a fatty acid) and one or more sugars. Galactocerebroside (galactosylceramide) and glucocerebroside are the most important glycolipids. The structure of galactosylceramide is given in ***Fig.3.4***. It contains the fatty acid cerebronic acid. Sulfagalactosylceramide is the ***sulfatide*** derived from galactosylceramide.

Gangliosides : These are predominantly found in ganglions and are the most complex form of glycosphingolipids. They are the derivatives of cerebrosides and contain one or more molecules of N-acetylneuraminic acid (NANA), the most important sialic acid. The structure of NANA is given in carbohydrate chemistry (***Refer Fig.2.11***).

The most important gangliosides present in the brain are GM_1, GM_2, GD, and GT, (***G*** represents ***g***anglioside while ***M***, ***D*** and ***T*** indicate ***m***ono-, ***d***i- or ***t***ri- sialic acid residues, and the number denotes the carbohydrate sequence attached to the ceramide). The ganglioside, GM_2 that accumulates in Tay-Sachs disease is represented next (outline structure).



LIPOPROTEINS

Lipoproteins are molecular complexes of lipids with proteins. They are the transport vehicles for lipids in the circulation. There are five types of lipoproteins, namely ***chylomicrons, very low density lipoproteins (VLDL), low density lipoproteins (LDL), high density lipoproteins (HDL) and free fatty acid-albumin complexes.*** Their structure, separation, metabolism and diseases are discussed together (***Chapter 14***).

STEROIDS

Steroids are the compounds containing a cyclic steroid nucleus (or ring) namely ***cyclopentanoperhydrophenanthrene (CPPP)***. It consists of a phenanthrene nucleus (rings A, B and C) to which a cyclopentane ring (D) is attached.

The structure and numbering of CPPP are shown in ***Fig.3.5***. The steroid nucleus represents saturated carbons, unless specifically shown as double bonds. The methyl side chains (19 and

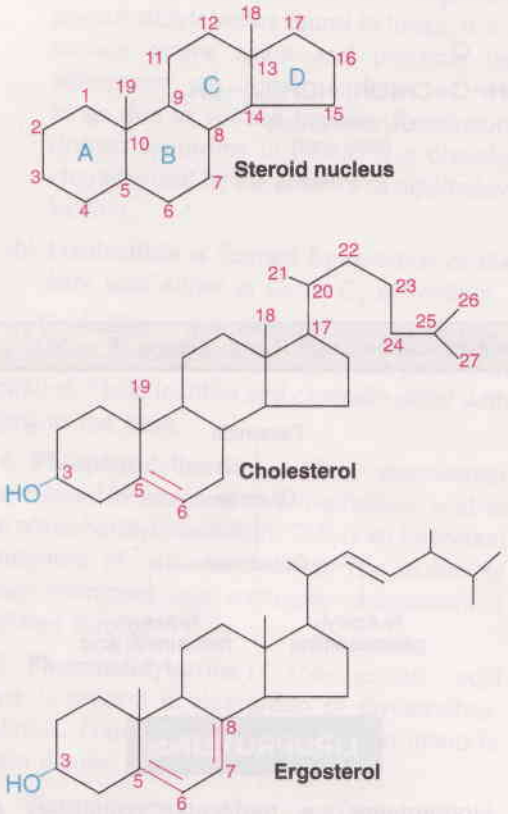


Fig. 3.5 : Structures of steroids (A, B, C—Perhydrophenanthrene; D-Cyclopentane).

18) attached to carbons 10 and 13 are shown as single bonds. At carbon 17, steroids usually contain a side chain.

There are several steroids in the biological system. These include ***cholesterol, bile acids, vitamin D, sex hormones, adrenocortical hormones,*** sitosterols, cardiac glycosides and alkaloids. If the steroid contains one or more hydroxyl groups it is commonly known as ***sterol*** (means solid alcohol).

CHOLESTEROL

Cholesterol, ***exclusively found in animals,*** is the most abundant animal sterol. It is widely distributed in all cells and is a major component of cell membranes and lipoproteins. Cholesterol (*Greek* : chole–bile) was first isolated from bile. Cholesterol literally means 'solid alcohol from bile.'

Structure and occurrence

The structure of cholesterol ($C_{27}H_{46}O$) is depicted in ***Fig.3.5.*** It has one hydroxyl group at C_3 and a double bond between C_5 and C_6. An 8 carbon aliphatic side chain is attached to C_{17}. Cholesterol contains a total of 5 methyl groups.

Due to the presence of an –OH group, cholesterol is weakly amphiphilic. As a structural component of plasma membranes, cholesterol is an important determinant of membrane permeability properties. The occurrence of cholesterol is much higher in the membranes of sub-cellular organelles.

Cholesterol is found in association with fatty acids to form cholesteryl esters (esterification occurs at the OH group of C_3).

Properties and reactions : Cholesterol is an yellowish crystalline solid. The crystals, under the microscope, show a notched (⊏) appearance. Cholesterol is insoluble in water and soluble in organic solvents such as chloroform, benzene, ether etc.

Several reactions given by cholesterol are useful for its qualitative identification and quantitative estimation. These include Salkowski's test, Liebermann-Burchard reaction and Zak's test.

Functions of cholesterol : Cholesterol is a poor conductor of heat and electricity, since it has a high dielectric constant. It is present in abundance in nervous tissues. It appears that cholesterol functions as an insulating cover for the transmission of electrical impulses in the nervous tissue. Cholesterol performs several other biochemical functions which include its role in membrane structure and function, in the synthesis of bile acids, hormones (sex and cortical) and vitamin D (for details, ***Refer Chapters 7*** and ***19***).

ERGOSTEROL

Ergosterol occurs in plants. It is also found as a structural constituent of membranes in yeast and fungi. Ergosterol (***Fig.3.5***) is an important precursor for vitamin D. When exposed to light,

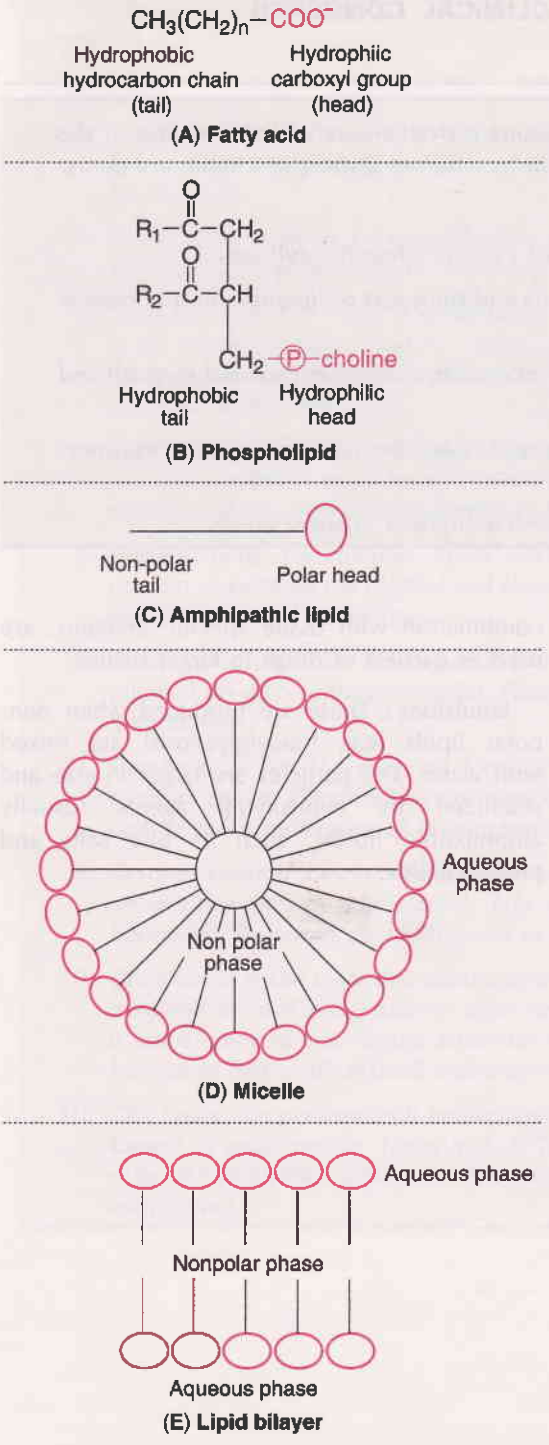


Fig. 3.6 : Summary of amphipathic lipids in the formation of micelle and lipid bilayer.

the ring B of ergosterol opens and it is converted to ergocalciferol, a compound containing vitamin D activity.

The other sterols present in plant cells include ***stigmasterol*** and ***β-sitosterol.***

AMPHIPATHIC LIPIDS

As per definition, lipids are insoluble (hydrophobic) in water. This is primarily due to the predominant presence of hydrocarbon groups. However, some of the lipids possess polar or hydrophilic groups which tend to be soluble in water. Molecules which contain both hydrophobic and hydrophilic groups are known as ***amphipathic*** (*Greek* : amphi-both, pathos—passion).

Examples of amphipathic lipids : Among the lipids, fatty acids, phospholipids, sphingolipids, bile salts and cholesterol (to some extent) are amphipathic in nature.

Phospholipids have a hydrophilic head (phosphate group attached to choline, ethanolamine, inositol etc.) and a long hydrophobic tail. The general structure of an amphipathic lipid may be represented as a polar or hydrophilic head with a non-polar or hydrophobic tail (***Fig.3.6***).

Fatty acids contain a hydrocarbon chain with a carboxyl (COO^-) group at physiological p^H. The carboxyl group is polar in nature with affinity to water (hydrophilic) while hydrocarbon chain of fatty acid is hydrophobic.

Orientation amphipathic lipids : When the amphipathic lipids are mixed in water (aqueous phase), the polar groups (heads) orient themselves towards aqueous phase while the non-polar (tails) orient towards the opposite directions. This leads to the formation of ***micelles*** (***Fig.3.6***). Micelle formation, facilitated by bile salts is very important for lipid digestion and absorption (***Chapter 8***).

Membrane bilayers

In case of biological membranes, a bilayer of lipids is formed orienting the polar heads to the

BIOMEDICAL / CLINICAL CONCEPTS

- *The phospholipid–dipalmitoyl lecithin—prevents the adherence of inner surface of the lungs, the absence of which is associated with respiratory distress syndrome in infants.*
- *Cephalins participate in blood clotting.*
- *The action of certain hormones is mediated through phosphatidylinositol.*
- *Phospholipids are important for the synthesis and transport of lipoproteins and reverse transport of cholesterol.*
- *Cholesterol is essential for the synthesis of bile acids, hormones (sex and cortical) and vitamin D.*
- *Lipoproteins occur in the membrane structure, besides serving as a means of transport vehicles for lipids.*
- *Lipids are associated with certain disorders—obesity and atherosclerosis.*

outer aqueous phase on either side and the nonpolar tails into the interior (***Fig.3.6***). The formation of a lipid bilayer is the basis of membrane structure.

Liposomes : They are produced when amphipathic lipids in aqueous medium are subjected to ***sonification***. They have intermittent aqueous phases in the lipid bilayer. Liposomes, in combination with tissue specific antigens, are used as carriers of drugs to target tissues.

Emulsions : These are produced when nonpolar lipids (e.g. triacylglycerols) are mixed with water. The particles are larger in size and stabilized by emulsifying agents (usually amphipathic lipids), such as bile salts and phospholipids.

SUMMARY

1. *Lipids are the organic substances relatively insoluble in water, soluble in organic solvents (alcohol, ether), actually or potentially related to fatty acids and are utilized by the body.*
2. *Lipids are classified into simple (fats and oils), complex (phospholipids, glycolipids), derived (fatty acids, steriod hormones) and miscellaneous (carotenoids).*
3. *Fatty acids are the major constituents of various lipids. Saturated and unsaturated fatty acids almost equally occur in natural lipids. The polyunsaturated fatty acids (PUFA) namely linoleic acid and linolenic acid are the essential fatty acids that need to be supplied in the diet.*
4. *Triacylglycerols (simply fats) are the esters of glycerol with fatty acids. They are found in adipose tissue and primarily function as fuel reserve of animals. Several tests (iodine number, RM number) are employed in the laboratory to test the purity of fats and oils.*
5. *Phospholipids are complex lipids containing phosphoric acid. Glycerophospholipids contain glycerol as the alcohol and these include lecithin, cephalin, phosphatidylinositol, plasmalogen and cardiolipin.*
6. *Sphingophospholipids (sphingomyelins) contain sphingosine as the alcohol in place of glycerol (in glycerophospholipids). Phospholipids are the major constituents of plasma membranes.*
7. *Cerebrosides are the simplest form of glycolipids which occur in the membranes of nervous tissue. Gangliosides are predominantly found in the ganglions. They contain one or more molecules of N-acetylneuraminic acid (NANA).*
8. *Steroids contain the ring cyclopentanoperhydrophenanthrene. The steroids of biological importance include cholesterol, bile acids, vitamin D, sex hormones and cortical hormones. A steroid containing one or more hydroxyl groups is known as sterol.*
9. *Cholesterol is the most abundant animal sterol. It contains one hydroxyl group (at C_3), a double bond (C_5–C_6) and an eight carbon side chain attached to C_{17}. Cholesterol is a constituent of membrane structure and is involved in the synthesis of bile acids, hormones (sex and cortical) and vitamin D.*
10. *The lipids that possess both hydrophobic (non -polar) and hydrophilic (polar) groups are known as amphipathic. These include fatty acids, phospholipids, sphingolipids and bile salts. Amphipathic lipids are important constituents in the bilayers of the biological membranes.*

SELF-ASSESSMENT EXERCISES

I. Essay questions

1. Write an account of classification of lipids with suitable examples.
2. Describe the structure and functions of phospholipids.
3. Discuss the saturated and unsaturated fatty acids of biological importance, along with their structures.
4. Describe the structure of steroids. Add a note on the functions of cholesterol.
5. Discuss the biological importance of amphipathic lipids.

II. Short notes

(a) Structure of triacylglycerols, (b) Glycolipids, (c) Essential fatty acids, (d) *Cis–trans* isomerism, (e) Rancidity, (f) Iodine number, (g) Phosphatidylinositol, (h) Sphingomyelins, (i) Steroid nucleus, (j) Micelles.

III. Fill in the blanks

1. The lipids that function as fuel reserve in animals _____.
2. The isomerism associated with unsaturated fatty acids _____.
3. The cyclic fatty acid employed in the treatment of leprosy _____.
4. The lipids that are not the structural components of biological membranes _____.
5. The prefix *sn* used to represent glycerol, *sn* stands for _____.
6. The number of mg of KOH required to hydrolyse 1 g fat or oil is known as _____.
7. The phospholipid that prevents the adherence of inner surfaces of lungs _____.
8. The phospholipid that produces second messengers in hormonal action _____.
9. Name the glycolipids containing N-acetylneuraminic acid _____.
10. The steroids contain a cyclic ring known as _____.

IV. Multiple choice questions

11. The nitrogenous base present in lecithin

    (a) Choline (b) Ethanolamine (c) Inositol (d) Serine.

12. The number of double bonds present in arachidonic acid

    (a) 1 (b) 2 (c) 3 (d) 4.

13. One of the following is an amphipathic lipid

    (a) Phospholipids (b) Fatty acid (c) Bile salts (d) All of the above.

14. Esterification of cholesterol occurs at carbon position

    (a) 1 (b) 2 (c) 3 (d) 4.

15. Name the test employed to check the purity of butter through the estimation of volatile fatty acids

    (a) Iodine number (b) Reichert-Meissl number (c) Saponification number (d) Acid number.

4 Proteins and Amino Acids

The proteins speak :

"We are the basis of structure and function of life;
Composed of twenty amino acids, the building blocks;
Organized into primary, secondary, tertiary
and quaternary structure;
Classified as simple, conjugated and derived proteins."

Proteins are the ***most abundant organic molecules of the living system***. They occur in every part of the cell and constitute about 50% of the cellular dry weight. Proteins form the fundamental basis of structure and function of life.

Origin of the word 'protein'

The term **protein** is derived from a *Greek* word ***proteios***, meaning *holding the first place*. Berzelius (Swedish chemist) suggested the name proteins to the group of organic compounds that are utmost important to life. Mulder (Dutch chemist) in 1838 used the term **proteins** for the high molecular weight nitrogen-rich and most abundant substances present in animals and plants.

Functions of proteins

Proteins perform a great variety of specialized and essential functions in the living cells. These functions may be broadly grouped as ***static*** (*structural*) and ***dynamic***.

Structural functions : Certain proteins perform *brick and mortar* roles and are primarily responsible for structure and strength of body. These include ***collagen*** and ***elastin*** found in bone matrix, vascular system and other organs and ***α-keratin*** present in epidermal tissues.

Dynamic functions : The dynamic functions of proteins are more diversified in nature. These include proteins acting as ***enzymes, hormones, blood clotting factors, immunoglobulins***, membrane receptors, storage proteins, besides their function in genetic control, muscle contraction, respiration etc. Proteins performing dynamic functions are appropriately regarded as ***the working horses*** of cell.

Elemental composition of proteins

Proteins are predominantly constituted by five major elements in the following proportion.

Carbon	:	50 – 55%
Hydrogen	:	6 – 7.3%
Oxygen	:	19 – 24%
Nitrogen	:	13 – 19%
Sulfur	:	0 – 4%

Besides the above, proteins may also contain other elements such as P, Fe, Cu, I, Mg, Mn, Zn etc.

The content of ***nitrogen,*** an essential component of proteins, on an average is ***16%.*** Estimation of nitrogen in the laboratory (mostly by ***Kjeldahl's method***) is also used to find out the amount of protein in biological fluids and foods.

Proteins are polymers of amino acids

Proteins on complete hydrolysis (with concentrated HCl for several hours) yield L-α-amino acids. This is a common property of all the proteins. Therefore, ***proteins are the polymers of L-α-amino acids.***

STANDARD AMINO ACIDS

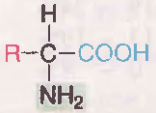
As many as 300 amino acids occur in nature—Of these, ***only 20***—known as standard amino acids are repeatedly ***found in the structure of proteins,*** isolated from different forms of life—animal, plant and microbial. This is because of the universal nature of the genetic code available for the incorporation of only 20 amino acids when the proteins are synthesized in the cells. The process in turn is controlled by DNA, the genetic material of the cell. After the synthesis of proteins, some of the incorporated amino acids undergo modifications to form their derivatives.

AMINO ACIDS

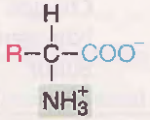
Amino acids are a group of organic compounds containing two ***functional groups—amino*** and ***carboxyl.*** The amino group ($-NH_2$) is basic while the carboxyl group (—COOH) is acidic in nature.

General structure of amino acids

The amino acids are termed as α-amino acids, if both the carboxyl and amino groups are attached to the same carbon atom, as depicted below



General structure



Exists as ion

The α-carbon atom binds to a side chain represented by R which is different for each of the 20 amino acids found in proteins. The amino acids mostly exist in the ionized form in the biological system (shown above).

Optical isomers of amino acids

If a carbon atom is attached to four different groups, it is asymmetric and therefore exhibits optical isomerism. The amino acids (except glycine) possess four distinct groups (R, H, COO^-, NH_3^+) held by α-carbon. Thus all the amino acids (except glycine where R = H) have optical isomers.

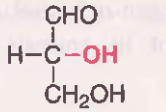
The structures of L- and D-amino acids are written based on the configuration of L- and D-glyceraldehyde as shown in ***Fig.4.1***. The proteins are composed of L-α-amino acids.

Classification of amino acids

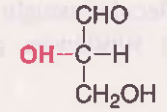
There are different ways of classifying the amino acids based on the structure and chemical nature, nutritional requirement, metabolic fate etc.

A. Amino acid classification based on the structure : A comprehensive classification of amino acids is based on their structure and chemical nature. Each amino acid is assigned a 3 letter or 1 letter symbol. These symbols are commonly used to represent the amino acids in protein structure. The 20 amino acids found in proteins are divided into seven distinct groups.

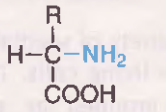
In ***Table 4.1***, the different groups of amino acids, their symbols and structures are given. The salient features of different groups are described next



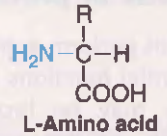
D-Glyceraldehyde



L-Glyceraldehyde



D-Amino acid



L-Amino acid

Fig. 4.1 : D- and L-forms of amino acid based on the structure of glyceraldehyde.

Table 4.1 Structural classification of L-α-amino acids found in proteins

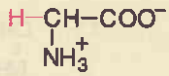
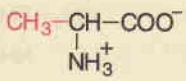
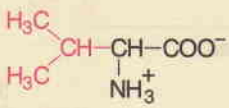
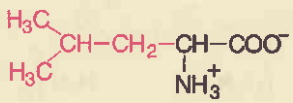
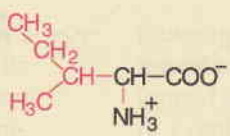
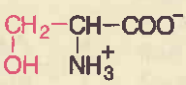
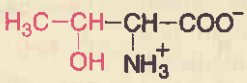
Name	Symbol		Structure	Special group present
	3 letters	1 letter		
I. Amino acids with aliphatic side chains				
1. Glycine	Gly	G		
2. Alanine	Ala	A		
3. Valine	Val	V		Branched chain
4. Leucine	Leu	L		Branched chain
5. Isoleucine	Ile	I		Branched chain
II. Amino acids containing hydroxyl (—OH) groups				
6. Serine	Ser	S		Hydroxyl
7. Threonine	Thr	T		Hydroxyl
Tyrosine	Tyr	Y	See under aromatic	Hydroxyl

Table 4.1 contd. next page

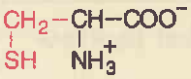
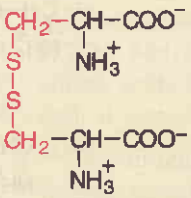
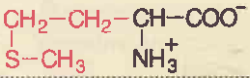
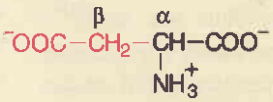
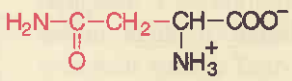
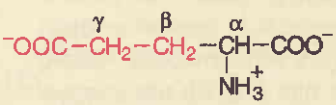
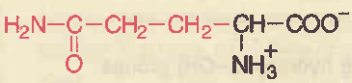
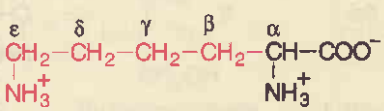
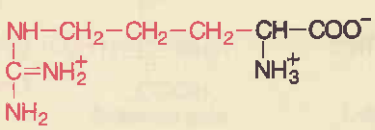
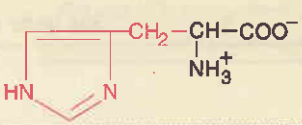
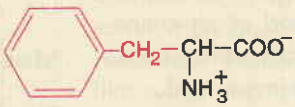
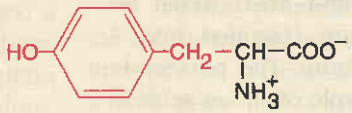
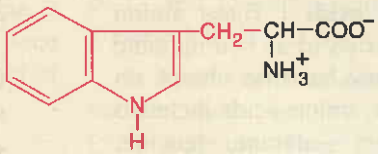
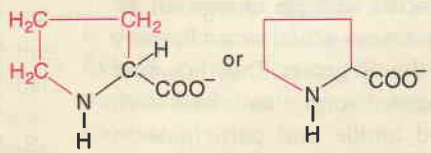
Name	Symbol		Structure	Special group present
	3 letters	1 letter		
III. Sulfur containing amino acids				
8. Cysteine	Cys	C		Sulfhydryl
Cystine	—	—		Disulfide
9. Methionine	Met	M		Thioether
IV. Acidic amino acids and their amides				
10. Aspartic acid	Asp	D		β-Carboxyl
11. Asparagine	Asn	N		Amide
12. Glutamic acid	Glu	E		γ-Carboxyl
13. Glutamine	Gln	Q		Amide
V. Basic amino acids				
14. Lysine	Lys	K		ε-Amino
15. Arginine	Arg	R		Guanidino
16. Histidine	His	H		Imidazole

Table 4.1 contd. next page

Name	Symbol		Structure	Special group present
	3 letters	1 letter		
VI. Aromatic amino acids				
17. Phenylalanine	Phe	F		Benzene or phenyl
18. Tyrosine	Tyr	Y		Phenol
19. Tryptophan	Trp	W		Indole
VII. Imino acid				
20. Proline	Pro	P		Pyrrolidine

(Note : R group is shown in red)

1. **Amino acids with aliphatic side chains :** These are monoamino monocarboxylic acids. This group consists of the most simple amino acids—glycine, alanine, valine, leucine and isoleucine. The last three amino acids (Leu, Ile, Val) contain branched aliphatic side chains, hence they are referred to as ***branched chain amino acids.***
2. **Hydroxyl group containing amino acids :** Serine, threonine and tyrosine are hydroxyl group containing amino acids. Tyrosine—being aromatic in nature—is usually considered under aromatic amino acids.
3. **Sulfur containing amino acids :** Cysteine with sulfhydryl group and methionine with thioether group are the two amino acids incorporated during the course of protein synthesis. Cystine, another important sulfur containing amino acid, is formed by condensation of two molecules of cysteine.
4. **Acidic amino acids and their amides :** Aspartic acid and glutamic acids are ***dicarboxylic monoamino acids*** while asparagine and glutamine are their respective amide derivatives. All these four amino acids possess distinct codons for their incorporation into proteins.
5. **Basic amino acids :** The three amino acids lysine, arginine (with guanidino group) and histidine (with imidazole ring) are dibasic monocarboxylic acids. They are highly basic in character.
6. **Aromatic amino acids :** Phenylalanine, tyrosine and tryptophan (with indole ring)

are aromatic amino acids. Besides these, histidine may also be considered under this category.

7. **Imino acids :** Proline containing pyrrolidine ring is a unique amino acid. It has an imino group (=NH), instead of an amino group ($-NH_2$) found in other amino acids. Therefore, proline is an α-imino acid.

B. Classification of amino acids based on polarity : Amino acids are classified into 4 groups based on their polarity. The polarity in turn reflects the functional role of amino acids in protein structure.

1. **Non-polar amino acids :** These amino acids are also referred to as hydrophobic (water hating). They have no charge on the 'R' group. The amino acids included in this group are — alanine, leucine, isoleucine, valine, methionine, phenylalanine, tryptophan and proline.
2. **Polar amino acids with no charge on 'R' group :** These amino acids, as such, carry no charge on the 'R' group. They however possess groups such as hydroxyl, sulfhydryl and amide and participate in hydrogen bonding of protein structure. The simple amino acid glycine (where R = H) is also considered in this category. The amino acids in this group are—glycine, serine, threonine, cysteine, glutamine, asparagine and tyrosine.
3. **Polar amino acids with positive 'R' group :** The three amino acids lysine, arginine and histidine are included in this group.
4. **Polar amino acids with negative 'R' group :** The dicarboxylic monoamino acids—aspartic acid and glutamic acid are considered in this group.

C. Nutritional classification of amino acids : The twenty amino acids (***Table 4.1***) are required for the synthesis of variety of proteins, besides other biological functions. However, all these 20 amino acids need not be taken in the diet. Based on the nutritional requirements, amino acids are grouped into two classes—essential and non-essential.

1. **Essential or indispensable amino acids :** The amino acids which ***cannot be synthesized by the body*** and, therefore, need to be supplied through the diet are called essential amino acids. They are required for proper growth and maintenance of the individual. The ten amino acids listed below are essential for humans (and also rats) :

   Arginine, **V**aline, **H**istidine, **I**soleucine, **L**eucine, **L**ysine, **M**ethionine, **P**henylalanine, **T**hreonine, **T**ryptophan.

   [The code ***A.V. HILL, MP., T. T.*** (first letter of each amino acid) may be memorized to recall essential amino acids. Other useful codes are H. VITTAL, LMP; PH. VILLMA, TT, PVT TIM HALL and MATTVILPhLy.]

   The two amino acids namely ***a***rginine and ***h***istidine can be synthesized by adults and not by growing children, hence these are considered as ***semi-essential amino acids*** (remember ***Ah***, to recall). Thus, 8 amino acids are absolutely essential while 2 are semi-essential.
2. **Non-essential or dispensable amino acids :** The body can synthesize about 10 amino acids to meet the biological needs, hence they need not be consumed in the diet. These are—glycine, alanine, serine, cysteine, aspartate, asparagine, glutamate, glutamine, tyrosine and proline.

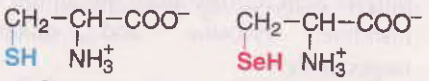
D. Amino acid classification based on their metabolic fate : The carbon skeleton of amino acids can serve as a precursor for the synthesis of glucose (glycogenic) or fat (ketogenic) or both. From metabolic view point, amino acids are divided into three groups (for details, ***Refer Chapter 15***).

1. **Glycogenic amino acids :** These amino acids can serve as precursors for the formation of glucose or glycogen. e.g. alanine, aspartate, glycine, methionine etc.
2. **Ketogenic amino acids :** Fat can be synthesized from these amino acids. Two amino acids leucine and lysine are exclusively ketogenic.

3. **Glycogenic and ketogenic amino acids :** The four amino acids isoleucine, phenylalanine, tryptophan, tyrosine are precursors for synthesis of glucose as well as fat.

Selenocysteine – the 21st amino acid

As already stated, 20 amino acids are commonly found in proteins. In recent years, a 21st amino acid namely selenocysteine has been added. It is found at the active sites of certain enzymes/proteins (***selenoproteins***). e.g. glutathione peroxidase, glycine reductase, 5'-deiodinase, thioredoxin reductase. Selenocysteine is an unusual amino acid containing the trace element selenium in place of the sulfur atom of cysteine.



Incorporation of selenocysteine into the proteins during translation is carried out by the codon namely UGA. It is interesting to note that UGA is normally a stop codon that terminates protein biosynthesis. Another unique feature is that selenocysteine is enzymatically generated from serine directly on the tRNA (selenocysteine-tRNA), and then incorporated into proteins.

Pyrrolysine – the 22nd amino acid? : In the year 2002, some researchers have described yet another amino acid namely pyrrolysine as the 22nd amino acid present in protein. The stop codon UAG can code for pyrrolysine.

Properties of amino acids

The amino acids differ in their physico–chemical properties which ultimately determine the characteristics of proteins.

A. Physical properties

1. **Solubility :** Most of the amino acids are usually soluble in water and insoluble in organic solvents.

2. **Melting points :** Amino acids generally melt at higher temperatures, often above 200°C.

3. **Taste :** Amino acids may be sweet (Gly, Ala, Val), tasteless (Leu) or bitter (Arg, Ile). ***Monosodium glutamate*** (MSG; ***ajinomoto***) is used as a flavoring agent in food industry, and Chinese foods to increase taste and flavor. In some individuals intolerant to MSG, ***Chinese restaurant syndrome*** (brief and reversible flu-like symptoms) is observed.

4. **Optical properties :** All the amino acids except glycine possess optical isomers due to the presence of asymmetric carbon atom. Some amino acids also have a second asymmetric carbon e.g. isoleucine, threonine. The structure of L- and D-amino acids in comparison with glyceraldehyde has been given (***See Fig.4.1***).

5. **Amino acids as ampholytes :** Amino acids contain both acidic (–COOH) and basic ($-NH_2$) groups. They can donate a proton or accept a proton, hence amino acids are regarded as ampholytes.

Zwitterion or dipolar ion : The name *zwitter* is derived from the German word which means ***hybrid***. Zwitter ion (or dipolar ion) is a hybrid molecule containing ***positive and negative ionic groups***.

The amino acids rarely exist in a neutral form with free carboxylic (–COOH) and free amino ($-NH_2$) groups. In strongly acidic pH (low pH), the amino acid is positively charged (cation) while in strongly alkaline pH (high pH), it is negatively charged (anion). Each amino acid has a characteristic pH (e.g. leucine, pH 6.0) at which it carries both positive and negative charges and exists as zwitterion (***Fig.4.2***).

Isoelectric pH (symbol pI) is defined as the pH at which a ***molecule exists as a zwitterion*** or ***dipolar ion*** and carries no net charge. Thus, the molecule is electrically neutral.

The pI value can be calculated by taking the average pKa values corresponding to the ionizable groups. For instance, leucine has two ionizable groups, and its pI can be calculated as follows.

$$pH = \frac{pK_1(COOH) + pK_2(NH_3^+)}{2}$$

$$pI = \frac{2.4 + 9.6}{2} = 6.0$$

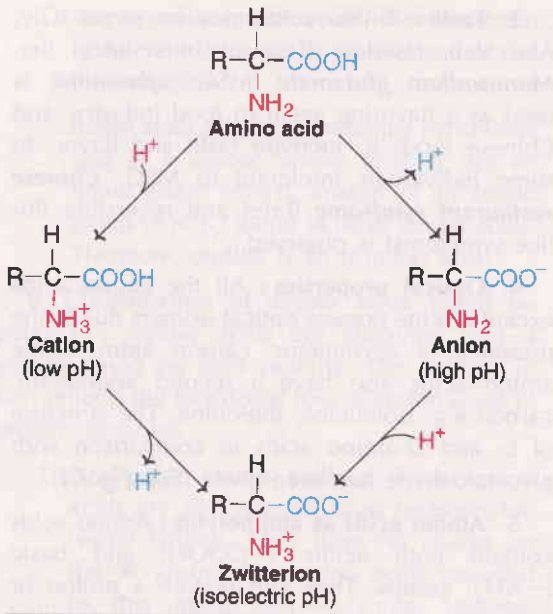


Fig. 4.2 : Existence of an amino acid as cation, anion and zwitterion.

Leucine exists as cation at pH below 6 and anion at pH above 6. At the isoelectric pH (pI = 6.0), leucine is found as zwitterion. Thus the pH of the medium determines the ionic nature of amino acids.

For the calculation of pI of amino acids with more than two ionizable groups, the pKas for all the groups have to be taken into account.

Titration of amino acids : The existence of different ionic forms of amino acids can be more easily understood by the titration curves. The graphic representation of leucine titration is depicted in ***Fig.4.3***. At low pH, leucine exists in a fully protonated form as cation. As the titration proceeds with NaOH, leucine loses its protons and at isoelectric pH (pI), it becomes a zwitterion. Further titration results in the formation of anionic form of leucine.

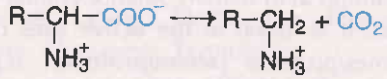
Some more details on isoelectric pH are discussed under the properties of proteins (p. 60).

B. Chemical properties

The general reactions of amino acids are mostly due to the presence of two functional groups namely carboxyl (−COOH) group and amino ($-NH_2$) group.

Reactions due to −COOH group

1. Amino acids form salts (−COONa) with bases and esters (−COOR′) with alcohols.
2. **Decarboxylation :** Amino acids undergo decarboxylation to produce corresponding amines.



This reaction assumes significance in the living cells due to the formation of many ***biologically important amines***. These include histamine, tyramine and γ-amino butyric acid (GABA) from the amino acids histidine, tyrosine and glutamate, respectively.

3. **Reaction with ammonia :** The carboxyl group of dicarboxylic amino acids reacts with NH_3 to form amide

   Aspartic acid + NH_3 ⟶ Asparagine

   Glutamic acid + NH_3 ⟶ Glutamine

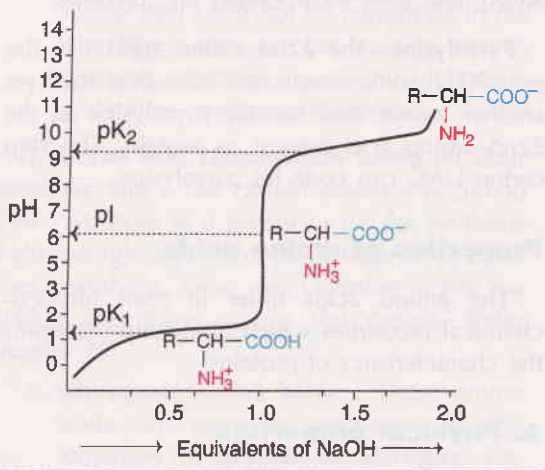


Fig. 4.3 : Titration curve of an amino acid-leucine ($R = (CH_3)_2—CH—CH_2—$; pK_1 = Dissociation constant for COOH; pI = Isoelectric pH; pK_2 = Dissociation constant for NH_3^+).

Reactions due to $-NH_2$ group

4. The amino groups behave as bases and combine with acids (e.g. HCl) to form salts ($-NH_3^+Cl^-$).

5. **Reaction with ninhydrin :** The α-amino acids react with ninhydrin to form a purple, blue or pink colour complex (***Ruhemann's purple***).

   Amino acid + Ninhydrin ⟶ Keto acid + NH_3 + CO_2 + Hydrindantin

   Hydrindantin + NH_3 + Ninhydrin ⟶ Ruhemann's purple

   Ninhydrin reaction is effectively used for the quantitative determination of amino acids and proteins. (***Note :*** Proline and hydroxyproline give yellow colour with ninhydrin).

6. **Colour reactions of amino acids :** Amino acids can be identified by specific colour reactions (***See Table 4.3***).

7. **Transamination :** Transfer of an amino group from an amino acid to a keto acid to form a new amino acid is a very important reaction in amino acid metabolism (details given in ***Chapter 15***).

8. **Oxidative deamination :** The amino acids undergo oxidative deamination to liberate free ammonia (***Refer Chapter 15***).

NON-STANDARD AMINO ACIDS

Besides the 20 standard amino acids (described above) present in the protein structure, there are several other amino acids which are biologically important. These include the amino acid derivatives found in proteins, non-protein amino acids performing specialized functions and the D-amino acids.

A. Amino acid derivatives in proteins : The 20 standard amino acids can be incorporated into proteins due to the presence of universal genetic code. Some of these amino acids undergo specific modification after the protein synthesis occurs. These derivatives of amino acids are very important for protein structure and functions. Selected examples are given hereunder.

- Collagen—the most abundant protein in mammals—contains ***4-hydroxyproline*** and ***5-hydroxylysine***.
- Histones—the proteins found in association with DNA—contain many methylated, phosphorylated or acetylated amino acids.
- ***γ-Carboxyglutamic acid*** is found in certain plasma proteins involved in blood clotting.
- Cystine is formed by combination of two cysteines. Cystine is also considered as derived amino acid.

B. Non-protein amino acids : These amino acids, although never found in proteins, perform several biologically important functions. They may be either α-or non-α-amino acids. A selected list of these amino acids along with their functions is given in ***Table 4.2***.

C. D-Amino acids : The vast majority of amino acids isolated from animals and plants are of L-category. Certain D-amino acids are also found in the antibiotics (actinomycin-D, valinomycin, gramicidin-S). D-serine and D-aspartate are found in brain tissue. D-Glutamic acid and D-alanine are present in bacterial cell walls.

Amino acids useful as drugs

There a certain non-standard amino acids that are used as drugs.

- D-Penicillamine (D-dimethylglycine), a metabolite of penicillin, is employed in the chelation therapy of Wilson's disease. This is possible since D-penicillamine can effectively chelate copper.
- N-Acetylcysteine is used in cystic fibrosis, and chronic renal insufficiency, as it can function as an antioxidant.
- Gabapentin (γ-aminobutyrate linked to cyclohexane) is used as an anticonvulsant.

Table 4.2 A selected list of important non-protein amino acids along with their functions

Amino acids	*Function(s)*
I. α-Amino acids	
Ornithine, Citrulline, Arginosuccinic acid	Intermediates in the biosynthesis of urea.
Thyroxine, Triiodothyronine	Thyroid hormones derived from tyrosine.
S-Adenosylmethionine	Methyl donor in biological system.
Homocysteine	Intermediate in methionine metabolism. A risk factor for coronary heart diseases
Homoserine	Intermediate in threonine, aspartate and methionine metabolisms.
3, 4-Dihydroxy phenylalanine (DOPA)	A neurotransmitter, serves as a precursor for melanin pigment.
Creatinine	Derived from muscle and excreted in urine
Ovothiol	Sulfur containing amino acid found in fertilized eggs, and acts as an antioxidant
Azaserine	An antibiotic
II. Non-α-amino acids	
β-Alanine	Component of vitamin pantothenic acid and coenzyme A
β-Aminoisobutyric acid	End product of pyrimidine metabolism.
γ-Aminobutyric acid (GABA)	A neurotransmitter produced from glutamic acid
δ-Aminolevulinic acid (ALA)	Intermediate in the synthesis of porphyrin (finally heme)
Taurine	Found in association with bile acids.

STRUCTURE OF PROTEINS

Proteins are the polymers of L-α-amino acids. The structure of proteins is rather complex which can be divided into 4 levels of organization (***Fig.4.4***) :

1. **Primary structure :** The linear sequence of amino acids forming the backbone of proteins (polypeptides).

2. **Secondary structure :** The spatial arrangement of protein by twisting of the polypeptide chain.

3. **Tertiary structure :** The three dimensional structure of a functional protein.

4. **Quaternary structure :** Some of the proteins are composed of two or more polypeptide chains referred to as subunits. The spatial arrangement of these subunits is known as quaternary structure.

[The structural hierarchy of proteins is comparable with the structure of a building. The amino acids may be considered as the bricks, the wall as the primary structure, the twists in a wall as the secondary structure, a full-fledged self-contained room as the tertiary structure. A building with similar and dissimilar rooms will be the quaternary structure].

The term ***protein*** is generally used for a polypeptide containing ***more than 50 amino acids.*** In recent years, however, some authors have been using '***polypeptide***' even if the number of amino acids is a few hundreds. They prefer to use protein to an assembly of polypeptide chains with quaternary structure.

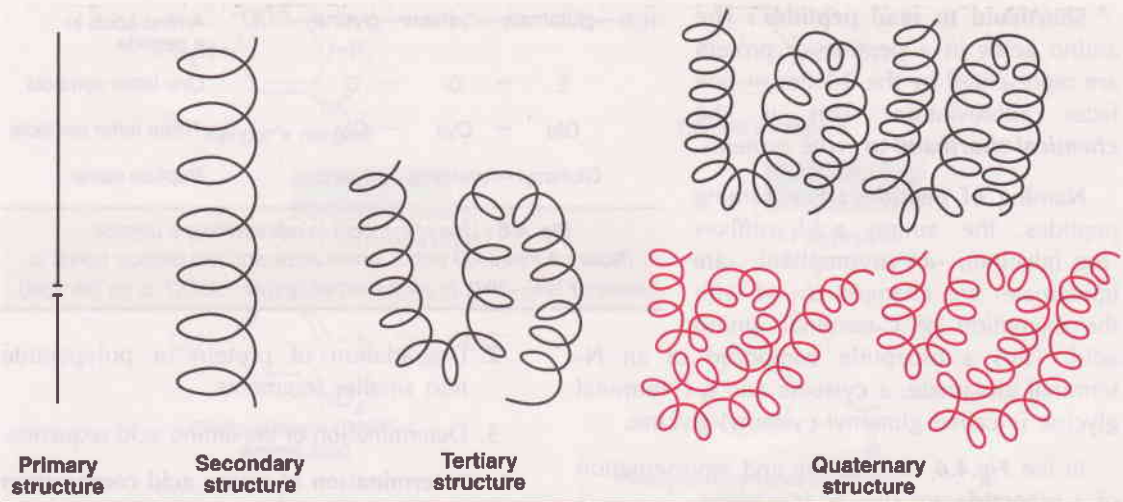


Fig. 4.4 : Diagrammatic representation of protein structure
(Note : The four subunits of two types in quaternary structure).

PRIMARY STRUCTURE OF PROTEIN

Each protein has a unique sequence of amino acids which is determined by the genes contained in DNA. The primary structure of a protein is largely responsible for its function. A vast majority of genetic diseases are due to abnormalities in the amino acid sequences of proteins i.e. changes associated with primary structure of protein.

The amino acid composition of a protein determines its physical and chemical properties.

Peptide bond

The amino acids are held together in a protein by covalent peptide bonds or linkages. These bonds are rather strong and serve as the cementing material between the individual amino acids (considered as bricks).

Formation of a peptide bond : When the ***amino group*** of an amino acid combines with the ***carboxyl group*** of another amino acid, a peptide bond is formed (***Fig.4.5***). Note that a dipeptide will have two amino acids and one peptide (not two) bond. Peptides containing more than 10 amino acids (decapeptide) are referred to as polypeptides.

Characteristics of peptide bonds : The peptide bond is rigid and planar with partial double bond in character. It generally exists in *trans* configuration. Both $-C=O$ and $-NH$ groups of peptide bonds are polar and are involved in hydrogen bond formation.

Writing of peptide structures : Conventionally, the peptide chains are written with the free amino end (N-terminal residue) at the left, and the free carboxyl end (C-terminal residue) at the right. The amino acid sequence is read from N-terminal end to C-terminal end. Incidentally, the protein biosynthesis also starts from the N-terminal amino acid.

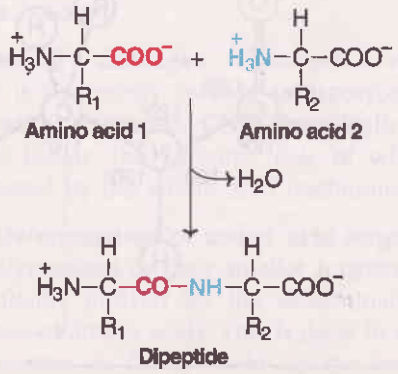


Fig. 4.5 : Formation of a peptide bond.

Shorthand to read peptides : The amino acids in a peptide or protein are represented by the 3-letter or one letter abbreviation. This is the ***chemical shorthand*** to write proteins.

H_3N^+—glutamate—cysteine—glycine—COO^-	Amino acids in a peptide
E — C — G	One letter symbols
Glu — Cys — Gly	Three letter symbols
Glutamyl—cysteinyl—glycine	Peptide name

Fig. 4.6 : Use of symbols in representing a peptide (Note : A tripeptide with 3 amino acids and two peptide bonds is shown; Free —NH_3^+ is on the left while free —COO^- is on the right).

Naming of peptides : For naming peptides, the amino acid suffixes ***-ine*** (glycine), ***-an*** (tryptophan), ***-ate*** (glutamate) are changed to ***-yl*** with the exception of C-terminal amino acid. Thus a tripeptide composed of an N-terminal glutamate, a cysteine and a C-terminal glycine is called glutamyl-cysteinyl-glycine.

In the ***Fig.4.6***, the naming and representation of a tripeptide are shown.

Dimensions of a peptide chain : The dimensions of a fully extended polypeptide chain are depicted in ***Fig.4.7***. The two adjacent α-carbon atoms are placed at a distance of 0.36 nm. The interatomic distances and bond angles are also shown in this figure.

Determination of primary structure

The primary structure comprises the identification of constituent amino acids with regard to their quality, quantity and sequence in a protein structure. A pure sample of a protein or a polypeptide is essential for the determination of primary structure which involves 3 stages :

1. Determination of amino acid composition.
2. Degradation of protein or polypeptide into smaller fragments.
3. Determination of the amino acid sequence.

1. Determination of amino acid composition in a protein : The protein or polypeptide is completely hydrolysed to liberate the amino acids which are quantitatively estimated. The hydrolysis may be carried out either by acid or alkali treatment or by enzyme hydrolysis. Treatment with enzymes, however results in smaller peptides rather than amino acids.

Pronase is a mixture of non-specific proteolytic enzymes that causes complete hydrolysis of proteins.

Separation and estimation of amino acids : The mixture of amino acids liberated by protein hydrolysis can be determined by chromatographic techniques. The reader must refer ***Chapter 41*** for the separation and quantitative determination of amino acids. Knowledge on

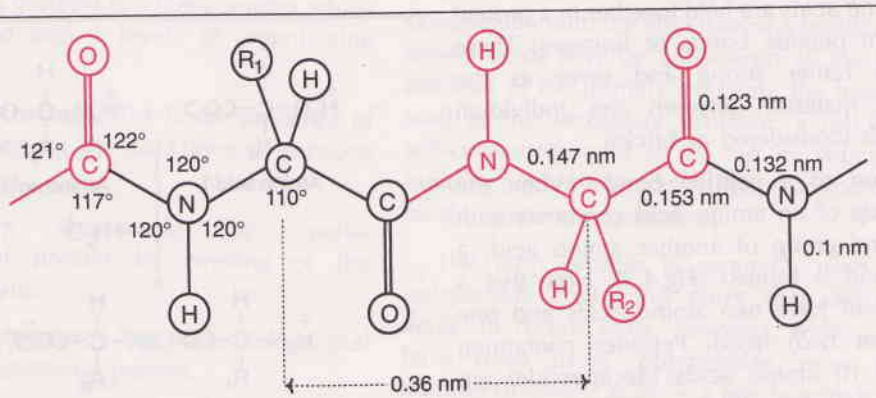


Fig. 4.7 : Dimensions of a fully extended polypeptide chain. (The distance between two adjacent α-carbon atoms is 0.36 nm).

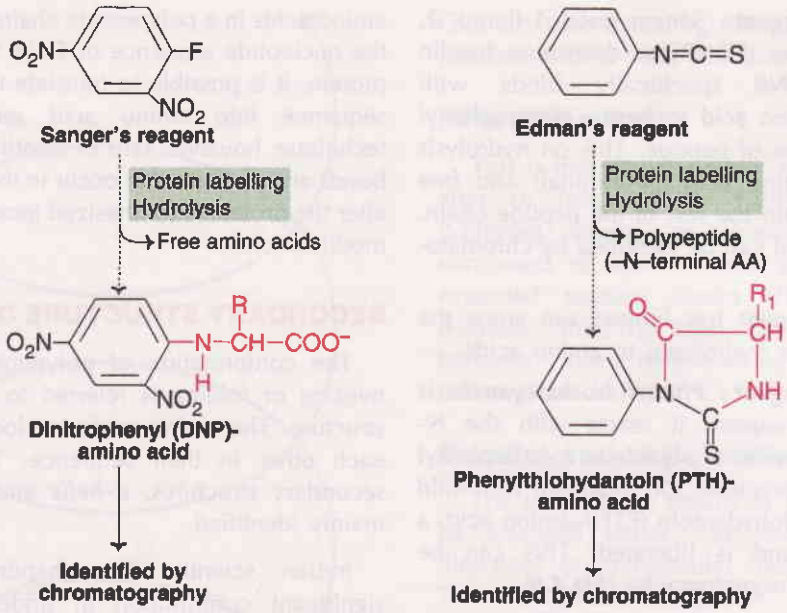


Fig. 4.8 : Sanger's reagent (1-fluoro 2,4-dinitrobenzene) and Edman's reagent (Phenyl isothiocyanate) in the determination of amino acid sequence of a protein (AA-Amino acid).

primary structure of proteins will be incomplete without a thorough understanding of chromatography.

2. Degradation of protein into smaller fragments : Protein is a large molecule which is sometimes composed of individual polypeptide chains. Separation of polypeptides is essential before degradation.

(a) **Liberation of polypeptides :** Treatment with urea or guanidine hydrochloride disrupts the non-covalent bonds and dissociates the protein into polypeptide units. For cleaving the disulfide linkages between the polypeptide units, treatment with performic acid is necessary.

(b) **Number of polypeptides :** The number of polypeptide chains can be identified by treatment of protein with ***dansyl chloride***. It specifically binds with N-terminal amino acids to form dansyl polypeptides which on hydrolysis yield N-terminal dansyl amino acid. The number of dansyl amino acids produced is equal to the number of polypeptide chains in a protein.

(c) **Breakdown of polypeptides into fragments :** Polypeptides are degraded into smaller peptides by enzymatic or chemical methods.

Enzymatic cleavage : The proteolytic enzymes such as trypsin, chymotrypsin, pepsin and elastase exhibit specificity in cleaving the peptide bonds (***Refer Fig.8.7***). Among these enzymes, trypsin is most commonly used. It hydrolyses the peptide bonds containing lysine or arginine on the carbonyl (−C=O) side of peptide linkage.

Chemical cleavage : Cyanogen bromide (CNBr) is commonly used to split polypeptides into smaller fragments. CNBr specifically splits peptide bonds, the carbonyl side of which is contributed by the amino acid methionine.

3. Determination of amino acid sequence : The polypeptides or their smaller fragments are conveniently utilized for the determination of sequence of amino acids. This is done in a stepwise manner to finally build up the order of amino acids in a protein. Certain reagents are employed for sequence determination (***Fig.4.8***).

Sanger's reagent : Sanger used 1-fluoro 2, 4-dinitrobenzene (FDNB) to determine insulin structure. ***FDNB*** specifically binds with N-terminal amino acid to form a dinitrophenyl (DNP) derivative of peptide. This on hydrolysis yields DNP-amino acid (N-terminal) and free amino acids from the rest of the peptide chain. DNP-amino acid can be identified by chromatography.

Sanger's reagent has limited use since the peptide chain is hydrolysed to amino acids.

Edman's reagent : ***Phenyl isothiocyanate*** is the Edman's reagent. It reacts with the N-terminal amino acid of peptide to form a phenyl thiocarbamyl derivative. On treatment with mild acid, phenyl thiohydantoin (PTH)–amino acid, a cyclic compound is liberated. This can be identified by chromatography (***Fig.4.8***).

Edman's reagent has an advantage since a peptide can be sequentially degraded liberating N-terminal amino acids one after another which can be identified. This is due to the fact that the peptide as a whole is not hydrolysed but only releases PTH-amino acid.

Sequenator : This is an ***automatic machine*** to determine the amino acid sequence in a polypeptide (with around 100 residues). It is based on the principle of Edman's degradation (described above). Amino acids are determined sequentially from N-terminal end. The PTH-amino acid liberated is identified by high-performance liquid chromatography (HPLC). Sequenator takes about 2 hours to determine each amino acid.

Overlapping peptides

In the determination of primary structure of protein, several methods (enzymatic or chemical) are simultaneously employed. This results in the formation of overlapping peptides. This is due to the specific action of different agents on different sites in the polypeptide. Overlapping peptides are very useful in determining the amino acid sequence.

Reverse sequencing technique

It is the genetic material (chemically DNA) which ultimately determines the sequence of amino acids in a polypeptide chain. By analysing the nucleotide sequence of DNA that codes for protein, it is possible to translate the nucleotide sequence into amino acid sequence. This technique, however, fails to identify the disulfide bonds and changes that occur in the amino acids after the protein is synthesized (post-translational modifications).

SECONDARY STRUCTURE OF PROTEIN

The conformation of polypeptide chain by twisting or folding is referred to as secondary structure. The amino acids are located close to each other in their sequence. Two types of secondary structures, ***α-helix*** and ***β-sheet***, are mainly identified.

Indian scientist Ramachandran made a significant contribution in understanding the spatial arrangement of polypeptide chains.

α-Helix

α-Helix is the ***most common*** spiral structure of protein. It has a rigid arrangement of polypeptide chain. α-Helical structure was proposed by Pauling and Corey (1951) which is regarded as one of the milestones in the biochemistry research. The salient features of α-helix (***Fig.4.9***) are given below

1. The α-helix is a tightly packed coiled structure with amino acid side chains extending outward from the central axis.

2. The α-helix is ***stabilized by*** extensive ***hydrogen bonding***. It is formed between H atom attached to peptide N, and O atom attached to peptide C. The hydrogen bonds are individually weak but collectively, they are strong enough to stabilize the helix.

3. All the peptide bonds, except the first and last in a polypeptide chain, participate in hydrogen bonding.

4. Each turn of α-helix contains 3.6 amino acids and travels a distance of 0.54 nm. The spacing of each amino acid is 0.15 nm.

5. α-Helix is a stable conformation formed spontaneously with the lowest energy.

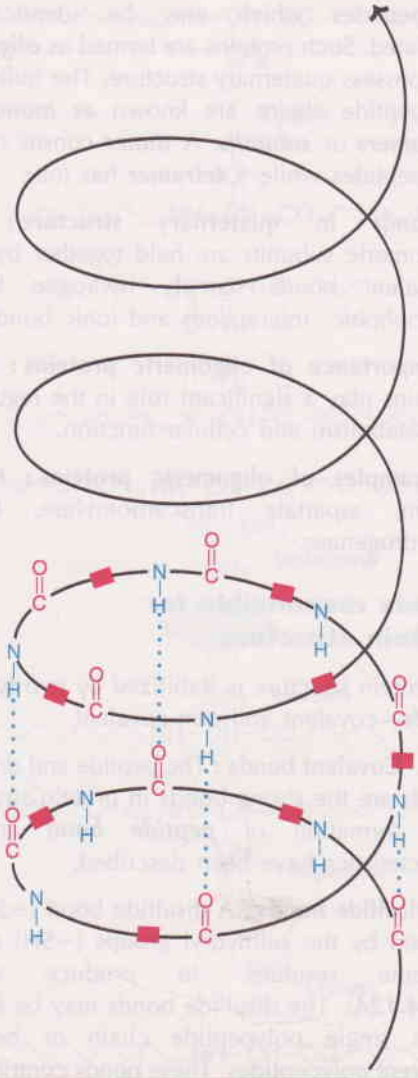


Fig. 4.9 : Diagrammatic representation of secondary structure of protein—a right handed α-helix

(■-Indicate *groups of amino acids; dotted blue lines are hydrogen bonds; Note that only a few hydrogen bonds shown for clarity).*

6. The right handed α-helix is more stable than left handed helix (a right handed helix turns in the direction that the fingers of right hand curl when its thumb points in the direction the helix rises).

7. Certain amino acids (particularly proline) disrupt the α-helix. Large number of acidic (Asp, Glu) or basic (Lys, Arg, His) amino acids also interfere with α-helix structure.

β-Pleated sheet

This is the second type of structure (hence β after α) proposed by Pauling and Corey. β-Pleated sheets (or simply β-sheets) are composed of two or more segments of fully extended peptide chains (***Fig.4.10***). In the β-sheets, the hydrogen bonds are formed between the neighbouring segments of polypeptide chain(s).

Parallel and anti-parallel β-sheets

The polypeptide chains in the β-sheets may be arranged either in parallel (the same direction) or anti-parallel (opposite direction). This is illustrated in ***Fig.4.10***.

β-Pleated sheet may be formed either by separate polypeptide chains (H-bonds are interchain) or a single polypeptide chain folding back on to itself (H-bonds are intrachain).

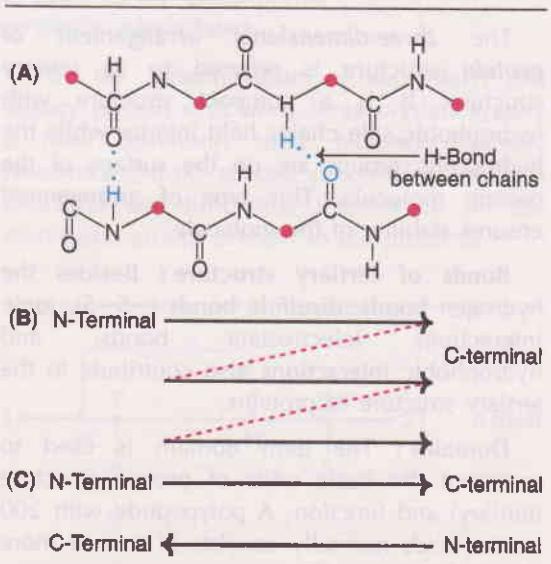


*Fig. 4.10 : Structure of β-pleated sheet (A) Hydrogen bonds between polypeptide chains (B) Parallel β-sheet (C) Antiparallel β-sheet. (**Note** : Red circles in A represent amino acid skeleton—*  *).*

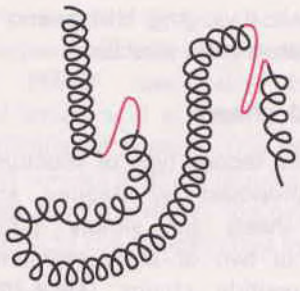


Fig. 4.11 : Diagrammatic representation of a protein containing α-helix and β-pleated sheet (blue).

Occurrence of β-sheets : Many proteins contain β-pleated sheets. As such, the α-helix and β-sheet are commonly found in the same protein structure (***Fig.4.11***). In the globular proteins, β-sheets form the core structure.

Other types of secondary structures : Besides the α-and β-structures described above, the β-bends and nonrepetitive (less organised structures) secondary structures are also found in proteins.

TERTIARY STRUCTURE OF PROTEIN

The ***three-dimensional arrangement of protein*** structure is referred to as tertiary structure. It is a compact structure with hydrophobic side chains held interior while the hydrophilic groups are on the surface of the protein molecule. This type of arrangement ensures stability of the molecule.

Bonds of tertiary structure : Besides the hydrogen bonds, disulfide bonds (−S−S), ionic interactions (electrostatic bonds) and hydrophobic interactions also contribute to the tertiary structure of proteins.

Domains : The term domain is used to represent the basic units of protein structure (tertiary) and function. A polypeptide with 200 amino acids normally consists of two or more domains.

QUATERNARY STRUCTURE OF PROTEIN

A great majority of the proteins are composed of single polypeptide chains. Some of the proteins, however, consist of two or more polypeptides which may be identical or unrelated. Such proteins are termed as ***oligomers*** and possess quaternary structure. The individual polypeptide chains are known as ***monomers, protomers*** or ***subunits***. A ***dimer*** consits of ***two*** polypeptides while a ***tetramer*** has four.

Bonds in quaternary structure : The monomeric subunits are held together by non-convalent bonds namely hydrogen bonds, hydrophobic interactions and ionic bonds.

Importance of oligomeric proteins : These proteins play a significant role in the regulation of metabolism and cellular function.

Examples of oligomeric proteins : Hemoglobin, aspartate transcarbomylase, lactate dehydrogenase.

Bonds responsible for protein structure

Protein structure is stabilized by two types of bonds—covalent and non-covalent.

1. **Covalent bonds :** The peptide and disulfide bonds are the strong bonds in protein structure. The formation of ***peptide bond*** and its chracteristics have been described.

Disulfide bonds : A disulfide bond (***−S−S***) is formed by the sulfhydryl groups (−SH) of two cysteine residues, to produce cystine (***Fig.4.12A***). The disulfide bonds may be formed in a single polypeptide chain or between different polypeptides. These bonds contribute to the structural conformation and stability of proteins.

2. **Non-covalent bonds :** There are, mainly, four types of non-covalent bonds.

(a) **Hydrogen bonds :** The hydrogen bonds are formed by sharing of hydrogen atoms between the nitrogen and carbonyl oxygen of different peptide bonds (***Fig.4.12B***). Each hydrogen bond is weak but collectively they are strong. A large number of hydrogen bonds significantly contribute to the protein structure.

(b) **Hydrophobic bonds :** The non-polar side chains of neutral amino acids tend to be

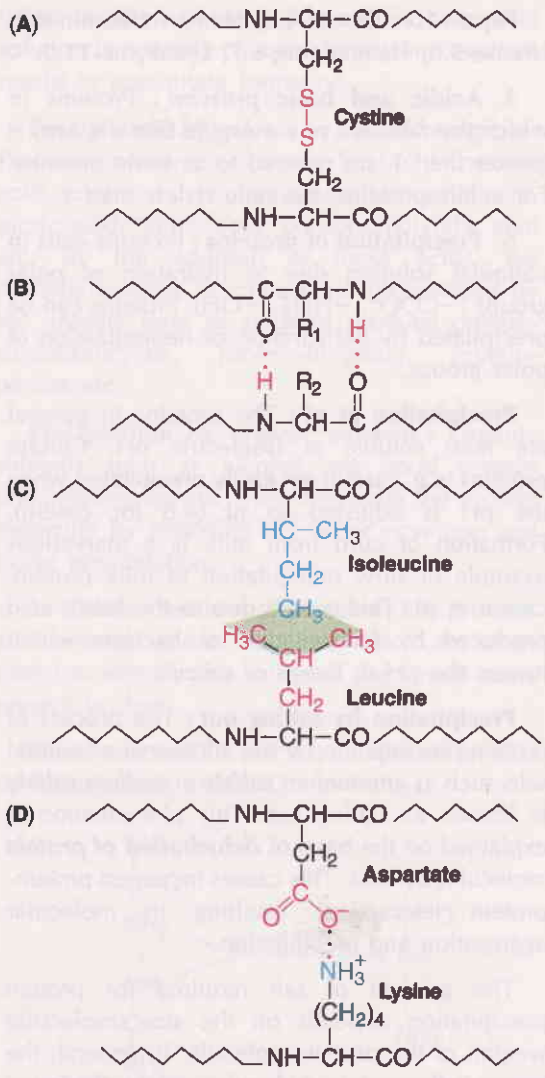


Fig. 4.12 : Major bonds in protein structure (A) Disulfide bond (B) Hydrogen bonds (C) Hydrophic bonds (D) Electrostatic bond.
(Note : See Fig. 4.5 for peptide bond).

closely associated with each other in proteins (***Fig.4.12C***). As such, these are not true bonds. The occurrence of hydrophobic forces is observed in aqueous environment wherein the molecules are forced to stay together.

(c) **Electrostatic bonds :** These bonds are formed by interactions between negatively charged groups (e.g. COO^-) of acidic amino acids with positively charged groups (e.g. $-NH_3^+$) of basic amino acids (***Fig.4.12D***).

(d) **Van der Waals forces :** These are the non-covalent associations between electrically neutral molecules. They are formed by the electrostatic interactions due to permanent or induced dipoles.

Examples of protein structure

Structure of human insulin : Insulin consists of two polypeptide chains, A and B (***Fig.4.13***). The A chain has glycine at the N-terminal end and asparagine at the C-terminal end. The B chain has phenylalanine and alanine at the N- and C-terminal ends, respectively. Originally, insulin is synthesized as a single polypeptide *preproinsulin* which undergoes proteolytic processing to give *proinsulin* and finally *insulin.*

The structural aspects of hemoglobin and collagen are respectively given in ***Chapters 10*** and ***22.***

Methods to determine protein structure

For the determination of secondary and tertiary protein structures, X-ray crystallography is most commonly used. Nuclear magnetic resonance (NMR) spectra of proteins provides structural and functional information on the atoms and groups present in the proteins.

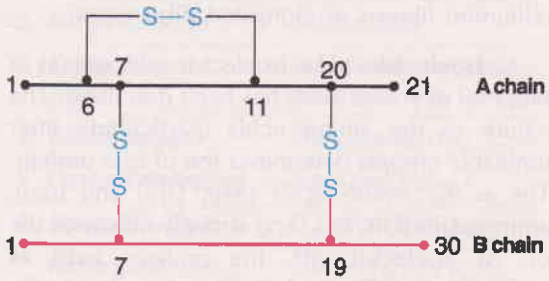


Fig. 4.13 : Diagrammatic representation of human insulin structure
(Note : A and B polypeptide chains are held by two disulfide linkages).

Methods for the isolation and purification of proteins

Several methods are employed to isolate and purify proteins. Initially, proteins are fractionated by using different concentrations of ammonium sulfate or sodium sulfate. Protein fractionation may also be carried out by ultracentrifugation.

Protein separation is achieved by utilizing electrophoresis, isoelectric focussing, immuno-electrophoresis, ion-exchange chromatography, gel-filtration, high performance liquid chromatography (HPLC) etc. The details of these techniques are described in ***Chapter 41***.

PROPERTIES OF PROTEINS

1. **Solubility :** Proteins form colloidal solutions instead of true solutions in water. This is due to huge size of protein molecules.

2. **Molecular weight :** The proteins vary in their molecular weights, which, in turn, is dependent on the number of amino acid residues. Each amino acid on an average contributes to a molecular weight of about 110. Majority of proteins/polypeptides may be composed of 40 to 4,000 amino acids with a molecular weight ranging from 4,000 to 440,000. A few proteins with their molecular weights are listed below :

Insulin-5,700; Myoglobin-1,700; Hemoglobin-64,450; Serum albumin-69,000.

3. **Shape :** There is a wide variation in the protein shape. It may be globular (insulin), oval (albumin) fibrous or elongated (fibrinogen).

4. **Isoelectric pH :** Isoelectric pH (pI) as a property of amino acids has been described. The nature of the amino acids (particularly their ionizable groups) determines the pI of a protein. The acidic amino acids (Asp, Glu) and basic amino acids (His, Lys, Arg) strongly influence the pI. At isoelectric pH, the proteins exist as ***zwitterions*** or ***dipolar ions***. They are electrically neutral (do not migrate in the electric field) with minimum solubility, maximum precipitability and least buffering capacity. The isoelectric pH(pI) for some proteins are given here

Pepsin-1.1; Casein-4.6; Human albumin-4.7; Urease-5.0; Hemoglobin-6.7; Lysozyme-11.0.

5. **Acidic and basic proteins :** Proteins in which the ratio (ε Lys + ε Arg)/(ε Glu + ε Asp) is greater than 1 are referred to as basic proteins. For acidic proteins, the ratio is less than 1.

6. **Precipitation of proteins :** Proteins exist in colloidal solution due to hydration of polar groups ($-COO^-$, $-NH_3^+$, $-OH$). Proteins can be precipitated by dehydration or neutralization of polar groups.

Precipitation at pI : The proteins in general are least soluble at isoelectric pH. Certain proteins (e.g. casein) get easily precipitated when the pH is adjusted to pI (4.6 for casein). Formation of curd from milk is a marvellous example of slow precipitation of milk protein, casein at pI. This occurs due to the lactic acid produced by fermentation of bacteria which lowers the pH to the pI of casein.

Precipitation by salting out : The process of protein precipitation by the additional of neutral salts such as ***ammonium sulfate*** or ***sodium sulfate*** is known as salting out. This phenomenon is explained on the basis of ***dehydration of protein*** molecules by salts. This causes increased protein-protein interaction, resulting in molecular aggregation and precipitation.

The amount of salt required for protein precipitation depends on the size (molecular weight) of the protein molecule. In general, the higher is the protein molecular weight, the lower is the salt required for precipitation. Thus, serum ***globulins*** are ***precipitated by half saturation*** with ammonium sulfate while ***albumin*** is precipitated ***by full saturation***. Salting out procedure is conveniently used for separating serum albumins from globulins.

The addition of small quantities of neutral salts increases the solubility of proteins. This process called as ***salting in*** is due to the diminished protein–protein interaction at low salt concentration.

Precipitation by salts of heavy metals : Heavy metal ions like Pb^{2+}, Hg^{2+}, Fe^{2+}, Zn^{2+}, Cd^{2+} cause precipitation of proteins. These metals

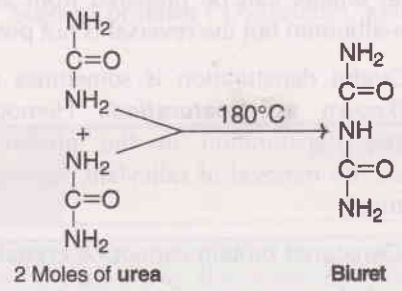
being positively charged, when added to protein solution (negatively charged) in alkaline medium results in precipitate formation.

Precipitation by anionic or alkaloid reagents : Proteins can be precipitated by trichloroacetic acid, sulphosalicylic acid, phosphotungstic acid, picric acid, tannic acid, phosphomolybdic acid etc. By the addition of these acids, the proteins existing as cations are precipitated by the anionic form of acids to produce protein-sulphosalicylate, protein-tungstate, protein-picrate etc.

Precipitation by organic solvents : Organic solvents such as alcohol are good protein precipitating agents. They dehydrate the protein molecule by removing the water envelope and cause precipitation.

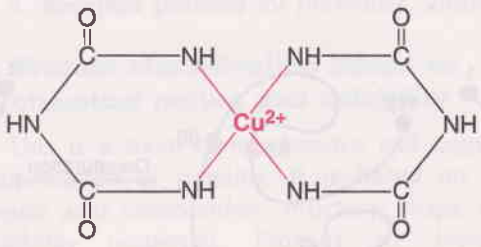
7. **Colour reactions of proteins :** The proteins give several colour reactions which are often useful to identify the nature of the amino acids present in them.

Biuret reaction : Biuret is a compound formed by heating urea to 180°C.



When biuret is treated with dilute copper sulfate in alkaline medium, a purple colour is obtained. This is the basis of biuret test widely used for identification of proteins and peptides.

Biuret test is answered by compounds containing two or more CO—NH groups i.e., peptide bonds. All proteins and peptides possessing at least two peptide linkages i.e., tripeptides (with 3 amino acids) give positive biuret test. Histidine is the only amino acid that answers biuret test. The principle of biuret test is conveniently used to detect the presence of proteins in biological fluids. The mechanism of biuret test is not clearly known. It is believed that the colour is due to the formation of a ***copper co-ordinated complex***, as shown below.



The presence of magnesium and ammonium ions interfere in the biuret test. This can be overcome by using excess alkali.

The colour reactions given by proteins due to the presence of specific amino acids are given in ***Table 4.3***. These reactions are often useful to know the presence or absence of the said amino acids in the given protein.

DENATURATION

The phenomenon of ***disorganization of native protein structure*** is known as denaturation. Denaturation results in the loss of secondary, tertiary and quaternary structure of proteins. This involves a change in physical, chemical and biological properties of protein molecules.

Table 4.3 Colour reactions of proteins/amino acids

Reaction	Specific group or amino acid
1. Biuret reaction	Two peptide linkages
2. Ninhydrin reaction	α-Amino acids
3. Xanthoproteic reaction	Benzene ring of aromatic amino acids (Phe, Tyr, Trp)
4. Millions reaction	Phenolic group (Tyr)
5. Hopkins-Cole reaction	Indole ring (Trp)
6. Sakaguchi reaction	Guanidino group (Arg)
7. Nitroprusside reaction	Sulfhydryl groups (Cys)
8. Sulfur test	Sulfhydryl groups (Cys)
9. Pauly's test	Imidazole ring (His)
10. Folin–Coicalteau's test	Phenolic groups (Tyr)

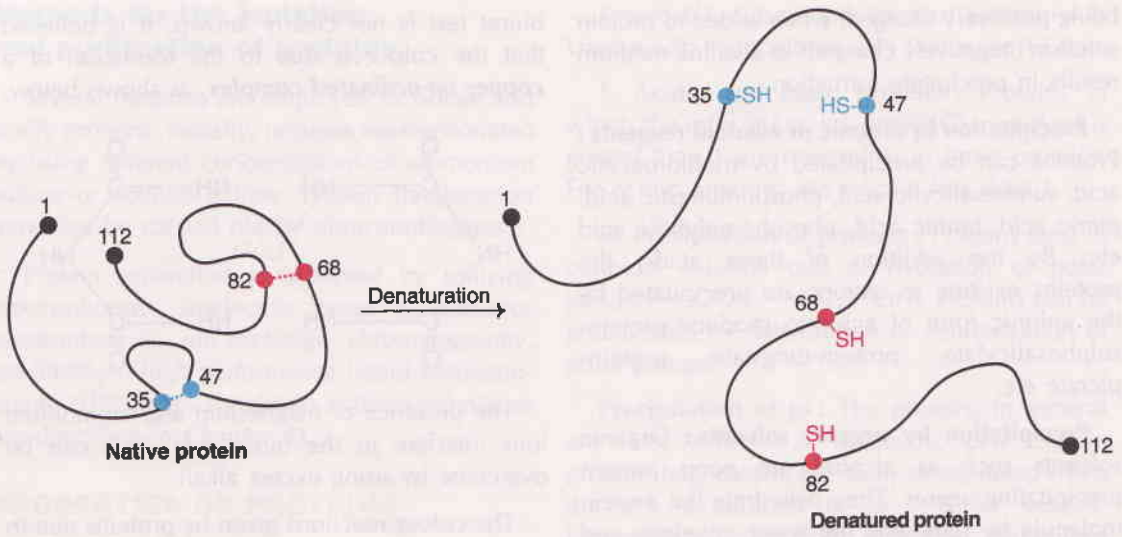


Fig. 4.14 : Denaturation of a protein.

Agents of denaturation

Physical agents : Heat, violent shaking, X-rays, UV radiation.

Chemical agents : Acids, alkalies, organic solvents (ether, alcohol), salts of heavy metals (Pb, Hg), urea, salicylate.

Characteristics of denaturation

1. The native helical structure of protein is lost (***Fig.4.14***).

2. The primary structure of a protein with peptide linkages remains intact i.e., peptide bonds are not hydrolysed.

3. The protein loses its biological activity.

4. Denatured protein becomes insoluble in the solvent in which it was originally soluble.

5 The viscosity of denatured protein (solution) increases while its surface tension decreases.

6. Denaturation is associated with increase in ionizable and sulfhydryl groups of protein. This is due to loss of hydrogen and disulfide bonds.

7. Denatured protein is more easily digested. This is due to increased exposure of peptide bonds to enzymes. Cooking causes protein denaturation and, therefore, cooked food (protein) is more easily digested.

8. Denaturation is usually irreversible. For instance, omelet can be prepared from an egg (protein-albumin) but the reversal is not possible.

9. Careful denaturation is sometimes reversible (known as ***renaturation***). Hemoglobin undergoes denaturation in the presence of salicylate. By removal of salicylate, hemoglobin is renatured.

10. Denatured protein cannot be crystallized.

Coagulation : The term 'coagulum' refers to a semi-solid viscous precipitate of protein. Irreversible denaturation results in coagulation. Coagulation is optimum and requires lowest temperature at isoelectric pH. Albumins and globulins (to a lesser extent) are coagulable proteins. ***Heat coagulation test is commonly used to detect the presence of albumin in urine.***

Flocculation : It is the process of protein precipitation at isoelectric pH. The precipitate is referred to as flocculum. Casein (milk protein) can be easily precipitated when adjusted to isoelectric pH (4.6) by dilute acetic acid. Flocculation is reversible. On application of

heat, flocculum can be converted into an irreversible mass, coagulum.

CLASSIFICATION OF PROTEINS

Proteins are classified in several ways. Three major types of classifying proteins based on their function, chemical nature and solubility properties and nutritional importance are discussed here.

A. Functional classification of proteins

Based on the functions they perform, proteins are classified into the following groups (with examples)

1. **Structural proteins :** Keratin of hair and nails, collagen of bone.
2. **Enzymes or catalytic proteins :** Hexokinase, pepsin.
3. **Transport proteins :** Hemoglobin, serum albumin.
4. **Hormonal proteins :** Insulin, growth hormone.
5. **Contractile proteins :** Actin, myosin.
6. **Storage proteins :** Ovalbumin, glutelin.
7. **Genetic proteins :** Nucleoproteins.
8. **Defense proteins :** Snake venoms, Immunoglobulins.
9. **Receptor proteins** for hormones, viruses.

B. Protein classification based on chemical nature and solubility

This is a more comprehensive and popular classification of proteins. It is based on the amino acid composition, structure, shape and solubility properties. Proteins are broadly classified into 3 major groups

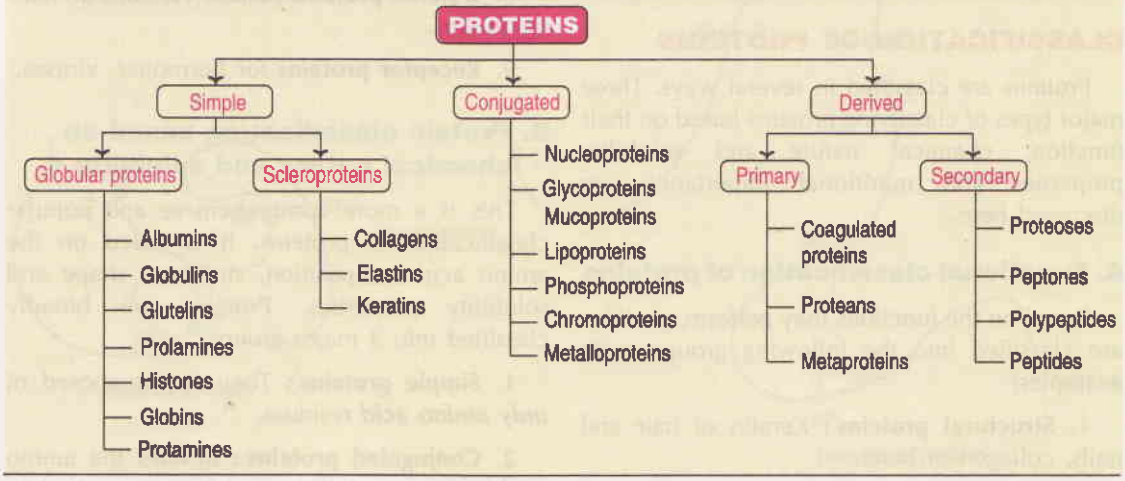
1. **Simple proteins :** They are composed of ***only amino acid*** residues.
2. **Conjugated proteins :** Besides the amino acids, these proteins contain a non-protein moiety known as ***prosthetic group*** or conjugating group.
3. **Derived proteins :** These are the denatured or degraded products of simple and conjugated proteins.

The above three classes are further subdivided into different groups. The summary of protein classification is given in the ***Table 4.4.***

BIOMEDICAL / CLINICAL CONCEPTS

- *Proteins are the most abundant organic molecules of life. They perform static (structural) and dynamic functions in the living cells.*
- *The dynamic functions of proteins are highly diversified such as enzymes, hormones, clotting factors, immunoglobulins, storage proteins and membrane receptors.*
- *Half of the amino acids (about 10) that occur in proteins have to be consumed by humans in the diet, hence they are essential.*
- *A protein is said to be complete (or first class) protein if all the essential amino acids are present in the required proportion by the human body e.g. egg albumin.*
- *Cooking results in protein denaturation exposing more peptide bonds for easy digestion.*
- *Monosodium glutamate (MSG) is used as a flavoring agent in foods to increase taste and flavour. In some individuals intolerant to MSG, Chinese restaurant syndrome (brief and reversible flu-like symptoms) is observed.*

Table 4.4 Summary of classification of proteins



1. Simple proteins

(a) **Globular proteins :** These are spherical or oval in shape, soluble in water or other solvents and digestible.

(i) **Albumins :** Soluble in water and dilute salt solutions and coagulated by heat. e.g. serum albumin, ovalbumin (egg), lactalbumin (milk).

(ii) **Globulins :** Soluble in neutral and dilute salt solutions e.g. serum globulins, vitelline (egg yolk).

(iii) **Glutelins :** Soluble in dilute acids and alkalies and mostly found in plants e.g. glutelin (wheat), oryzenin (rice).

(iv) **Prolamines :** Soluble in 70% alcohol e.g. *gliadin (wheat)*, zein (maize).

(v) **Histones :** Strongly basic proteins, soluble in water and dilute acids but insoluble in dilute ammonium hydroxide e.g. thymus histones, histones of codfish sperm.

(vi) **Globins :** These are generally considered along with histones. However, globins are not basic proteins and are not precipitated by NH_4OH.

(vii) **Protamines :** They are strongly basic and resemble histones but smaller in size and soluble in NH_4OH. Protamines are also found in association with nucleic acids e.g. sperm proteins.

(b) **Fibrous proteins :** These are fiber like in shape, insoluble in water and resistant to digestion. Albuminoids or scleroproteins constitute the most predominant group of fibrous proteins.

(i) **Collagens** are connective tissue proteins lacking tryptophan. Collagens, on boiling with water or dilute acids, yield gelatin which is soluble and digestible.

(ii) **Elastins :** These proteins are found in elastic tissues such as tendons and *arteries*.

(iii) **Keratins :** These are present in exoskeletal structures e.g. hair, nails, horns. Human hair keratin contains as much as 14% cysteine.

2. Conjugated proteins

(a) **Nucleoproteins :** Nucleic acid (DNA or RNA) is the prosthetic group e.g. nucleohistones, nucleoprotamines.

(b) **Glycoproteins :** The prosthetic group is carbohydrate, which is less than 4% of protein. The term ***mucoprotein*** is used if the carbohydrate content is more than 4%. e.g. mucin (saliva), ovomucoid (egg white).

(c) **Lipoproteins :** Protein found in combination with lipids as the prosthetic group e.g. serum lipoproteins, membrane lipoproteins.

(d) **Phosphoproteins :** Phosphoric acid is the prosthetic group e.g. casein (milk), vitelline (egg yolk).

(e) **Chromoproteins :** The prosthetic group is coloured in nature e.g. hemoglobins, cytochromes.

(f) **Metalloproteins :** These proteins contain metal ions such as Fe, Co, Zn, Cu, Mg etc., e.g. ceruloplasmin (Cu), carbonic anhydrase (Zn).

3. Derived proteins : The derived proteins are of two types. The primary derived are the denatured or coagulated or first hydrolysed products of proteins. The secondary derived are the degraded (due to breakdown of peptide bonds) products of proteins.

(a) **Primary derived proteins**

(i) **Coagulated proteins :** These are the denatured proteins produced by agents such as heat, acids, alkalies etc. e.g. cooked proteins, coagulated albumin (egg white).

(ii) **Proteans :** These are the earliest products of protein hydrolysis by enzymes, dilute acids, alkalies etc. which are insoluble in water. e.g. fibrin formed from fibrinogen.

(iii) **Metaproteins :** These are the second stage products of protein hydrolysis obtained by treatment with slightly stronger acids and alkalies e.g. acid and alkali metaproteins.

(b) **Secondary derived proteins :** These are the progressive hydrolytic products of protein hydrolysis. These include proteoses, peptones, polypeptides and peptides.

C. Nutritional classification of proteins

The nutritive value of proteins is determined by the composition of essential amino acids (described already). From the nutritional point of view, proteins are classified into 3 categories.

1. **Complete proteins :** These proteins have all the ten essential amino acids in the required proportion by the human body to promote good growth. e.g. ***egg albumin***, milk casein.

2. **Partially incomplete proteins :** These proteins are partially lacking one or more essential amino acids and hence can promote moderate growth. e.g. wheat and rice proteins (limiting Lys, Thr).

3. **Incomplete proteins :** These proteins completely lack one or more essential amino acids. Hence they do not promote growth at all e.g. ***gelatin*** (lacks Trp), zein (lacks Trp, Lys).

BIOLOGICALLY IMPORTANT PEPTIDES

Several peptides occur in the living organisms that display a wide spectrum of biological functions. Generally, the term 'peptide' is applied when the number of constituent amino acids is less than 10. Some examples of biologically active peptides and their functions are described here.

1. **Glutathione :** It is a tripeptide composed of 3 amino acids. Chemically, glutathione is γ-glutamyl-cysteinyl-glycine. It is widely distributed in nature and exists in reduced or oxidized states.

$$\underset{\text{Reduced}}{2G-SH} \rightleftharpoons \underset{\text{Oxidized}}{G=S-S-G}$$

Functions : In a steady state, the cells generally maintain a ratio of about 100/1 of GSH to G–S–S–G. The reversible oxidation-reduction of glutathione is important for many of its biological functions.

- Glutathione serves as a coenzyme for certain enzymes e.g. prostaglandin PGE_2 synthetase, glyoxylase.
- It prevents the oxidation of sulfhydryl (–SH) groups of several proteins to disulfide (–S–S–) groups. This is essential for the protein function, including that of enzymes.

- It is believed that glutathione in association with glutathione reductase participates in the formation of correct disulfide bonds in several proteins.
- Glutathione (reduced) performs specialized functions in erythrocytes
  - (i) It maintains RBC membrane structure and integrity.
  - (ii) It protects hemoglobin from getting oxidized by agents such as H_2O_2.
- Glutathione is involved in the transport of amino acids in the intestine and kidney tubules via ***γ-glutamyl cycle*** or ***Meister cycle*** (***Refer Chapter 8***).
- Glutathione is involved in the detoxication process. The toxic substances (organophosphates, nitro compounds) are converted to mercapturic acids.
- Toxic amounts of peroxides and free radicals produced in the cells are scavanged by glutathione peroxidase (a selenium containing enzyme).

$$2\,GSH + H_2O_2 \xrightarrow{\text{Peroxidase}} G-S-S-G + 2\,H_2O$$

2. **Thyrotropin releasing hormone** (*TRH*) **:** It is a tripeptide secreted by hypothalamus. TRH stimulates pituitary gland to release thyrotropic hormone.

3. **Oxytocin :** It is a hormone secreted by posterior pituitary gland and contains 9 amino acids (nonapeptide). Oxytocin causes contraction of uterus.

4. **Vasopressin** (***antidiuretic hormone, ADH***) **:** ADH is also a nonapeptide produced by posterior pituitary gland. It stimulates kidneys to retain water and thus increases the blood pressure.

5. **Angiotensins :** Angiotensin I is a decapeptide (10 amino acids) which is converted to angiotensin II (8 amino acids). The later has more hypertensive effect. Angiotensin II also stimulates the release of aldosterone from adrenal gland.

BIOMEDICAL / CLINICAL CONCEPTS

- *Collagen is the most abundant protein in mammals. It is rich in hydroxyproline and hydroxylysine.*
- *Several biologically important peptides are known in the living organism. These include glutathione for the maintenance of RBC structure and integrity; oxytocin that causes uterus contraction; vasopressin that stimulates retention of water by kidneys; enkephalins that inhibit the sense of pain in the brain.*
- *Antibiotics such as actinomycin, gramicidin, bacitracin and tyrocidin are peptide in nature.*
- *γ-Carboxyglutamic acid is an amino acid derivative found in certain plasma proteins involved in blood clotting.*
- *Homocysteine has been implicated as a risk factor in the onset of coronary heart diseases.*
- *Several non-protein amino acids of biological importance are known. These include ornithine, citrulline and arginosuccinic acid (intermediates of urea synthesis), thyroxine and triiodothyronine (hormones), and β-alanine (of coenzyme A).*
- *The protein-free filtrate of blood, required for biochemical investigations (e.g. urea, sugar) can be obtained by using protein precipitating agents such as phosphotungstic acid and trichloroacetic acid.*
- *Heat coagulation test is most commonly employed to detect the presence of albumin in urine.*

6. **Methionine enkephalin :** It is a pentapeptide found in the brain and has opiate like function. It inhibits the sense of a pain.

7. **Bradykinin and kallidin :** They are nona- and decapeptides, respectively. Both of them act as powerful vasodilators. They are produced from plasma proteins by snake venom enzymes.

8. **Peptide antibiotics :** Antibiotics such as gramicidin, bacitracin, tyrocidin and actinomycin are peptide in nature.

9. **Aspartame :** It is a dipeptide (aspartyl-phenylalanine methyl ester), produced by a combination of aspartic acid and phenylalanine. Aspartame is about 200 times sweeter than sucrose, and is used as a low-calorie artificial sweetner in softdrink industry.

10. **Gastrointestinal hormones :** Gastrin, secretin etc. are the gastrointestinal peptides which serve as hormones.

SUMMARY

1. *Proteins are nitrogen containing, most abundant organic macromolecules widely distributed in animals and plants. They perform structural and dynamic functions in the organisms.*
2. *Proteins are polymers composed of L-α-amino acids. They are 20 in number and classified into different groups based on their structure, chemical nature, nutritional requirement and metabolic fate. Selenocysteine has been recently identified as the 21st amino acid, and is found in certain proteins.*
3. *Amino acids possess two functional groups namely carboxyl (−COOH) and amino ($-NH_2$). In the physiological system, they exist as dipolar ions commonly referred to as zwitterions.*
4. *Besides the 20 standard amino acids present in proteins, there are several non-standard amino acids. These include the amino acid derivatives found in proteins (e.g. hydroxyproline, hydroxylysine) and, non-protein amino acids (e.g. ornithine, citrulline).*
5. *The structure of protein is divided into four levels of organization. The primary structure represents the linear sequence of amino acids. The twisting and spatial arrangement of polypeptide chain is the secondary structure. Tertiary structure constitutes the three dimensional structure of a functional protein. The assembly of similar or dissimilar polypeptide subunits comprises quaternary structure.*
6. *The determination of primary structure of a protein involves the knowledge of quality, quantity and the sequence of amino acids in the polypeptide. Chemical and enzymatic methods are employed for the determination of primary structure.*
7. *The secondary structure of protein mainly consists of α-helix and/or β-sheet. α-Helix is stabilized by extensive hydrogen bonding. β-Pleated sheet is composed of two or more segments of fully extended polypeptide chains.*
8. *The tertiary and quaternary structures of protein are stabilized by non-covalent bonds such as hydrogen bonds, hydrophobic interactions, ionic bonds etc.*
9. *Proteins are classified into three major groups. Simple proteins contain only amino acid residues (e.g. albumin). Conjugated proteins contain a non-protein moiety known as prosthetic group, besides the amino acids (e.g. glycoproteins). Derived proteins are obtained by degradation of simple or conjugated proteins.*
10. *In addition to proteins, several peptides perform biologically important functions. These include glutathione, oxytocin and vasopressin.*

SELF-ASSESSMENT EXERCISES

I. Essay questions

1. Describe the classification of amino acids along with their structures.
2. Discuss the organization of protein structure. Give an account of the determination of primary structure of protein.
3. Describe the classification of proteins with suitable examples.
4. Write an account of non-standard amino acids.
5. Discuss the important biologically active peptides.

II. Short notes

(a) Essential amino acids, (b) Zwitterion, (c) Peptide bond, (d) Edman's reagent, (e) α-Helix, (f) β-Pleated sheet, (g) Denaturation, (h) Isoelectric point, (i) Glutathione, (j) Quaternary structure of protein.

III. Fill in the blanks

1. The average nitrogen content of proteins _____.
2. Proteins are the polymers of _____.
3. Name the sulfur containing essential amino acid _____.
4. The charged molecule which is electrically neutral is known as _____.
5. The non –α amino acid present in coenzyme A _____.
6. The bonds forming the backbone of protein structure _____.
7. The amino acid that is completely destroyed by acid hydrolysis of protein _____.
8. The number of peptide bonds present in a decapeptide _____.
9. The chemical name of Sanger's reagent _____.
10. The phenomenon of disorganization of native protein structure is known as _____.

IV. Multiple choice questions

11. The imino acid found in protein structure

    (a) Arginine (b) Proline (c) Histidine (d) Lysine.

12. The following is a non-protein amino acid

    (a) Ornithine (b) Homocysteine (c) Histamine (d) All of them.

13. The bonds in protein structure that are not broken on denaturation.

    (a) Hydrogen bonds (b) Peptide bonds (c) Ionic bond (d) Disulfide bonds.

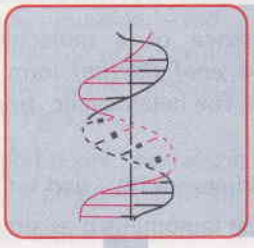
14. Sequenator is an automatic machine to determine amino acid sequence in a polypeptide chain. The reagent used in sequenator is

    (a) Sanger's reagent (b) CNBr (c) Trypsin (d) Edman's reagent.

15. The reaction given by two or more peptide linkages is

    (a) Biuret test (b) Ninhydrin test (c) Xanthoproteic reaction (d) Pauley's test.

5 Nucleic Acids and Nucleotides



DNA, the bank of genetic information speaks :

"I am the chemical basis of life and heredity!
Organized into genes that control every function;
Composed of repeating units of deoxyribonucleotides;
Arrranged in a double helix, held by hydrogen bonds."

There are two types of nucleic acids, namely ***deoxyribonucleic acid*** (DNA) and ***ribonucleic acid*** (RNA). Primarily, nucleic acids serve as repositories and transmitters of genetic information.

Brief history

DNA was discovered in 1869 by Johann Friedrich Miescher, a Swiss researcher. The demonstration that DNA contained genetic information was first made in 1944, by Avery, Macleod and MacCary.

Functions of nucleic acids

DNA is the chemical basis of heredity and may be regarded as the reserve bank of genetic information. DNA is exclusively responsible for maintaining the identity of different species of organisms over millions of years. Further, every aspect of cellular function is under the control of DNA. The ***DNA*** is organized into ***genes***, the fundamental units of ***genetic information***. The genes control the protein synthesis through the mediation of RNA, as shown below

The interrelationship of these three classes of biomolecules (DNA, RNA and proteins) constitutes the ***central dogma of molecular biology*** or more commonly the ***central dogma of life***.

Components of nucleic acids

Nucleic acids are the polymers of nucleotides (polynucleotides) held by 3′ and 5′ phosphate bridges. In other words, nucleic acids are built up by the monomeric units—nucleotides (It may be recalled that protein is a polymer of amino acids).

NUCLEOTIDES

Nucleotides are composed of a ***nitrogenous base***, a ***pentose sugar*** and a ***phosphate***. Nucleotides perform a wide variety of functions in the living cells, besides being the building blocks or

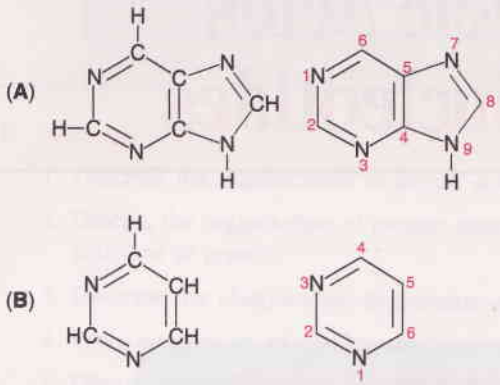


Fig. 5.1 : General structure of nitrogen bases (A) Purine (B) Pyrimidine (The positions are numbered according to the international system).

monomeric units in the nucleic acid (DNA and RNA) structure. These include their role as structural components of some coenzymes of B-complex vitamins (e.g. FAD, NAD^+), in the energy reactions of cells (ATP is the energy currency), and in the control of metabolic reactions.

STRUCTURE OF NUCLEOTIDES

As already stated, the nucleotide essentially consists of ***nucleobase***, ***sugar*** and ***phosphate***. The term nucleoside refers to base + sugar. Thus, nucleotide is nucleoside + phosphate.

Purines and pyrimidines

The nitrogenous bases found in nucleotides (and, therefore, nucleic acids) are ***aromatic heterocyclic compounds***. The bases are of two types—purines and pyrimidines. Their general structures are depicted in ***Fig.5.1***. Purines are numbered in the anticlockwise direction while pyrimidines are numbered in the clockwise direction. And this is an internationally accepted system to represent the structure of bases.

Major bases in nucleic acids

The structures of major purines and pyrimidines found in nucleic acids are shown in ***Fig.5.2***. DNA and RNA contain the same purines namely adenine (A) and guanine (G). Further, the pyrimidine cytosine (C) is found in both DNA and RNA. However, the nucleic acids differ with respect to the second pyrimidine base. ***DNA contains thymine (T) whereas RNA contains uracil (U)***. As is observed in the ***Fig.5.2***, thymine and uracil differ in structure by the presence (in T) or absence (in U) of a methyl group.

Tautomeric forms of purines and pyrimidines

The existence of a molecule in a ***keto (lactam)*** and ***enol (lactim)*** form is known as tautomerism. The heterocyclic rings of purines and pyrimidines with ***oxo*** functional groups exhibit tautomerism as simplified below.

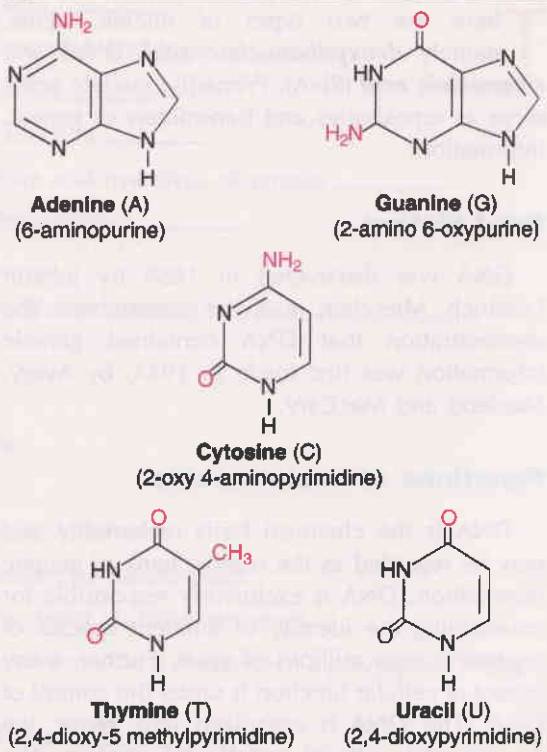


Fig. 5.2 : Structures of major purines (A, G) and pyrimidines (C, T, U) found in nucleic acids.

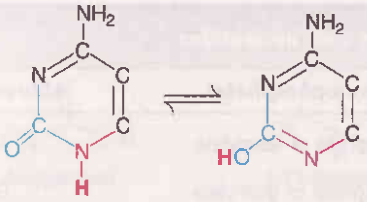


Fig. 5.3 : The tautomeric forms of cytosine.

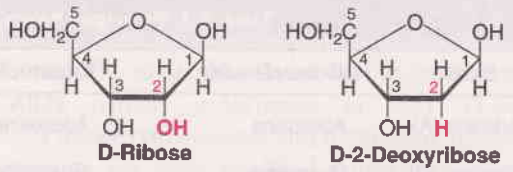


Fig. 5.5 : Structures of sugars present in nucleic acids (ribose is found in RNA and deoxyribose in DNA; Note the structural difference at C_2).

The purine—guanine and pyrimidines-cytosine, thymine and uracil exhibit tautomerism. The lactam and lactim forms of cytosine are represented in ***Fig.5.3***.

At physiological pH, the lactam (keto) tautomeric forms are predominantly present.

Minor bases found in nucleic acids : Besides the bases described above, several minor and unusual bases are often found in DNA and RNA. These include 5-methylcytosine, N^4-acetylcytosine, N^6-methyladenine, N^6, N^6-dimethyladenine, pseudouracil etc. It is believed that the unusual bases in nucleic acids will help in the recognition of specific enzymes.

Other biologically important bases : The bases such as hypoxanthine, xanthine and uric acid (***Fig.5.4***) are present in the free state in the cells. The former two are the intermediates in purine synthesis while uric acid is the end product of purine degradation.

Purine bases of plants : Plants contain certain methylated purines which are of pharmacological interest. These include caffeine (of coffee), theophylline (of tea) and theobromine (of cocoa).

Fig. 5.4 : Structures of some biologically important purines.

Sugars of nucleic acids

The five carbon monosaccharides (pentoses) are found in the nucleic acid structure. ***RNA*** contains ***D-ribose*** while ***DNA*** contains ***D-deoxyribose***. Ribose and deoxyribose differ in structure at C_2. Deoxyribose has one oxygen less at C_2 compared to ribose (***Fig.5.5***).

Nomenclature of nucleotides

The addition of a pentose sugar to base produces a nucleoside. If the sugar is ribose, ribonucleosides are formed. Adenosine, guanosine, cytidine and uridine are the ribonucleosides of A, G, C and U respectively. If the sugar is a deoxyribose, deoxyribonucleosides are produced.

The term mononucleotide is used when a single phosphate moiety is added to a nucleoside. Thus adenosine monophosphate (AMP) contains adenine + ribose + phosphate.

The principal bases, their respective nucleosides and nucleotides found in the structure of nucleic acids are given in ***Table 5.1***. Note that the prefix 'd' is used to indicate if the sugar is deoxyribose (e.g. dAMP).

The binding of nucleotide components

The atoms in the purine ring are numbered as 1 to 9 and for pyrimidine as 1 to 6 (***See Fig.5.1***). The carbons of sugars are represented with an associated prime (') for differentiation. Thus the pentose carbons are 1' to 5'.

Table 5.1 Principal bases, nucleosides and nucleotides

Base	*Ribonucleoside*	*Ribonucleotide (5′-monophosphate)*	*Abbreviation*
Adenine (A)	Adenosine	Adenosine 5′-monophosphate or adenylate	AMP
Guanine (G)	Guanosine	Guanosine 5′-monophosphate or guanylate	GMP
Cytosine (C)	Cytidine	Cytidine 5′-monophosphate or cytidylate	CMP
Uracil (U)	Uridine	Uridine 5′-monophosphate or uridylate	UMP
Base	***Deoxyribonucleoside***	***Deoxyribonucleotide (5′-monophosphate)***	***Abbreviation***
Adenine (A)	Deoxyadenosine	Deoxyadenosine 5′-monophosphate or deoxyadenylate	dAMP
Guanine (G)	Deoxyguanosine	Deoxyguanosine 5′-monophosphate or deoxyguanylate	dGMP
Cytosine (C)	Deoxycytidine	Deoxycytidine 5′-monophosphate or deoxycytidylate	dCMP
Thymine (T)	Deoxythymidine	Deoxythymidine 5′-monophosphate or deoxythymidylate	dTMP

The pentoses are bound to nitrogenous bases by β-N-glycosidic bonds. The N^9 of a purine ring binds with $C_{1(1')}$ of a pentose sugar to form a covalent bond in the purine nucleoside. In case of pyrimidine nucleosides, the glycosidic linkage is between N^1 of a pyrimidine and C'_1 of a pentose.

The hydroxyl groups of adenosine are esterified with phosphates to produce 5′- or 3′-monophosphates. 5′-Hydroxyl is the most commonly esterified, hence 5′ is usually omitted while writing nucleotide names. Thus AMP represents adenosine 5′-monophosphate. However, for adenosine 3′-monophosphate, the abbreviation 3′-AMP is used.

The structures of two selected nucleotides namely AMP and TMP are depicted in ***Fig.5.6***.

Nucleoside di- and triphosphates

Nucleoside monophosphates possess only one phosphate moiety (AMP, TMP). The addition of second or third phosphates to the nucleoside results in nucleoside diphosphate (e.g. ADP) or triphosphate (e.g. ATP), respectively.

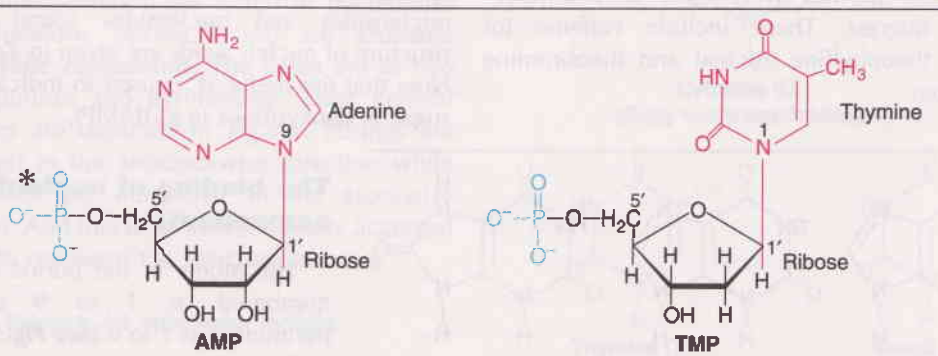


Fig. 5.6 : *The structures of adenosine 5′-monophosphate (AMP) and thymidine 5′-monophosphate (TMP) [*-Addition of second or third phosphate gives adenosine diphosphate (ADP) and adenosine triphosphate (ATP) respectively].*

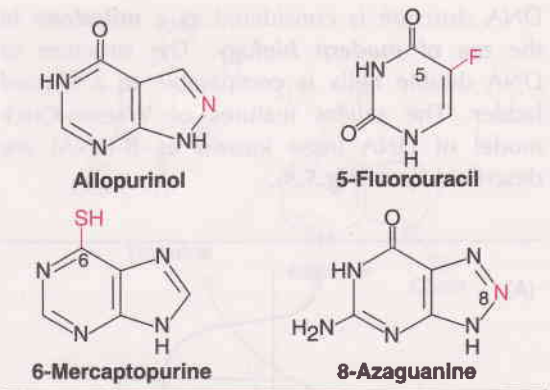


Fig. 5.7 : Structures of selected purine and pyrimidine analogs.

The anionic properties of nucleotides and nucleic acids are due to the negative charges contributed by phosphate groups.

PURINE, PYRIMIDINE AND NUCLEOTIDE ANALOGS

It is possible to alter heterocyclic ring or sugar moiety, and produce synthetic analogs of purines, pyrimidines, nucleosides and nucleotides. Some of the synthetic analogs are highly useful in clinical medicine. The structures of selected purine and pyrimidine analogs are given in ***Fig.5.7***.

The pharmacological applications of certain analogs are listed below

1. ***Allopurinol*** is used in the treatment of hyperuricemia and gout (For details, ***Refer Chapter 17***).

2. ***5-Fluorouracil***, 6-mercaptopurine, 8-azaguanine, 3-deoxyuridine, 5- or 6-azauridine, 5- or 6-azacytidine and 5-idouracil are employed in the treatment of cancers. These compounds get incorporated into DNA and block cell proliferation.

3. ***Azathioprine*** (which gets degraded to 6-mercaptopurine) is used to suppress immunological rejection during transplantation.

4. ***Arabinosyladenine*** is used for the treatment of neurological disease, viral encephalitis.

5. Arabinosylcytosine is being used in cancer therapy as it interferes with DNA replication.

6. The drugs employed in the treatment of AIDS namely zidovudine or AZT (3-azido 2′,3′-dideoxythymidine) and didanosine (dideoxyinosine) are sugar modified ***synthetic nucleotide analogs*** (For their structure and more details ***Refer Chapter 38***).

STRUCTURE OF DNA

DNA is a ***polymer of deoxyribonucleotides*** (or simply deoxynucleotides). It is composed of monomeric units namely deoxyadenylate (dAMP), deoxyguanylate (dGMP), deoxycytidylate (dCMP) and deoxythymidylate (dTMP) (It may be noted here that some authors prefer to use TMP for deoxythymidylate, since it is found only in DNA). The details of the nucleotide structure are given above.

Schematic representation of polynucleotides

The monomeric deoxynucleotides in DNA are held together by 3′,5′-phosphodiester bridges (***Fig.5.8***). DNA (or RNA) structure is often represented in a short-hand form. The horizontal line indicates the carbon chain of sugar with base attached to $C_{1'}$. Near the middle of the horizontal line is $C_{3'}$ phosphate linkage while at the other end of the line is $C_{5'}$ phosphate linkage (***Fig.5.8***).

Chargaff's rule of DNA composition

Erwin Chargaff in late 1940s quantitatively analysed the DNA hydrolysates from different species. He observed that in all the species he studied, DNA had equal numbers of adenine and thymine residues (A = T) and equal numbers of guanine and cytosine residues (G = C). This is known as Chargaff's rule of ***molar equivalence between the purines and pyrimidines in DNA*** structure. The significance of Chargaff's rule was not immediately realised. The double helical structure of DNA derives its strength from Chargaff's rule (discussed later).

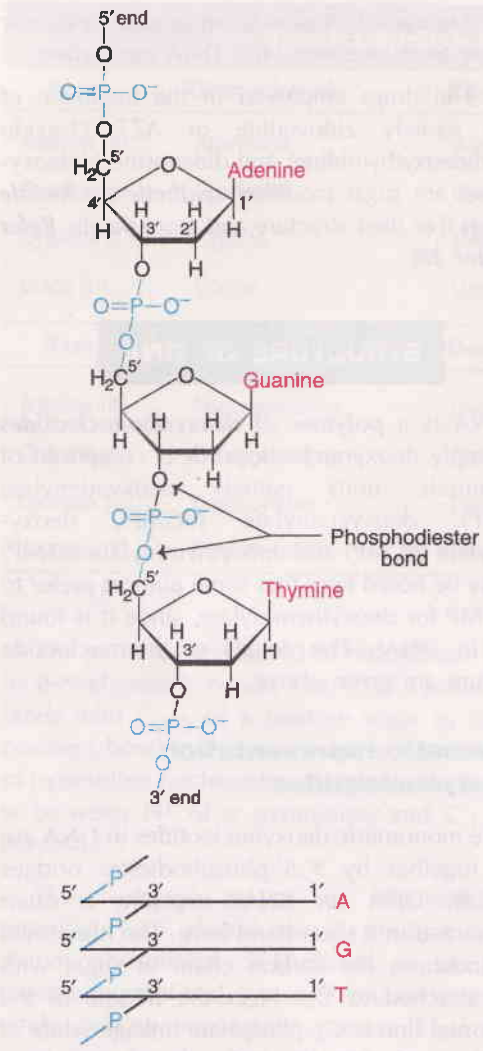


Fig. 5.8 : *Structure of a polydeoxyribonucleotide segment held by phosphodiester bonds. On the lower part is the representation of short hand form of oligonucleotides.*

Single-stranded DNA, and RNAs which are usually single-stranded, do not obey Chargaff's rule. However, double-stranded RNA which is the genetic material in certain viruses satisfies Chargaff's rule.

DNA DOUBLE HELIX

The double helical structure of DNA was proposed by ***James Watson*** and ***Francis Crick*** in 1953 (Nobel Prize, 1962). The elucidation of DNA structure is considered as a ***milestone in*** the era of ***modern biology***. The structure of DNA double helix is comparable to a twisted ladder. The salient features of Watson-Crick model of DNA (now known as B-DNA) are described next (***Fig.5.9***).

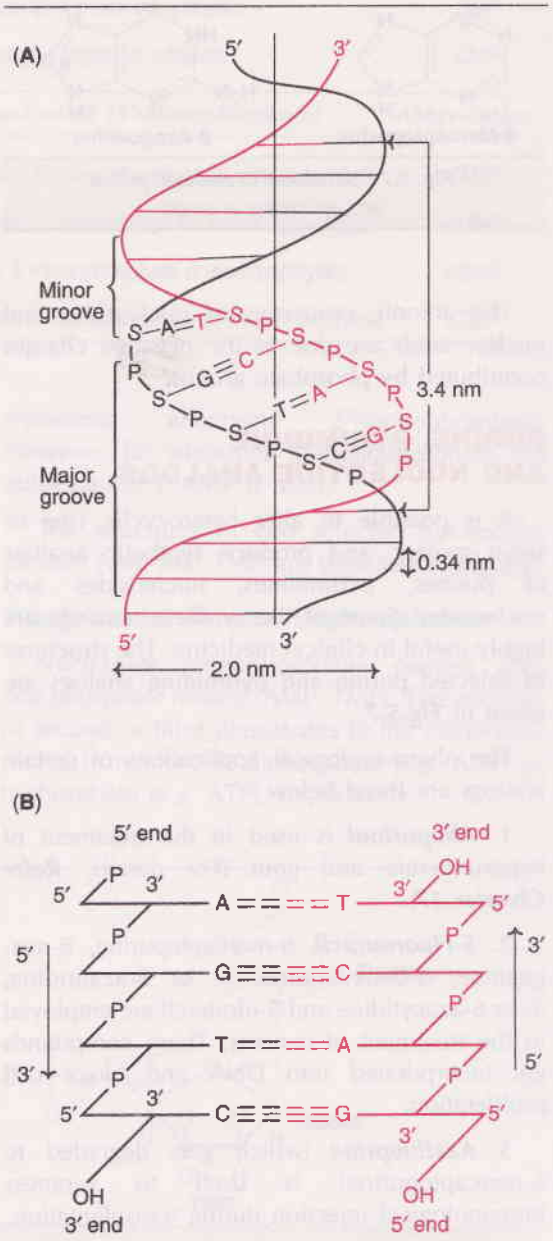


Fig. 5.9 : *(A) Watson–Crick model of DNA helix (B) Complementary base pairing in DNA helix.*

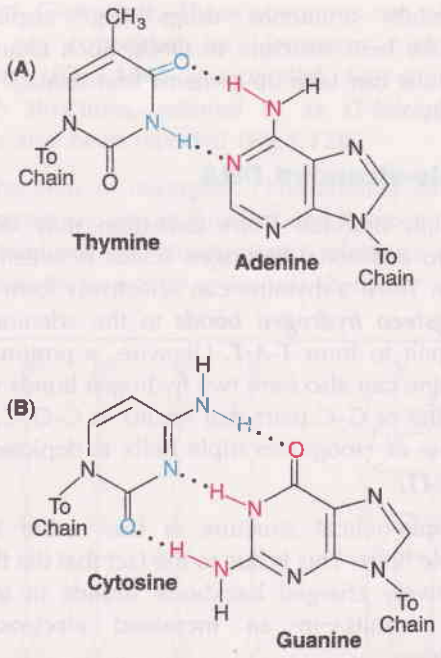


Fig. 5.10 : Complementary base pairing in DNA
(A) Thymine pairs with adenine by 2 hydrogen bonds
(B) Cytosine pairs with guanine by 3 hydrogen bonds.

1. The DNA is a right handed double helix. It consists of ***two polydeoxyribonucleotide chains*** (strands) twisted around each other on a common axis.

2. The two strands are ***antiparallel***, i.e., one strand runs in the 5′ to 3′ direction while the other in 3′ to 5′ direction. This is comparable to two parallel adjacent roads carrying traffic in opposite direction.

3. The width (or diameter) of a double helix is 20 A° (2 nm).

4. Each turn (pitch) of the helix is 34 A° (3.4 nm) with 10 pairs of nucleotides, each pair placed at a distance of about 3.4 A°.

5. Each strand of DNA has a hydrophilic deoxyribose phosphate backbone (3′-5′ phosphodiester bonds) on the outside (periphery) of the molecule while the hydrophobic bases are stacked inside (core).

6. The two polynucleotide chains are not identical but complementary to each other due to base pairing.

7. The two strands are ***held together by hydrogen bonds*** formed by complementary base pairs (***Fig.5.10***). The A-T pair has 2 hydrogen bonds while G-C pair has 3 hydrogen bonds. The G ≡ C is stronger by about 50% than A = T.

8. The hydrogen bonds are formed between a purine and a pyrimidine only. If two purines face each other, they would not fit into the allowable space. And two pyrimidines would be too far to form hydrogen bonds. The only base arrangement possible in DNA structure, from spatial considerations is A-T, T-A, G-C and C-G.

9. The complementary base pairing in DNA helix proves ***Chargaff's rule***. The content of adenine equals to that of thymine (A = T) and guanine equals to that of cytosine (G = C).

10. The ***genetic information resides on*** one of the two strands known as ***template strand*** or sense strand. The opposite strand is antisense strand. The double helix has (wide) major grooves and (narrow) minor grooves along the phosphodiester backbone. Proteins interact with DNA at these grooves, without disrupting the base pairs and double helix.

Conformations of DNA double helix

Variation in the conformation of the nucleotides of DNA is associated with conformational variants of DNA. The double helical structure of DNA exists in at least 6 different forms-A to E and Z. Among these, B, A and Z forms are important (***Table 5.2***). The ***B-form of DNA*** double helix, described by Watson and Crick (discussed above), is the most predominant form ***under physiological conditions***. Each turn of the B-form has 10 base pairs spanning a distance of 3.4 nm. The width of the double helix is 2 nm.

The A-form is also a right-handed helix. It contains 11 base pairs per turn. There is a tilting of the base pairs by 20° away from the central axis.

The Z-form (Z-DNA) is a left-handed helix and contains 12 base pairs per turn. The

Table 5.2 Comparison of structural features of different conformations of DNA double helix

Feature	*B-DNA*	*A-DNA*	*Z-DNA*
Helix type	Right-handed	Right-handed	Left-handed
Helical diameter (nm)	2.37	2.55	1.84
Distance per each complete turn (nm)	3.4	3.2	4.5
Rise per base pair (nm)	0.34	0.29	0.37
Number of base pairs per complete turn	10	11	12
Base pair tilt	+19°	−1.2° (variable)	−9°
Helix axis rotation	Major groove	Through base pairs (variable)	Minor groove

polynucleotide strands of DNA move in a somewhat 'zig zag' fashion, hence the name Z-DNA.

It is believed that transition between different helical forms of DNA plays a significant role in regulating gene expression.

OTHER TYPES OF DNA STRUCTURE

It is now recognized that besides double helical structure, DNA also exists in certain unusual structures. It is believed that such structures are important for molecular recognition of DNA by proteins and enzymes. This is in fact needed for the DNA to discharge its functions in an appropriate manner. Some selected ***unusual structures of DNA*** are briefly described.

Bent DNA

In general, adenine base containing DNA tracts are rigid and straight. Bent conformation of DNA occurs when A-tracts are replaced by other bases or a collapse of the helix into the minor groove of A-tract. Bending in DNA structure has also been reported due to photochemical damage or mispairing of bases.

Certain antitumor drugs (e.g. cisplatin) produce bent structure in DNA. Such changed structure can take up proteins that damage the DNA.

Triple-stranded DNA

Triple-stranded DNA formation may occur due to additional hydrogen bonds between the bases. Thus, a thymine can selectively form two ***Hoogsteen hydrogen bonds*** to the adenine of A-T pair to form ***T-A-T***. Likewise, a protonated cytosine can also form two hydrogen bonds with guanine of G–C pairs that results in C–G–C. An outline of Hoogsteen triple helix is depicted in ***Fig.5.11***.

Triple-helical structure is less stable than double helix. This is due to the fact that the three negatively charged backbone strands in triple helix results in an increased electrostatic repulsion.

Four-stranded DNA

Polynucleotides with very high contents of guanine can form a novel tetrameric structure

Fig. 5.11 : An outline of Hoogsteen triple helical structure of DNA.

called ***G-quartets***. These structures are planar and are connected by Hoogsteen hydrogen bonds (***Fig.5.12A***). Antiparallel four-stranded DNA structures, referred to as ***G-tetraplexes*** have also been reported (***Fig.5.12B***).

The ends of eukaryotic chromosomes namely ***telomeres*** are rich in guanine, and therefore form G-tetraplexes. In recent years, telomeres have become the targets for anticancer chemotherapies.

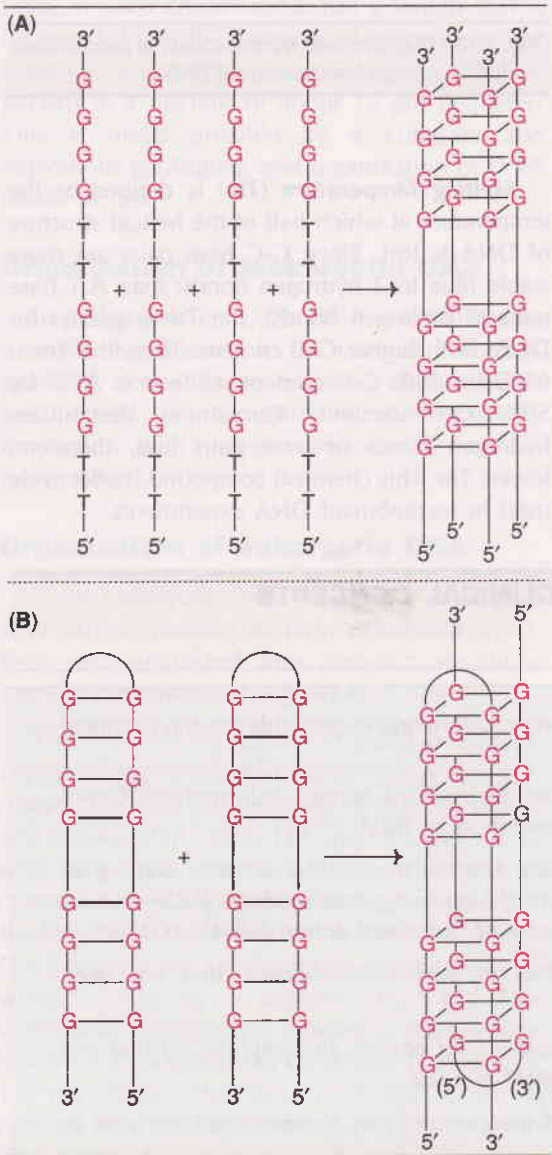


Fig. 5.12 : *Four-stranded DNA structure (A) Parallel G-quartets (B) Antiparallel G-tetraplex.*

G-tetraplexes have been implicated in the recombination of immunoglobulin genes, and in dimerization of double-stranded genomic RNA of the human immunodeficiency virus (HIV).

THE SIZE OF DNA MOLECULE —UNITS OF LENGTH

DNA molecules are huge in size. On an average, a pair of B-DNA with a thickness of 0.34 nm has a molecular weight of 660 daltons.

For the measurement of lengths, DNA double-stranded structure is considered, and expresssed in the form of ***base pairs*** (***bp***). A ***kilobase pair (kb)*** is 10^3 bp, and a ***megabase pair (Mb)*** is 10^6 bp and a gigabase pair (Gb) is 10^9 bp. The kb, Mb and Gb relations may be summarized as follows :

1 kb = 1000 bp

1 Mb = 1000 kb = 1,000,000 bp

1 Gb = 1000 Mb = 1,000,000,000 bp

It may be noted here that the lengths of RNA molecules (like DNA molecules) cannot be expressed in bp, since most of the RNAs are single-stranded.

The length of DNA varies from species to species, and is usually expressed in terms of base pair composition and ***contour length***. Contour length represents the total length of the genomic DNA in a cell. Some examples of organisms with bp and contour lengths are listed.

- λ phage virus — 4.8×10^4 bp — contour length 16.5 mm.
- *E. coli* — 4.6×10^6 bp — contour length 1.5 mm.
- Diploid human cell (46 chromosomes) — 6.0×10^9 bp — contour length 2 meters.

It may be noted that the genomic DNA size is usually much larger the size of the cell or nucleus containing it. For instance, in humans, a 2-meter long DNA is packed compactly in a nucleus of about 10μm diameter.

The genomic DNA may exist in linear or circular forms. Most DNAs in bacteria exist as closed circles. This includes the DNA of bacterial chromosomes and the extra-chromosomal DNA of plasmids. Mitochondria and chloroplasts of eukaryotic cells also contain circular DNA.

Chromosomal DNAs in higher organisms are mostly linear. Individual human chromosomes contain a single DNA molecule with variable sizes compactly packed. Thus the smallest chromosome contains 34 Mb while the largest one has 263 Mb.

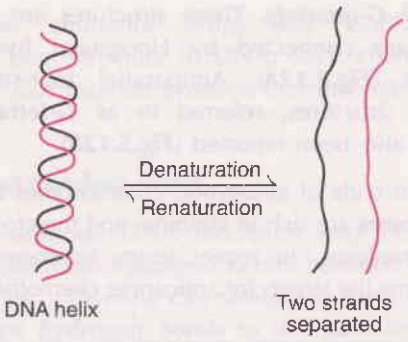


Fig. 5.13 : Diagrammatic representation of denaturation and renaturation of DNA.

DENATURATION OF DNA STRANDS

The two strands of DNA helix are held together by hydrogen bonds. Disruption of hydrogen bonds (by change in pH or increase in temperature) results in the separation of polynucleotide strands. This phenomenon of ***loss of helical structure of DNA*** is known as ***denaturation*** (***Fig.5.13***). The phosphodiester bonds are not broken by denaturation. Loss of helical structure can be measured by increase in absorbance at 260 nm (in a spectrophotometer).

Melting temperature (***Tm***) is defined as the temperature at which half of the helical structure of DNA is lost. Since G-C base pairs are more stable (due to 3 hydrogen bonds) than A-T base pairs (2 hydrogen bonds), the Tm is greater for DNAs with higher G-C content. Thus, the Tm is 65°C for 35% G-C content while it is 70°C for 50% G-C content. Formamide destabilizes hydrogen bonds of base pairs and, therefore, lowers Tm. This chemical compound is effectively used in recombinant DNA experiments.

BIOMEDICAL / CLINICAL CONCEPTS

- *DNA is the reserve bank of genetic information, ultimately responsible for the chemical basis of life and heredity.*
- *DNA is organized into genes, the fundamental units of genetic information. Genes control protein biosynthesis through the mediation of RNA.*
- *Nucleic acids are the polymers of nucleotides. Certain nucleotides serve as B-complex vitamin coenzymes (FAD, NAD^+, CoA), carriers of high energy intermediates (UDP-glucose, S-adenosylmethionine) and second messengers of hormonal action (cAMP, cGMP).*
- *Uric acid is a purine, and the end product of purine metabolism, that has been implicated in the disorder gout.*
- *Certain purine bases from plants such as caffeine (of coffee), theophylline (of tea) and theobromine (of cocoa) are of pharmacological interest.*
- *Synthetic analogs of bases (5-fluorouracil, 6-mercaptopurine, 6-azauridine) are used to inhibit the growth of cancer cells.*
- *Certain antitumor drugs (e.g. cisplatin) can produce bent DNA structure and damage it.*

Renaturation (or reannealing) is the process in which the separated complementary DNA strands can form a double helix.

ORGANIZATION OF DNA IN THE CELL

As already stated, the double-stranded DNA helix in each chromosome has a length that is thousands times the diameter of the nucleus. For instance, in humans, a 2-meter long DNA is packed in a nucleus of about 10 µm diameter! This is made possible by a compact and marvellous packaging, and organization of DNA inside in cell.

Organization of prokaryotic DNA

In prokaryotic cells, the DNA is organized as a single chromosome in the form of a double-stranded circle. These bacterial chromosomes are packed in the form of nucleoids, by interaction with proteins and certain cations (polyamines).

Organization of eukaryotic DNA

In the eukaryotic cells, the DNA is associated with various proteins to form ***chromatin*** which then gets ***organized into*** compact structures namely ***chromosomes*** (***Fig.5.14***).

The DNA double helix is wrapped around the core proteins namely ***histones*** which are basic in nature. The core is composed of two molecules of histones (H2A, H2B, H3 and H4). Each core with two turns of DNA wrapped round it (approximately with 150 bp) is termed as a ***nucleosome***, the basic unit of chromatin. Nucleosomes are separated by spacer DNA to which histone H_1 is attached (***Fig.5.15***). This continuous string of nucleosomes, representing beads-on-a string form of chromatin is termed as 10 nm fiber. The length of the DNA is considerably reduced by the formation of 10 nm fiber. This 10-nm fiber is further coiled to produce 30-nm fiber which has a solenoid structure with six nucleosomes in every turn. These 30-nm fibers are further organized into loops by anchoring the fiber at A/T-rich regions namely scaffold-associated regions (SARS) to a protein scafold. During the course of mitosis, the loops are further coiled, the chromosomes condense and become visible.

STRUCTURE OF RNA

RNA is a polymer of ribonucleotides held together by 3′,5′-phosphodiester bridges. Although RNA has certain similarities with DNA structure, they have specific differences

1. **Pentose :** The sugar in RNA is ribose in contrast to deoxyribose in DNA.

2. **Pyrimidine :** RNA contains the pyrimidine uracil in place of thymine (in DNA).

3. **Single strand :** RNA is usually a single-stranded polynucleotide. However, this strand may fold at certain places to give a double-stranded structure, if complementary base pairs are in close proximity.

4. **Chargaff's rule—not obeyed :** Due to the single-stranded nature, there is no specific relation between purine and pyrimidine contents. Thus the guanine content is not equal to cytosine (as is the case in DNA).

5. **Susceptibility to alkali hydrolysis :** Alkali can hydrolyse RNA to 2′,3′-cyclic diesters. This is possible due to the presence of a hydroxyl group at 2′ position. DNA cannot be subjected to alkali hydrolysis due to lack of this group.

6. **Orcinol colour reaction :** RNAs can be histologically identified by orcinol colour reaction due to the presence of ribose.

TYPES OF RNA

The three major types of RNAs with their respective cellular composition are given below

1. **Messenger RNA** (mRNA) : 5–10%
2. **Transfer RNA** (tRNA) : 10–20%
3. **Ribosomal RNA** (rRNA) : 50–80%

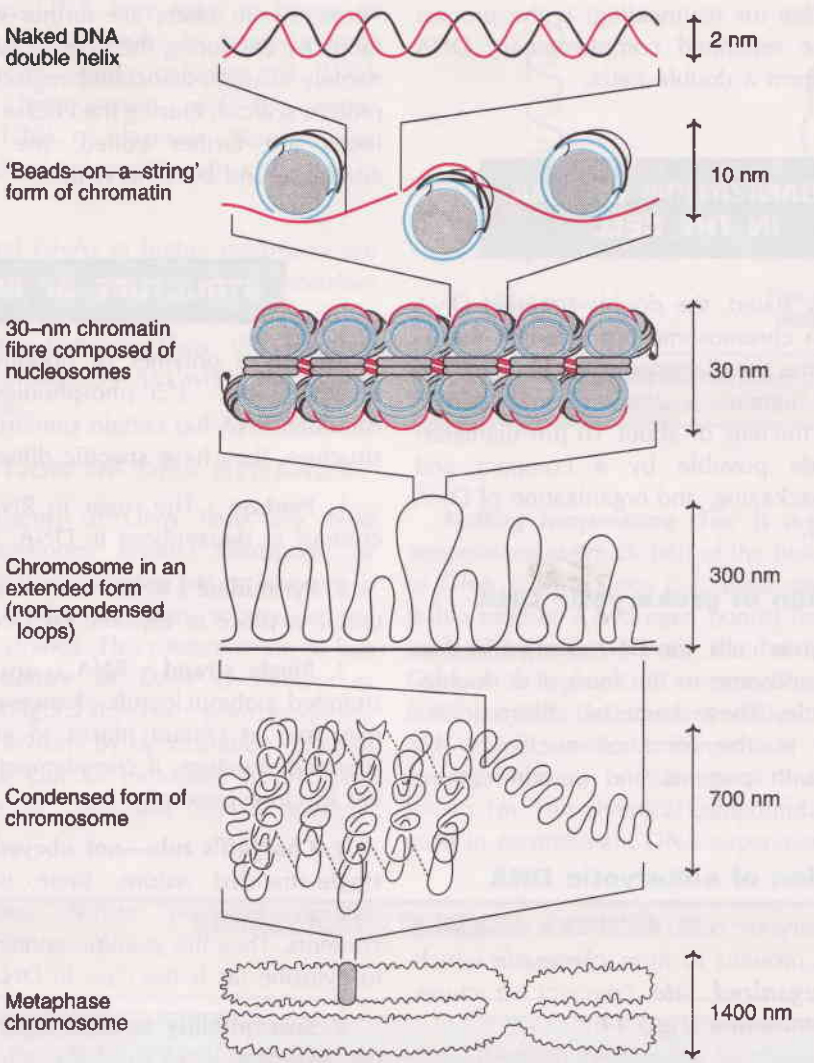


***Fig. 5.14** : Organization of eukaryotic DNA structure in the form of chromatin and chromosomes.*

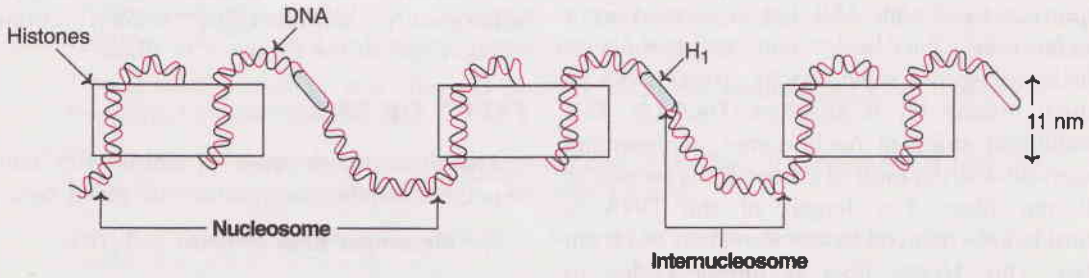


***Fig. 5.15** : Structure of nucleosomes.*

Table 5.3 Cellular RNAs and their function(s)

Type of RNA	*Abbreviation*	*Function(s)*
Messenger RNA	mRNA	Transfers genetic information from genes to ribosomes to synthesize proteins.
Heterogeneous nuclear RNA	hnRNA	Serves as precursor for mRNA and other RNAs.
Transfer RNA	tRNA	Transfers amino acid to mRNA for protein biosynthesis.
Ribosomal RNA	rRNA	Provides structural framework for ribosomes.
Small nuclear RNA	snRNA	Involved in mRNA processing.
Small nucleolar RNA	snoRNA	Plays a key role in the processing of rRNA molecules.
Small cytoplasmic RNA	scRNA	Involved in the selection of proteins for export.
Transfer-messenger RNA	tmRNA	Mostly present in bacteria. Adds short peptide tags to proteins to facilitate the degradation of incorrectly synthesized proteins.

Besides the three RNAs referred above, other RNAs are also present in the cells. These include heterogeneous nuclear RNA (hnRNA), small nuclear RNA (snRNA), small nucleolar RNA (snoRNA) and small cytoplasmic RNA (scRNA). The major functions of these RNAs are given in ***Table 5.3***.

The RNAs are synthesized from DNA, and are primarily involved in the process of protein biosynthesis (***Chapter 25***). The RNAs vary in their structure and function. A brief description on the major RNAs is given.

Messenger RNA (mRNA)

The mRNA is synthesized in the nucleus (in eukaryotes) as ***heterogeneous nuclear RNA*** (***hnRNA***). hnRNA, on processing, liberates the functional mRNA which enters the cytoplasm to participate in ***protein synthesis***. mRNA has high molecular weight with a short half-life.

The eukaryotic mRNA is capped at the 5'-terminal end by 7-methylguanosine triphosphate. It is believed that this cap helps to prevent the hydrolysis of mRNA by 5'-exo-nucleases. Further, the cap may be also involved in the recognition of mRNA for protein synthesis.

The 3'-terminal end of mRNA contains a polymer of adenylate residues (20-250 nucleotides) which is known as ***poly (A) tail***. This tail may provide stability to mRNA, besides preventing it from the attack of 3'-exonucleases.

mRNA molecules often contai certain modified bases such as 6-methyladenylates in the internal structure.

Transfer RNA (tRNA)

Transfer RNA (***soluble RNA***) molecule contains 71-80 nucleotides (mostly 75) with a molecular weight of about 25,000. There are at least 20 species of tRNAs, corresponding to 20 amino acids present in protein structure. The structure of tRNA (for alanine) was first elucidated by Holley.

The structure of tRNA, depicted in ***Fig.5.16***, resembles that of a clover leaf. tRNA contains mainly four arms, each arm with a base paired stem.

1. **The acceptor arm :** This arm is capped with a sequence CCA (5' to 3'). The amino acid is attached to the acceptor arm.

2. **The anticodon arm :** This arm, with the three specific nucleotide bases (anticodon), is responsible for the recognition of triplet codon of mRNA. The codon and anticodon are complementary to each other.

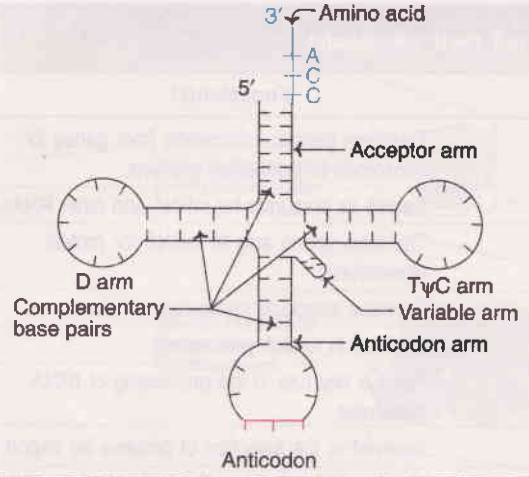


Fig. 5.16 : Structure of transfer RNA.

3. **The D arm :** It is so named due to the presence of dihydrouridine.

4. **The TΨC arm :** This arm contains a sequence of T, pseudouridine (represented by psi, Ψ) and C.

5. **The variable arm :** This arm is the most variable in tRNA. Based on this variability, tRNAs are classified into 2 categories :

(a) **Class I tRNAs :** The most predominant (about 75%) form with 3-5 base pairs length.

(b) **Class II tRNAs :** They contain 13-20 base pair long arm.

Base pairs in tRNA : The structure of tRNA is maintained due to the complementary base pairing in the arms. The four arms with their respective base pairs are given below

The acceptor arm	– 7 bp
The TΨC arm	– 5 bp
The anticodon arm	– 5 bp
The D arm	– 4 bp

Ribosomal RNA (rRNA)

The ribosomes are the factories of protein synthesis. The eukaryotic ribosomes are composed of two major nucleoprotein complexes–60S subunit and 40S subunit. The 60S subunit contains 28S rRNA, 5S rRNA and 5.8S rRNA while the 40S subunit contains 18S rRNA. The function of rRNAs in ribosomes is not clearly known. It is believed that they play a significant role in the binding of mRNA to ribosomes and protein synthesis.

Other RNAs

The various other RNAs and their functions are summarised in ***Table 5.3***.

CATALYTIC RNAs—RIBOZYMES

In certain instances, the RNA component of a ribonucleoprotein (RNA in association with protein) is catalytically active. Such RNAs are termed as ribozymes. At least five distinct species of RNA that act as catalysts have been identified. Three are involved in the self processing reactions of RNAs while the other two are regarded as true catalysts (RNase P and rRNA).

Ribonuclease P (RNase P) is a ribozyme containing protein and RNA component. It cleaves tRNA precursors to generate mature tRNA molecules.

RNA molecules are known to adapt tertiary structure just like proteins (i.e. enzymes). The specific conformation of RNA may be responsible for its function as biocatalyst. It is believed that ***ribozymes*** (RNAs) were functioning as ***catalysts before the occurrence of protein enzymes***, during the course of evolution.

SUMMARY

1. *DNA is the chemical basis of heredity organized into genes, the basic units of genetic information.*
2. *RNAs (mRNA, tRNA and rRNA) are produced by DNA which in turn carry out protein synthesis.*
3. *Nucleic acids are the polymers of nucleotides (polynucleotides) held by 3′ and 5′ phosphodiester bridges. A nucleotide essentially consists of base + sugar (nucleoside) and phosphate.*
4. *Besides being the constituents of nucleic acid structure, nucleotides perform a wide variety of cellular functions (e.g. energy carriers, metabolic regulators, second messengers etc.)*
5. *Both DNA and RNA contain the purines-adenine (A) and guanine (G) and the pyrimidine-cytosine (C). The second pyrimidine is thymine (T) in DNA while it is uracil (U) in RNA. The pentose sugar, D-deoxyribose is found in DNA while it is D-ribose in RNA.*
6. *The structure of DNA is a double helix (Watson-Crick model) composed of two antiparallel strands of polydeoxynucleotides twisted around each other. The strands are held together by 2 or 3 hydrogen bonds formed between the bases i.e. A = T; G ≡ C. DNA structure satisfies Chargaff's rule that the content of A is equal to T, and that of G equal to C.*
7. *Besides the double helical structure, DNA also exists in certain unusual structures — bent DNA, triple-strand DNA, four-strand DNA.*
8. *RNA is usually a single stranded polyribonucleotide. mRNA is capped at 5′ terminal end by 7-methylGTP while at the 3′-terminal end, it contains a poly A tail. mRNA specifies the sequence of amino acids in protein synthesis.*
9. *The structure of tRNA resembles that of a clover leaf with four arms (acceptor, anticodon, D-, and TΨC) held by complementary base pairs. tRNA delivers amino acids for protein synthesis.*
10. *Certain RNAs that can function as enzymes are termed as ribozymes. Ribozymes were probably functioning as catalysts before the occurrence of protein enzymes during evolution.*

SELF-ASSESSMENT EXERCISES

I. Essay questions

1. Describe the structure of DNA.
2. Name different RNAs and discuss their structure.
3. Write an account of structure, function and nomenclature of nucleotides.
4. Describe the structure of nitrogenous bases present in nucleic acids. Add a note on tautomerism.
5. "The backbone of nucleic acid structure is 3'-5' phosphodiester bridge."—justify.

II. Short notes

(a) Chargaff's rule, (b) Ribose and deoxyribose, (c) Hydrogen bonds in DNA, (d) Nucleoside, (e) Different forms of DNA, (f) Transfer RNA, (g) Purine bases of plants, (h) Complementary base pairs, (i) DNA denaturation, (j) hnRNA.

III. Fill in the blanks

1. The fundamental unit of genetic information is known as _____.
2. DNA controls protein synthesis through the mediation of _____.
3. Nucleic acids are the polymers of _____.
4. The pyrimidine present in DNA but absent in RNA _____.
5. Ribose and deoxyribose differ in their structure around carbon atom _____.
6. Nucleotide is composed of _____.
7. The scientist who observed that there exists a relationship between the contents of purines and pyrimidines in DNA structure (A = T; G = C) _____.
8. The base pair G-C is more stable and stronger than A-T due to _____.
9. Under physiological condition, the DNA structure is predominantly in the form _____.
10. The acceptor arm of tRNA contains a capped nucleotide sequence _____.

IV. Multiple choice questions

11. The nitrogenous base not present in DNA structure

    (a) Adenine (b) Guanine (c) Cytosine (d) Uracil.

12. The number of base pairs present in each turn (pitch) of B-form of DNA helix

    (a) 9 (b) 10 (c) 11 (d) 12.

13. The backbone of nucleic acid structure is constructed by

    (a) Peptide bonds (b) Glycosidic bonds (c) Phosphodiester bridges (d) All of them.

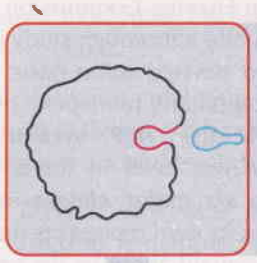
14. The following coenzyme is a nucleotide

    (a) FAD (b) NAD^+ (c) CoASH (d) All of them.

15. The nucleotide that serves as an intermediate for biosynthetic reaction

    (a) UDP-glucose (b) CDP-acylglycerol (c) S-Adenosylmethionine (d) All of them.

6 Enzymes



The enzymes speak :

"We are the catalysts of the living world!
Protein in nature, and in action specific,
rapid and accurate;
Huge in size but with small active centres;
Highly exploited for disease diagnosis in lab centres."

Enzymes are biocatalysts – the catalysts of life. A ***catalyst*** is defined as a ***substance that increases the velocity*** or rate of a chemical reaction without itself undergoing any change in the overall process.

The student-teacher relationship may be a good example to understand how a catalyst works. The students often find it difficult to learn from a text-book on their own. The teacher explains the subject to the students and increases their understanding capability. It is no wonder that certain difficult things which the students take days together to understand, and sometimes do not understand at all – are easily learnt under the guidance of the teacher. Here, the teacher acts like a catalyst in enhancing the understanding ability of students. A good teacher is always a good catalyst in students' life!

Enzymes may be defined as biocatalysts synthesized by living cells. They are protein in nature (exception – RNA acting as ribozyme), colloidal and thermolabile in character, and specific in their action.

In the laboratory, hydrolysis of proteins by a strong acid at 100°C takes at least a couple of days. The same protein is fully digested by the enzymes in gastrointestinal tract at body temperature (37°C) within a couple of hours. This remarkable difference in the chemical reactions taking place in the living system is exclusively due to enzymes. The very existence of life is unimaginable without the presence of enzymes.

HISTORICAL BACKGROUND

Berzelius in 1836 coined the term ***catalysis*** (*Greek* : to dissolve). In 1878, Kuhne used the word ***enzyme*** (*Greek* : in yeast) to indicate the catalysis taking place in the biological systems. Isolation of enzyme system from cell-free extract of yeast was achieved in 1883 by Buchner. He named the active principle as zymase (later found to contain a mixture of enzymes), which could convert sugar to alcohol. In 1926, James

Sumner ***first*** achieved the ***isolation*** and crystallization of the enzyme ***urease*** from jack bean and identified it as a protein.

NOMENCLATURE AND CLASSIFICATION

In the early days, the enzymes were given names by their discoverers in an arbitrary manner. For example, the names pepsin, trypsin and chymotrypsin convey no information about the function of the enzyme or the nature of the substrate on which they act. Sometimes, the suffix-***ase*** was added to the substrate for naming the enzymes e.g. lipase acts on lipids; nuclease on nucleic acids; lactase on lactose. These are known as ***trivial names*** of the enzymes which, however, fail to give complete information of enzyme reaction (type of reaction, cofactor requirement etc.)

Enzymes are sometimes considered under two broad categories : (a) **Intracellular enzymes** – They are functional within cells where they are synthesized. (b) **Extracellular enzymes** – These enzymes are active outside the cell; all the digestive enzymes belong to this group.

The International Union of Biochemistry (IUB) appointed an Enzyme Commission in 1961. This committee made a thorough study of the existing enzymes and devised some basic principles for the classification and nomenclature of enzymes. Since 1964, the ***IUB system of enzyme classification*** has been in force. Enzymes are divided into ***six major classes*** (in that order). Each class on its own represents the general type of reaction brought about by the enzymes of that class (***Table 6.1***).

Table 6.1 Classification of enzymes

*Enzyme class with examples**	*Reaction catalysed*
1. **Oxidoreductases**	
Alcohol dehydrogenase (alcohol : NAD^+ oxidoreductase E.C. 1.1.1.1.), cytochrome oxidase, L- and D-amino acid oxidases	Oxidation $\longrightarrow$ Reduction $AH_2 + B \longrightarrow A + BH_2$
2. **Transferases**	
Hexokinase (ATP : D-hexose 6-phosphotransferase, E.C. 2.7.1.1.), transaminases, transmethylases, phosphorylase	Group transfer $A - X + B \longrightarrow A + B - X$
3. **Hydrolases**	
Lipase (triacylglycerol acyl hydrolase E.C. 3.1.1.3), choline esterase, acid and alkaline phosphatases, pepsin, urease	Hydrolysis $A - B + H_2O \longrightarrow AH + BOH$
4. **Lyases**	
Aldolase (ketose 1-phosphate aldehyde lyase, E.C. 4.1.2.7), fumarase, histidase	Addition $\longrightarrow$ Elimination $A - B + \dot{X} - \acute{Y} \longrightarrow A\dot{X} - B\acute{Y}$
5. **Isomerases**	
Triose phosphate isomerase (D-glyceraldehyde 3-phosphate ketoisomerase, E.C. 5.3.1.1), retinol isomerase, phosphohexose isomerase	Interconversion of isomers $A \longrightarrow A'$
6. **Ligases**	
Glutamine synthetase (L-glutamate ammonia ligase, E.C. 6.3.1.2), acetyl CoA carboxylase, succinate thiokinase	Condensation (usually dependent on ATP) $A + B \xrightarrow[ADP + Pi]{ATP} A - B$

**For one enzyme in each class, systematic name along with E.C. number is given in the brackets.*

1. **Oxidoreductases :** Enzymes involved in oxidation-reduction reactions.

2. **Transferases :** Enzymes that catalyse the transfer of functional groups.

3. **Hydrolases :** Enzymes that bring about hydrolysis of various compounds.

4. **Lyases :** Enzymes specialised in the addition or removal of water, ammonia, CO_2 etc.

5. **Isomerases :** Enzymes involved in all the isomerization reactions.

6. **Ligases :** Enzymes catalysing the synthetic reactions (*Greek* : ligate—to bind) where two molecules are joined together and ATP is used.

[The word ***OTHLIL*** (first letter in each class) may be memorised to remember the six classes of enzymes in the correct order].

Each class in turn is subdivided into many sub-classes which are further divided. A four digit ***E***nzyme ***C***ommission (***E.C.***) number is assigned to each enzyme representing the class (first digit), sub-class (second digit), sub-sub class (third digit) and the individual enzyme (fourth digit). Each enzyme is given a specific name indicating the substrate, coenzyme (if any) and the type of the reaction catalysed by the enzyme. Although the IUB names for the enzymes are specific and unambiguous, they have not been accepted for general use as they are complex and cumbersome to remember. Therefore, the trivial names, along with the E.C. numbers as and when needed, are commonly used and widely accepted.

CHEMICAL NATURE AND PROPERTIES OF ENZYMES

All the enzymes are invariably proteins. In recent years, however, a few RNA molecules have been shown to function as enzymes. Each enzyme has its own tertiary structure and specific conformation which is very essential for its catalytic activity. The functional unit of the enzyme is known as ***holoenzyme*** which is often made up of ***apoenzyme*** (the protein part) and a ***coenzyme*** (non-protein organic part).

Holoenzyme ⟶ Apoenzyme + Coenzyme
(active enzyme) (protein part) (non-protein part)

The term ***prosthetic group*** is used when the non-protein moiety tightly (covalently) binds with the apoenzyme. The coenzyme can be separated by dialysis from the enzyme while the prosthetic group cannot be.

The word ***monomeric enzyme*** is used if it is made up of a single polypeptide e.g. ribonuclease, trypsin. Some of the enzymes which possess more than one polypeptide (subunit) chain are known as ***oligomeric enzymes*** e.g. lactate dehydrogenase, aspartate transcarbamoylase etc. There are certain ***multienzyme complexes*** possessing specific sites to catalyse different reactions in a sequence. Only the native intact multienzyme complex is functionally active and not the individual units, if they are separated e.g. pyruvate dehydrogenase, fatty acid synthase, prostaglandin synthase etc. The enzymes exhibit all the general properties of proteins (***Chapter 4***).

Genetic engineering and modified enzymes

Recent advances in biotechnology have made it possible to modify the enzymes with desirable characters-improved catalytic abilities, activities under unusual conditions. This approach is required since enzymes possess enormous potential for their use in medicine and industry.

Hybrid enzymes : It is possible to rearrange genes and produce ***fusion proteins***. e.g. a hybrid enzyme (of glucanase and cellulase) that can more efficiently hydrolyse barley β-glucans in beer manufacture.

Site-directed mutagenesis : This is a technique used to produce a specified mutation at a predetermined position in a DNA molecule. The result is incorporation of a desired amino acid (of one's choice) in place of the specified amino acid in the enzyme. By this approach, it is possible to produce an enzyme with desirable characteristics. e.g. tissue plasminogen activator (used to lyse blood clots in myocardial

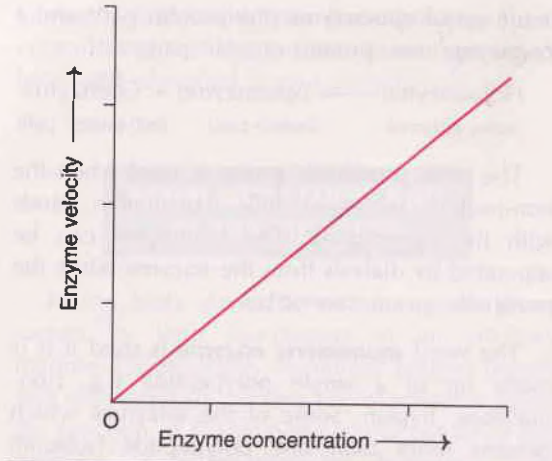


Fig. 6.1 : Effect of enzyme concentration on enzyme velociy.

infarction) with increased half-life. This is achieved by replacing asparagine (at position 120) by glutamine.

In recent years, it has also become possible to produce ***hybrid enzymes*** by rearrangement of genes. Another innovative approach is the production of ***abzymes*** or catalytic antibodies, the antibody enzymes.

FACTORS AFFECTING ENZYME ACTIVITY

The contact between the enzyme and substrate is the most essential pre-requisite for enzyme activity. The important factors that influence the velocity of the enzyme reaction are discussed hereunder

1. Concentration of enzyme

As the concentration of the enzyme is increased, the velocity of the reaction proportionately increases (***Fig.6.1***). In fact, this property of enzyme is made use in determining the serum enzymes for the diagnosis of diseases. By using a known volume of serum, and keeping all the other factors (substrate, pH, temperature etc.) at the optimum level, the enzyme could be assayed in the laboratory.

2. Concentration of substrate

Increase in the substrate concentration gradually ***increases the velocity of enzyme reaction*** within the limited range of substrate levels. A rectangular hyperbola is obtained when velocity is plotted against the substrate concentration (***Fig.6.2***). Three distinct phases of the reaction are observed in the graph (A-linear; B-curve; C-almost unchanged).

Order of reaction : When the velocity of the reaction is almost proportional to the substrate concentration (i.e. [S] is less than K_m), the rate of the reaction is said to be ***first order*** with respect to substrate. When the [S] is much greater than K_m, the rate of reaction is independent of substrate concentration, and the reaction is said to be ***zero order***.

Enzyme kinetics and K_m value : The enzyme (E) and substrate (S) combine with each other to form an unstable enzyme-substrate complex (ES) for the formation of product (P).

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P$$

Here k_1, k_2 and k_3 represent the velocity constants for the respective reactions, as indicated by arrows.

K_m, the Michaelis-Menten constant (or ***Brig's and Haldane's constant***), is given by the formula

$$K_m = \frac{k_2 + k_3}{k_1}$$

The following equation is obtained after suitable algebraic manipulation.

$$v = \frac{V_{max}[S]}{K_m + [S]} \quad \text{equation (1)}$$

where v = Measured velocity,
V_{max} = Maximum velocity,
S = Substrate concentration,
K_m = Michaelis – Menten constant.

Let us assume that the measured velocity (v) is equal to $\frac{1}{2}V_{max}$. Then the equation (1) may be substituted as follows

$$\frac{1}{2}V_{max} = \frac{V_{max}[S]}{K_m + [S]}$$

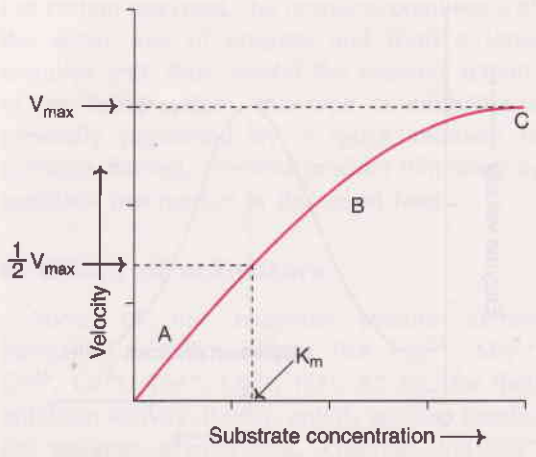


Fig. 6.2 : Effect of substrate concentration on enzyme velocity (A-linear; B-curve; C-almost unchanged).

$$K_m + [S] = \frac{2V_{max}[S]}{V_{max}}$$

$$K_m + [S] = 2[S]$$

$$K_m = [S]$$

K stands for a constant and ***m*** stands for ***M***ichaelis (in K_m).

K_m or the ***Michaelis-Menten constant*** is defined as the ***substrate concentration*** (expressed in moles/l) ***to produce half-maximum velocity*** in an enzyme catalysed reaction. It indicates that half of the enzyme molecules (i.e. 50%) are bound with the substrate molecules when the substrate concentration equals the K_m value.

K_m value is a constant and a characteristic feature of a given enzyme (comparable to a thumb impression or signature). It is a representative for measuring the strength of ES complex. A ***low K_m value indicates a strong affinity between enzyme and substrate***, whereas a high K_m value reflects a weak affinity between them. For majority of enzymes, the K_m values are in the range of 10^{-5} to 10^{-2} moles. It may however, be noted that K_m is not dependent on the concentration of enzyme.

Lineweaver-Burk double reciprocal plot : For the determination of K_m value, the substrate saturation curve (***Fig.6.2***) is not very accurate since V_{max} is approached asymptotically. By taking the reciprocals of the equation (1), a straight line graphic representation is obtained.

$$\frac{1}{v} = \frac{K_m + [S]}{V_{max}[S]}$$

$$\frac{1}{v} = \frac{K_m}{V_{max}} \times \frac{1}{[S]} + \frac{[S]}{V_{max}[S]}$$

$$\frac{1}{v} = \frac{K_m}{V_{max}} \times \frac{1}{[S]} + \frac{1}{V_{max}}$$

The above equation is similar to y = ax + b. Therefore, a plot of the reciprocal of the velocity $\left(\frac{1}{v}\right)$ vs. the reciprocal of the substrate concentration $\left(\frac{1}{[S]}\right)$ gives a straight line. Here the slope is K_m/V_{max} and whose y intercept is $1/V_{max}$.

The Lineweaver-Burk plot is shown in ***Fig.6.3***. It is much easier to calculate the K_m from the intercept on x-axis which is $-(1/K_m)$. Further, the double reciprocal plot is useful in understanding the effect of various inhibitions (discussed later).

Enzyme reactions with two or more substrates : The above discussion is based on the presumption of a single substrate-enzyme reaction. In fact, a majority of the enzyme-catalysed reactions involve two or more substrates. Even in case of ***multisubstrate***

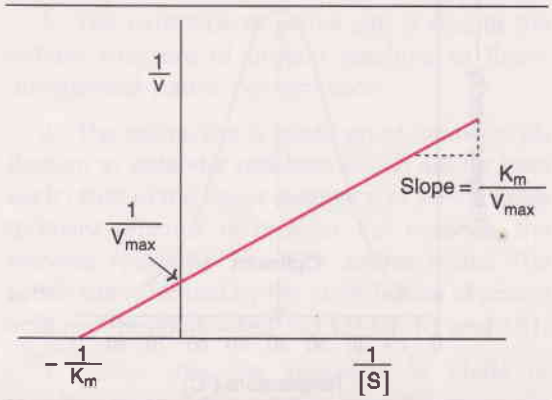


Fig. 6.3 : Lineweaver-Burk double reciprocal plot.

enzymes, despite the complex mathematical expressions, the fundamental principles conform to Michaelis-Menten Kinetics.

3. Effect of temperature

Velocity of an enzyme reaction increases with increase in temperature up to a maximum and then declines. A ***bell-shaped curve*** is usually observed (***Fig.6.4***).

Temperature coefficient or Q_{10} is defined as increase in enzyme velocity when the temperature is increased by 10°C. For a majority of enzymes, Q_{10} is 2 between 0°C and 40°C. Increase in temperature results in higher activation energy of the molecules and more molecular (enzyme and substrate) collision and interaction for the reaction to proceed faster.

The optimum temperature for most of the enzymes is between 40°C–45°C. However, a few enzymes (e.g. venom phosphokinases, muscle adenylate kinase) are active even at 100°C. Some plant enzymes like urease have optimum activity around 60°C. This may be due to very stable structure and conformation of these enzymes.

In general, when the enzymes are exposed to a temperature above 50°C, ***denaturation*** leading to derangement in the native (tertiary) structure of the protein and active site are seen. Majority of the enzymes become inactive at higher temperature (above 70°C).

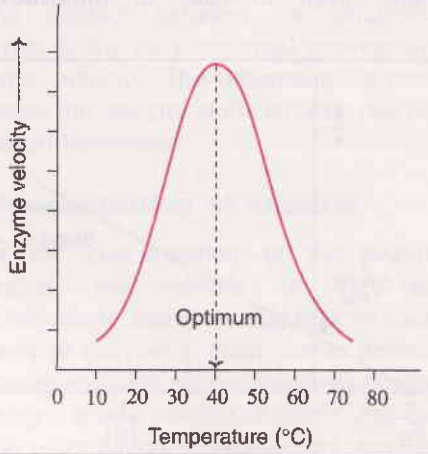


Fig. 6.4 : Effect of temperature on enzyme velocity.

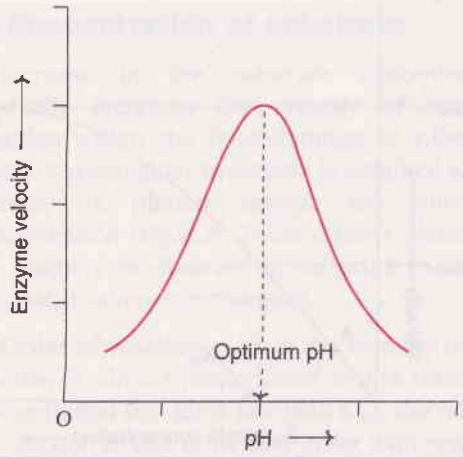


Fig. 6.5 : Effect of pH on enzyme velocity.

It is worth noting here that the enzymes have been assigned optimal temperatures based on the laboratory work. These temperatures, however, may have less relevance and biological significance in the living system.

4. Effect of pH

Increase in the hydrogen ion concentration (pH) considerably influences the enzyme activity and a ***bell-shaped curve*** is normally obtained (***Fig.6.5***). Each enzyme has an optimum pH at which the velocity is maximum. Below and above this pH, the enzyme activity is much lower and at extreme pH, the enzyme becomes totally inactive.

Most of the enzymes of higher organisms show optimum activity around neutral pH (6-8). There are, however, many exceptions like pepsin (1-2), acid phosphatase (4-5) and alkaline phosphatase (10-11). Enzymes from fungi and plants are most active in acidic pH (4-6).

Hydrogen ions influence the enzyme activity by altering the ionic charges on the amino acids (particularly at the active site), substrate, ES complex etc.

5. Effect of product concentration

The accumulation of reaction products generally decreases the enzyme velocity.

For certain enzymes, the products combine with the active site of enzyme and form a loose complex and, thus, inhibit the enzyme activity. In the living system, this type of inhibition is generally prevented by a quick removal of products formed. The end product inhibition by feedback mechanism is discussed later.

6. Effect of activators

Some of the enzymes require certain inorganic ***metallic cations*** like Mg^{2+}, Mn^{2+}, Zn^{2+}, Ca^{2+}, Co^{2+}, Cu^{2+}, Na^{+}, K^{+} etc. for their optimum activity. Rarely, anions are also needed for enzyme activity e.g. chloride ion (Cl^{-}) for amylase. Metals function as activators of enzyme velocity through various mechanisms—combining with the substrate, formation of ES-metal complex, direct participation in the reaction and bringing a conformational change in the enzyme.

Two categories of enzymes requiring metals for their activity are distinguished

- **Metal-activated enzymes :** The metal is not tightly held by the enzyme and can be exchanged easily with other ions

  e.g. ATPase (Mg^{2+} and Ca^{2+})

  Enolase (Mg^{2+})

- **Metalloenzymes :** These enzymes hold the metals rather tightly which are not readily exchanged. e.g. alcohol dehydrogenase, carbonic anhydrase, alkaline phosphatase, carboxypeptidase and aldolase contain zinc.

  Phenol oxidase (copper);

  Pyruvate oxidase (manganese);

  Xanthine oxidase (molybdenum);

  Cytochrome oxidase (iron and copper).

7. Effect of time

Under ideal and optimal conditions (like pH, temperature etc.), the time required for an enzyme reaction is less. Variations in the time of the reaction are generally related to the alterations in pH and temperature.

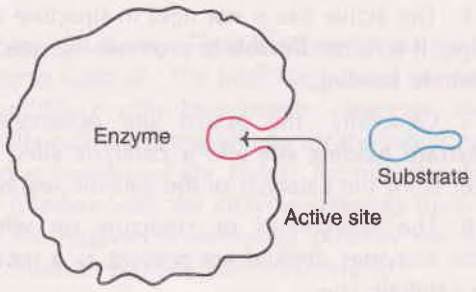


Fig. 6.6 : A diagrammatic representation of an enzyme with active site.

8. Effect of light and radiation

Exposure of enzymes to ultraviolet, beta, gamma and X-rays inactivates certain enzymes due to the formation of peroxides. e.g. UV rays inhibit salivary amylase activity.

ACTIVE SITE

Enzymes are big in size compared to substrates which are relatively smaller. Evidently, a small portion of the huge enzyme molecule is directly involved in the substrate binding and catalysis (***Fig.6.6***).

The active site (or active centre) of an enzyme represents as the small region at which the substrate(s) binds and participates in the catalysis.

Salient features of active site

1. The existence of active site is due to the tertiary structure of protein resulting in three-dimensional native conformation.

2. The active site is made up of amino acids (known as ***catalytic residues***) which are far from each other in the linear sequence of amino acids (primary structure of protein). For instance, the enzyme lysozyme has 129 amino acids. The active site is formed by the contribution of amino acid residues numbered 35, 52, 62, 63 and 101.

3. Active sites are regarded as ***clefts*** or ***crevices*** or pockets occupying a small region in a big enzyme molecule.

4. The active site is not rigid in structure and shape. It is rather ***flexible*** to promote the specific substrate binding.

5. Generally, the active site possesses a ***substrate binding site*** and a ***catalytic site.*** The latter is for the catalysis of the specific reaction.

6. The coenzymes or cofactors on which some enzymes depend are present as a part of the catalytic site.

7. The substrate(s) binds at the active site by weak noncovalent bonds.

8. Enzymes are specific in their function due to the existence of active sites.

9. The commonly found amino acids at the active sites are serine, aspartate, histidine, cysteine, lysine, arginine, glutamate, tyrosine etc. Among these amino acids, ***serine*** is the most frequently found.

10. The substrate[S] binds the enzyme (E) at the active site to form enzyme-substrate complex (ES). The product (P) is released after the catalysis and the enzyme is available for reuse.

$$E + S \rightleftharpoons ES \rightarrow E + P$$

ENZYME INHIBITION

Enzyme inhibitor is defined as a substance which binds with the enzyme and brings about a ***decrease in catalytic activity*** of that enzyme. The inhibitor may be organic or inorganic in nature. There are three broad categories of enzyme inhibition

1. Reversible inhibition.
2. Irreversible inhibition.
3. Allosteric inhibition.

1. Reversible inhibition

The inhibitor binds non-covalently with enzyme and the enzyme inhibition can be reversed if the inhibitor is removed. The reversible inhibition is further sub-divided into

I. Competitive inhibition (***Fig.6.7A***)

II. Non-competitive inhibition (***Fig.6.7B***)

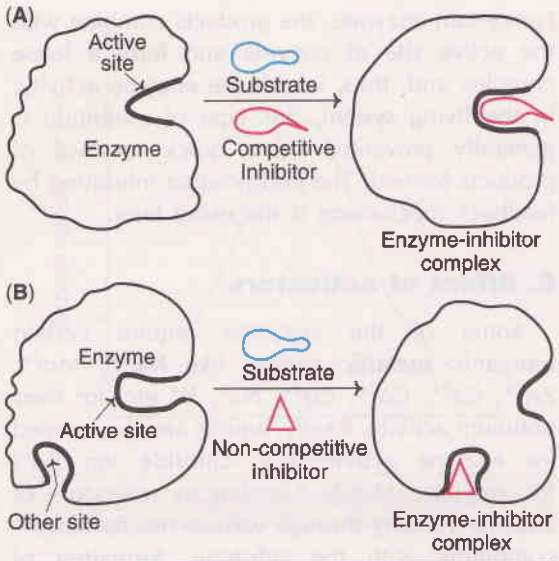


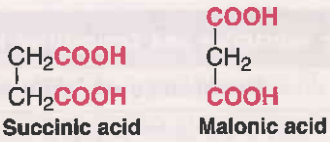
Fig. 6.7 : A diagrammatic representation of (A) Competitive and (B) Non-competitive inhibition.

I. **Competitive inhibition :** The inhibitor (I) which closely resembles the real substrate (S) is regarded as a ***substrate analogue***. The inhibitor competes with substrate and binds at the active site of the enzyme but does not undergo any catalysis. As long as the competitive inhibitor holds the active site, the enzyme is not available for the substrate to bind. During the reaction, ES and EI complexes are formed as shown below

$$E \xrightarrow{+S} ES \rightarrow E + P \qquad E \xrightarrow{+I} EI$$

The relative concentration of the substrate and inhibitor and their respective affinity with the enzyme determines the degree of competitive inhibition. The inhibition could be overcome by a high substrate concentration. In competitive inhibition, the ***K_m value increases*** whereas ***V_{max}*** remains ***unchanged*** (***Fig.6.8***).

The enzyme succinate dehydrogenase (SDH) is a classical example of competitive inhibition with succinic acid as its substrate. The compounds, namely, malonic acid, glutaric acid and oxalic acid, have structural similarity with succinic acid and compete with the substrate for binding at the active site of SDH.



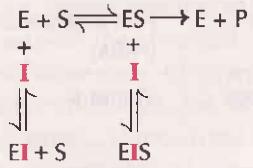
Methanol is toxic to the body when it is converted to formaldehyde by the enzyme alcohol dehydrogenase (ADH). Ethanol can compete with methanol for ADH. Thus, ethanol can be used in the treatment of methanol poisoning.

Some more examples of the enzymes with substrates and competitive inhibitors (of clinical and pharmacological significance) are given in ***Table 6.2.***

Antimetabolites : These are the chemical compounds that block the metabolic reactions by their inhibitory action on enzymes. Antimetabolites are usually structural analogues of substrates and thus are competitive inhibitors (***Table 6.2***). They are in use for cancer therapy, gout etc. The term ***antivitamins*** is used for the antimetabolites which block the biochemical actions of vitamins causing deficiencies, e.g. sulphonilamide, dicumarol.

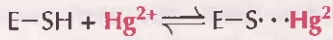
II. **Non-competitive inhibition :** The inhibitor binds at a site other than the active site on the enzyme surface. This binding impairs the enzyme function. The inhibitor has no structural resemblance with the substrate. However, there usually exists a strong affinity for the inhibitor to bind at the second site. In fact, the inhibitor does not interfere with the enzyme-substrate binding. But the catalysis is prevented, possibly due to a distortion in the enzyme conformation.

The inhibitor generally binds with the enzyme as well as the ES complex. The overall relation in non-competitive inhibition is represented below



For non-competitive inhibition, the ***K_m value is unchanged*** while ***V_{max} is lowered*** (***Fig.6.9***).

Heavy metal ions (Ag^+, Pb^{2+}, Hg^{2+} etc.) can non-competitively inhibit the enzymes by binding with cysteinyl sulfhydryl groups. The general reaction for Hg^{2+} is shown below.



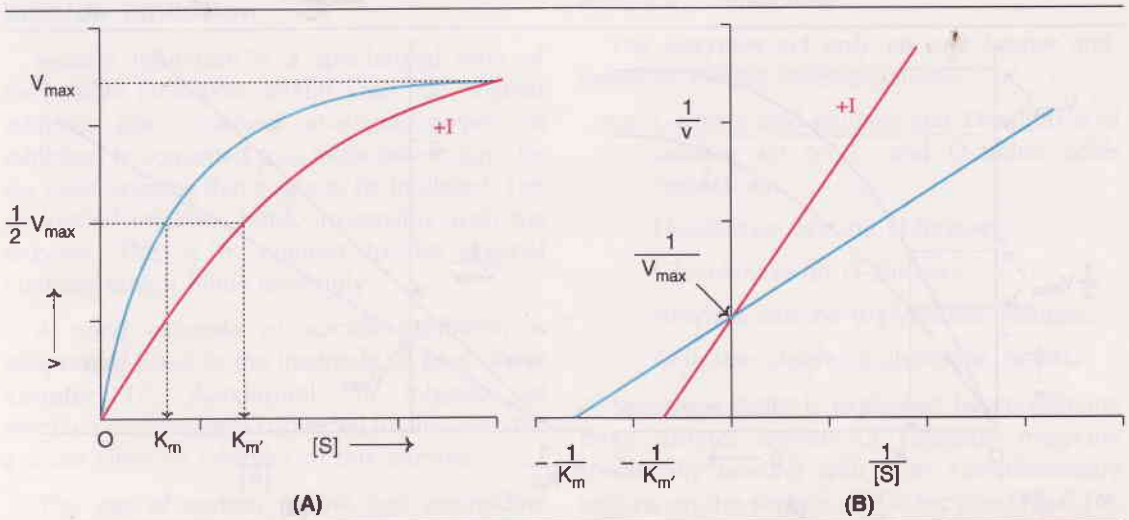


Fig. 6.8 : Effect of competitive inhibitor (i) on enzyme velocity. (A) Velocity (v) versus substrate (S) plot. (B) Lineweaver-Burk plot (Red lines with inhibitor; competitive inhibitor increases K_m, unalters V_{max}).

Table 6.2 Selected examples of enzymes with their respective substrates and competitive inhibitors

Enzyme	*Substrate*	*Inhibitor(s)*	*Significance of inhibitor(s)*
Xanthine oxidase	Hypoxanthine xanthine	Allopurinol	Used in the control of gout to reduce excess production of uric acid from hypoxanthine.
Monoamine oxidase	Catecholamines (epinephrine, norepinephrine)	Ephedrine, amphetamine	Useful for elevating catecholamine levels.
Dihydrofolate reductase	Dihydrofolic acid	Aminopterin, amethopterin, methotrexate	Employed in the treatment of leukemia and other cancers.
Acetylcholine esterase	Acetylcholine	Succinyl choline	Used in surgery for muscle relaxation, in anaesthetised patients.
Dihydropteroate synthase	Para aminobenzoic acid (PABA)	Sulfonilamide	Prevents bacterial synthesis of folic acid.
Vitamin K epoxide reductase	Vitamin K	Dicumarol	Acts as an anticoagulant.
HMG CoA reductase	HMG CoA	Lovastatin, compactin	Inhibit cholesterol biosynthesis

Heavy metals also lead to the formation of covalent bonds with carboxyl groups and histidine, often resulting in irreversible inhibition.

2. Irreversible inhibition

The inhibitors bind covalently with the enzymes and inactivate them, which is irreversible. These inhibitors are usually toxic poisonous substances.

Iodoacetate is an irreversible inhibitor of the enzymes like papain and glyceraldehyde 3-phosphate dehydrogenase. Iodoacetate combines with sulfhydryl (−SH) groups at the active site of these enzymes and makes them inactive.

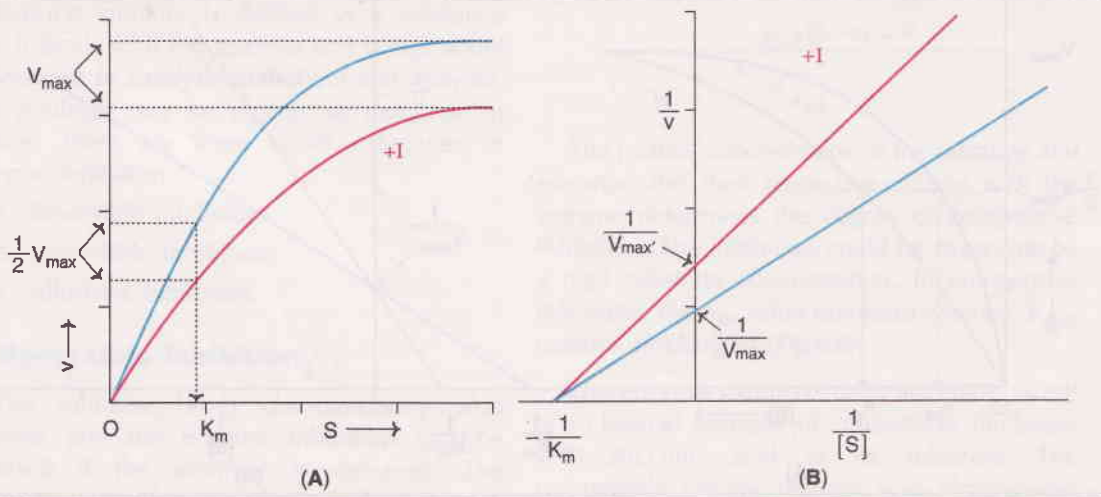


Fig. 6.9 : Effect of non-competitive inhibitor (I) on enzyme velocity (A) Velocity (v) versus substrate (S) (B) Lineweaver-Burk plot (Red lines with inhibitor, non-competitive inhibitor does not change K_m but decreases V_{max}).

Diisopropyl fluorophosphate (DFP) is a ***nerve gas*** developed by the Germans during Second World War. DFP irreversibly binds with enzymes containing serine at the active site, e.g. ***serine proteases, acetylcholine esterase.***

Many organophosphorus insecticides like melathion are toxic to animals (including man) as they block the activity of acetylcholine esterase (essential for nerve conduction), resulting in paralysis of vital body functions.

Disulfiram (Antabuse®) is a drug used in the treatment of alcoholism. It irreversibly inhibits the enzyme aldehyde dehydrogenase. Alcohol addicts, when treated with disulfiram become sick due to the accumulation of acetaldehyde, leading to alcohol avoidance. (***Note :*** Alcohol is metabolized by two enzymes. It is first acted upon by alcohol dehydrogenase to yield acetaldehyde. The enzyme aldehyde dehydrogenase converts acetaldehyde to acetic acid.)

The penicillin antibiotics act as irreversible inhibitors of serine – containing enzymes, and block the bacterial cell wall synthesis.

Irreversible inhibitors are frequently used to identify amino acid residues at the active site of the enzymes, and also to understand the mechanism of enzyme action.

Suicide inhibition

Suicide inhibition is a specialized form of irreversible inhibition. In this case, the original inhibitor (the structural analogue/competitive inhibitor) is converted to a more potent form by the same enzyme that ought to be inhibited. The so formed inhibitor binds irreversibly with the enzyme. This is in contrast to the original inhibitor which binds reversibly.

A good example of suicide inhibition is ***allopurinol*** (used in the treatment of gout, ***Refer Chapter 17***). Allopurinol, an inhibitor of xanthine oxidase, gets converted to alloxanthine, a more effective inhibitor of this enzyme.

The use of certain purine and pyrimidine analogues in cancer therapy is also explained on the basis suicide inhibition. For instance, ***5-fluorouracil*** gets converted to fluorodeoxyuridylate which inhibits the enzyme thymidylate synthase, and thus nucleotide synthesis.

3. Allosteric inhibition

The details of this type of inhibition are given under allosteric regulation as a part of the regulation of enzyme activity in the living system.

ENZYME SPECIFICITY

Enzymes are highly specific in their action when compared with the chemical catalysts. The occurrence of thousands of enzymes in the biological system might be due to the specific nature of enzymes. Three types of enzyme specificity are well-recognised

1. Stereospecificity,
2. Reaction specificity,
3. Substrate specificity,

Specificity is a characteristic property of the active site.

1. **Stereospecificity or optical specificity :** Stereoisomers are the compounds which have the same molecular formula, but differ in their structural configuration.

The ***enzymes act only on one isomer*** and, therefore, exhibit stereospecificity.

e.g. L-amino acid oxidase and D-amino acid oxidase act on L- and D-amino acids respectively.

Hexokinase acts on D-hexoses;

Glucokinase on D-glucose;

Amylase acts on α-glycosidic linkages;

Cellulase cleaves β-glycosidic bonds.

Stereospecificity is explained by considering three distinct regions of substrate molecule specifically binding with three complementary regions on the surface of the enzyme (***Fig.6.10***). The class of enzymes belonging to ***isomerases do not exhibit stereospecificity***, since they are specialized in the interconversion of isomers.

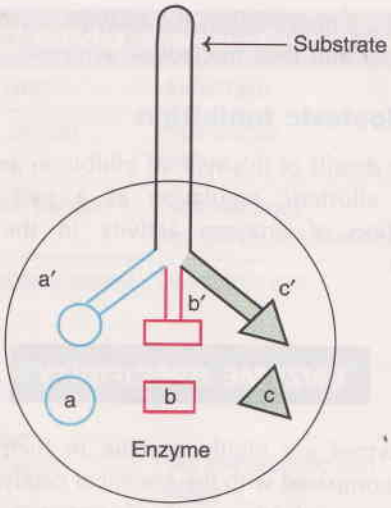


Fig. 6.10 : Diagrammatic representation of stereo-specificity (a', b', c')—three point attachment of substrate to the enzyme (a, b, c).

2. **Reaction specificity :** The same substrate can undergo different types of reactions, each catalysed by a separate enzyme and this is referred to as reaction specificity. An amino acid can undergo transamination, oxidative deamination, decarboxylation, racemization etc. The enzymes however, are different for each of these reactions *(For details, **refer Chapter 15**).*

3. ***Substrate specificity*** **:** The substrate specificity varies from enzyme to enzyme. It may be either absolute, relative or broad.

- **Absolute substrate specificity :** Certain enzymes act only on one substrate e.g. glucokinase acts on glucose to give glucose 6-phosphate, urease cleaves urea to ammonia and carbon dioxide.
- **Relative substrate specificity :** Some enzymes act on structurally related substances. This, in turn, may be dependent on the specific group or a bond present. The action of trypsin is a good example for ***group specificity*** **(*Refer Fig.8.7*).** Trypsin hydrolyses peptide linkage involving arginine or lysine. Chymotrypsin cleaves peptide bonds attached to aromatic amino acids (phenylalanine, tyrosine and tryptophan). Examples of ***bond specificity-*** glycosidases acting on glycosidic bonds of carbohydrates, lipases cleaving ester bonds of lipids etc.
- **Broad specificity :** Some enzymes act on closely related substrates which is commonly known as broad substrate specificity, e.g. hexokinase acts on glucose, fructose, mannose and glucosamine and not on galactose. It is possible that some structural similarity among the first four compounds makes them a common substrate for the enzyme hexokinase.

COENZYMES

The protein part of the enzyme, on its own, is not always adequate to bring about the catalytic activity. Many enzymes require certain non-protein small additional factors, collectively referred to as cofactors for catalysis. The cofactors may be organic or inorganic in nature.

The non-protein, organic, low molecular weight and dialysable substance associated with enzyme function is known as coenzyme.

The functional enzyme is referred to as ***holoenzyme*** *which is made up of a protein part* ***(apoenzyme)*** *and a non-protein part* ***(coenzyme).*** The term prosthetic group is used when a non-protein moiety is tightly bound to the enzyme which is not easily separable by dialysis. The term ***activator*** is referred to the inorganic cofactor (like Ca^{2+}, Mg^{2+}, Mn^{2+} etc.) necessary to enhance enzyme activity. It may, however, be noted that some authors make no distinction between the terms cofactor, coenzyme and prosthetic group and use them interchangeably.

Coenzymes are second substrates : Coenzymes are often regarded as the second substrates or ***co-substrates***, since they have affinity with the enzyme comparable with that of the substrates. Coenzymes undergo alterations during the enzymatic reactions, which are later regenerated. This is in contrast to the substrate which is converted to the product.

Table 6.3 Coenzymes of B-complex vitamins*

Coenzyme (abbreviation)	*Derived from vitamin*	*Atom or group transferred*	*Dependent enzyme (example)*
Thiamine pyrophosphate (TPP)	Thiamine	Aldehyde or keto	Transketolase
Flavin mononucleotide (FMN)	Riboflavin	Hydrogen and electron	L - Amino acid oxidase
Flavin adenine dinucleotide (FAD)	Riboflavin	"	D - Amino acid oxidase
Nicotinamide adenine dinucleotide(NAD^+)	Niacin	"	Lactate dehydrogenase
Nicotinamide adenine dinucleotide phosphate ($NADP^+$)	"	"	Glucose 6-phosphate dehydrogenase
Lipoic acid	Lipoic acid	"	Pyruvate dehydrogenase complex
Pyridoxal phosphate (PLP)	Pyridoxine	Amino or keto	Alanine transaminase
Coenzyme A (CoA)	Pantothenic acid	Acyl	Thiokinase
Tetrahydrofolate (FH_4)	Folic acid	One carbon (formyl, methenyl etc.)	Formyl transferase
Biocytin	Biotin	CO_2	Pyruvate carboxylase
Methylcobalamin; Deoxyadenosyl cobalamin	Cobalamin	Methyl/isomerisation	Methylmalonyl CoA mutase

* *Details for each coenzyme are given in Chapter 7 on vitamins*

Coenzymes participate in various reactions involving transfer of atoms or groups like hydrogen, aldehyde, keto, amino, acyl, methyl, carbon dioxide etc. Coenzymes play a decisive role in enzyme function.

Coenzymes from B-complex vitamins : Most of the coenzymes are the derivatives of water soluble B-complex vitamins. In fact, the biochemical functions of B-complex vitamins are exerted through their respective coenzymes. The chapter on vitamins gives the details of structure and function of the coenzymes (***Chapter 7***). In ***Table. 6.3***, a summary of the vitamin related coenzymes with their functions is given.

Non-vitamin coenzymes : Not all coenzymes are vitamin derivatives. There are some other organic substances, which have no relation with vitamins but function as coenzymes. They may be considered as non-vitamin coenzymes e.g. ATP, CDP, UDP etc. The important non-vitamin coenzymes along with their functions are given in ***Table 6.4***.

Nucleotide coenzymes : Some of the coenzymes possess nitrogenous base, sugar and

Table 6.4 Coenzymes not related to B-complex vitamins

Coenzyme	*Abbreviation*	*Biochemical functions*
Adenosine triphosphate	ATP	Donates phosphate, adenosine and adenosine monophosphate (AMP) moieties.
Cytidine diphosphate	CDP	Required in phospholipid synthesis as carrier of choline and ethanolamine.
Uridine diphosphate	UDP	Carrier of monosaccharides (glucose, galactose), required for glycogen synthesis.
S - Adenosylmethionine (active methionine)	SAM	Donates methyl group in biosynthetic reactions.
Phosphoadenosine phosphosulfate (active sulfate)	PAPS	Donates sulfate for the synthesis of mucopolysaccharides.

phosphate. Such coenzymes are, therefore, regarded as nucleotides e.g. NAD^+, $NADP^+$, FMN, FAD, coenzyme A, UDPG etc.

Coenzymes do not decide enzyme specificity : A particular coenzyme may participate in catalytic reactions along with different enzymes. For instance, NAD^+ acts as a coenzyme for lactate *dehydrogenase and alcohol dehydrogenase. In* both the enzymatic reactions, NAD^+ is involved in hydrogen transfer. The ***specificity of the enzyme is mostly dependent on the apoenzyme and not on the coenzyme.***

MECHANISM OF ENZYME ACTION

Catalysis is the prime function of enzymes. The nature of catalysis taking place in the biological system is similar to that of non-biological catalysis. For any chemical reaction to occur, the reactants have to be in an activated state or transition state.

Enzymes lower activation energy : The energy required by the reactants to undergo the reaction is known as ***activation energy.*** The reactants when heated attain the activation energy. The catalyst (or the enzyme in the biological system) reduces the activation energy and this causes the reaction to proceed at a lower temperature. Enzymes do not alter the equilibrium constants, they only enhance the velocity of the reaction.

The role of catalyst or enzyme is comparable with a tunnel made in a mountain to reduce the barrier as illustrated in ***Fig.6.11.*** The enzyme lowers energy barrier of reactants, thereby making the reaction go faster. The enzymes reduce the activation energy of the reactants in such a way that all the biological systems occur at body temperature (below 40°C).

Enzyme-substrate complex formation

The prime requisite for enzyme catalysis is that the substrate (S) must combine with the enzyme (E) at the active site to form enzyme-substrate complex (ES) which ultimately results in the product formation (P).

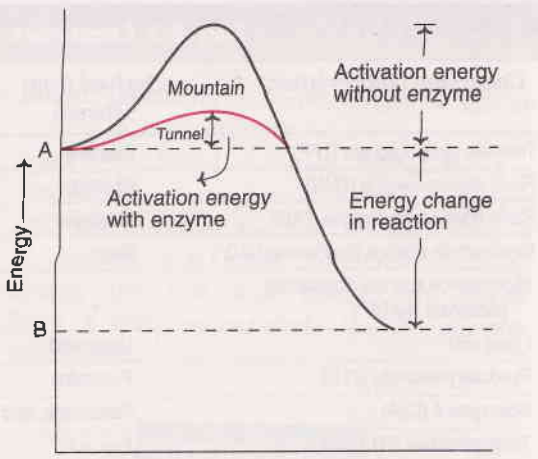


Fig. 6.11 : Effect of enzyme on activation energy of a reaction (A is the substrate and B is the product. Enzyme decreases activation energy).

$$E + S \rightleftharpoons ES \longrightarrow E + P$$

A few theories have been put forth to explain mechanism of enzyme-substrate complex formation.

Lock and key model or Fischer's template theory

This theory was proposed by a German biochemist, Emil Fischer. This is in fact the very first model proposed to explain an enzyme catalysed reaction.

According to this model, the structure or conformation of the enzyme is rigid. The substrate fits to the binding site (now active site) just as a key fits into the proper lock or a hand into the proper glove. Thus the active site of an enzyme is a rigid and pre-shaped template where only a specific substrate can bind. This model does not give any scope for the flexible nature of enzymes, hence the model totally fails to explain many facts of enzymatic reactions, the most important being the effect of allosteric modulators.

Induced fit theory or Koshland's model

Koshland, in 1958, proposed a more acceptable and realistic model for enzyme-substrate complex formation. As per this model,

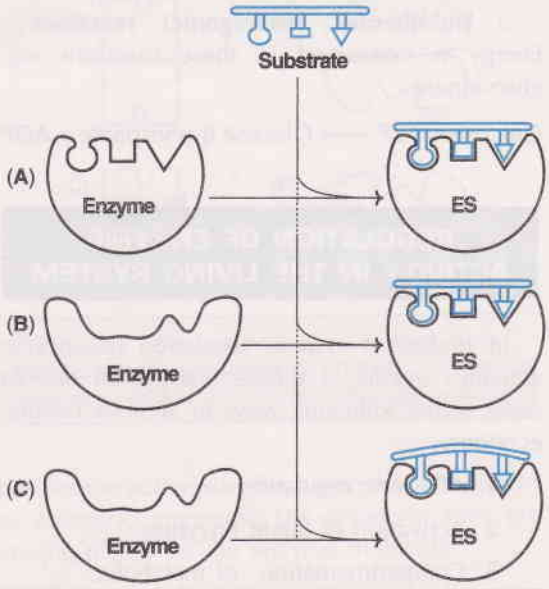


Fig. 6.12 : Mechanism of enzyme-substrate (ES) complex formation (A) Lock and key model (B) Induced fit theory (C) Substrate strain theory.

the active site is not rigid and pre-shaped. The essential features of the substrate binding site are present at the nascent active site. The interaction of the substrate with the enzyme induces a fit or a conformation change in the enzyme, resulting in the formation of a strong substrate binding site. Further, due to induced fit, the appropriate amino acids of the enzyme are repositioned to form the active site and bring about the catalysis (***Fig.6.12***).

Induced fit model has sufficient experimental evidence from the X-ray diffraction studies. Koshland's model also explains the action of allosteric modulators and competitive inhibition on enzymes.

Substrate strain theory

In this model, the substrate is strained due to the induced conformation change in the enzyme. It is also possible that when a substrate binds to the preformed active site, the enzyme induces a strain to the substrate. The strained substrate leads to the formation of product.

In fact, a combination of the induced fit model with the substrate strain is considered to be operative in the enzymatic action.

MECHANISM OF ENZYME CATALYSIS

The formation of an enzyme-substrate complex (ES) is very crucial for the catalysis to occur, and for the product formation. It is estimated that an enzyme catalysed reaction proceeds 10^6 to 10^{12} times faster than a non-catalysed reaction. The enhancement in the rate of the reaction is mainly due to four processes :

1. Acid-base catalysis;
2. Substrate strain;
3. Covalent catalysis;
4. Entropy effects.

1. **Acid-base catalysis** : Role of acids and bases is quite important in enzymology. At the physiological pH, histidine is the most important amino acid, the protonated form of which functions as an acid and its corresponding conjugate as a base. The other acids are −OH group of tyrosine, −SH group of cysteine, and ε-amino group of lysine. The conjugates of these acids and carboxyl ions (COO^-) function as bases.

Ribonuclease which cleaves phosphodiester bonds in a pyrimidine loci in RNA is a classical example of the role of acid and base in the catalysis.

2. **Substrate strain** : Induction of a strain on the substrate for ES formation is discussed above. During the course of strain induction, the energy level of the substrate is raised, leading to a transition state.

The mechanism of lysozyme (an enzyme of tears, that cleaves β-1,4 glycosidic bonds) action is believed to be due to a combination of substrate strain and acid-base catalysis.

3. **Covalent catalysis** : In the covalent catalysis, the negatively charged (nucleophilic) or positively charged (electrophilic) group is present at the active site of the enzyme. This group attacks the substrate that results in the covalent binding of the substrate to the enzyme. In the serine proteases (so named due to the presence of serine at active site), covalent catalysis along with acid-base catalysis occur, e.g. chymotrypsin, trypsin, thrombin etc.

4. **Entropy effect :** Entropy is a term used in thermodynamics. It is defined as the extent of disorder in a system. The enzymes bring about a decrease in the entropy of the reactants. This enables the reactants to come closer to the enzyme and thus increase the rate of reaction.

In the actual catalysis of the enzymes, more than one of the processes – acid-base catalysis, substrate strain, covalent catalysis and entropy are simultaneously operative. This will help the substrate(s) to attain a transition state leading to the formation of products.

THERMODYNAMICS OF ENZYMATIC REACTIONS

The enzyme catalysed reactions may be broadly grouped into three types based on thermodynamic (energy) considerations.

1. **Isothermic reactions :** The energy exchange between reactants and products is negligible e.g. glycogen phosphorylase

Glycogen + Pi ⟶ Glucose 1-phosphate

2. **Exothermic (exergonic) reactions :** Energy is liberated in these reactions e.g. urease

Urea ⟶ NH_3 + CO_2 + energy

3. **Endothermic (endergonic) reactions :** Energy is consumed in these reactions e.g. glucokinase

Glucose + ATP ⟶ Glucose 6-phosphate + ADP

REGULATION OF ENZYME ACTIVITY IN THE LIVING SYSTEM

In biological system, regulation of enzyme activities occurs at different stages in one or more of the following ways to achieve cellular economy.

1. Allosteric regulation
2. Activation of latent enzymes
3. Compartmentation of metabolic pathways
4. Control of enzyme synthesis
5. Enzyme degradation
6. Isoenzymes

1. Allosteric regulation and allosteric inhibition

Some of the enzymes possess additional sites, known as allosteric sites (*Greek* : allo–other),

BIOMEDICAL / CLINICAL CONCEPTS

- *The existence of life is unimaginable without the presence of enzymes—the biocatalysts.*
- *Majority of the coenzymes (TPP, NAD^+, FAD, CoA) are derived from B-complex vitamins in which form the latter exert their biochemical functions.*
- *Competitive inhibitors of certain enzymes are of great biological significance. Allopurinol, employed in the treatment of gout, inhibits xanthine oxidase to reduce the formation of uric acid. The other competitive inhibitors include aminopterin used in the treatment of cancers, sulfanilamide as antibactericidal agent and dicumarol as an anticoagulant.*
- *The nerve gas (diisopropyl fluorophosphate), first developed by Germans during Second World War, inhibits acetylcholine esterase, the enzyme essential for nerve conduction and paralyses the vital body functions. Many organophosphorus insecticides (e.g. melathion) also block the activity of acetylcholine esterase.*
- *Penicillin antibiotics irreversibly inhibit serine containing enzymes of bacterial cell wall synthesis.*

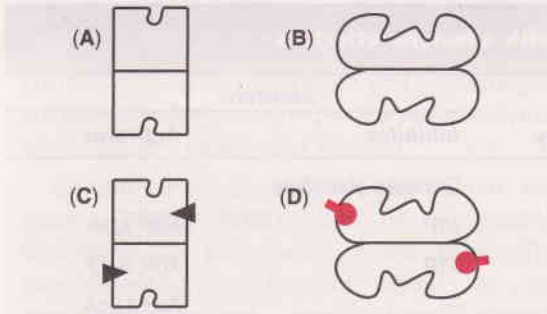


Fig. 6.13 : Diagrammatic representation of an allosteric enzyme (A) T-form; (B) R-form; (C) Effect of allosteric inhibitor; (D) Effect of allosteric activator.

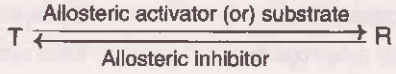
besides the active site. Such enzymes are known as allosteric enzymes. The allosteric sites are unique places on the enzyme molecule.

Allosteric effectors : Certain substances referred to as allosteric modulators (effectors or modifiers) bind at the allosteric site and regulate the enzyme activity. The enzyme activity is increased when a positive (+) allosteric effector binds at the allosteric site known as activator site. On the other hand, a negative (–) allosteric effector binds at the allosteric site called inhibitor site and inhibits the enzyme activity.

Classes of allosteric enzymes : Enzymes that are regulated by allosteric mechanism are referred to as allosteric enzymes. They are divided into two classes based on the influence of allosteric effector on K_m and V_{max}.

- **K-class of allosteric enzymes,** the effector changes the K_m and not the V_{max}. Double reciprocal plots, similar to competitive inhibition are obtained e.g. phospho-fructokinase.
- **V-class of allosteric enzymes,** the effector alters the V_{max} and not the K_m. Double reciprocal plots resemble that of non-competitive inhibition e.g. acetyl CoA carboxylase.

Conformational changes in allosteric enzymes : Most of the allosteric enzymes are oligomeric in nature. The subunits may be identical or different. The non-covalent reversible binding of the effector molecule at the allosteric site brings about a conformational change in the active site of the enzyme, leading to the inhibition or activation of the catalytic activity (***Fig.6.13***). In the concerted model, allosteric enzymes exist in two conformational states – the T (tense or taut) and the R (relaxed). The T and R states are in equilibrium.



Allosteric inhibitors favour T state whereas activators and substrates favour R state. The substrate can bind only with the R form of the enzyme. The concentration of enzyme molecule in the R state increases as more substrate is added, therefore the binding of the substrate to the allosteric enzyme is said to be cooperative. Allosteric enzymes give a sigmoidal curve (instead of hyperbola) when the velocity (v) versus substrate(S) concentration are plotted (***Fig.6.14***).

The term ***homotropic effect*** is used if the substrate influences the substrate binding through allosteric mechanism, their effect is always positive. ***Heterotropic effect*** is used when an allosteric modulator effects the binding of substrate to the enzyme. Heterotropic interactions are either positive or negative. Selected examples of allosteric enzymes responsible for rapid control of biochemical pathways are given in ***Table 6.5***.

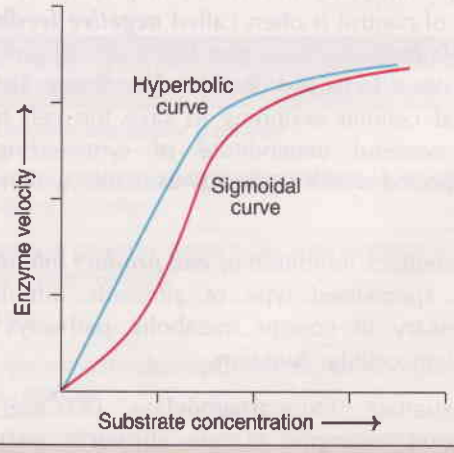


Fig. 6.14 : Effect of substrate concentration on allosteric enzyme (red line-sigmoidal curve) in comparison with normal enzyme (blue line-hyperbolic curve).

Table 6.5 Some enzymes with allosteric effectors

Enzyme	Metabolic pathway	Allosteric	
		Inhibitor	*Activator*
Hexokinase	Glycolysis	Glucose 6-phosphate	—
Phosphofructokinase	Glycolysis	ATP	AMP, ADP
Isocitrate dehydrogenase	Krebs cycle	ATP	ADP, NAD^+
Pyruvate carboxylase	Gluconeogenesis	—	Acetyl CoA
Fructose 1, 6 - bisphosphatase	Gluconeogenesis	AMP	—
Carbamoyl phosphate synthetase I	Urea cycle	—	N - Acetylglutamate
Tryptophan oxygenase	Tryptophan metabolism	—	L - Tryptophan
Acetyl CoA carboxylase	Fatty acid synthesis	Palmitate	Isocitrate

Feedback regulation

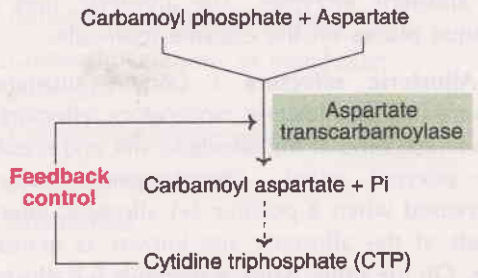
The process of ***inhibiting the first step by the final product***, in a series of enzyme catalysed reactions of a metabolic pathway is referred to as feedback regulation. Look at the series of reactions given below

$$A \xrightarrow{e_1} B \xrightarrow{e_2} C \xrightarrow{e_3} D \xrightarrow{e_4} E$$

A is the initial substrate, B, C, and D are the intermediates and E is the end product, in a pathway catalysed by four different enzymes (e_1, e_2, e_3, e_4). The very first step (A → B by the enzyme e_1) is the most effective for regulating the pathway, by the final end product E. This type of control is often called ***negative feedback regulation*** since increased levels of end product will result in its (e_1) decreased synthesis. This is a real cellular economy to save the cell from the wasteful expenditure of synthesizing a compound which is already available within the cell.

Feedback inhibition or ***end product inhibition*** is a specialised type of allosteric inhibition necessary to control metabolic pathways for efficient cellular function.

Aspartate transcarbamoylase (ATCase) is a good example of an allosteric enzyme inhibited by a feedback mechanism. ATCase catalyses the very first reaction in pyrimidine biosynthesis.



Carbamoyl phosphate undergoes a sequence of reactions for synthesis of the end product, CTP. When CTP accumulates, it allosterically inhibits the enzyme aspartate transcarbamoylase by a feedback mechanism.

Feedback regulation or feedback inhibition? Sometimes a distinction is made between these two usages. Feedback regulation represents a phenomenon while feedback inhibition involves the mechanism of regulation. Thus, in a true sense, they are not synonymous. For instance, dietary cholesterol decreases hepatic cholesterol biosynthesis through feedback regulation. This does not involve feedback inhibition, since dietary cholesterol does not directly inhibit the regulatory enzyme HMG CoA reductase. However, the activity of gene encoding this enzyme is reduced (repression) by cholesterol.

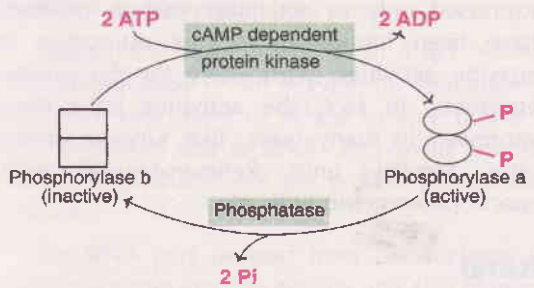
2. Activation of latent enzymes

Latent enzymes, as such, are inactive. Some enzymes are synthesized as ***proenzymes*** or ***zymogens*** which undergo irreversible covalent

activation by the breakdown of one or more peptide bonds. For instance, proenzymes –namely chymotrypsinogen, pepsinogen and plasminogen, are respectively – converted to the active enzymes chymotrypsin, pepsin and plasmin.

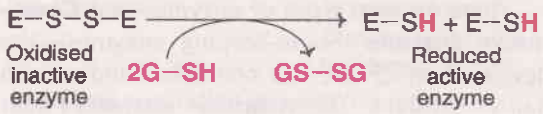
Certain enzymes exist in the active and inactive forms which are interconvertible, depending on the needs of the body. The interconversion is brought about by the reversible covalent modifications, namely phosphorylation and dephosphorylation, and oxidation and reduction of disulfide bonds.

Glycogen phosphorylase is a muscle enzyme that breaks down glycogen to provide energy. This enzyme is a homodimer (two identical subunits) and exists in two interconvertible forms. Phosphorylase b (dephospho enzyme) is inactive which is converted by phosphorylation of serine residues to active form phosphorylase a. The inactive enzyme phosphorylase b is produced on dephosphorylation as illustrated below.



There are some enzymes which are active in dephosphorylated state and become inactive when phosphorylated e.g. glycogen synthase, acetyl CoA carboxylase.

A few enzymes are active only with sulfhydryl (–SH) groups, e.g. succinate dehydrogenase, urease. Substances like glutathione bring about the stability of these enzymes.



3. Compartmentation

There are certain substances in the body (e.g., fatty acids, glycogen) which are synthesized and also degraded. There is no point for simultaneous occurrence of both the pathways. Generally, the ***synthetic*** (anabolic) and ***breakdown*** (catabolic) ***pathways are operative in different cellular organelles*** to achieve maximum economy. For instance, enzymes for fatty acid synthesis are found in the cytosol whereas enzymes for fatty acid oxidation are present in the mitochondria. Depending on the needs of the body — through the mediation of hormonal and other controls — fatty acids are either synthesized or oxidized.

The intracellular location of certain enzymes and metabolic pathways is given in ***Table 6.6***.

Table 6.6 Distribution of certain enzymes and metabolic pathways in cellular organelles

Organelle	*Enzyme/metabolic pathway*
Cytoplasm	Aminotransferases; peptidases; glycolysis; hexose monophosphate shunt; fatty acid synthesis; purine and pyrimidine catabolism.
Mitochondria	Fatty acid oxidation; amino acid oxidation; Krebs cycle; urea synthesis; electron transport chain and oxidative phosphorylation.
Nucleus	Biosynthesis of DNA and RNA.
Endoplasmic reticulum (microsomes)	Protein biosynthesis; triacylglycerol and phospholipid synthesis; steroid synthesis and reduction; cytochrome P_{450}; esterase.
Lysosomes	Lysozyme; phosphatases; phospholipases; hydrolases; proteases; lipases; nucleases.
Golgi apparatus	Glucose 6-phosphatase; 5′-nucleotidase; glucosyl- and galactosyl-transferases.
Peroxisomes	Catalase; urate oxidase; D-amino acid oxidase; long chain fatty acid oxidation.

4. Control of enzyme synthesis

Most of the enzymes, particularly the rate limiting ones, are present in very low concentration. Nevertheless, the amount of the enzyme directly controls the velocity of the reaction, catalysed by that enzyme. ***Many rate limiting enzymes have short half-lives***. This helps in the efficient regulation of the enzyme levels.

There are two types of enzymes—(a) ***Constitutive enzymes*** (house-keeping enzymes)—the levels of which are not controlled and remain fairly constant. (b) ***Adaptive enzymes***—their concentrations increase or decrease as per body needs and are well-regulated. The synthesis of enzymes (proteins) is regulated by the genes (***Refer Chapter 26***).

Induction and repression : The term induction is used to represent increased synthesis of enzyme while repression indicates its decreased synthesis. Induction or repression which ultimately determines the enzyme concentration at the gene level through the mediation of hormones or other substances.

Examples of enzyme induction : The hormone insulin induces the synthesis of ***glycogen synthetase***, glucokinase, phosphofructokinase and pyruvate kinase. All these enzymes are involved in the utilization of glucose. The hormone cortisol induces the synthesis of many enzymes e.g. pyruvate carboxylase, tryptophan oxygenase and tyrosine aminotransferase.

Examples of repression : In many instances, substrate can repress the synthesis of enzyme. Pyruvate carboxylase is a key enzyme in the synthesis of glucose from non-carbohydrate sources like pyruvate and amino acids. If there is sufficient glucose available, there is no necessity for its synthesis. This is achieved through repression of ***pyruvate carboxylase by glucose***.

5. Enzyme degradation

Enzymes are not immortal, since it will create a series of problems. There is a lot of variability in the half-lives of individual enzymes. For some, it is in days while for others in hours or in minutes, e.g. LDH_4 — 5 to 6 days; LDH_1 — 8 to 12 hours; amylase — 3 to 5 hours.

In general, the key and regulatory enzymes are most rapidly degraded. If not needed, they immediately disappear and, as and when required, they are quickly sysnthesized. Though not always true, an enzyme with long half-life is usually sluggish in its catalytic activity.

6. Isoenzymes

Multiple forms of the same enzyme will also help in the regulation of enzyme activity, Many of the isoenzymes are tissue-specific. Although isoenzymes of a given enzyme catalyse the same reaction, they differ in K_m, V_{max} or both. e.g. isoenzymes of LDH and CPK.

UNITS OF ENZYME ACTIVITY

Enzymes are never expressed in terms of their concentration (as mg or µg etc.), but are expressed only as activities. Various methods have been introduced for the estimation of enzyme activities (particularly for the plasma enzymes). In fact, the activities have been expressed in many ways, like King-Armstrong units, Somogyi units, Reitman-Frankel units, spectrophotometric units etc.

Katal

In order to maintain uniformity in the expression of enzyme activities (as units) worldover, the Enzyme Commission of IUB has suggested radical changes. A new unit – namely katal (abbreviated as kat) – was introduced. ***One kat denotes the conversion of one mole substrate per second*** (mol/sec). Activity may also be expressed as millikatals (mkat), microkatals (µkat) and so on.

International Units (IU)

Some workers prefer to use standard units or ***SI units*** (System International). One SI unit or International Unit (IU) is defined as the amount of enzyme activity that catalyses the ***conversion of one micromol of substrate per minute***. SI units and katal are interconvertible.

1 IU = 60 μkatal
(or)
1 nkatal = 1.67 IU

Laboratory use of enzyme units

In the clinical laboratories, however, the units— namely katal or SI units—are yet to find a place. Many investigators still use the old units like King-Armstrong units, Somogyi units etc. while expressing the enzyme activities. It is therefore, essential that the units of enzyme activity, along with the normal values, be invariably stated while expressing the enzymes for comparison.

NON-PROTEIN ENZYMES

Ribozymes

Ribozymes are a group of ***ribonucleic acids*** that function as biological ***catalysts***, and they are regarded as non-protein enzymes.

Altman and his coworkers, in 1983, found that ***ribonuclease P*** – an enzyme till then known to cleave precursors of tRNAs to give tRNAs – was functional ***due to RNA*** component present in the enzyme and not the protein part of the enzyme.

The RNA part isolated from ribonuclease P exhibited a true enzyme activity and also obeyed Michaelis-Menten kinetics. Later studies have proved that RNA, in fact, can function as an enzyme and bring about the catalysis.

RNA molecules are known to adapt a tertiary structure just as in the case of proteins (i.e. enzymes). The specific conformation of RNA may be responsible for its function as biocatalyst. It is believed that ribozymes (RNAs) were functioning as catalysts before the occurrence of protein enzymes during evolution.

APPLICATIONS OF ENZYMES

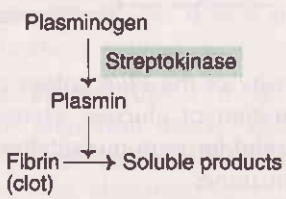
Certain enzymes are useful as therapeutic agents, analytical reagents, in genetic manipulations and for industrial applications (***Table 6.7***).

Table 6.7 A selected list of applications of enzymes

Enzyme	Application
Therapeutic applications	
Streptokinase/urokinase	To remove blood clots
Asparaginase	In cancer therapy
Papain	Anti-inflammatory
α_1-Antitrypsin	To treat emphysema (breathing difficulty due to distension of lungs)
Analytical application reagents (for estimation)	
Glucose oxidase and peroxidase	Glucose
Urease	Urea
Cholesterol oxidase	Cholesterol
Uricase	Uric acid
Lipase	Triacylglycerols
Luciferase	To detect bacterial contamination of foods
Alkaline phosphatase/ horse radish peroxidase	In the analytical technique ELISA
Applications in genetic engineering	
Restriction endonucleases	Gene transfer, DNA finger printing
Taq DNA polymerase	Polymerase chain reaction
Industrial applications	
Rennin	Cheese preparation
Glucose isomerase	Production of high fructose syrup
α-Amylase	In food industry to convert starch to glucose
Proteases	Washing powder

Enzymes as therapeutic agents

1. **Streptokinase** prepared from streptococcus is useful for clearing the blood clots. Streptokinase activates plasma plasminogen to plasmin which, in turn, attacks fibrin to convert into soluble products.



2. The enzyme **asparaginase** is used in the treatment of leukemias. Tumor cells are dependent on asparagine of the host's plasma for their multiplication. By administering asparaginase, the host's plasma levels of asparagine are drastically reduced. This leads to depression in the viability of tumor cells.

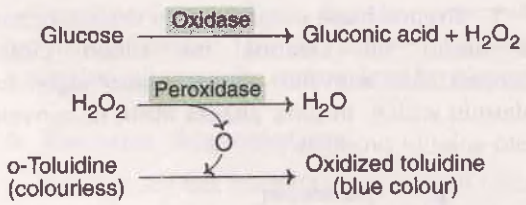
Enzymes as analytical reagents

Some enzymes are useful in the clinical laboratory for the measurement of substrates, drugs, and even the activities of other enzymes. The biochemical compounds (e.g. glucose, urea, uric acid, cholesterol) can be more accurately and specifically estimated by enzymatic procedures compared to the conventional chemical methods. A good example is the estimation of plasma glucose by glucose oxidase and peroxidase method.

Immobilized enzymes

Enzymes can be used as catalytic agents in industrial and medical applications. Some of these enzymes are immobilized by binding them to a solid, insoluble matrix which will not affect the enzyme stability or its catalytic activity. Beaded gels and cyanogen bromide activated sepharose are commonly used for immobilization of enzymes. The bound enzymes can be preserved for long periods without loss of activity.

Glucose oxidase and peroxidase, immobilized and coated on a strip of paper, are used in the clinical laboratory for the detection of glucose in urine.



The intensity of the blue colour depends on the concentration of glucose. Hence, the strip method is useful for semi-quantitative estimation of glucose in urine.

DIAGNOSTIC IMPORTANCE OF ENZYMES

Estimation of enzyme activities in biological fluids (particularly plasma/serum) is of great clinical importance. Enzymes in the circulation are divided into two groups – plasma functional and plasma non-functional.

1. Plasma specific or plasma functional enzymes

Certain enzymes are normally present in the plasma and they have specific functions to perform. Generally, these enzyme activities are higher in plasma than in the tissues. They are mostly synthesized in the liver and enter the circulation e.g. lipoprotein lipase, plasmin, thrombin, choline esterase, ceruloplasmin etc.

Impairment in liver function or genetic disorders often leads to a fall in the activities of plasma functional enzymes e.g. deficiency of ceruloplasmin in Wilson's disease.

2. Non-plasma specific or plasma non-functional enzymes

These enzymes are either totally absent or present at a low concentration in plasma compared to their levels found in the tissues. The digestive enzymes of the gastrointestinal tract (e.g. amylase, pepsin, trypsin, lipase etc.) present in the plasma are known as ***secretory enzymes.*** All the other plasma enzymes associated with metabolism of the cell are collectively referred to as ***constitutive enzymes*** (e.g. lactate dehydrogenase, transaminases, acid and alkaline phosphatases, creatine phosphokinase).

Estimation of the activities of non-plasma specific enzymes is very important for the diagnosis and prognosis of several diseases.

The normal serum level of an enzyme indicates the balance between its synthesis and release in the routine cell turnover. The raised enzyme levels could be due to cellular damage, increased rate of cell turnover, proliferation of cells, increased synthesis of enzymes etc. Serum

Table 6.8 Important enzymes in the diagnosis of diseases

Serum enzyme (elevated)	*Disease (most important)*
Amylase	Acute pancreatitis
Serum glutamate pyruvate transaminase (SGPT)	Liver diseases (hepatitis)
Serum glutamate oxaloacetate transaminase (SGOT)	Heart attacks (myocardial infarction)
Alkaline phosphatase	Rickets, obstructive jaundice
Acid phosphatase	Cancer of prostate gland
Lactate dehydrogenase (LDH)	Heart attacks, liver diseases
Creatine phosphokinase (CPK)	Myocardial infarction (early marker)
Aldolase	Muscular dystrophy
5′-Nucleotidase	Hepatitis
γ-Glutamyl transpeptidase (GGT)	Alcoholism

enzymes are conveniently used as ***markers*** to detect the cellular damage which ultimately helps ***in the diagnosis of diseases***.

A summary of the important enzymes useful for the diagnosis of specific diseases is given in ***Table 6.8***. Detailed information on the diagnostic enzymes including reference values is provided in ***Table 6.9***. A brief account of selected diagnostic enzymes is discussed

Amylase : The activity of serum amylase is increased in ***acute pancreatitis*** (normal 80-180 Somogyi units/dl). The peak value is observed within 8-12 hours after the onset of disease which returns to normal by 3rd or 4th day. Elevated activity of amylase is also found in urine of the patients of acute pancreatitis. Serum amylase is also important for the diagnosis of chronic pancreatitis, acute parotitis (mumps) and obstruction of pancreatic duct.

Alanine transaminase (ALT/SGPT) : SGPT is elevated in ***acute hepatitis*** of viral or toxic origin, jaundice and cirrhosis of liver (normal 3-40 IU/l).

Aspartate transaminase (AST/SGOT) : SGOT activity in serum is increased in ***myocardial infarction*** and also in liver diseases (normal 4-45 IU/l).

It may be noted that SGPT is more specific for the diagnosis of liver diseases while SGOT is for heart diseases. This is mainly because of their cellular distribution – SGPT is a cytosomal enzyme while SGOT is found in cytosol and mitochondria.

Alkaline phosphatase (ALP) : It is elevated in certain bone and liver diseases (normal 3-13 KA units/dl). ALP is useful for the diagnosis of ***rickets***, hyperparathyroidism, carcinoma of bone, and ***obstructive jaundice.***

Acid phosphatase (ACP) : It is increased in the ***cancer of prostate gland*** (normal 0.5-4 KA units/dl). The tartarate labile ACP (normal <1 KA units/dl) is useful for the diagnosis and prognosis of prostate cancers i.e. ACP is a good tumor marker.

Lactate dehydrogenase (LDH) : LDH is useful for the diagnosis of ***myocardial infarction, infective hepatitis***, leukemia and muscular dystrophy (serum LDH normal 50-200 IU/l). LDH has five isoenzymes, the details of which are described later.

Creatine kinase (CK) : It is elevated in ***myocardial infarction*** (early detection) and muscular dystrophy (normal 10-50 IU/l). CK has three isoenzymes (described later).

Table 6.9 Increase in plasma (serum) enzymes in the diagnosis of diseases

Enzymes	Reference value	Disease(s) in which increased
I. Digestive enzymes		
Amylase	80–180 Somogyi units/dl or 2.5–5.5 µKat	Acute pancreatitis, mumps (acute parotitis), obstruction in pancreatic duct, severe diabetic ketoacidosis.
Lipase	0.2–1.5 IU/l	Acute pancreatitis, moderate elevation in carcinoma of pancreas.
II. Transaminases		
Alanine transaminase (ALT) or serum glutamate pyruvate transaminase (SGPT)	3–40 IU/l or 40–250 nKat	Acute hepatitis (viral or toxic), jaundice, cirrhosis of liver.
Aspartate transaminase (AST) or serum glutamate oxaloacetate transaminase (SGOT)	4–45 IU/l or 50–320 nKat	Myocardial infarction, liver diseases, liver cancer, cirrhosis of liver.
III. Phosphatases		
Alkaline phosphatase (ALP) (pH optimum 9–10)	In adults-3–13 King Armstrong (KA) units/dl or 25–90 IU/l or 500–1400 nKat. In children–15–30 KA/dl	Bone diseases (related to higher osteoblastic activity)-rickets, Pagets' disease, hyperparathyroidism, carcinoma of bone. Liver diseases-obstructive jaundice (cholestasis), infective hepatitis, cirrhosis of liver.
Acid phosphatase (ACP) (pH optimum 4–6)	0.5–4 KA units/dl or 2.5–12 IU/l or 10–100 nKat. Tartarate labile ACP-0–0.9 KA units/dl	Prostatic carcinoma i.e. cancer of prostate gland (tartarate liabile ACP serves as a marker for diagnosis and follow up), Pagets' disease.
IV. Enzymes of carbohydrate metabolism		
Aldolase	2–6 IU/l	Muscular dystrophy, liver diseases, myocardial infarction, myasthenia gravis, leukemias
Isocitrate dehydrogenase (ICD)	1–4 IU/l	Liver diseases (inflammatory toxic or malignant)
Lactate dehydrogenase (LDH)	50–200 IU/l or 1–5 µKat	Myocardial infarction, acute infective hepatitis, muscular dystrophy, leukemia, pernicious anaemia.
V. Miscellaneous enzymes		
Creatine kinase (CK) or creatine phosphokinase (CPK)	10–50 IU/l	Myocardial infarction (CK useful for early detection), muscular dystrophy, hypothyroidism, alcoholism.
Cholinesterase (ChEI)	2–10 IU/l	Nephrotic syndrome, myocardial infarction
5′-Nucleotidase or nucleotide phosphatase (NTP)	2–15 IU/l	Hepatitis, obstructive jaundice, tumors
γ-Glutamyl transpeptidase (GGT)	5–40 IU/l	Alcoholism, infective hepatitis, obstructive jaundice.
Ceruloplasmin (ferrooxidase)	20–50 mg/dl	Bacterial infections, collagen diseases, cirrhosis, pregnancy.

Table 6.10 Decrease in plasma (serum) enzymes in certain diseases

Enzyme	*Reference values*	*Disease(s) in which decreased*
Amylase	80–180 Somogyi units/dl	Liver diseases
Pseudocholinesterase (ChE II)	10–20 IU/dl	Viral hepatitis, malnutrition, liver cancer, cirrhosis of liver
Ceruloplasmin	20–50 mg/dl	Wilson's disease (hepatolenticular degeneration)
Glucose 6-phosphate dehydrogenase (G6PD) in RBC	120–260 IU/10^{12} RBC	Congenital deficiency with hemolytic anemia

γ-Glutamyl transpeptidase (GGT) : It is a sensitive diagnostic marker for the detection of ***alcoholism***. GGT is also increased in infective hepatitis and obstructive jaundice.

Decreased plasma enzyme activities

Sometimes, the plasma activities of the enzymes may be lower than normal which could be due to decreased enzyme synthesis or congenital deficiency. In ***Table 6.10***, the decreased plasma enzymes in certain disorders are given.

ISOENZYMES

The ***multiple forms of an enzyme*** catalysing the same reaction are ***isoenzymes*** or ***isozymes.*** They, however, differ in their physical and chemical properties which include the structure, electrophoretic and immunological properties, K_m and V_{max} values, pH optimum, relative susceptibility to inhibitors and degree of denaturation.

Explanation for the existence of isoenzymes

Many possible reasons are offered to explain the presence of isoenzymes in the living systems.

1. Isoenzymes synthesized from different genes e.g. malate dehydrogenase of cytosol is different from that found in mitochondria.

2. Oligomeric enzymes consisting of more than one type of subunits e.g. lactate dehydrogenase and creatine phosphokinase.

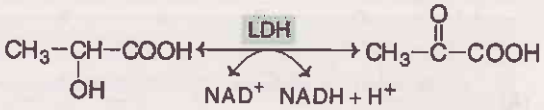
3. An enzyme may be active as monomer or oligomer e.g. glutamate dehydrogenase.

4. In glycoprotein enzymes, differences in carbohydrate content may be responsible for isoenzymes e.g. alkaline phosphatase.

Isoenzymes of lactate dehydrogenase (LDH)

Among the isoenzymes, LDH has been the most thoroughly investigated.

LDH whose systematic name is L-lactate-NAD^+ oxidoreductase (E.C. 1.1.1.27) catalyses the interconversion of lactate and pyruvate as shown below



Lactic acid **Pyruvic acid**

LDH has five distinct isoenzymes LDH_1, LDH_2, LDH_3, LDH_4 and LDH_5. They can be separated by electrophoresis (cellulose or starch gel or agarose gel). LDH_1 has more positive charge and fastest in electrophoretic mobility while LDH_5 is the slowest.

Structure of LDH isoenzymes : LDH is an oligomeric (tetrameric) enzyme made up of four polypeptide subunits. Two types of subunits namely M (for muscle) and H (for heart) are produced by different genes. M–subunit is basic while H subunit is acidic. The isoenzymes contain either one or both the subunits giving LDH_1 to LDH_5. The characteristic features of LDH isoenzymes are given in ***Table 6.11***.

Table 6.11 Lactate dehydrogenase (LDH) isoenzymes and their characteristics

Isoenzyme	*Subunit constitution*	*Principal tissue of origin*	*Electrophoretic mobility*	*Whether destroyed by heat (at 60°C)*	*Percentage of normal serum in humans*
LDH_1	H_4	Heart and RBC	Fastest	No	25%
LDH_2	H_3M	Heart and RBC	Faster	No	35%
LDH_3	H_2M_2	Brain and kidney	Fast	Partially	27%
LDH_4	HM_3	Liver and skeletal muscle	Slow	Yes	8%
LDH_5	M_4	Skeletal muscle and liver	Slowest	Yes	5%

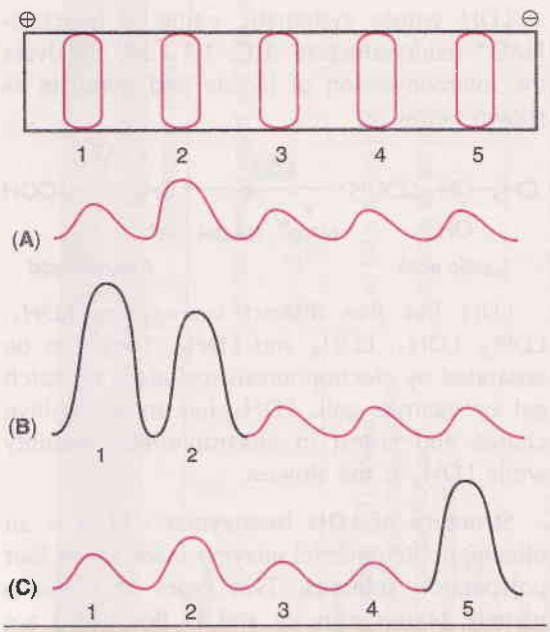


Fig. 6.15 : Electrophoresis of lactate dehydrogenase with relative proportions of isoenzymes (A) Normal serum (B) Serum from a patient of myocardial infarction (LDH_1 and $LDH_2\uparrow$) (C) Serum from a patient of liver disease ($LDH_5\uparrow$).

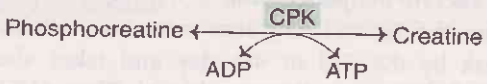
Significance of differential catalytic activity : LDH_1 (H_4) is predominantly found in heart muscle and is inhibited by pyruvate – the substrate. Hence, pyruvate is not converted to lactate in cardiac muscle but is converted to acetyl CoA which enters citric acid cycle. LDH_5 (M_4) is mostly present in skeletal muscle and the inhibition of this enzyme by pyruvate is minimal, hence pyruvate is converted to lactate. Further, LDH_5 has low K_m (high affinity) while LDH_1 has high K_m (low affinity) for pyruvate. The differential catalytic activities of LDH_1 and LDH_5 in heart and skeletal muscle, respectively, are well suited for the aerobic (presence of oxygen) and anaerobic (absence of oxygen) conditions, prevailing in these tissues.

Diagnostic importance of LDH : Isoenzymes of LDH have immense value in the diagnosis of heart and liver related disorders (***Fig.6.15***). In healthy individuals, the activity of LDH_2 is higher than that of LDH_1 in serum. In the case of ***myocardial infarction***, ***LDH_1*** is much greater than LDH_2 and this happens within 12 to 24 hours after infarction. Increased activity of ***LDH_5***

in serum is an indicator of ***liver diseases***. LDH activity in the RBC is 80–100 times more than that in the serum. Hence for estimation of LDH or its isoenzymes, serum should be totally free from hemolysis or else false positive results will be obtained.

Isoenzymes of creatine phosphokinase

Creatine kinase (CK) or creatine phosphokinase (CPK) catalyses the inter-conversion of phosphocreatine (or creatine phosphate) to creatine.



CPK exists as three isoenzymes. Each isoenzyme is a dimer composed of two subunits—M (muscle) or B (brain) or both.

Isoenzyme	*Subunit*	*Tissue of origin*
CPK_1	BB	Brain
CPK_2	MB	Heart
CPK_3	MM	Skeletal muscle

In healthy individuals, the isoenzyme CPK_2 (MB) is almost undetectable in serum with less than 2% of total CPK. After the myocardial infarction (MI), within the first 6-18 hours, CPK_2 increases in the serum to as high as 20% (against 2% normal). CPK_2 isoenzyme is not elevated in skeletal muscle disorders. Therefore, estimation of the enzyme ***CPK_2 (MB) is the earliest reliable indication of myocardial infarction.***

Isoenzymes of alkaline phosphatase

As many as six isoenzymes of alkaline phosphatase (ALP) have been identified. ALP is a monomer, the isoenzymes are due to the ***difference in the carbohydrate content*** (sialic acid residues). The most important ALP isoenzymes are α_1-ALP, α_2-heat labile ALP, α_2-heat stable ALP, pre-β ALP, γ-ALP etc.

Increase in α_2-heat labile ALP suggests hepatitis whereas pre β-ALP indicates bone diseases.

BIOMEDICAL / CLINICAL CONCEPTS

- *In the living system, the regulation of enzyme activities occurs through allosteric inhibition, activation of latent enzymes, compartmentation of metabolic pathways, control of enzyme synthesis and degradation.*
- *Feedback (or end product) inhibition is a specialized form of allosteric inhibition that controls several metabolic pathways e.g. CTP inhibits aspartate transcarbamoylase; Cholesterol inhibits HMG CoA reductase. The end product inhibition is utmost important to cellular economy since a compound is synthesized only when required.*
- *Certain RNA molecules (ribozymes) function as non-protein enzymes. It is believed that ribozymes were functioning as biocatalysts before the occurrence of protein enzymes during evolution.*
- *Certain enzymes are utilized as therapeutic agents. Streptokinase in used to dissolve blood clots in circulation while asparaginase is employed in the treatment of leukemias.*
- *Determination of serum enzyme activities is of great importance for the diagnosis of several diseases (refer Table 6.8).*
- *Lowered body temperature (hypothermia) is accompained by a decrease in enzyme activities. This principle is exploited to reduce metabolic demand during open heart surgery or transportation of organs for transplantation surgery.*

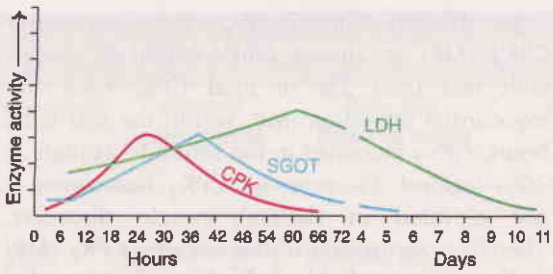


***Fig. 6.16** : Enzyme pattern in myocardial infarction (CPK-Creatine phosphokinase; SGOT-Serum glutamate oxaloacetate transaminase; LDH-Lactate dehydrogenase).*

Isoenzymes of alcohol dehydrogenase

Alcohol dehydrogenase (ADH) has two heterodimer isoenzymes. Among the white Americans and Europeans, $\alpha\beta_1$ isoenzyme is predominant whereas in Japanese and Chinese (Orientals) $\alpha\beta_2$ is mostly present. The isomer $\alpha\beta_2$ more rapidly converts alcohol to acetaldehyde.

Accumulation of acetaldehyde is associated with tachycardia (increase in heart rate) and facial flushing among Orientals which is not commonly seen in whites. It is believed that Japanese and Chinese have increased sensitivity to alcohol due to the presence of $\alpha\beta_2$–isoenzyme of ADH.

ENZYME PATTERN IN DISEASES

For the right diagnosis of a particular disease, it is always better to estimate a few (three or more) serum enzymes, instead of a single enzyme. Examples of enzyme patterns in important diseases are given here.

Enzymes in myocardial infarction

The enzymes – namely creatine phosphokinase (CPK), aspartate transaminase (AST) and lactate dehydrogenase (LDH)—are important for the diagnosis of myocardial infarction (MI). The elevation of these enzymes in serum in relation to hours/days of MI is given in the ***Fig.6.16***.

Creatine phosphokinase (precisely isoenzyme MB) is the first enzyme to be released into circulation within 6-18 hours after the infarction. Therefore, CPK estimation is highly useful for the early diagnosis of MI. This enzyme reaches a peak value within 24-30 hours, and returns to normal level by the 2nd or 3rd day.

Aspartate transaminase (AST or SGOT) rises sharply after CPK, and reaches a peak within 48 hours of the myocardial infarction. AST takes 4-5 days to return to normal level.

Lactate dehydrogenase (LDH_1) generally rises from the second day after infarction, attains a peak by the 3rd or 4th day and takes about 10-15 days to reach normal level. Thus, LDH is the last enzyme to rise and also the last enzyme to return to normal level in MI.

Cardiac troponins (CT) : Although not enzymes, the proteins cardiac troponins are highly useful for the early diagnosis of MI. Among these, ***troponin I*** (*i*nhibitory element of actomysin ATPase) and ***troponin T*** (*t*ropomysin binding element) are important. *C*ardiac troponin I (CTI) is released into circulation within four hours after the onset of MI, reaches a peak value by 12–24 hours, and remains elevated for about a week.

The protein ***myoglobin*** is also an early marker for the diagnosis of MI. Myoglobin is however, not commonly used as it is not specific to cardiac diseases. In the ***Table 6.12***, a summary of the diagnostic markers used in MI is given.

Enzymes in liver diseases

The following enzymes—when elevated in serum – are useful for the diagnosis of liver dysfunction due to viral hepatitis (jaundice), toxic hepatitis, cirrhosis and hepatic necrosis

1. Alanine transaminase;
2. Aspartate transaminase;
3. Lactate dehydrogenase;

The enzymes that markedly increase in intrahepatic and extrahepatic cholestasis are :

1. Alkaline phosphatase, 2. 5′-Nucleotidase

Table 6.12 Summary of diagnostic markers used for the evaluation of acute myocardial infarction

Diagnostic marker	*Time of peak elevation*	*Time of return to normal level*	*Diagnostic importance*
Myoglobin	4-6 hrs	20–25 hrs	Earliest marker, however not cardiac specific.
Cardiac troponin I	12-24 hrs	5-9 days	Early marker and cardiac specific.
Cardiac troponin T	18-36 hrs	5-14 days	Relatively early marker and cardiac specific. However, elevated in other degenerative diseases.
Creatine phosphokinase (MB)	20-30 hrs	24-48 hrs	Cardiac specific and early marker.
Lactate dehydrogenase (LDH I)	48-72 hrs	10-15 days	Relatively late marker and cardiac specific.
Aspartate transaminase	30-48 hrs	4-6 days	Not cardiac specific.

Serum-γ-glutamyl transpeptidase is useful in the diagnosis of alcoholic liver diseases.

Enzymes in muscle diseases

In the muscular dystrophies, probably due to the increased leakage of enzymes from the damaged cells, serum levels of certain muscle enzymes are increased. These include creatine phosphokinase, aldolase and aspartate transaminase. Of these, CPK is the most reliable indicator of muscular diseases, followed by aldolase.

Enzymes in cancers

Increase in the serum acid phosphatase (tartarate labile) is specific for the detection of prostatic carcinoma.

[**Note : *Prostate specific antigen*** (PSA; mol wt. 32 KD), though not an enzyme, is a more reliable marker for the detection of prostate cancer. Normal serum concentration of PSA is 1-4 ng/ml].

A non-specific increase in certain enzymes like LDH, alkaline phosphatase and transaminase may be associated with malignancy in any part of the body.

β-Glucuronidase estimation in urine is useful for detecting the cancers of urinary bladder, pancreas etc.

SUMMARY

1. *Enzymes are the protein biocatalysts synthesized by the living cells. They are classified into six major classes—oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases.*

2. *An enzyme is specific in its action, possessing active site, where the substrate binds to form enzyme-substrate complex, before the product is formed.*

3. *Factors like concentration of enzyme, substrate, temperature, pH etc. influence enzyme activity. The substrate concentration to produce half-maximal velocity is known as Michaelis constant (K_m value).*

4. *Enzyme activities are inhibited by reversible (competitive, and non-competitive), irreversible and allosteric manner.*

5. *Many enzymes require the presence of non-protein substances called cofactors (coenzymes) for their action. Most of the coenzymes are derivatives of B-complex vitamins (e.g. NAD^+, FAD, TPP etc.)*

6. *The mechanism of enzyme action is explained by lock and key model (of Fischer), more recently induced fit model (of Koshland) and substrate strain theory.*

7. *The enzymes enhance the rate of reaction through acid-base catalysis, covalent catalysis and/or entropy effects.*

8. *In the living system, there is a constant regulation of enzyme levels brought about by allosteric mechanism, activation of proenzymes, synthesis and degradation of enzymes etc.*

9. *Estimation of serum enzymes is of great help in the diagnosis of several diseases. Serum amylase and lipase are increased in acute pancreatitis; alanine transaminase in hepatitis; aspartate transaminase, lactate dehydrogenase (LDH) and creatine phosphokinase (CPK) in myocardial infarction; alkaline phosphatase in rickets and hyperparathyroidism; acid phosphatase in prostatic carcinoma; γglutamyl transpeptidase in alcoholism.*

10. *Isoenzymes are the multiple forms of an enzyme catalysing the same reaction which however, differ in their physical and chemical properties. LDH has five isoenzymes while CPK has three. LDH_1 and CPK_2 are very important in the diagnosis of myocardial infarction.*

SELF-ASSESSMENT EXERCISES

I. Essay questions

1. What are enzymes? Describe their classification and nomenclature.
2. Write an account of the various factors affecting enzyme activity.
3. Describe the mechanism of enzyme action.
4. What are coenzymes? Write briefly on the role of coenzymes in enzyme action.
5. Write an account of the importance of serum enzymes in the diagnosis of diseases.

II. Short notes

(a) Enzyme specificity, (b) Competitive inhibition, (c) Coenzymes, (d) Allosteric enzymes, (e) Isoenzymes, (f) K_m value, (g) Serum enzymes in myocardial infarction, (h) Lactate dehydrogenase, (i) Role of metals in enzyme action, (j) Active site.

III. Fill in the blanks

1. The literal meaning of enzyme is _____.
2. The class of enzymes involved in synthetic reactions are _____.
3. The non-protein part of holoenzyme _____.
4. Enzymes lose the catalytic activity at temperature above 70°C due to _____.
5. Examples of two enzymes containing zinc are _____ and _____.
6. The place at which substrate binds with the enzyme _____.
7. The enzyme glucose 6-phosphate dehydrogenase requires the coenzyme _____.
8. The E.C. number for alcohol dehydrogenase is _____.
9. Phsophofructokinase is allosterically activated by _____.
10. The very first enzyme elevated in serum in myocardial infarction _____.

IV. Multiple choice questions

11. Pepsin is an example for the class of enzymes namely

    (a) Oxidoreductases (b) Transferases (c) Hydrolases (d) Ligases.

12. The coenzyme not involved in hydrogen transfer

    (a) FMN (b) FAD (c) $NADP^+$ (d) FH_4.

13. In the feedback regulation, the end product binds at

    (a) Active site (b) Allosteric site (c) E-S complex (d) None of these.

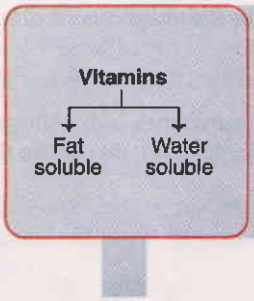
14. γ-Glutamyl transpeptidase activity in serum is elevated in

    (a) Pancreatitis (b) Muscular dystrophy (c) Myocardial infarction (d) Alcoholism.

15. In recent years, a non-protein compound has been identified to bring about catalysis in biological system. The name of the compound is

    (a) DNA (b) RNA (c) Lipids (d) Carbohydrates.

7 Vitamins



The vitamins speak :

"We are for growth, health and welfare of organisms;
Discharge our duties directly or through coenzymes;
Deficiency symptoms are our alert signals;
Satisfied we shall be, with additional supplements."

It is difficult to define vitamins precisely. ***Vitamins may be regarded as organic compounds required in the diet in small amounts to perform specific biological functions for normal maintenance of optimum growth and health of the organism.*** The bacterium *E.coli* does not require any vitamin, as it can synthesize all of them. It is believed that during the course of evolution, the ability to synthesize vitamins was lost. Hence, the higher organisms have to obtain them from diet. The vitamins are required in small amounts, since their degradation is relatively slow.

History and nomenclature

In the beginning of 20th century, it was clearly understood that the diets containing purified carbohydrate, protein, fat and minerals were not adequate to maintain the growth and health of experimental rats, which the natural foods (such as milk) could do.

Hopkins coined the term ***accessory factors*** to the unknown and essential nutrients present in the natural foods. Funk (1913) isolated an active principle (an amine) from rice polishings and, later in yeast, which could cure beri-beri in pigeons. He coined the term ***vitamine*** (*Greek* : vita-life) to the accessory factors with a belief that all of them were ***amines.*** It was later realised that only few of them are amines. The term *vitamin*, however, is continued without the final letter 'e'.

The usage of A, B and C to vitamins was introduced in 1915 by McCollum and Davis. They first felt there were only two vitamins— ***fat soluble A*** and ***water soluble B*** (anti-beriberi factor). Soon another water soluble anti-scurvy factor named vitamin C was described. Vitamin A was later found to possess two components- one that prevents night blindness (vitamin A) and another anti-ricket factor named as vitamin D. A fat soluble factor called vitamin E, in the absence of which rats failed to reproduce properly, was discovered. Yet another fat soluble vitamin concerned with coagulation was discovered in mid 1930s. It was named as vitamin K. In the

sequence of alphabets it should have been F, but K was preferred to reflect its function (koagulation).

As regards the water soluble factors, vitamin C was identified as a pure substance and named as ascorbic acid. Vitamin B was found to be a complex mixture and nomenclature also became complex. B_1 was clearly identified as anti-beri-beri factor. Many investigators carried out intensive research between 1920 and 1930 and went on naming them as the water soluble vitamins B_2, B_3, B_4, B_5, B_6, B_7, B_8, B_9, B_{10}, B_{11} and B_{12}. Some of them were found to be mixtures of already known vitamins. And for this reason, a few members (numbers!) of the B-complex series disappeared from the scene. Except for B_1, B_2, B_6 and B_{12}, names are more commonly used for other B-complex vitamins.

Classification of vitamins

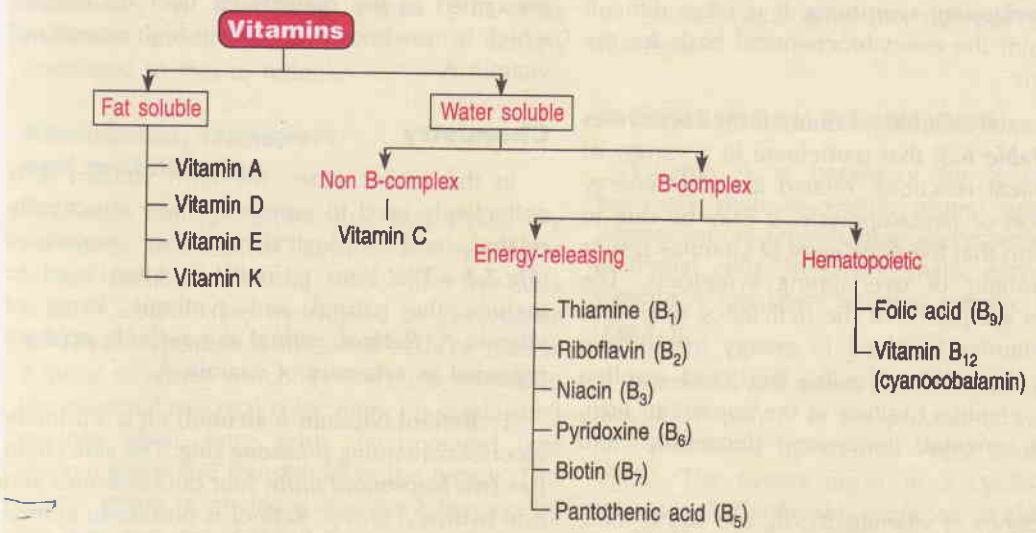
There are about 15 vitamins, essential for humans. They are classified as ***fat soluble*** (A, D, E and K) and ***water soluble*** (C and B-group) vitamins as shown in the ***Table 7.1***. The B-complex vitamins may be sub-divided into ***energy-releasing*** (B_1, B_2, B_6, biotin etc.) and ***hematopoietic*** (folic acid and B_{12}). Most of the water soluble vitamins exert the functions through their respective coenzymes while only one fat soluble vitamin (K) has been identified to function as a coenzyme.

Synthesis of vitamins by intestinal bacteria

Vitamins, as per the definition, are not synthesized in the body. However, the bacteria of the gut can produce some of the vitamins, required by man and animals. The bacteria mainly live and synthesize vitamins in the colon region, where the absorption is relatively poor. Some of the animals (e.g. rat, deer etc.) eat their own feces, a phenomenon known as ***coprophagy***.

As far as humans are concerned, it is believed that the normal intestinal ***bacterial synthesis***, and absorption of ***vitamin K*** and ***biotin may be sufficient*** to meet the body requirements. For other B-complex vitamins, the synthesis and absorption are relatively less. Administration of anitibiotics often kills the vitamin synthesizing bacteria present in the gut, hence additional consumption of vitamins is recommended.

Table 7.1 Classification of vitamins



Fat soluble vitamins—general

The four vitamins, namely vitamin A, D, E, and K are known as fat or lipid soluble. Their availability in the diet, absorption and transport are associated with fat. They are soluble in fats and oils and also the fat solvents (alcohol, acetone etc.). Fat soluble vitamins can be stored in liver and adipose tissue. They are not readily excreted in urine. Excess consumption of these vitamins (particularly A and D) leads to their accumulation and toxic effects.

All the fat soluble vitamins are ***isoprenoid compounds***, since they are made up of one or more of five carbon units namely ***isoprene units*** ($-CH=C.CH_3-CH=CH-$). Fat soluble vitamins perform diverse functions. Vitamin K has a specific coenzyme function.

Water soluble vitamins—general

The water soluble vitamins are a heterogenous group of compounds since they differ chemically from each other. The only common character shared by them is their solubility in water. Most of these vitamins are readily excreted in urine and they are not toxic to the body. Water soluble vitamins are not stored in the body in large quantities (except B_{12}). For this reason, they must be continuously supplied in the diet. ***Generally, vitamin deficiencies are multiple rather than individual with overlapping symptoms.*** It is often difficult to pinpoint the exact biochemical basis for the symptoms.

The water soluble vitamins form coenzymes (***Refer Table 6.3***) that participate in a variety of biochemical reactions, related to either energy generation or hematopoiesis. It may be due to this reason that the deficiency of vitamins results in a number of overlapping symptoms. The common symptoms of the deficiency of one or more vitamins involved in energy metabolism include dermatitis, glossitis (red and swollen tongue), cheilitis (rupture at the corners of lips), diarrhea, mental confusion, depression and malaise.

Deficiency of vitamins B_1, B_6 and B_{12} is more closely associated with neurological manifestations.

Vitamers

The term ***vitamers*** represents the chemically similar substances that possess qualitatively similar vitamin activity. Some good examples of vitamers are given below

- Retinol, retinal and retinoic acid are vitamers of vitamin A.
- Pyridoxine, pyridoxal and pyridoxamine are vitamers of vitamin B_6.

INDIVIDUAL VITAMINS

In the following pages, the individual members of the fat soluble and water soluble vitamins are discussed with regard to the chemistry, biochemical functions, recommended dietary/daily allowances (RDA), dietary sources, deficiency manifestations etc.

VITAMIN A

The fat soluble vitamin A, as such is present only in foods of animal origin. However, its ***provitamins carotenes*** are found ***in plants.***

It is recorded in the history that Hippocrates (about 500 B.C.) cured night blindness. He prescribed to the patients ox liver (in honey), which is now known to contain high quantity of vitamin A.

Chemistry

In the recent years, the term vitamin A is collectively used to represent many structurally related and biologically active molecules (***Fig.7.1***). The term retinoids is often used to include the natural and synthetic forms of vitamin A. ***Retinol, retinal*** and ***retinoic acid*** are regarded as ***vitamers*** of vitamin A.

1. **Retinol (vitamin A alcohol) :** It is a primary alcohol containing ***β-ionone ring***. The side chain has ***two isoprenoid units***, four double bonds and one hydroxyl group. Retinol is present in animal tissues as retinyl ester with long chain fatty acids.

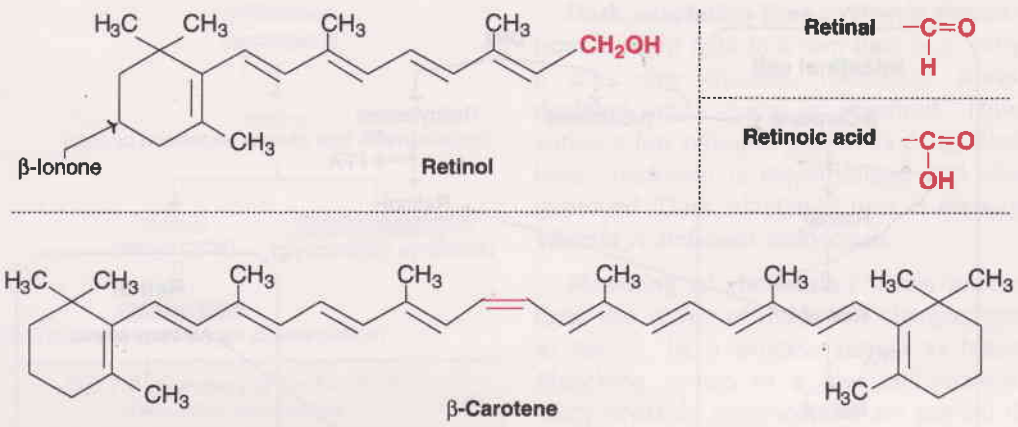


Fig. 7.1 : Structures of vitamin A and related compounds (Red colour represents the substituent groups in the respective compounds).

2. **Retinal (vitamin A aldehyde) :** This is an aldehyde form obtained by the oxidation of retinol. Retinal and retinol are interconvertible. Previously, the name retinine was used for retinal.

3. **Retinoic acid (vitamin A acid) :** This is produced by the oxidation of retinal. However, retinoic acid cannot give rise to the formation of retinal or retinol.

4. **β-Carotene (provitamin A) :** This is found in plant foods. It is cleaved in the intestine to produce two moles of retinal. In humans, this conversion is inefficient, hence β-carotene possesses about one-sixth vitamin A activity compared to that of retinol.

Absorption, transport and mobilization

Dietary retinyl esters are hydrolysed by pancreatic or intestinal brush border hydrolases in the intestine, releasing retinol and free fatty acids. Carotenes are hydrolysed by ***β-carotene 15-15′-dioxygenase*** of intestinal cells to release 2 moles of retinal which is reduced to retinol. In the intestinal mucosal cells, retinol is reesterified to long chain fatty acids, incorporated into chylomicrons and transferred to the lymph. The retinol esters of chylomicrons are taken up by the liver and stored (***Fig.7.2***).

As and when needed, vitamin A is released from the liver as free retinol. It is believed that zinc plays an important role in retinol mobilization. Retinol is transported in the circulation by the ***plasma retinol binding protein*** (***RBP***; mol. wt. 21,000) in association with pre-albumin. The retinol-RBP complex binds to specific receptors on the cell membrane of peripheral tissue and enters the cells. Many cells of target tissues contain a ***cellular retinol-binding protein*** that carries retinol to the nucleus and binds to the chromatin (DNA). It is here that retinol exerts its function in a manner analogous to that of a steroid hormone.

BIOCHEMICAL FUNCTIONS

Vitamin A is necessary for a variety of functions such as vision, proper growth and differentiation, reproduction and maintenance of epithelial cells. In recent years, each form of vitamin A has been assigned specific functions (***Fig.7.3***).

Vitamin A and vision : The biochemical function of vitamin A in the process of vision was first elucidated by George Wald (Nobel Prize 1968). The events occur in a cyclic process known as ***Rhodopsin cycle*** or ***Wald's visual cycle*** (***Fig.7.4***).

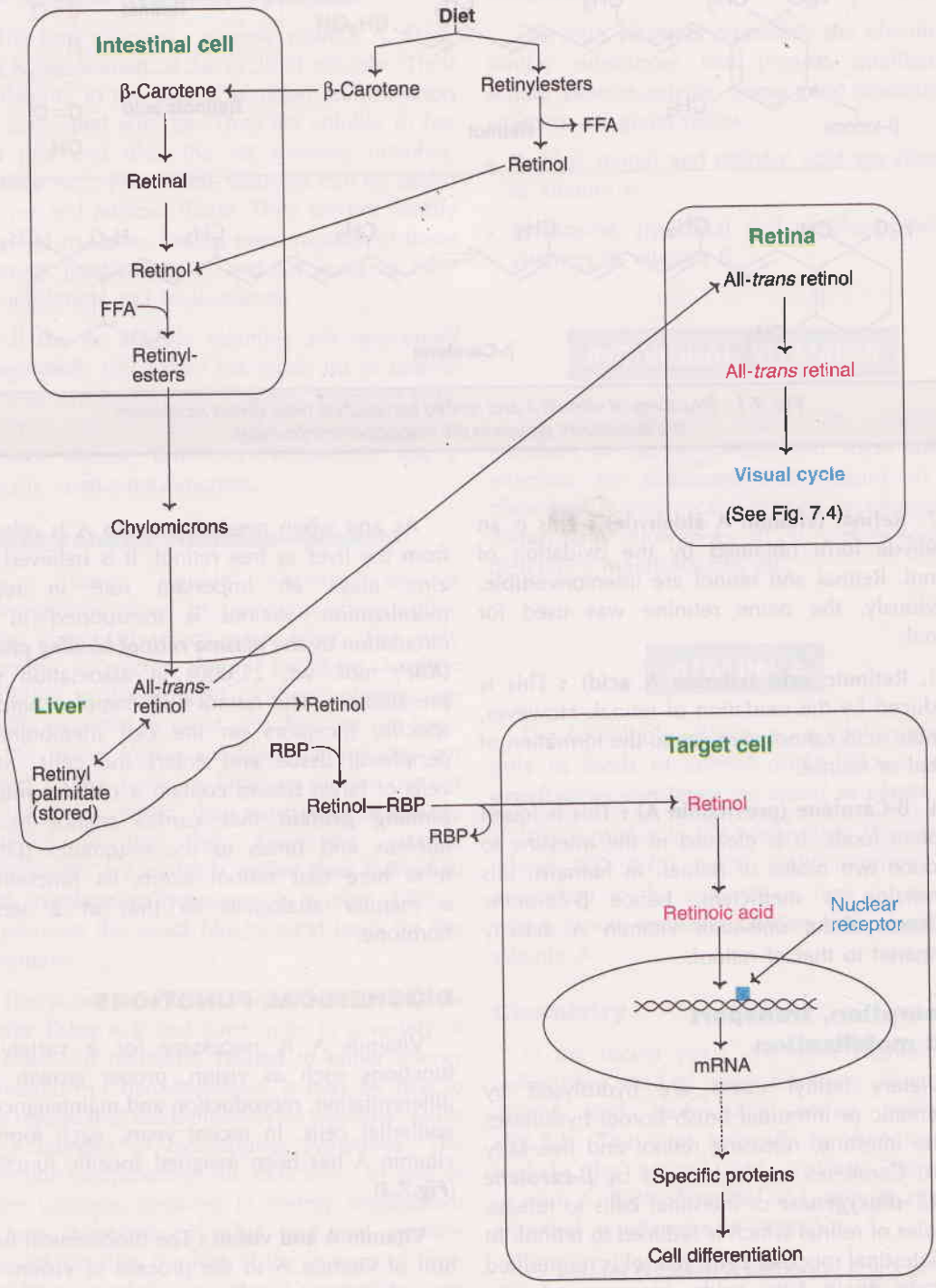


Fig. 7.2 : *Summary of vitamin A absorption, transport and biochemical functions (FFA-Free fatty acid; RBP-Retinol binding protein).*

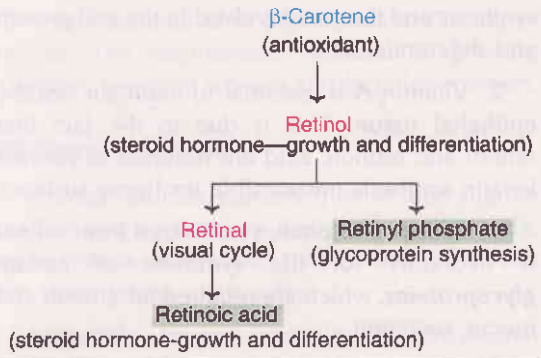


Fig. 7.3 : Summary of the functions of vitamin A compounds.

Rods and cones

The retina of the eye possesses two types of cells—rods and cones. The human eye has about 10 million rods and 5 million cones. The rods are in the periphery while cones are at the centre of retina. ***Rods*** are involved in ***dim light vision*** whereas cones are responsible for bright light and colour vision. Animals—such as owls and cats for which night vision is more important—possess mostly rods.

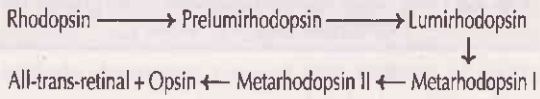
Wald's visual cycle

Rhodopsin (mol. wt. 35,000) is a conjugated protein present in rods. It contains 11-*cis* retinal and the protein opsin. The aldehyde group (of retinal) is linked to ε-amino group of lysine (of opsin).

The primary event in visual cycle, on exposure to light, is the isomerization of 11-*cis*-retinal to all-*trans* retinal. This leads to a conformational change in opsin which is responsible for the generation of nerve impulse. The all-*trans*-retinal is immediately isomerized by retinal isomerase (of retinal epithelium) to 11-*cis*-retinal. This combines with opsin to regenerate rhodopsin and complete the visual cycle (***Fig.7.4***). However, the conversion of all *trans*-retinal to 11-*cis* retinal is incomplete. Therefore, most of the all-*trans*-retinal is transported to the liver and converted to all-*trans* retinol by alcohol dehydrogenase. The all-*trans*-retinol undergoes isomerization to 11-*cis* retinol which is then oxidized to 11-*cis* retinal to participate in the visual cycle.

Dark adaptation time : When a person shifts from a bright light to a dim light (e.g. entry into a dim cine theatre), rhodopsin stores are depleted and vision is impaired. However, within a few minutes, known as dark adaptation time, rhodopsin is resynthesized and vision is improved. Dark adaptation time is ***increased in vitamin A deficient individuals.***

Bleaching of rhodopsin : When exposed to light, the colour of rhodopsin changes from red to yellow, by a process known as bleaching. Bleaching occurs in a few milliseconds and many unstable intermediates are formed during this process.



Visual cascade and cGMP : When light strikes the retina, a number of biochemical changes leading to membrane hyperpolarization occur resulting in the genesis of nerve impulse. The hyperpolarization of the membrane is brought about by a visual cascade involving cyclic GMP. When a photon (from light) is absorbed by rhodopsin, metarhodopsin II is produced. The protein ***transducin*** is activated by metarhodopsin II. This involves an exchange of GTP for GDP on inactive transducin. The activated transducin activates cyclic GMP phosphodiesterase. This

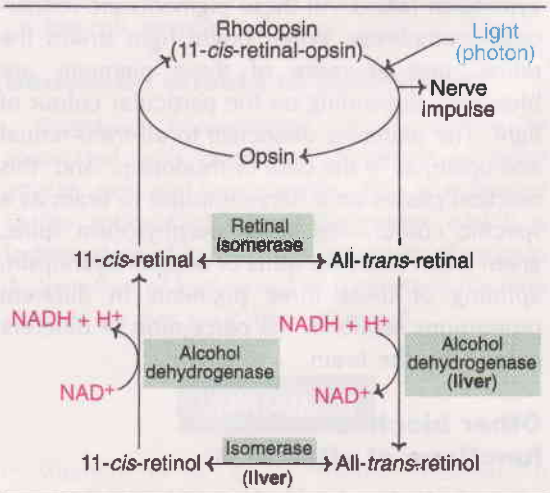


Fig. 7.4 : Wald's visual cycle.

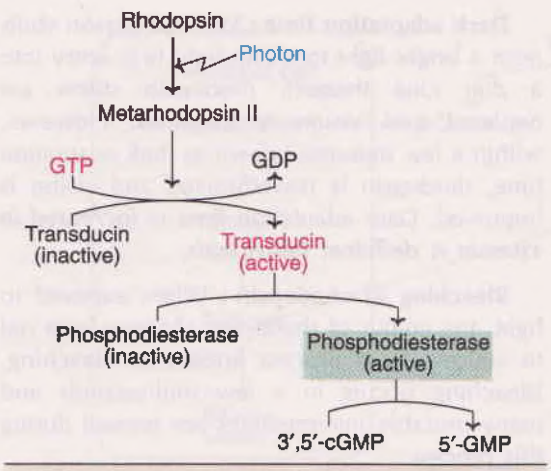


Fig. 7.5 : The visual cascade involving cyclic guanosine monophosphate (3',5'-cGMP).

enzyme degrades cyclic GMP in the rod cells (***Fig.7.5***). A rapid decrease in cyclic GMP closes the Na^+ channels in the membranes of the rod cells. This results in hyperpolarization which is an excitatory response transmitted through the neuron network to the visual cortex of the brain.

Colour vision

Cones are specialized in bright and colour vision. Visual cycle comparable to that present in rods is also seen in cones. The colour vision is governed by colour sensitive pigments–***porphyropsin (red), iodopsin (green) and cyanopsin (blue)***. All these pigments are retinal-opsin complexes. When bright light strikes the retina, one or more of these pigments are bleached, depending on the particular colour of light. The pigments dissociate to all-*trans*-retinal and opsin, as in the case of rhodopsin. And this reaction passes on a nerve impulse to brain as a specific colour—red when porphyropsin splits, green when iodopsin splits or blue for cyanopsin. Splitting of these three pigments in different proportions results in the perception of different colours by the brain.

Other biochemical functions of vitamin A

1. ***Retinol and retinoic acid*** function almost like ***steroid hormones***. They regulate the protein synthesis and thus are involved in the cell growth and differentiation.

2. Vitamin A is essential to maintain healthy epithelial tissue. This is due to the fact that retinol and retinoic acid are required to prevent keratin synthesis (responsible for horny surface).

3. Retinyl phosphate synthesized from retinol is necessary for the ***synthesis of*** certain ***glycoproteins***, which are required for growth and mucus secretion.

4. Retinol and retinoic acid are involved in the synthesis of transferrin, the iron transport protein.

5. Vitamin A is considered to be essential for the maintenance of proper immune system to fight against various infections.

6. Cholesterol synthesis requires vitamin A. Mevalonate, an intermediate in the cholesterol biosynthesis, is diverted for the synthesis of coenzyme Q in vitamin A deficiency. It is pertinent to note that the discovery of coenzyme Q was originally made in vitamin A deficient animals.

7. ***Carotenoids*** (most important β-carotene) function as ***antioxidants*** and reduce the risk of cancers initiated by free radicals and strong oxidants. β-Carotene is found to be beneficial to prevent heart attacks. This is also attributed to the antioxidant property.

Recommended dietary allowance (RDA)

The daily requirement of vitamin A is expressed as ***retinol equivalents (RE)*** rather than International Units (IU).

1 retinol equivalent = 1 µg retinol
= 6 µg β-carotene
= 12 µg other carotenoids
= 3.33 IU of vitamin A activity from retinol
= 10 IU of vitamin A activity from β-carotene

The RDA of vitamin A for adults is around ***1,000*** retinol equivalents (3,500 IU) for man and ***800*** retinol equivalents (2,500 IU) for woman.

One International Unit (IU) equals to 0.3 mg of retinol. The requirement increases in growing children, pregnant women and lactating mothers.

Dietary sources

Animal sources contain (preformed) vitamin A. The best sources are liver, kidney, egg yolk, milk, cheese, butter. Fish (cod or shark) liver oils are very rich in vitamin A.

Vegetable sources contain the provitamin A-carotenes. Yellow and dark green vegetables and fruits are good sources of carotenes e.g. carrots, spinach, amaranthus, pumpkins, mango, papaya etc.

Vitamin A deficiency

The deficiency symptoms of vitamin A are not immediate, since the hepatic stores can meet the body requirements for quite sometime (2-4 months). The deficiency manifestations are related to the eyes, skin and growth.

Deficiency manifestations of the eyes : *Night blindness* (nyctalopia) is one of the earliest symptoms of vitamin A deficiency. The individuals have difficulty to see in dim light since the dark adaptation time is increased. Prolonged deficiency irreversibly damages a number of visual cells.

Severe deficiency of vitamin A leads to ***xerophthalmia***. This is characterized by dryness in conjunctiva and cornea, and keratinization of epithelial cells. In certain areas of conjunctiva, white triangular plaques known as ***Bitot's spots*** are seen.

If xerophthalmia persisits for a long time, corneal ulceration and degeneration occur. This results in the destruction of cornea, a condition referred to as ***keratomalacia***, causing total blindness. Vitamin A deficiency blindness is mostly common in children of the developing countries.

Other deficiency manifestations

Effect on growth : Vitamin A deficiency results in growth retardation due to impairment in skeletal formation.

Effect on reproduction : The reproductive system is adversely affected in vitamin A deficiency. Degeneration of germinal epithelium leads to sterility in males.

Effect on skin and epithelial cells : The skin becomes rough and dry. Keratinization of epithelial cells of gastrointestinal tract, urinary tract and respiratory tract is noticed. This leads to increased bacterial infection. Vitamin A deficiency is associated with formation of urinary stones.

The plasma level of retinol binding protein is decreased in vitamin A deficiency.

Hypervitaminosis A

Excessive consumption of vitamin A leads to toxicity. The symptoms of hypervitaminosis A include dermatitis (drying and redness of skin), enlargement of liver, skeletal decalcification, tenderness of long bones, loss of weight, irritability, loss of hair, joint pains etc.

Total serum vitamin A level (normal 20–50 µg/dl) is elevated in hypervitaminosis A. Free retinol or retinol bound to plasma lipoproteins is actually harmful to the body. It is now believed that the vitamin A toxicosis symptoms appear only after retinol binding capacity of retinol binding protein exceeds.

Higher concentration of retinol increases the synthesis of lysosomal hydrolases. The manifestations of hypervitaminosis A are attributed to the destructive action of hydrolases, particularly on the cell membranes.

Beneficial effects of β-carotene

Increased consumption of β-carotene is associated with decreased incidence of heart attacks, skin and lung cancers. This is attributed to the antioxidant role of β-carotene which is independent of its role as a precursor of vitamin A. Ingestion of high doses of β-carotene for long periods are not toxic like vitamin A.

VITAMIN D

Vitamin D is a fat soluble vitamin. It resembles sterols in structure and functions like a hormone.

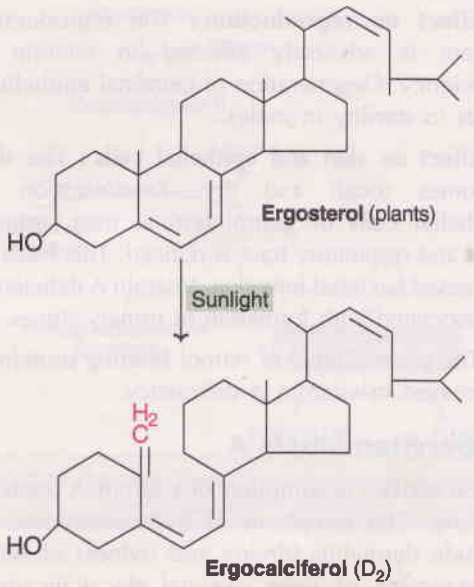


Fig. 7.6 : Formation of ergocalciferol from ergosterol.

The symptoms of rickets and the benefical effects of sunlight to prevent rickets have been known for centuries. Hess (1924) reported that irradiation with ultraviolet light induced anti-rachitic activity in some foods. Vitamin D was isolated by Angus (1931) who named it calciferol.

Chemistry

Ergocalciferol (vitamin D_2) is formed from ergosterol and is present in plants (***Fig.7.6***). Cholecalciferol (vitamin D_3) is found in animals. Both the sterols are similar in structure except that ergocalciferol has an additional methyl group and a double bond. ***Ergocalciferol*** and ***cholecalciferol*** are sources for vitamin D activity and are referred to as ***provitamins***.

During the course of cholesterol biosynthesis (***Chapter 14***), 7-dehydrocholesterol is formed as an intermediate. On exposure to sunlight, 7-dehydrocholesterol is converted to cholecalciferol in the skin (dermis and epidermis) (***Fig.2.7***) Vitamin D is regarded as ***sun-shine vitamin***.

The synthesis of vitamin D_3 in the skin is proportional to the exposure to sunlight. Dark skin pigment (melanin) adversly influences the synthesis of cholecalciferol.

Absorption, transport and storage

Vitamin D is absorbed in the small intestine for which bile is essential. Through lymph, vitamin D enters the circulation bound to plasma α_2-globulin and is distributed throughout the body. Liver and other tissues store small amounts of vitamin D.

METABOLISM AND BIOCHEMICAL FUNCTIONS

Vitamins D_2 and D_3, as such, are not biologically active. They are metabolized identically in the body and converted to active forms of vitamin D. The metabolism and biochemical functions of vitamin D are depicted in ***Fig.7.8***.

Synthesis of 1,25-DHCC

Cholecalciferol is first hydroxylated at 25th position to 25-hydroxycholecalciferol (25-OH D_3) by a specific hydroxylase present in liver. 25-OH D_3 is the major storage and circulatory form of vitamin D. Kidney possesses a specific enzyme, ***25-hydroxycholecalciferol (calciol) 1-hydroxylase*** which hydroxylates 25-hydroxy-cholecalciferol at position 1 to produce ***1,25-dihydroxycholecalciferol (1,25-DHCC)***. 1,25 DHCC contains 3 hydroxyl groups (1,3 and 25 carbon) hence referred to as ***calcitriol***. Both the hydroxylase enzymes (of liver and kidney) require cytochrome P_{450}, NADPH and molecular oxygen for the hydroxylation process. The synthesis of calcitriol is depicted in ***Figs.7.7*** and ***7.8***.

Regulation of the synthesis of 1,25-DHCC

The concentration of 1,25-DHCC is regulated by plasma levels of ***calcium*** and ***phosphate***. They control hydroxylation reaction at position 1. Low plasma phosphate increases the activity of 25-hydroxycholecalciferol 1-hydroxylase. Low plasma calcium enhances the production of ***parathyroid hormone*** which in turn activates 1-hydroxylase. Thus the action of phosphate is direct while that of calcium is indirect on kidney 1-hydroxylase.

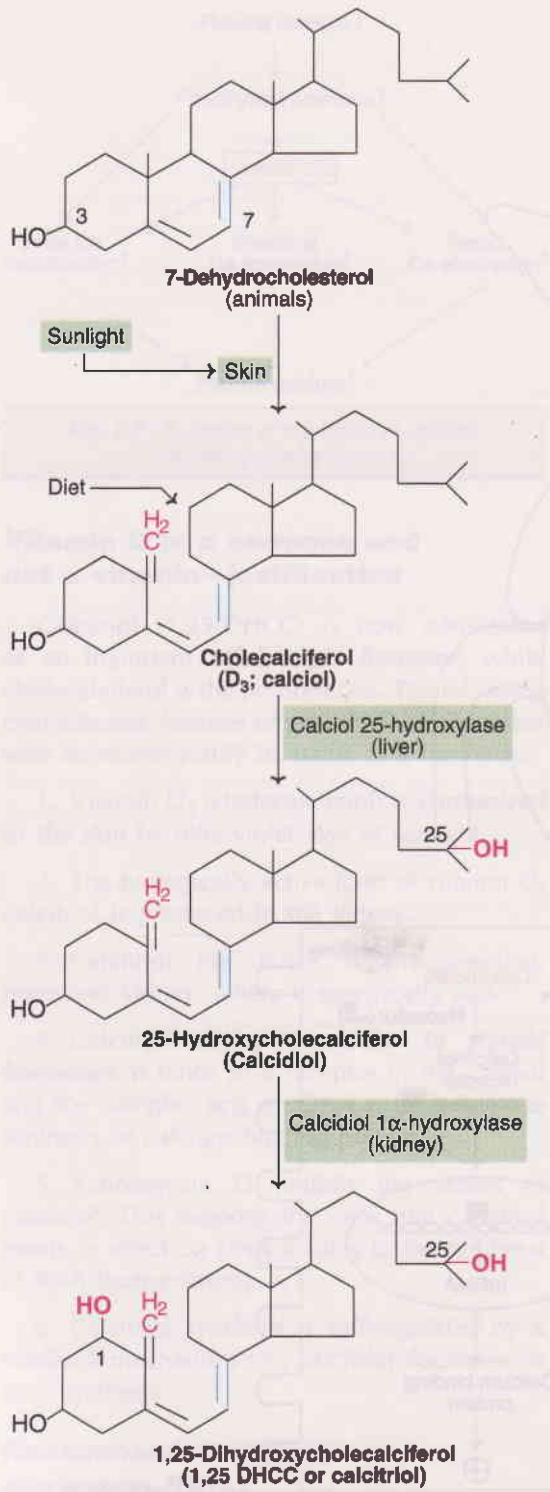


Fig. 7.7 : Biosynthesis of active form of vitamin D-calcitriol (1,25 DHCC).

Biochemical functions

Calcitriol (1,25-DHCC) is the biologically ***active*** form of vitamin D. It ***regulates the plasma levels of calcium and phosphate***. Calcitriol acts at 3 different levels (intestine, kidney and bone) to maintain plasma calcium (normal 9–11 mg/dl).

1. **Action of calcitriol on the intestine :** Calcitriol ***increases the intestinal absorption of calcium*** and phosphate. In the intestinal cells, calcitriol binds with a cytosolic receptor to form a calcitriol-receptor complex. This complex then approaches the nucleus and interacts with a specific DNA leading to the synthesis of a specific ***calcium binding protein***. This protein increases the calcium uptake by the intestine. The mechanism of action of calcitriol on the target tissue (intestine) is similar to the action of a steroid hormone.

2. **Action of calcitriol on the bone :** In the osteoblasts of bone, calcitriol stimulates calcium uptake for deposition as calcium phosphate. Thus calcitriol is essential for ***bone formation***. The bone is an important reservoir of calcium and phosphate. Calcitriol along with parathyroid hormone increases the mobilization of calcium and phosphate from the bone. This causes elevation in the plasma calcium and phosphate levels.

3. **Action of calcitriol on the kidney :** Calcitriol is also involved in minimizing the excretion of calcium and phosphate through the kidney, by decreasing their excretion and enhancing reabsorption.

The sequence of events that take place in response to low plasma calcium concentration and the action of calcitriol on intestine, kidney and bone, ultimately leading to the ***increase in plasma calcium*** is given in ***Fig.7.9***.

24,25-Dihydroxycholecalciferol (24,25-DHCC) is another metabolite of vitamin D. It is also synthesized in the kidney by 24-hydroxylase. The exact function of 24,25-DHCC is not known. It is believed that when calcitriol concentration is adequate, 24-hydroxylase acts leading to the synthesis of a less important compound 24, 25-DHCC. In this way, to maintain the homeostasis of calcium, synthesis of 24,25-DHCC is also important.

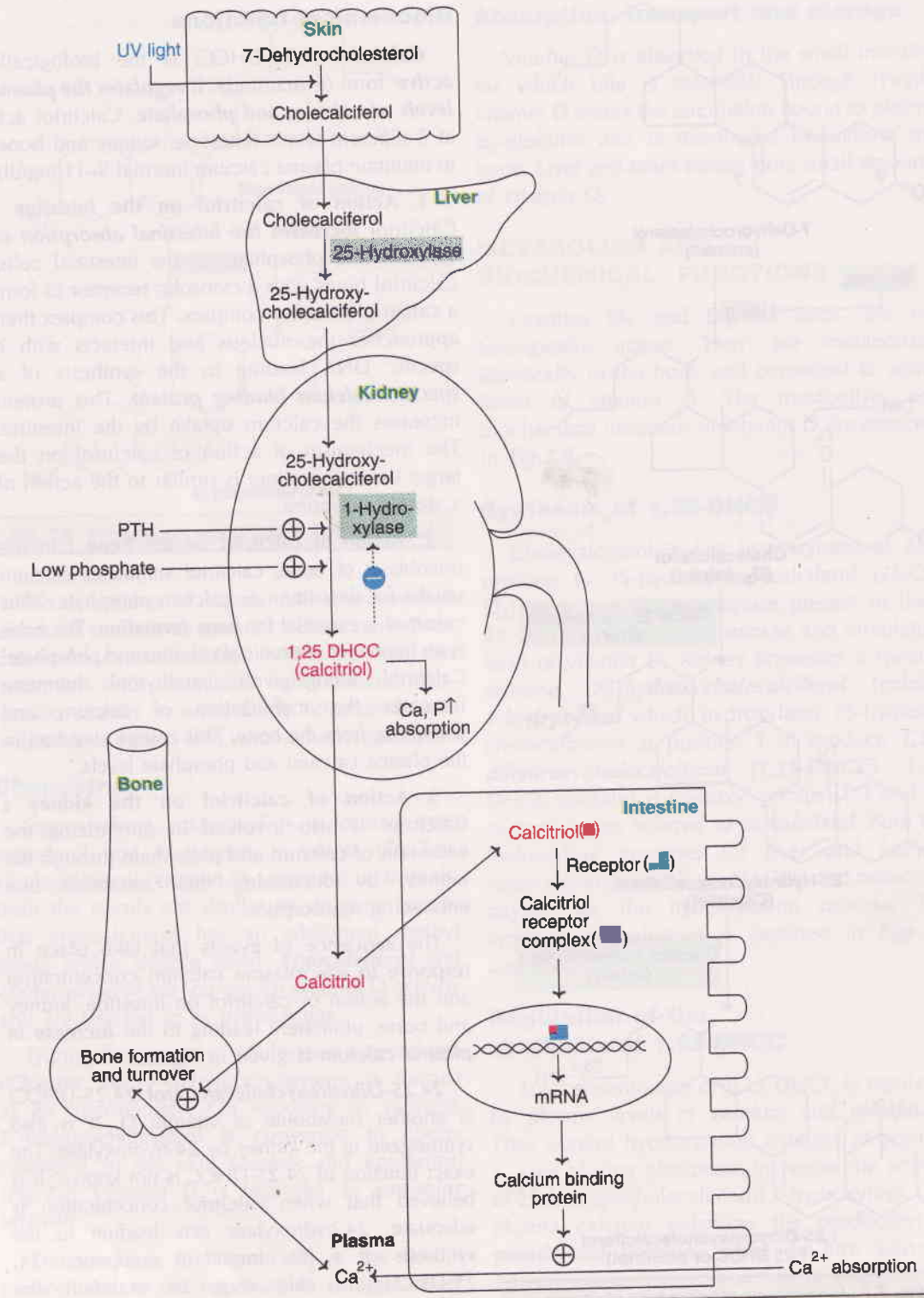


Fig. 7.8 : Metabolism and biochemical functions of vitamin D (1, 25 DHCC-1, 25-Dihydroxycholecalciferol, also called as calcitriol is the active form of vitamin D; PTH-Parathyroid hormone).

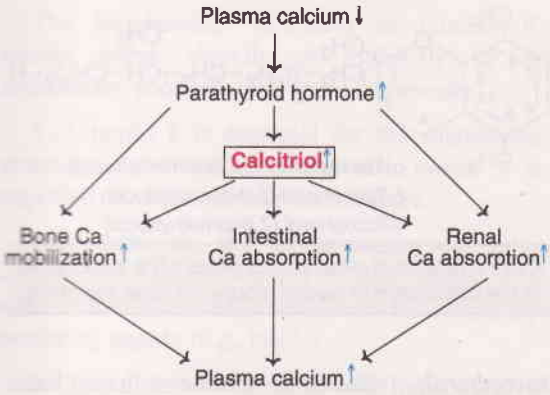


Fig. 7.9 : Summary of the action of calcitriol in elevating plasma calcium.

Vitamin D is a hormone and not a vitamin—justification

Calcitriol (1,25-DHCC) is now considered as an important ***calciotropic hormone***, while cholecalciferol is the prohormone. The following characteristic features of vitamin D (comparable with hormone) justify its status as a hormone.

1. Vitamin D_3 (cholecalciferol) is ***synthesized in the skin*** by ultra-violet rays of sunlight.

2. The biologically active form of vitamin D, calcitriol is produced in the kidney.

3. Calcitriol has target organs—intestine, bone and kidney, where it specifically acts.

4. Calcitriol ***action*** is ***similar to steroid hormones***. It binds to a receptor in the cytosol and the complex acts on DNA to stimulate the synthesis of calcium binding protein.

5. Actinomycin D inhibits the action of calcitriol. This supports the view that calcitriol exerts its effect on DNA leading to the synthesis of RNA (transcription).

6. Calcitriol ***synthesis*** is ***self-regulated*** by a feedback mechanism i.e., calcitriol decreases its own synthesis.

Recommended dietary allowance (RDA)

The daily requirement of vitamin D is ***400 International Units*** or 10 mg of cholecalciferol. In countries with good sunlight (like India), the RDA for vitamin D is 200 IU (or 5 mg cholecalciferol).

Dietary sources

Good sources of vitamin D include fatty fish, fish liver oils, egg yolk etc. Milk is not a good source of vitamin D.

Vitamin D can be provided to the body in three ways

1. Exposure of skin to sunlight for synthesis of vitamin D;

2. Consumption of natural foods;

3. By irradiating foods (like yeast) that contain precursors of vitamin D and fortification of foods (milk, butter etc.).

Deficiency symptoms

Vitamin D deficiency is relatively less common, since this vitamin can be synthesized in the body. However, ***insufficient exposure to sunlight*** and consumption of diet lacking vitamin D ***results*** in its ***deficiency***.

Vitamin D deficiency occurs in strict vegetarians, chronic alcoholics, individuals with liver and kidney diseases or fat malabsorption syndromes. In some people, who cover the entire body (purdah) for religious customs, vitamin D deficiency is also observed, if the requirement is not met through diet.

Deficiency of vitamin D causes ***rickets in children and osteomalacia in adults***. Rickets is derived from an old *English* word 'wrickken', meaning to twist. Osteomalacia is derived from Greek (osteon-bone; malakia-softness). Vitamin D is often called as antirachitic vitamin.

Rickets in children is characterized by bone deformities due to incomplete mineralization, resulting in soft and pliable bones and delay in teeth formation. The weight-bearing bones are bent to form ***bow-legs***. In rickets, the plasma level of calcitriol is decreased and ***alkaline phosphatase activity is elevated***. Alkaline phosphatase is concerned with the process of bone formation. There is an overproduction of

alkaline phosphatase related to more cellular activity of the bone. It is believed to be due to a vain attempt to result in bone formation.

In case of osteomalacia (adult rickets) demineralization of the bones occurs (bones become softer), increasing their susceptibility to fractures.

Renal rickets (renal osteodystrophy)

This is seen in patients with chronic renal failure. Renal rickets is mainly due to decreased synthesis of calcitriol in kidney. It can be treated by administration of calcitriol.

Hypervitaminosis D

Vitamin D is stored mostly in liver and slowly metabolised. Among the vitamins, vitamin D is the ***most toxic in overdoses*** (10-100 times RDA). Toxic effects of hypervitaminosis D include demineralization of bone (resorption) and increased calcium absorption from the intestine, leading to elevated calcium in plasma (hypercalcemia). Prolonged hypercalcemia is associated with deposition of calcium in many soft tissues such as kidney and arteries. Hypervitaminosis D may lead to formation of stones in kidneys (renal calculi). High consumption of vitamin D is associated with loss of appetite, nausea, increased thirst, loss of weight etc.

VITAMIN E

Vitamin E (tocopherol) is a naturally occurring antioxidant. It is essential for normal reproduction in many animals, hence known as ***anti-sterility vitamin***. Vitamin E is described as a *'vitamin in search of a disease.'* This is due to the lack of any specific vitamin E deficiency disease in humans.

Evans and his associates (1936) isolated the compounds of vitamin E activity and named them as tocopherols (*Greek* : tokos-child birth; pheros-to bear; ol-alcohol).

Chemistry

Vitamin E is the name given to a group of tocopherols and tocotrienols. About eight

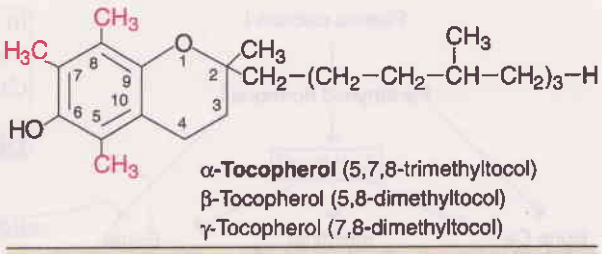


*Fig. 7.10 : Structure of α-tocopherol (**Note** : The tocopherols differ in the substitution of methyl groups, represented in red).*

tocopherols (vitamin E vitamers) have been identified–*α, β, γ, δ* etc. Among these, ***α-tocopherol*** is the most active. The tocopherols are derivatives of ***6-hydroxy chromane (tocol) ring*** with isoprenoid (3 units) side chain. The antioxidant property is due to the chromane ring.

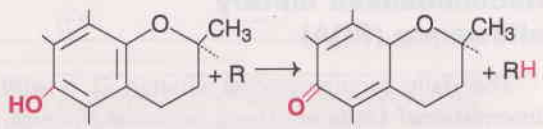
Absorption, transport and storage

Vitamin E is absorbed along with fat in the small intestine. Bile salts are necessary for the absorption. In the liver, it is incorporated into lipoproteins (VLDL and LDL) and transported. Vitamin E is stored in adipose tissue, liver and muscle. The normal plasma level of tocopherol is less than 1 mg/dl.

Biochemical functions

Most of the functions of vitamin E are related to its ***antioxidant*** property. It prevents the non-enzymatic oxidations of various cell components (e.g. unsaturated fatty acids) by molecular oxygen and free radicals such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2). The element ***selenium helps*** in these functions.

Vitamin E is lipophilic in character and is found in association with lipoproteins, fat deposits and cellular membranes. ***It protects the polyunsaturated fatty acids (PUFA) from peroxidation reactions.*** Vitamin E acts as a scavenger and gets itself oxidized (to quinone form) by free radicals (R) and spares PUFA, as shown below



The biochemical functions of vitamin E, related either directly or indirectly to its antioxidant property, are given hereunder

1. Vitamin E is essential for the membrane structure and integrity of the cell, hence it is regarded as a ***membrane antioxidant.***

2. It prevents the peroxidation of poly-unsaturated fatty acids in various tissues and membranes. It protects RBC from hemolysis by oxidizing agents (e.g. H_2O_2).

3. It is closely associated with reproductive functions and prevents sterility. Vitamin E preserves and maintains germinal epithelium of gonads for proper reproductive function.

4. It increases the synthesis of heme by enhancing the activity of enzymes δ-aminolevulinic acid (ALA) synthase and ALA dehydratase.

5. It is required for cellular respiration– through electron transport chain (believed to stabilize coenzyme Q).

6. Vitamin E prevents the oxidation of vitamin A and carotenes.

7. It is required for proper storage of creatine in skeletal muscle.

8. Vitamin E is needed for optimal absorption of amino acids from the intestine.

9. It is involved in proper synthesis of nucleic acids.

10. Vitamin E protects liver from being damaged by toxic compounds such as carbon tetrachloride.

11. It works in association with vitamins A, C and β-carotene, to delay the onset of cataract.

12. Vitamin E has been recommended for the prevention of chronic diseases such as cancer and heart diseases. Clinical trials in this regard are rather disappointing, hence it is no more recommended. However, some clinicians continue to use it particularly in subjects susceptible to heart attacks. It is believed that vitamin E prevents the oxidation of LDL. (**Note :** The oxidized LDL have been implicated to promote heart diseases.)

Vitamin E and selenium

The element selenium is found in the enzyme ***glutathione peroxidase*** that destroys free radicals. Thus, Se is also involved in antioxidant functions like vitamin E, and both of them act synergistically. To a certain extent, Se can spare the requirement vitamin E, and vice versa.

Recommended dietary allowance (RDA)

Intake of vitamin E is directly related to the consumption of polyunsaturated fatty acids (PUFA) i.e., requirement increases with increased intake of PUFA. A daily consumption of about ***10 mg*** (15 IU) of α-tocopherol for man and ***8 mg*** (12 IU) for woman is recommended. One mg of α-tocopherol is equal to 1.5 IU. Vitamin E supplemented diet is advised for pregnant and lactating women.

Dietary sources

Many vegetable oils are rich sources of vitamin E. Wheat germ oil, cotton seed oil, peanut oil, corn oil and sunflower oil are the good sources of this vitamin. It is also present in meat, milk, butter and eggs.

Deficiency symptoms

The symptoms of vitamin E deficiency vary from one animal species to another. In many animals, the deficiency is associated with sterility, degenerative changes in muscle, megaloblastic anaemia and changes in central nervous system. Severe symptoms of vitamin E deficiency are not seen in humans except increased fragility of erythrocytes and minor neurological symptoms.

Toxicity of vitamin E

Among the fat soluble vitamins (A, D, E, K), vitamin E is the ***least toxic***. No toxic effect has been reported even after ingestion of 300 mg/day for 23 years.

VITAMIN K

Vitamin K is the only fat soluble vitamin with a specific coenzyme function. It is required for

the production of blood clotting factors, essential for coagulation (in *German–K*oagulation; hence the name K for this vitamin).

Chemistry

Vitamin K exists in different forms (***Fig.7.11***). Vitamin K_1 (phylloquinone) is present in plants. Vitamin K_2 (menaquinone) is produced by the intestinal bacteria and also found in animals. Vitamin K_3 (menadione) is a synthetic form.

All the three vitamins (K_1, K_2, K_3) are ***naphthoquinone derivatives.*** Isoprenoid side chain is present in vitamins K_1 and K_2. The three vitamins are stable to heat. Their activity is, however, lost by oxidizing agents, irradiation, strong acids and alkalies.

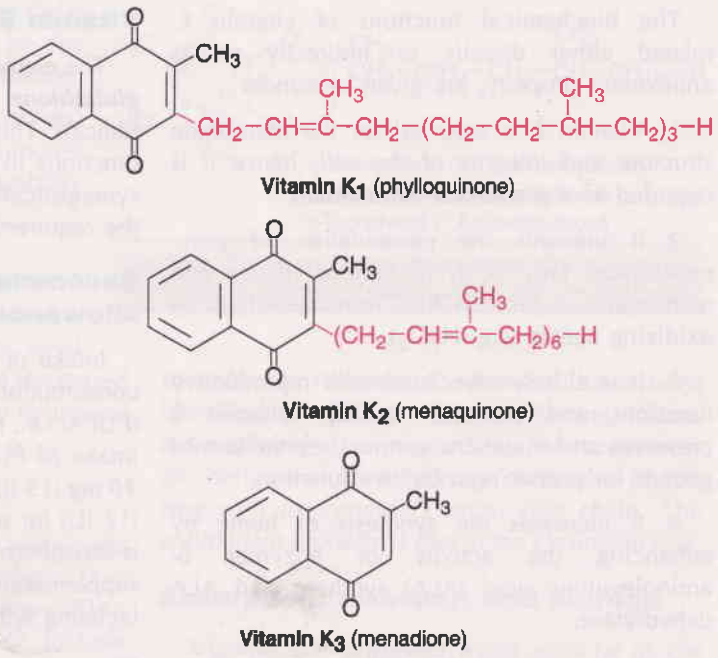


Fig. 7.11 : Structures of vitamin K.

Absorption, transport and storage

Vitamin K is taken in the diet or synthesized by the intestinal bacteria. Its absorption takes place along with fat (chylomicrons) and is dependent on bile salts. Vitamin K is transported along with LDL and is stored mainly in liver and, to a lesser extent, in other tissues.

Biochemical functions

The functions of vitamin K are concerned with blood clotting process. It brings about the ***post-translational*** (after protein biosynthesis in the cell) ***modification of certain blood clotting factors***. The clotting factors ***II*** (prothrombin), ***VII***, ***IX*** and ***X*** are synthesized as inactive precursors (zymogens) in the liver. Vitamin K acts as a coenzyme for the ***carboxylation of glutamic acid*** residues present in the proteins and this reaction is catalysed by a carboxylase (microsomal). It involves the conversion of glutamate (Glu) to γ-carboxyglutamate (Gla) and requires vitamin K, O_2 and CO_2 (***Fig.7.12***). The formation of ***γ-carboxyglutamate*** is inhibited by ***dicumarol***, an anticoagulant found in spoilt sweet clover. ***Warfarin*** is a synthetic analogue that can inhibit vitamin K action (***Fig.7.13***).

Vitamin K is also required for the carboxylation of glutamic acid residues of ***osteocalcin***, a calcium binding protein present in the bone.

The mechanism of carboxylation is not fully understood. It is known that a 2,3-epoxide derivative of vitamin K is formed as an intermediate during the course of the reaction. Dicumarol inhibits the enzyme (reductase) that converts epoxide to active vitamin K.

Role of Gla in clotting : The γ-carboxy-glutamic acid (Gla) residues of clotting factors are negatively charged (COO^-) and they combine with positively charged calcium ions (Ca^{2+}) to form a complex. The mechanism of action has been studied for prothrombin. The prothrombin —Ca complex binds to the phospholipids on the membrane surface of the platelets (***Fig.7.14***). This leads to the increased conversion of prothrombin to thrombin.

Recommended dietary allowance (RDA)

Strictly speaking, there is no RDA for vitamin K, since it can be adequately synthesized in the

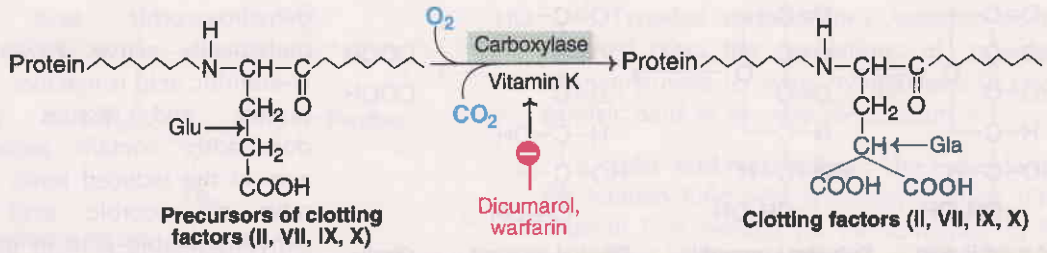


Fig. 7.12 : Vitamin K dependent carboxylation of the precursors of clotting factors.

gut. It is however, recommended that half of the body requirement is provided in the diet, while the other half is met from the bacterial synthesis. Accordingly, the suggested RDA for an adult is ***70-140 µg/day***.

Dietary sources

Cabbage, cauliflower, tomatoes, alfa alfa, spinach and other green vegetables are good sources. It is also present in egg yolk, meat, liver, cheese and dairy products.

Deficiency symptoms

The deficiency of vitamin K is uncommon, since it is present in the diet in sufficient quantity and/or is adequately synthesized by the intestinal bacteria. However, vitamin K deficiency may occur due to its faulty absorption (lack of bile salts), loss of vitamin into feces (diarrheal diseases) and administration of antibiotics (killing of intestinal flora).

Deficiency of vitamin K leads to the lack of active prothrombin in the circulation. The result is that blood coagulation is adversely affected. The individual bleeds profusely even for minor injuries. ***The blood clotting time is increased.***

Hypervitaminosis K

Administration of large doses of vitamin K produces hemolytic anaemia and jaundice, particularly in infants. The toxic effect is due to increased breakdown of RBC.

Antagonists of vitamin K

The compounds---namely heparin, bishydroxy-coumarin—act as anticoagulants and are antagonists to vitamin K. The salicylates and dicumarol are also antagonists to vitamin K.

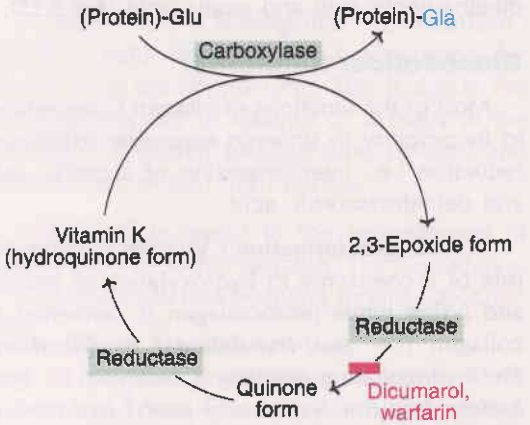


Fig. 7.13 : Summary of vitamin K cycle in carboxylation reaction.

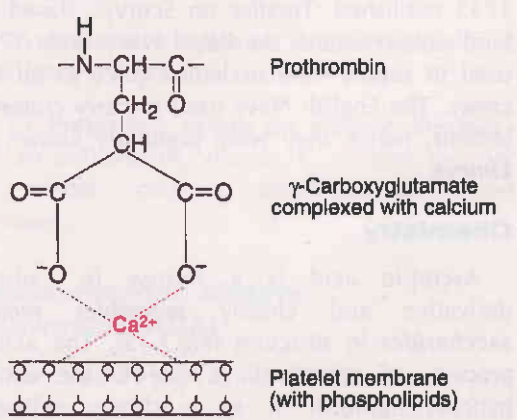


Fig. 7.14 : Mechanism of action of γ-carboxyglutamate of prothrombin in blood clotting.

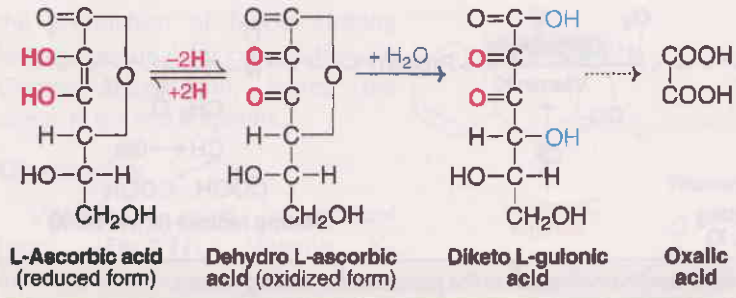


Fig. 7.15 : Structures of vitamin C (ascorbic acid) and its related compounds.

Dicumarol is structurally related to vitamin K and acts as a competitive inhibitor in the synthesis of active prothrombin.

VITAMIN C (ASCORBIC ACID)

Vitamin C is a water soluble versatile vitamin. It plays an important role in human health and disease. Vitamin C has become the most controversial vitamin in recent years. This is because of the claims and counter-claims on the use of vitamin C in megadoses to cure everything from common cold to cancer.

Scurvy has been known to man for centuries. It was the first disease found to be associated with diet. In the sixteenth century about 10,000 mariners died of a miraculous disease (scurvy) due to lack of fresh vegetables in their diet. James Lind, a surgeon of the English Navy, in 1753 published 'Treatise on Scurvy'. Based on Lind's observations, the Royal Navy since 1795 used to supply lime or lemon juice to all the crews. The English Navy used to carry crates of lemons, hence they were popularly known as ***Limeys***.

Chemistry

Ascorbic acid is a hexose (6 carbon) derivative and closely ***resembles monosaccharides*** in structure (***Fig.7.15***). The acidic property of vitamin C is due to the enolic hydroxyl groups. It is a strong reducing agent. L-Ascorbic acid undergoes oxidation to form dehydroascorbic acid and this reaction is reversible. Both ascorbic acid and dehydroascorbic acid are biologically active. However, D-ascorbic acid is inactive. The plasma and tissues predominantly contain ascorbic acid in the reduced form. The ratio of ascorbic acid to dehydroascorbic acid in many tissues is 15 : 1. On hydration, dehydroascorbic acid is irreversibly converted to 2,3-diketogulonic acid which is inactive. Hydration reaction is almost spontaneous, in alkaline or neutral solution. It is for this reason that oxidation of vitamin C is regarded as biological inactivation (formation of diketogulonic acid). Oxidation of ascorbic acid is rapid in the presence of copper. Hence vitamin C becomes inactive if the foods are prepared in copper vessels.

Biosynthesis and metabolism

Many animals can synthesize ascorbic acid from glucose via uronic acid pathway (***Chapter 13***). However, ***man,*** other primates, guinea pigs and bats cannot synthesize ascorbic acid due to the ***deficiency of*** a single enzyme namely ***L-gulonolactone oxidase***.

Vitamin C is rapidly absorbed from the intestine. It is not stored in the body to a significant extent. Ascorbic acid is excreted in urine as such, or as its metabolites—diketogulonic acid and oxalic acid (***Fig.7.15***).

Biochemical functions

Most of the functions of vitamin C are related to its property to undergo reversible oxidation–reduction i.e., interconversion of ascorbic acid and dehydroascorbic acid.

1. **Collagen formation :** Vitamin C plays the role of a coenzyme in hydroxylation of proline and lysine while protocollagen is converted to collagen (i.e. ***post-translational modification***). The hydroxylation reaction is catalysed by ***lysyl hydroxylase*** (for lysine) and ***prolyl hydroxylase*** (for proline). This reaction is dependent on vitamin C, molecular oxygen and α-ketoglutarate (***Fig.7.16***).

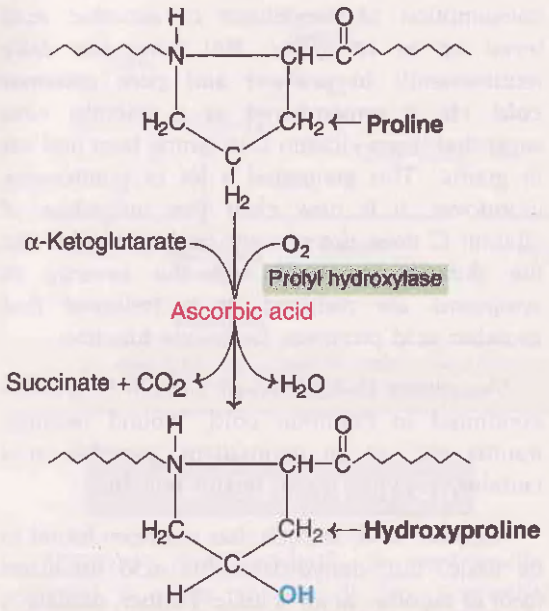


Fig. 7.16 : Ascorbic acid dependent hydroxylation of proline of protocollagen.

Hydroxyproline and hydroxylysine are essential for the collagen cross-linking and the strength of the fiber. In this way, vitamin C is necessary for maintenance of normal connective tissue and wound healing process.

2. **Bone formation :** Bone tissues possess an organic matrix, collagen and the inorganic calcium, phosphate etc. Vitamin C is required for bone formation.

3. **Iron and hemoglobin metabolism :** Ascorbic acid enhances iron absorption by keeping it in the ferrous form. This is due to the reducing property of vitamin C. It helps in the formation of ferritin (storage form of iron) and mobilization of iron from ferritin.

Vitamin C is useful in the reconversion of methemoglobin to hemoglobin. The degradation of hemoglobin to bile pigments requires ascorbic acid.

4. **Tryptophan metabolism :** Vitamin C is essential for the hydroxylation of tryptophan (enzyme-hydroxylase) to hydroxytryptophan in the synthesis of serotonin.

5. **Tyrosine metabolism :** Ascorbic acid is required for the oxidation of p-hydroxy phenylpyruvate (enzyme hydroxylase) to homogentisic acid in tyrosine metabolism.

6. **Folic acid metabolism :** The active form of the vitamin folic acid is tetrahydrofolate (FH_4). Vitamin C is needed for the formation of FH_4 (enzyme-folic acid reductase). Further, in association with FH_4, ascorbic acid is involved in the maturation of erythrocytes.

7. **Peptide hormone synthesis :** Many peptide hormones contain carboxyl terminal amide which is derived from terminal glycine. Hydroxylation of glycine is carried out by peptidylglycine hydroxylase which requires vitamin C.

8. **Synthesis of corticosteroid hormones :** Adrenal gland possesses high levels of ascorbic acid, particularly in periods of stress. It is believed that vitamin C is necessary for the hydroxylation reactions in the synthesis of corticosteroid hormones.

9. **Sparing action of other vitamins :** Ascorbic acid is a strong antioxidant. It spares vitamin A, vitamin E, and some B-complex vitamins from oxidation.

10. **Immunological function :** Vitamin C enhances the synthesis of immunoglobulins (antibodies) and increases the phagocytic action of leucocytes.

11. **Preventive action on cataract :** Vitamin C reduces the risk of cataract formation.

12. **Preventive action on chronic diseases :** As an antioxidant, vitamin C reduces the risk of cancer, cataract, and coronary heart diseases.

Recommended dietary allowance (RDA)

About ***60-70 mg*** vitamin C intake per day will meet the adult requirement. Additional intakes (20-40% increase) are recommended for women during pregnancy and lactation.

Dietary sources

Citrus fruits, gooseberry (amla), guava, green vegetables (cabbage, spinach), tomatoes, potatoes (particularly skin) are rich in ascorbic acid. High content of vitamin C is found in adrenal gland and gonads. Milk is a poor source of ascorbic acid.

Deficiency symptoms

The deficiency of ascorbic acid results in ***scurvy***. This disease is characterized by spongy and ***sore gums***, ***loose teeth***, anemia, swollen joints, fragile blood vessels, decreased immunocompetence, delayed wound healing, sluggish hormonal function of adrenal cortex and gonads, haemorrhage, osteoporosis etc. Most of these symptoms are related to impairment in the synthesis of collagen and/or the antioxidant property of vitamin C.

Megadoses of vitamin C and its controversy

Linus Pauling (1970) first advocated the consumption of megadoses of ascorbic acid (even up to 18 g/day, 300 times the daily requirement!) ***to prevent and cure common cold***. He is remembered as a scientist who suggested 'keep vitamin C in gunny bags and eat in grams.' This generated a lot of controversy worldover. It is now clear that megadose of vitamin C does not prevent common cold. But the duration of cold and the severity of symptoms are reduced. It is believed that ascorbic acid promotes leukocyte function.

Megadoses (1-4 g/day) of vitamin C are still continued in common cold, wound healing, trauma etc. As an antioxidant, ascorbic acid certainly provides some health benefits.

Ascorbic acid, as such, has not been found to be toxic. But, dehydroascorbic acid (oxidized form of ascorbic acid) is toxic. Further, oxalate is a major metabolite of vitamin C. Oxalate has been implicated in the formation of kidney stones. However, there are controversial reports on the megadoses of vitamin C leading to urinary stones.

BIOMEDICAL / CLINICAL CONCEPTS

- *It is believed that during the course of evolution, the ability to synthesize vitamins was lost by the higher organisms, hence they should be supplied through the diet.*
- *For humans, the normal intestinal bacterial synthesis of vitamin K and biotin is almost sufficient to meet the body requirements.*
- *Administration of antibiotics often destroys the vitamin synthesizing bacteria in the gut, hence additional supplementation of vitamins is recommended during antibiotic therapy.*
- *Vitamin A deficiency causes night blindness; vitamin D deficiency rickets (in children) or osteomalacia (in adults); vitamin E deficiency minor neurological symptoms; vitamin K deficiency bleeding.*
- *Fat soluble vitamins are not readily excreted in urine, hence excess consumption leads to their accumulation and toxic effects.*
- *Vitamin C deficiency causes scurvy. The manifestations of scurvy are related to the impairment in the synthesis of collagen and/or the antioxidant property of vitamin C.*
- *Megadoses of vitamin C are used in common cold, wound healing, trauma etc.*
- *β-Carotene, vitamin E and ascorbic acid serve as antioxidants and reduce the risk of heart attacks and cancers.*

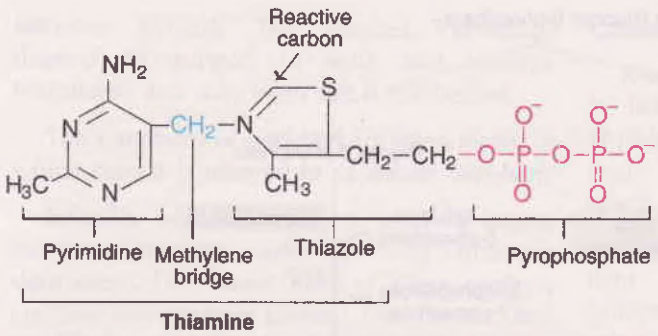


Fig. 7.17 : Structures of thiamine and thiamine pyrophosphate (TPP).

THIAMINE (VITAMIN B_1)

Thiamine (anti-beri-beri or antineuritic vitamin) is water soluble. It has a specific coenzyme, ***thiamine pyrophosphate (TPP)*** which is mostly associated with carbohydrate metabolism.

Chemistry

Thiamine contains a pyrimidine ring and a thiazole ring held by a methylene bridge (***Fig.7.17***). Thiamine is the only natural compound with thiazole ring.

The alcohol (OH) group of thiamine is esterfied with phosphate (2 moles) to form the ***coenzyme, thiamine pyrophosphate*** (TPP or ***cocarboxylase***). The pyrophosphate moiety is donated by ATP and the reaction is catalysed by the enzyme thiamine pyrophosphate transferase.

Biochemical functions

The coenzyme, thiamine pyrophosphate or cocarboxylase is intimately connected with the energy releasing reactions in the carbohydrate metabolism (***Fig.7.18***).

1. The enzyme ***pyruvate dehydrogenase*** catalyses (oxidative decarboxylation) the irreversible conversion of pyruvate to acetyl CoA. This reaction is dependent on TPP, besides the other coenzymes (details given in carbohydrate metabolism, ***Chapter 13***).

2. **α-Ketoglutarate dehydrogenase** is an enzyme of the citric acid cycle. This enzyme is comparable with pyruvate dehydrogenase and requires TPP.

3. **Transketolase** is dependent on TPP. This is an enzyme of the hexose monophosphate shunt (HMP shunt).

4. **The branched chain α-keto acid dehydrogenase (decarboxylase)** catalyses the oxidative decarboxylation of branched chain amino acids (valine, leucine and isoleucine) to the respective keto acids. This enzyme also requires TPP.

5. TPP plays an important role in the ***transmission of nerve impulse***. It is believed that TPP is required for acetylcholine synthesis and the ion translocation of neural tissue.

Recommended dietary allowance (RDA)

The daily requirement of thiamine depends on the intake of carbohydrate. A dietary supply of ***1-1.5 mg/day*** is recommended for adults (about 0.5 mg/1,000 Cals of energy). For children RDA is 0.7-1.2 mg/day. The requirement marginally increases in pregnancy and lactation (2 mg/day), old age and alcoholism.

Dietary sources

Cereals, pulses, oil seeds, nuts and yeast are good sources. Thiamine is mostly concentrated in the outer layer (bran) of cereals. Polishing of rice removes about 80% of thiamine. Vitamin B_1 is also present in animal foods like pork, liver, heart, kidney, milk etc. In the parboiled (boiling of paddy with husk) and milled rice, thiamine is not lost in polishing. Since thiamine is a water soluble vitamin, it is extracted into the water during cooking process. Such water should not be discarded.

Deficiency symptoms

The deficiency of vitamin B_1 results in a condition called ***beri-beri*** [*Sinhalese* : I cannot

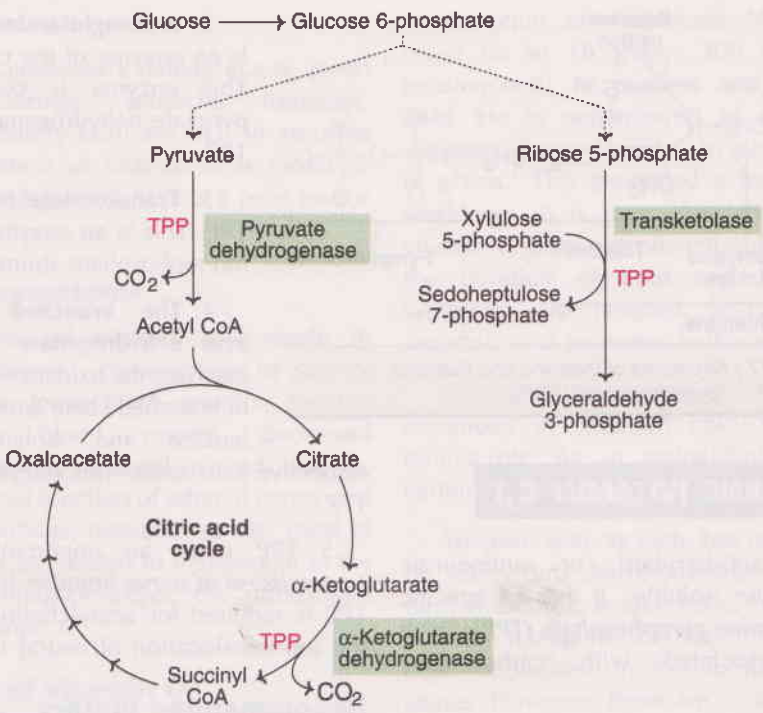


Fig. 7.18 : Summary of the reactions dependent on thiamine pyrophosphate (TPP).

(said twice)]. Beri-beri is mostly seen in populations consuming exclusively polished rice as staple food. The early symptoms of thiamine deficiency are loss of appetite (anorexia), weakness, constipation, nausea, mental depression, ***peripheral neuropathy***, irritability etc. Numbness in the legs complaints of 'pins and needles sensations' are reported.

Biochemical changes in B_1 deficiency

1. Carbohydrate metabolism is impaired. Accumulation of ***pyruvate*** occurs in the tissues which is harmful. Pyruvate concentration in plasma is elevated and it is also excreted ***in urine***.

2. Normally, pyruvate does not cross the blood-brain barrier and enter the brain. However, in thiamine deficiency, an alteration occurs in the blood-brain barrier permitting the pyruvate to enter the brain directly. It is believed that pyruvate accumulation in brain results in disturbed metabolism that may be responsible for polyneuritis.

3. Thiamine deficiency leads to impairment in nerve impulse transmission due to lack of TPP.

4. The transketolase activity in erythrocytes is decreased. Measurement of ***RBC transketolase*** activity is a reliable diagnostic test to assess thiamine deficiency.

In adults, two types of beri-beri, namely wet beri-beri and dry beri-beri occur. Infantile beri-beri that differs from adult beri-beri is also seen.

Wet beri-beri : This is characterized by ***edema*** of legs, face, trunk and serous cavities. Breathlessness and palpitation are present. The calf muscles are slightly swollen. The systolic blood pressure is elevated while diastolic is decreased. Fast and bouncing pulse is observed. The heart becomes weak and death may occur due to heart failure.

Dry beri-beri : This is associated with ***neurological manifestations*** resulting in peripheral neuritis. Edema is not commonly seen. The muscles become progressively weak and walking

becomes difficult. The affected individuals depend on support to walk and become bedridden, and may even ***die*** if not treated.

The symptoms of ***beri-beri*** are often mixed in which case it is referred to as ***mixed beri-beri***.

Infantile beri-beri : This is seen in infants born to mothers suffering from thiamine deficiency. The breast milk of these mothers contains low thiamine content. Infantile beri-beri is characterized by sleeplessness, restlessness, vomiting, convulsions and bouts of screaming that resemble abdominal colic. Most of these symptoms are due to cardiac dilatation. Death may occur suddenly due to cardiac failure.

Wernicke-Korsakoff syndrome

This is a disorder mostly ***seen in chronic alcoholics***. The body demands of thiamine increase in alcoholism. Insufficient intake or impaired intestinal absorption of thiamine will lead to this syndrome. It is characterized by loss of memory, apathy and a rhythmical to and fro motion of the eye balls.

Thiamine deficiency due to thiaminase and pyrithiamine

The enzyme thiaminase is present in certain seafoods. Their inclusion in the diet will destroy thiamine by a cleavage action (pyrimidine and thiazole rings are separated) and lead to deficiency. Pyrithiamine, a structural analogue and an antimetabolite of thiamine; is found in certain plants like ferns. Horses and cattle often develop thiamine deficiency (fern poisoning) due to the overconsumption of the plant fern.

Thiamine antagonists

Pyrithiamine and ***oxythiamine*** are the two important antimetabolites of thiamine.

RIBOFLAVIN (VITAMIN B_2)

Riboflavin through its coenzymes takes part in a variety of cellular oxidation–reduction reactions.

Chemistry

Riboflavin contains ***6,7-dimethyl isoalloxazine*** (a heterocyclic 3 ring structure) attached to ***D-ribitol*** by a nitrogen atom. Ribitol is an open chain form of sugar ribose with the aldehyde group (CHO) reduced to alcohol (CH_2OH).

Riboflavin is stable to heat but sensitive to light. When exposed to ultra-violet rays of sunlight, it is converted to ***lumiflavin*** which exhibits yellow fluorescence. The substances namely ***lactoflavin*** (from milk), ***hepatoflavin*** (from liver) and ***ovoflavin*** (from eggs) which were originally thought to be different are structurally identical to riboflavin.

Coenzymes of riboflavin

Flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) are the two coenzyme forms of riboflavin. The ribitol (5 carbon) is linked to a phosphate in FMN. FAD is formed from FMN by the transfer of an AMP moiety from ATP (***Fig.7.19***).

Biochemical functions

The flavin coenzymes (mostly FAD and to a lesser extent FMN) participate in many ***redox reactions*** responsible for energy production. The functional unit of both the coenzymes is isoalloxazine ring which serves as an acceptor of two hydrogen atoms (with electrons). FMN or FAD undergo identical reversible reactions accepting two hydrogen atoms forming $FMNH_2$ or $FADH_2$ (***Fig.7.20***).

Enzymes that use flavin coenzymes (FMN or FAD) are called ***flavoproteins***. The coenzymes (prosthetic groups) often bind rather tightly, to the protein (apoenzyme) either by non-covalent bonds (mostly) or covalent bonds in the holoenzyme. Many flavoproteins contain metal atoms (iron, molybdenum etc.) which are known as ***metalloflavoproteins***.

The coenzymes, FAD and FMN are associated with certain enzymes involved in carbohydrate, lipid, protein and purine metabolisms, besides the electron transport chain. A few examples are listed in ***Table 7.2***. Further details are given in the respective chapters.

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Milk and milk products, meat, eggs, liver, kidney are rich sources. Cereals, fruits, vegetables and fish are moderate sources.

Deficiency symptoms

Riboflavin deficiency symptoms include ***cheilosis*** (fissures at the corners of the mouth), ***glossitis*** (tongue smooth and purplish) and ***dermatitis***. Riboflavin deficiency as such is uncommon. It is mostly seen along with other vitamin deficiencies. Chronic alcoholics are susceptible to B_2 deficiency. Assay of the enzyme ***glutathione reductase*** in erythrocytes will be useful in assessing riboflavin deficiency.

Antimetabolite : Galactoflavin is an antimetabolite of riboflavin.

NIACIN

Niacin or ***nicotinic acid*** is also known as ***pellagra preventive (P.P.) factor*** of Goldberg. The coenzymes of niacin (NAD^+ and $NADP^+$) can be synthesized by the essential amino acid, tryptophan.

The disease pellagra (*Italian* : rough skin) has been known for centuries. However, its relation to the deficiency of a dietary factor was first identified by Goldberger. Goldberger and his associates conducted an interesting experiment

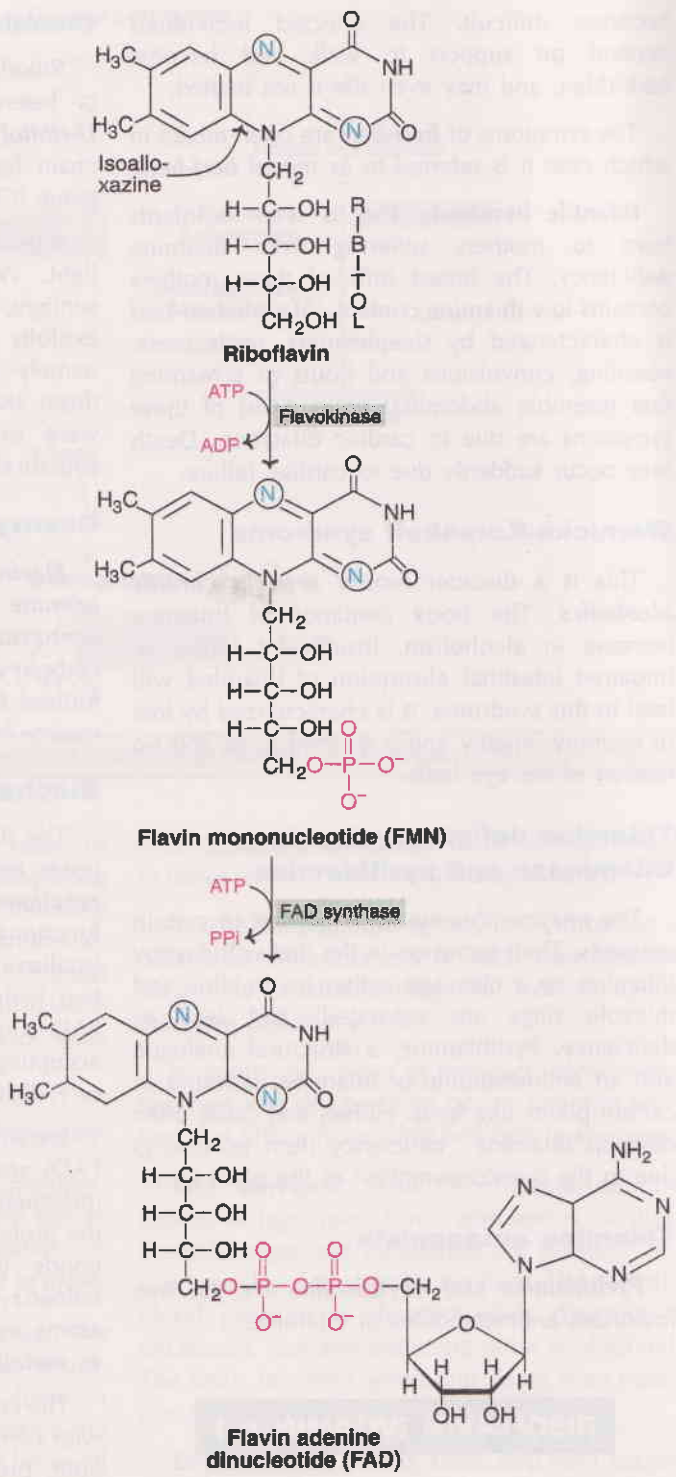
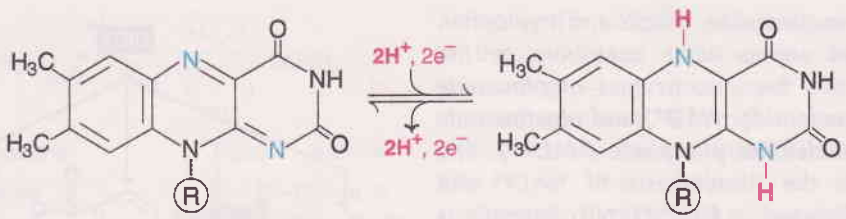


Fig. 7.19 : Structures and biosynthesis of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD).



Oxidized flavin (FMN or FAD)

Reduced flavin ($FMNH_2$ or $FADH_2$)

Fig. 7.20 : Participation of FMN or FAD in oxidation-reduction reactions (R-represents the rest of the structure of FMN or FAD as depicted in ***Fig. 7.19****).*

for this purpose. Twelve convicts were promised pardon if they consumed diet of pellagrous families for one year. The diet consisted of corn meal, corn starch, rice, sweet potato and pork fat. More than half of the subjects showed symptoms of pellagra in less than an year, while no such symptoms were observed in other prisoners on a regular diet. Administration of dried meat or liver to the patients cured pellagra (Goldberger, 1928).

Much before it was recognized as a vitamin, nicotinic acid was well known as a chemical compound, produced by the oxidation of nicotine (present in tobacco leaves). The term 'niacin' was coined and more commonly used for nicotinic acid. This was done to emphasize the role of niacin as a vitamin and avoid the impression that nicotinic acid is an oxidized form of nicotine. However, most of the authors use niacin and nicotinic acid synonymously.

Chemistry and synthesis of coenzymes

Niacin is a ***pyridine derivative***. Structurally, it is pyridine 3-carboxylic acid. The amide form of niacin is known as niacinamide or nicotinamide.

Table 7.2 Selected examples of FAD and FMN dependent enzymes along with their respective reactions

Enzyme	*Reaction*
FAD dependent	
I. ***Carbohydrate metabolism***	
(a) Pyruvate dehydrogenase complex*	Pyruvate ⟶ Acetyl CoA
(b) α-Ketoglutarate dehydrogenase complex*	α-Ketoglutarate ⟶ Succinyl CoA
(c) Succinate dehydrogenase	Succinate ⟶ Fumarate
II. ***Lipid metabolism***	
(d) Acyl CoA dehydrogenase	Acyl CoA ⟶ α, β-Unsaturated acyl CoA
III. ***Protein metabolism***	
(e) Glycine oxidase	Glycine ⟶ Glyoxylate + NH_3
(f) D-Amino acid oxidase	D-Amino acid ⟶ α-Keto acid + NH_3
IV. ***Purine metabolism***	
(g) Xanthine oxidase	Xanthine ⟶ Uric acid
FMN dependent	
L-Amino acid oxidase	L-Amino acid ⟶ α-Keto acid + NH_3

* *Dihydrolipoyl dehydrogenase component requires FAD*

Dietary nicotinamide, niacin and tryptophan (an essential amino acid) contribute to the synthesis of the coenzymes–***nicotinamide adenine dinucleotide (NAD^+) and nicotinamide adenine dinucleotide phosphate ($NADP^+$)***. The pathway for the biosynthesis of NAD^+ and $NADP^+$ is depicted in ***Fig.7.21***. Nicotinamide is deaminated in the body to niacin. Niacin then undergoes a series of reactions to produce NAD^+ and $NADP^+$. Tryptophan produces quinolinate which then forms nicotinate mononucleotide and, ultimately, NAD^+ and $NADP^+$. ***Sixty milligrams of tryptophan is equivalent to 1 mg of niacin for the synthesis of niacin coenzymes***. Phosphoribosyl pyrophosphate and ATP, respectively, provide ribose phosphate and AMP moieties for the synthesis of NAD^+. Glutamine donates amide group. In the structure of the coenzymes, nitrogen atom of nicotinamide carries a positive charge (due to the formation of an extra bond, N is in quaternary state), hence the coenzymes are NAD^+ and $NADP^+$. Nicotinamide, liberated on the degradation of NAD^+ and $NADP^+$ is mostly ***excreted in urine as N-methylnicotinamide***.

(**Note :** Some authors prefer to use NAD/NADP without a positive charge to represent general or oxidized form of niacin coenzymes.)

Biochemical functions

The coenzymes NAD^+ and $NADP^+$ are involved in a variety of ***oxidation-reduction reactions.*** They accept hydride ion (hydrogen atom and one electron $:H^-$) and undergo reduction in the pyridine ring. This results in the neutralization of positive charges. The nitrogen atom and the fourth carbon atom of nicotinamide ring participate in the reaction. While one atom of hydrogen (as hydride ion) from the substrate (AH_2) is accepted by the coenzyme, the other hydrogen ion (H^+) is released into the surrounding medium.

This reaction is reversed when NADH is oxidized to NAD^+. $NADP^+$ also functions like NAD^+ in the oxidation-reduction reactions.

A large number of enzymes (about 40) belonging to the class ***oxidoreductases*** are

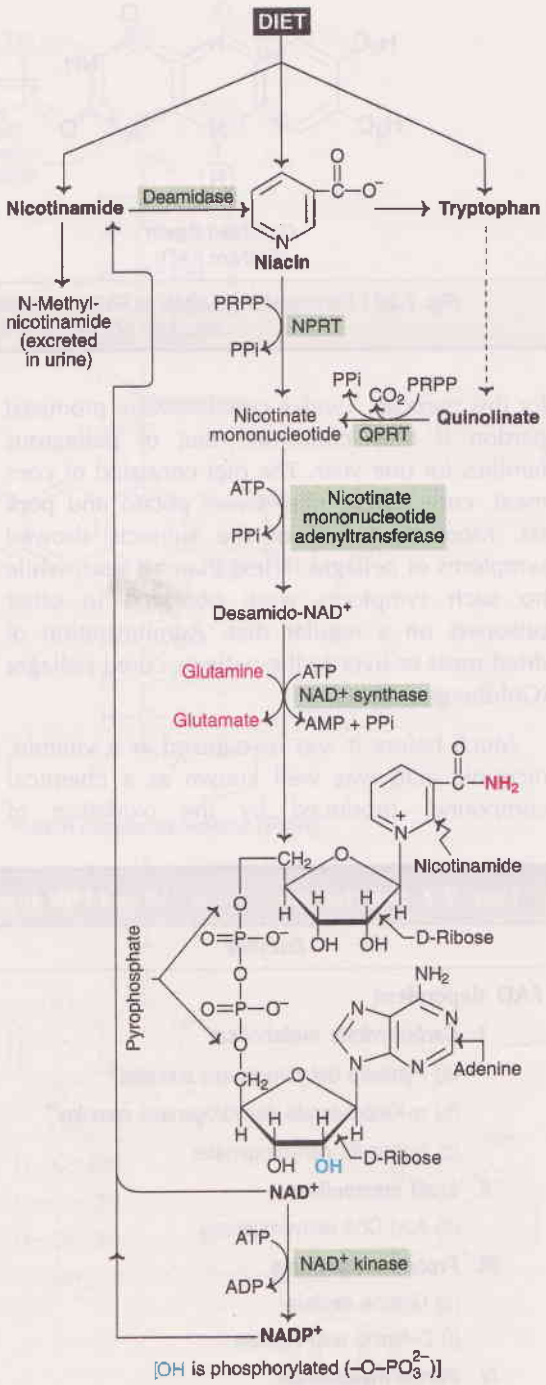


***Fig. 7.21** : Outline of the biosynthesis of nicotinamide nucleotides, NAD^+ and $NADP^+$ (NPRT-Nicotinate phosphoribosyltransferase; QPRT-Quinolinate phosphoribosyltransferase; PPi-Pyrophosphate; PRPP-Phosphoribosyl pyrophosphate).*

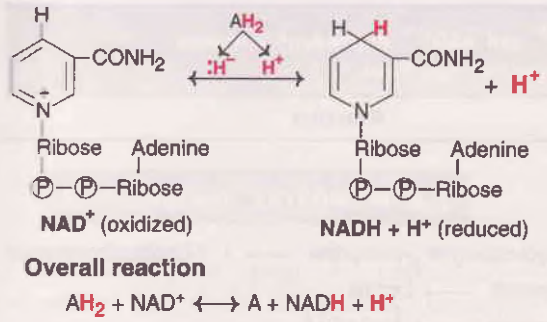


Fig. 7.22 : Mechanism of oxidation and reduction of nicotinamide coenzyme—NAD⁺
*(**Note** : Similar mechanism operates for NADP⁺ also).*

dependent on NAD^+ or $NADP^+$. The coenzymes are loosely bound to the enzymes and can be separated easily by dialysis. NAD^+ and $NADP^+$ participate in almost all the metabolisms (carbohydrate, lipid, protein etc.). Some enzymes are exclusively dependent on NAD^+ whereas some require only $NADP^+$. A few enzymes can use either NAD^+ or $NADP^+$. Selected examples of enzymes and the reactions they catalyse are given in ***Table 7.3***.

NADH produced is oxidized in the electron transport chain ***to generate ATP***. ***NADPH*** is also important for many ***biosynthetic reactions*** as it donates reducing equivalents.

Recommended dietary allowance (RDA)

The daily requirement of niacin for an adult is ***15-20 mg*** and for children, around 10-15 mg. Very often, the term ***niacin equivalents (NE)*** is used while expressing its RDA. One NE = 1 mg niacin or 60 mg of tryptophan. Instead of mg, the daily requirements are known as niacin equivalents. Pregnancy and lactation in women impose an additional metabolic burden and increase the niacin requirement.

Dietary sources

The rich natural sources of niacin include liver, yeast, whole grains, cereals, pulses like beans and peanuts. Milk, fish, eggs and vegetables are moderate sources. The essential amino acid tryptophan can serve as a precursor for the synthesis of nicotinamide coenzymes. On an average, 1 g of a good quality protein containing about 60 mg of tryptophan is equivalent to 1 mg of niacin ***(conversion ratio 60 : 1)*** for the synthesis of nicotinamide coenzymes. Tryptophan has many other essential and important functions in the body, hence dietary ***tryptophan cannot totally replace niacin***. Increased conversion of tryptophan to niacin has been reported in low protein diet and starvation. Tryptophan can replace niacin to an extent of 10% for the synthesis of coenzymes. Therefore, both niacin and tryptophan have to be invariably provided in the diet.

Deficiency symptoms

Niacin deficiency results in a condition called ***pellagra*** (*Italian*: rough skin). This disease involves skin, gastrointestinal tract and central nervous system. The symptoms of pellagra are commonly referred to as ***three Ds***. The disease also progresses in that order ***dermatitis***, ***diarrhea***, ***dementia***, and if not treated may rarely lead to ***death*** (4th D).

Dermatitis (inflammation of skin) is usually found in the areas of the skin exposed to sunlight (neck, dorsal part of feet, ankle and parts of face). Diarrhea may be in the form of loose stools, often with blood and mucus. Prolonged diarrhea leads to weight loss. Dementia is associated with degeneration of nervous tissue. The symptoms of dementia include anxiety, irritability, poor memory, insomnia (sleeplessness) etc.

Pellagra is mostly seen among people whose staple diet is corn or maize. Niacin present in maize is unavailable to the body as it is in bound form. Further, tryptophan content is low in maize.

Therapeutic uses of niacin

Administration of niacin in pharmacological doses (2-4 g/day, 200 times the RDA) results in a number of biochemical effects in the body, not related to its function as a vitamin. Most of the effects are believed to be due to the influence of niacin on cyclic AMP levels.

Table 7.3 Selected examples of NAD^+ and $NADP^+$ dependent enzymes along with their respective reactions

Enzyme	Reaction
NAD^+ dependent	
I. Carbohydrate metabolism	
(a) Glyceraldehyde 3-phosphate dehydrogenase	Glyceraldehyde 3-phosphate ⟶ 1, 3-Bisphosphoglycerate
(b) Lactate dehydrogenase	Pyruvate ⟶ Lactate
(c) Pyruvate dehydrogenase complex	Pyruvate ⟶ Acetyl CoA
(d) α-Ketoglutarate dehydrogenase complex	α-Ketoglutarate ⟶ Succinyl CoA
II. Lipid metabolism	
(e) β-Hydroxy acyl CoA dehydrogenase	β-Hydroxy acyl CoA ⟶ β-Keto acyl CoA
(f) β-Hydroxybutyrate dehydrogenase	β-Hydroxybutyrate ⟶ Acetoacetate
(g) Alcohol dehydrogenase	Alcohol ⟶ Acetaldehyde
III. Protein metabolism	
(h) Branched chain α-keto acid dehydrogenase	α-Keto acids of branched chain amino acids (Leu, Ile, Val) ⟶ Corresponding acyl CoA thioesters
(i) Tyramine dehydrogenase	Tyramine ⟶ p-Hydroxyphenyl acetate
NAD^+ or $NADP^+$ dependent	
(a) Glutamate dehydrogenase	Glutamate ⟶ α-Ketoglutarate + NH_3
(b) Isocitrate dehydrogenase	Isocitrate ⟶ Oxalosuccinate
$NADP^+$ dependent	
(a) Glucose 6-phosphate dehydrogenase	Glucose 6-phosphate ⟶ 6-Phosphogluconolactone
(b) Malic enzyme	Malate ⟶ Pyruvate
NADPH dependent	
(a) 3-Ketoacyl reductase	3-Ketoacyl enzyme ⟶ 3-Hydroxy acyl enzyme
(b) HMG CoA reductase	HMG CoA ⟶ Mevalonate
(c) Squalene epoxidase	Squalene ⟶ Squalene oxide
(d) Cholesterol 7α-hydroxylase	Cholesterol ⟶ 7α-Hydroxy cholesterol
(e) Phenylalanine hydroxylase	Phenylalanine ⟶ Tyrosine
(f) Dihydrofolate reductase	Folic acid ⟶ Tetrahydrofolic acid.

1. Niacin ***inhibits lipolysis*** in the adipose tissue and decreases the circulatory free fatty acids.

2. Triacylglycerol synthesis in the liver is decreased.

3. The serum levels of low density lipoproteins (LDL), very low density lipoproteins (VLDL), triacylglycerol and cholesterol are lowered. Hence niacin is used in the treatment of ***hyperlipoproteinemia type II b*** (elevation of LDL and VLDL).

Although megadoses of niacin are useful for the treatment of hyperlipidemia, there are certain harmful side effects also.

1. Glycogen and fat reserves of skeletal and cardiac muscle are depleted.

2. There is a tendency for the increased levels of glucose and uric acid in the circulation.

3. Prolonged use of niacin results in elevated serum levels of certain enzymes, suggesting liver damage.

PYRIDOXINE (VITAMIN B_6)

Vitamin B_6 is used to collectively represent the three compounds namely ***pyridoxine, pyridoxal and pyridoxamine*** (the ***vitamers of B_6***).

Chemistry

Vitamin B_6 compounds are ***pyridine derivatives***. They differ from each other in the structure of a functional group attached to 4th carbon in the pyridine ring. Pyridoxine is a primary alcohol, pyridoxal is an aldehyde form while pyridoxamine is an amine (***Fig.7.23***). Pyridoxamine is mostly present in plants while pyridoxal and pyridoxamine are found in animal foods. Pyridoxine can be converted to pyridoxal and pyridoxamine, but the latter two cannot form pyridoxine.

Synthesis of coenzyme

The active form of vitamin B_6 is the coenzyme ***pyridoxal phosphate (PLP)***. PLP can be synthesized from the three compounds pyridoxine, pyridoxal and pyridoxamine. B_6 is excreted in urine as 4-pyridoxic acid. The different forms of B_6 and their inter-relationship are depicted in ***Fig.7.23***.

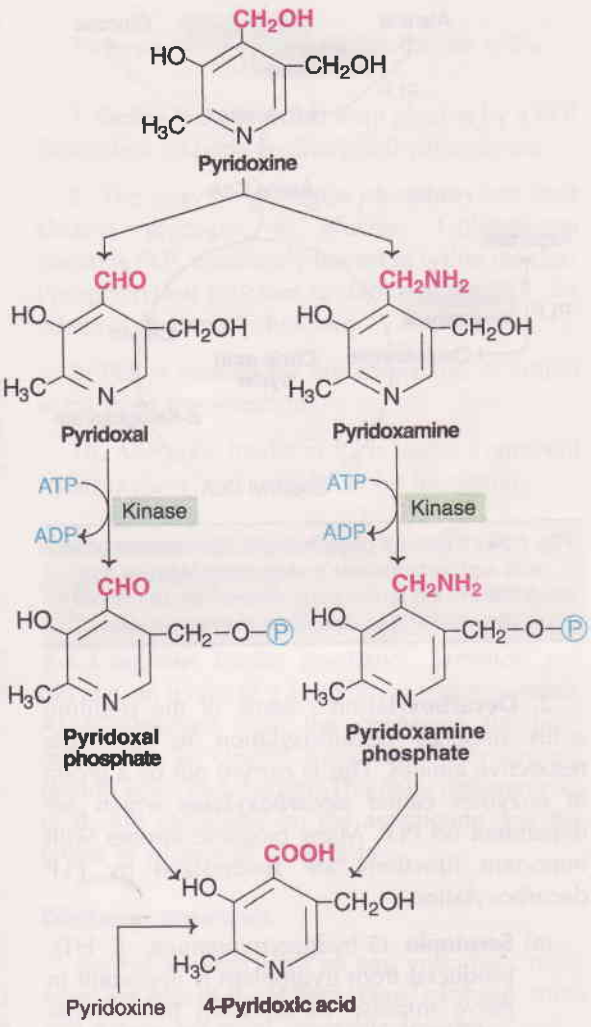


Fig. 7.23 : Pyridoxine, its derivatives and coenzyme.

Biochemical functions

Pyridoxal phosphate (PLP), the coenzyme of vitamin B_6 is found attached to the ε-amino group of lysine in the enzyme. PLP is closely associated with the metabolism of amino acids. The synthesis of certain specialized products such as serotonin, histamine, niacin coenzymes from the amino acids is dependent on pyridoxine. Pyridoxal phosphate participates in reactions like ***transamination, decarboxylation, deamination, transsulfuration, condensation*** etc.

1. **Transamination :** Pyridoxal phosphate is involved in the transamination reaction (by transaminase) converteing amino acids to keto acids. The keto acids enter the citric acid cycle and get oxidized to generate energy. Thus B_6 is an energy releasing vitamin. It integrates carbohydrate and amino acid metabolisms (***Fig.7.24***).

During the course of transamination, PLP interacts with amino acid to form a ***Schiff base (Fig.7.25)***. The amino group is handed over to PLP to form pyridoxamine phosphate and the keto acid is liberated.

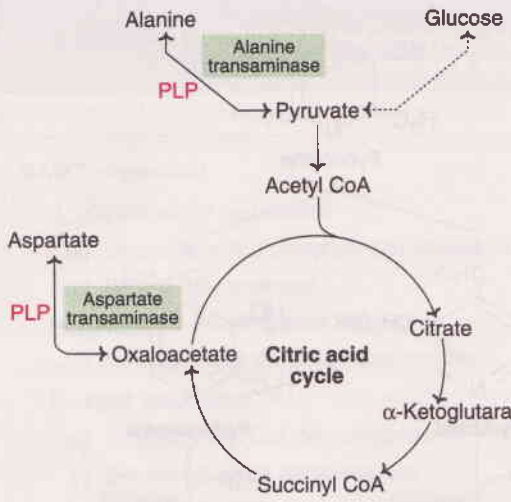
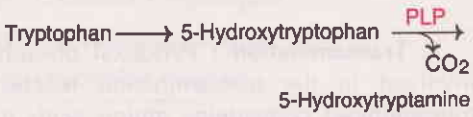


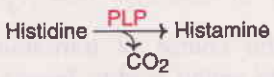
Fig. 7.24 : Pyridoxal phosphate (PLP) integrates amino acid and carbohydrate metabolisms (Alanine and aspartate are the amino acids respectively converted to pyruvate and oxaloacetate, the keto acids).

2. **Decarboxylation :** Some of the α-amino acids undergo decarboxylation to form the respective amines. This is carried out by a group of enzymes called ***decarboxylases*** which are dependent on PLP. Many biogenic amines with important functions are synthesized by PLP decarboxylation.

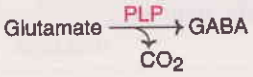
(a) **Serotonin** (5-hydroxytryptamine, 5 HT), produced from tryptophan is important in nerve impulse transmission (neurotransmitter). It regulates sleep, behaviour, blood pressure etc.



(b) **Histamine** is a vasodilator and lowers blood pressure. It stimulates gastric HCl secretion and is involved in inflammation and allergic reactions.



(c) Glutamate on decarboxylation gives ***γ-amino butyric acid*** (GABA). GABA inhibits the transmission of nerve impulses, hence it is an inhibitory neurotransmitter.



(d) The synthesis of ***catecholamines*** (dopamine, norepinephrine and epinephrine) from tyrosine require PLP. Catecholamines are involved in metabolic and nervous regulation.

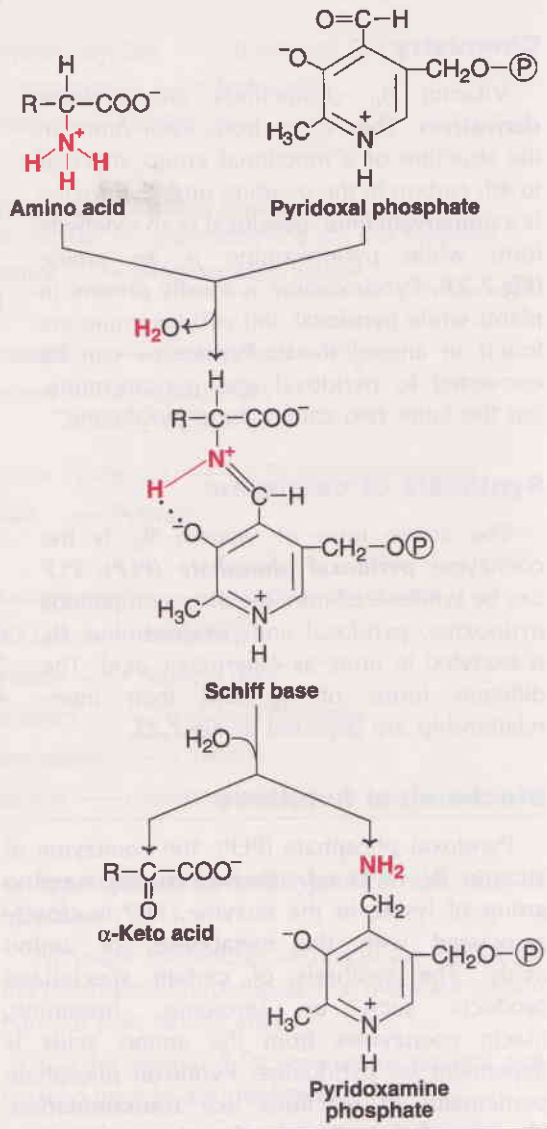


Fig. 7.25 : Formation of Schiff base in transamination.

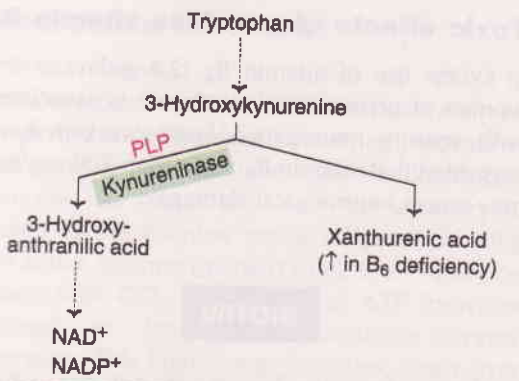
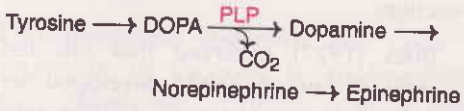
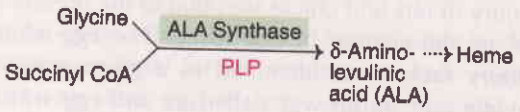


Fig. 7.26 : Role of pyridoxine in tryptophan metabolism (PLP-Pyridoxal phosphate).



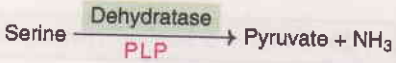
3. Pyridoxal phosphate is required for the synthesis of ***δ-amino levulinic acid***, the precursor for heme synthesis.

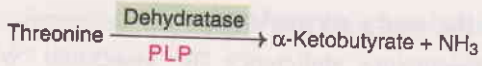


4. The synthesis of ***niacin coenzymes*** (NAD^+ and $NADP^+$) from tryptophan is dependent on PLP. The enzyme ***kynureninase requires PLP***. In B_6 deficiency, 3-hydroxy anthranilic acid is diverted to xanthurenic acid (***Fig.7.26***). Increased excretion of xanthurenate in urine is an indication of B_6 deficiency.

5. PLP plays an important role in the metabolism of sulfur containing amino acids (***Fig.7.27***). ***Transsulfuration*** (transfer of sulfur) from homocysteine to serine occurs in the synthesis of cysteine. This is carried out by a PLP dependent cystathionine synthase. Taurine, a decarboxylated (PLP dependent) product of cysteine, is involved in the conjugation of bile acids.

6. **Deamination** of hydroxyl group containing amino acids requires PLP.





7. **Serine** is synthesized from glycine by a PLP dependent enzyme hydroxymethyltransferase.

8. The enzyme ***glycogen phosphorylase*** (that cleaves glycogen to glucose 1-phosphate) contains PLP, covalently bound to lysine residue. Phosphorylase structure is stabilized by PLP, for effective enzymatic function.

9. PLP is needed for the absorption of amino acids from the intestine.

10. Adequate intake of B_6 is useful to prevent hyperoxaluria and urinary stone formation.

Recommended dietary allowance (RDA)

The requirement of pyridoxine for an adult is ***2-2.2 mg/day***. During pregnancy, lactation and old age, an intake of 2.5 mg/day is recommended. As is observed from the coenzyme function, pyridoxine is closely associated with protein (amino acid) metabolism. The daily requirements of B_6 are calculated on the assumption that the intake of protein is <100 g/day.

Dietary sources

Animal sources such as egg yolk, fish, milk, meat are rich in B_6. Wheat, corn, cabbage, roots and tubers are good vegetable sources.

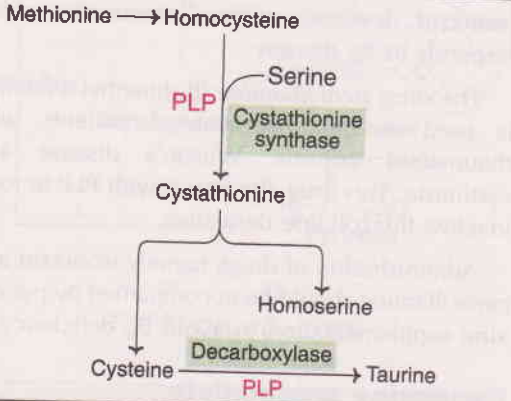


Fig. 7.27 : Role of pyridoxine in the metabolism of sulfur amino acids (PLP-Pyridoxal phosphate).

Deficiency symptoms

Pyridoxine deficiency is associated with ***neurological symptoms*** such as depression, irritability, nervousness and mental confusion. Convulsions and peripheral neuropathy are observed in severe deficiency. These symptoms are related to the decreased synthesis of biogenic amines (serotonin, GABA, norepinephrine and epinephrine). In children, B_6 deficiency with a drastically reduced GABA production results in convulsions (epilepsy).

Decrease in hemoglobin levels, associated with hypochromic microcytic anaemia, is seen in B_6 deficieny. This is due to a ***reduction in heme production.***

The synthesis of niacin coenzymes (NAD^+ and $NADP^+$) from tryptophan is impaired. ***Xanthurenic acid***, produced in high quantities is ***excreted in urine***, which serves as a reliable index (particularly after tryptophan load test) for ***B_6 deficiency.***

Dietary deficiency of pyridoxine is rather rare and is mostly observed in women taking oral contraceptives, alcoholics and infants.

Drug induced B_6 deficiency

Isoniazid (isonicotinic acid hydrazide, INH) is a drug frequently used for the treatment of ***tuberculosis***. It combines with pyridoxal phosphate to form inactive hydrazone derivatives which inhibit PLP dependent enzymes. Tuberculosis patients, on long term use of isoniazid, develop peripheral neuropathy which responds to B_6 therapy.

The drug ***penicillamine*** (β-dimethyl cysteine) is used in the treatment of patients with rheumatoid arthritis, Wilson's disease and cystinuria. This drug also reacts with PLP to form inactive thiazolidine derivative.

Administration of drugs namely isoniazid and penicillamine should be accompanied by pyridoxine supplementation to avoid B_6 deficiency.

Pyridoxine antagonists

Isoniazid, deoxypyridoxine and methoxy pyridoxine are the antagonists of vitamin B_6.

Toxic effects of overdose vitamin B_6

Excess use of vitamin B_6 (2.5 g/day) in the women of premenstrual syndrome is associated with sensory neuropathy. Some workers have suggested that vitamin B_6 more than 200 mg/day may cause neurological damage.

BIOTIN

Biotin (formerly known as anti-egg white injury factor, vitamin B_7 or vitamin H) is a sulfur containing B-complex vitamin. It directly participates as a coenzyme in the carboxylation reactions.

Boas (1927) observed that rats fed huge quantity of raw egg white developed dermatitis and nervous manifestations, besides retardation in growth. She however, found that feeding cooked egg did not produce any of these symptoms. It was later shown that the egg white injury in rats and chicks was due to the presence of an anti-vitamin in egg white. The ***egg-white injury factor*** was identified as a glycoprotein–***avidin*** and ***biotin*** was called as ***anti-egg white injury factor.***

Chemistry

Biotin is a heterocyclic sulfur containing monocarboxylic acid. The structure is formed by fusion of ***imidazole*** and ***thiophene rings*** with a valeric acid side chain (***Fig.7.28***). Biotin is covalently bound to ε-amino group of lysine to form biocytin in the enzymes. ***Biocytin*** may be regarded as the ***coenzyme*** of biotin.

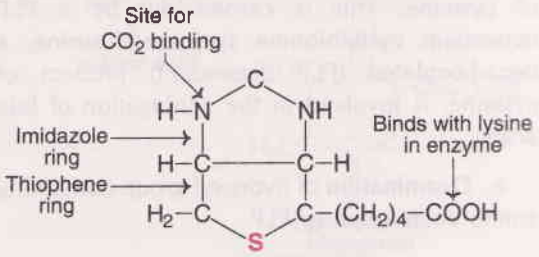


***Fig. 7.28** : Structure of biotin with binding sites.*

Biochemical functions

Biotin serves as a carrier of CO_2 in ***carboxylation reactions.*** The reaction catalysed by ***pyruvate carboxylase***, converting pyruvate to oxaloacetate has been investigated in detail. This enzyme has biotin bound to the apoenzyme linked to the ε-amino group of lysine, forming the active enzyme (holoenzyme). Biotin–enzyme reacts with CO_2 in presence of ATP (provides energy) to form a carboxybiotin–enzyme complex. This high energy complex hands over the CO_2 to pyruvate (carboxylation reaction) to produce oxaloacetate (***Fig.7.29***).

As a coenzyme, biotin is involved in various metabolic reactions.

1. **Gluconeogenesis and citric acid cycle :** The conversion of pyruvate to oxaloacetate by biotin dependent pyruvate carboxylase (described above) is essential for the synthesis of glucose from many non-carbohydrate sources. Oxaloacetate so formed is also required for the continuous operation of citric acid cycle.

2. **Fatty acid synthesis :** Acetyl CoA is the starting material for the synthesis of fatty acids. The very first step in fatty acid synthesis is a carboxylation reaction.

Acetyl CoA —(Biotin; Acetyl CoA carboxylase)→ Malonyl CoA

3. **Propionyl CoA** is produced in the metabolism of certain amino acids (valine, isoleucine, threonine etc.) and degradation of odd chain fatty acids. Its further metabolism is dependent on biotin.

Propionyl CoA —(Biotin; Propionyl CoA carboxylase)→ Methylmalonyl CoA

4. In the metabolism of ***leucine***, the following reaction is dependent on biotin.

β-Methylcrotonyl CoA —(Biotin; β-Methylcrotonyl CoA carboxylase)→ β-Methyl glutaconyl CoA

[**Note :** It was once believed that all the carboxylation reactions in the biological system are dependent on biotin. This was later proved to be wrong. There are a few carboxylation reactions which do not require biotin e.g. formation of carbamoyl phosphate in urea cycle, incorporation of CO_2 in purine synthesis.]

Recommended dietary allowance (RDA)

A daily intake of about ***100-300 mg*** is recommended for adults. In fact, biotin is normally synthesized by the intestinal bacteria. However, to what extent the synthesized biotin contributes to the body requirements is not clearly known.

Dietary sources

Biotin is widely distributed in both animal and plant foods. The rich sources are liver, kidney, egg yolk, milk, tomatoes, grains etc.

Deficiency symptoms

The symptoms of biotin deficiency include anemia, loss of appetite, nausea, dermatitis,

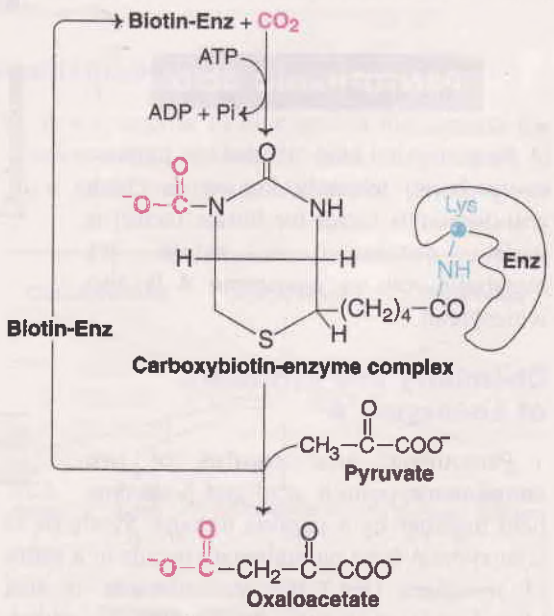


Fig. 7.29 : *Role of biotin in the carboxylation reaction, catalysed by the enzyme pyruvate carboxylase (Enz-Enzyme).*

glossitis etc. Biotin deficiency may also result in depression, hallucinations, muscle pain and dermatitis.

Biotin ***deficiency is uncommon***, since it is well distributed in foods and also supplied by the intestinal bacteria. The deficiency may however, be associated with the following two causes.

1. Destruction of intestinal flora due to prolonged use of drugs such as ***sulfonamides***.

2. High consumption of raw eggs. The raw egg white contains a glycoprotein–***avidin***, which tightly binds with biotin and blocks its absorption from the intestine. An intake of about 20 raw eggs per day is needed to produce biotin deficiency symptoms in humans. Consumption of an occasional raw egg will not result in deficiency.

Antagonists

Desthiobiotin, biotin sulphonic acid are biotin antagonists.

PANTOTHENIC ACID

Pantothenic acid (*Greek* : pantos–everywhere), formerly known as chick anti-dermatitis factor (or filtrate factor) is widely distributed in nature. It's metabolic role as ***coenzyme A*** is also widespread.

Chemistry and synthesis of coenzyme A

Pantothenic acid consists of two components, pantoic acid and β-alanine, held together by a peptide linkage. Synthesis of coenzyme A from pantothenate occurs in a series of reactions (***Fig.7.30***). Pantothenate is first phosphorylated to which cysteine is added. Decarboxylation, followed by addition of AMP moiety and a phosphate (each from ATP) results in coenzyme A. The structure of coenzyme A consists of pantothenic acid joined to β-mercaptoethanolamine (thioethanolamine) at one end. On the other side, pantothenic acid is held by a phosphate bridge to adenylic acid. The adenylic acid is made up of adenine, and a phosphate linked to carbon-3 of ribose.

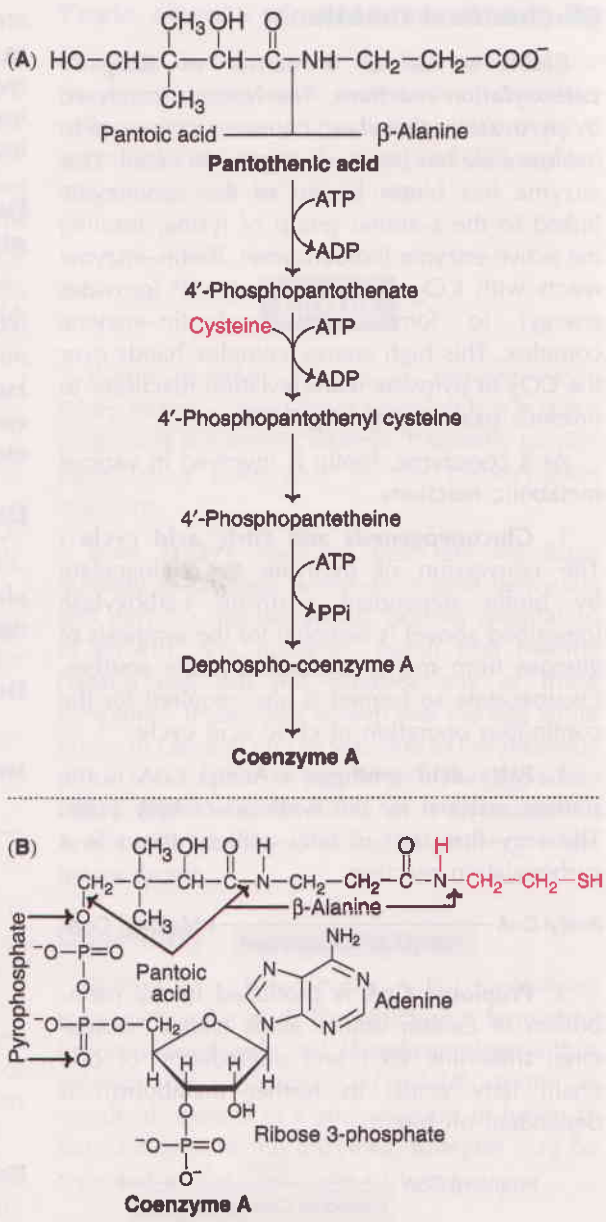


Fig. 7.30 : (A) Summary of the synthesis of coenzyme A from pantothenic acid (B) Structure of coenzyme A.

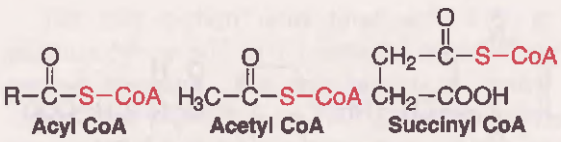


Fig. 7.31 : Selected examples of compounds bound to coenzyme A.

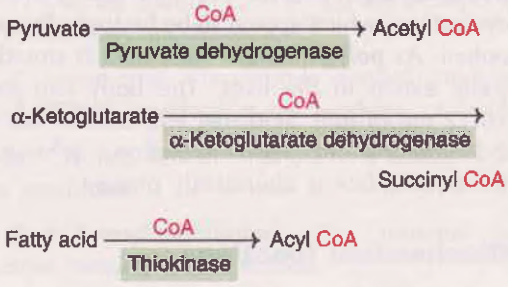
Biochemical functions

The functions of pantothenic acid are exerted through ***coenzyme A or CoA (A for acetylation)***. Coenzyme A is a central molecule involved in all the metabolisms (carbohydrate, lipid and protein). It plays a unique role in integrating various metabolic pathways. More than 70 enzymes that depend on coenzyme A are known.

Coenzyme A has a terminal thiol or sulfhydryl group (–SH) which is the reactive site, hence CoA-SH is also used. Acyl groups (free fatty acids) are linked to coenzyme A by a thioester bond, to give acyl CoA. When bound to acetyl unit, it is called acetyl CoA. With succinate, succinyl CoA is formed. There are many other compounds bound to coenzyme A.

Coenzyme A serves as a ***carrier of activated acetyl or acyl groups*** (as thiol esters). This is comparable with ATP which is a carrier of activated phosphoryl groups.

A few examples of enzymes involved the participation of coenzyme A are given below.



In some of the metabolic reactions, group transfer is important which occurs in a coenzyme A bound form.

Acetyl CoA + Choline ⟶ Acetylcholine + CoA

Acetyl CoA + Oxaloacetate ⟶ Citrate + CoA

Succinyl CoA + Acetoacetate ⟶ Acetoacetyl CoA + Succinate

Coenzyme A may be regarded as a ***coenzyme of metabolic integration***, since acetyl CoA is a central molecule for a wide variety of biochemical reactions, as illustrated in ***Fig.7.32***.

Succinyl CoA is also involved in many reactions, including the synthesis of porphyrins of heme.

Besides the various functions through coenzyme A, ***pantothenic acid*** itself is a ***component of fatty acid synthase complex*** and is involved in the formation of fatty acids.

Recommended dietary allowance (RDA)

The requirement of pantothenic acid for humans is not clearly known. A daily intake of about ***5-10 mg*** is advised for adults.

Dietary sources

Pantothenic acid is one of the most widely distributed vitamins found in plants and animals. The rich sources are egg, liver, meat, yeast, milk etc.

Deficiency symptoms

It is a surprise to biochemists that despite the involvement of pantothenic acid (as coenzyme A) in a great number of metabolic reactions, its

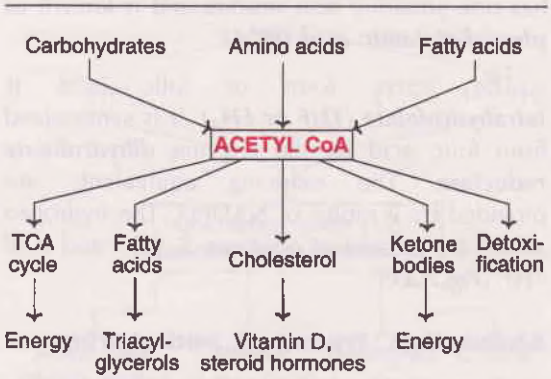


Fig. 7.32 : An overview of formation and utilization of acetyl CoA.

deficiency manifestations have not been reported in humans. This may be due to the widespread distribution of this vitamin or the symptoms of pantothenic acid may be similar to other vitamin deficiencies. Dr. Gopalan, a world renowned nutritionist from India, linked the ***burning feet syndrome*** (pain and numbness in the toes, sleeplessness, fatigue etc.) with pantothenic acid deficiency.

Pantothenic acid deficiency in experimental animals results in anemia, fatty liver, decreased steroid synthesis etc.

FOLIC ACID

Folic acid or folacin (*Latin* : folium-leaf) is abundantly found in green leafy vegetables. It is important for ***one carbon metabolism*** and is required for the synthesis of certain amino acids, purines and the pyrimidine-thymine.

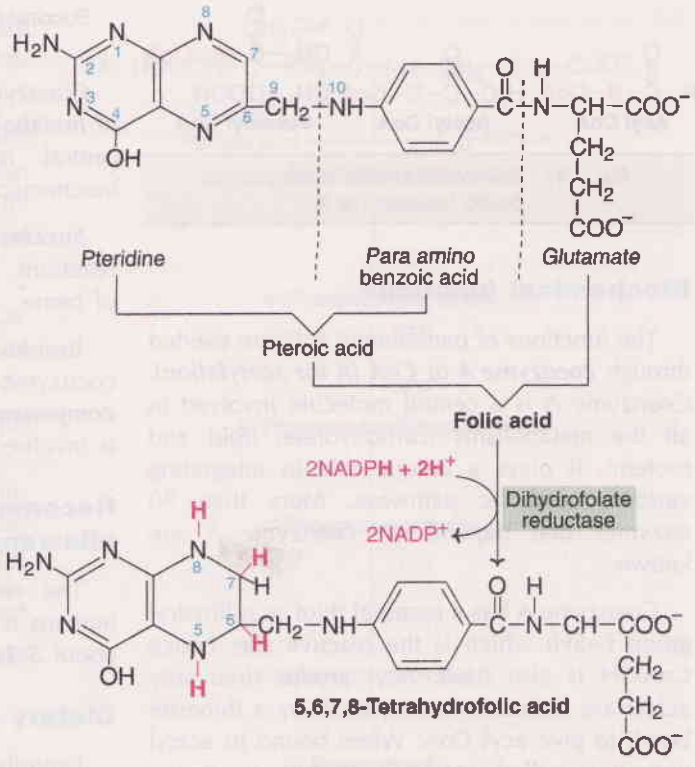


Fig. 7.33 : Conversion of folic acid to tetrahydrofolic acid (THF).

Chemistry

Folic acid consists of three components–pteridine ring, p-amino benzoic acid (PABA) and glutamic acid (1 to 7 residues). Folic acid mostly has one glutamic acid residue and is known as ***pteroyl-glutamic acid (PGA)***.

The active form of folic acid is ***tetrahydrofolate (THF or FH_4)***. It is synthesized from folic acid by the enzyme ***dihydrofolate reductase***. The reducing equivalents are provided by 2 moles of NADPH. The hydrogen atoms are present at positions 5, 6, 7 and 8 of THF (***Fig.7.33***).

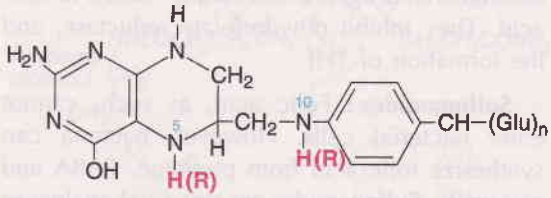
Absorption, transport and storage

Most of the dietary folic acid found as polyglutamate with 3-7 glutamate residues (held by peptide bonds) is not absorbed in the intestine. The enzyme ***folate conjugase*** present in duodenum and jejunum splits the glutamate residues. Only the monoglutamate of folic acid is absorbed from the intestine. However, inside the cells, tetrahydrofolates are found as polyglutamates (with 5-6 amino acid residues) derivatives, which appear to be biologically most potent. As ***polyglutamate***, folic acid is stored to some extent in the liver. The body can store 10-12 mg of folic acid that will usually last for 2-3 months. In the circulation, N^5-methyl tetrahydrofolate is abundantly present.

Biochemical functions

Tetrahydrofolate (THF or FH_4), the coenzyme of folic acid, is actively involved in the ***one carbon metabolism***. THF serves as an acceptor or donor of one carbon units (formyl, methyl etc.) in a variety of reactions involving amino acid and nucleotide metabolism.

The one carbon units bind with THF at position N^5 or N^{10} or on both N^5 and N^{10} of pteroyl structure. The attachment of formyl (−CHO) at position 5 of THF gives N^5-formyl tetrahydrofolate which is commonly known as ***folinic acid or citrovorum factor***. The other commonly found one carbon moieties and their binding with THF are given below.



THF-1 carbon derivative	R group (one carbon unit)
N^5-Formyl THF	−CHO
N^{10}-Formyl THF	−CHO
N^5-Formimino THF	−CH=NH
N^5, N^{10}-Methenyl THF	≡CH
N^5, N^{10}-Methylene THF	=CH_2
N^5 – Methyl THF	−CH_3

The essential functions of THF in one carbon metabolism are summarized in ***Fig.7.34***.

The interrelationship between the various 1-carbon THF derivatives along with their involvement in the synthesis of different compounds is given in ***Fig.15.32*** (***Chapter 15***). Many important compounds are synthesized in one carbon metabolism.

1. Purines (carbon 2, 8) which are incorporated into DNA and RNA.

2. Pyrimidine nucleotide–deoxythymidylic acid (dTMP), involved in the synthesis of DNA.

3. Glycine, serine, ethanolamine and choline are produced.

4. N-Formylmethionine, the initiator of protein biosynthesis is formed.

Tetrahydrofolate is mostly trapped as N^5-methyl THF in which form it is present in the circulation. Vitamin B_{12} is needed for the conversion of N^5-methyl THF to THF, in a reaction wherein homocysteine is converted to methionine. This step is essential for the liberation of free THF and for its repeated use in one carbon metabolism. In B_{12} deficiency, conversion of N^5-methyl THF to THF is blocked (more details given under vitamin B_{12}).

Recommended dietary allowance (RDA)

The daily requirement of folic acid is around ***200 µg***. In the women, higher intakes are recommended during pregnancy (400 µg/day) and lactation (300 µg/day).

Dietary sources

Folic acid is widely distributed in nature. The rich sources are green leafy vegetables, whole grains, cereals, liver, kidney, yeast and eggs. Milk is rather a poor source of folic acid.

Deficiency symptoms

Folic acid deficiency is probably the ***most common vitamin deficiency***, observed primarily in the pregnant women, in both developed (including USA) and developing countries (including India). The pregnant women, lactating women, women on oral contraceptives, and alcoholics are also susceptible to folate

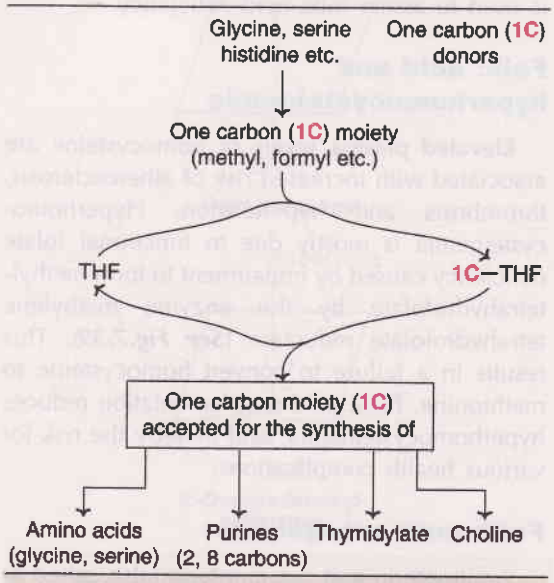


Fig. 7.34 : An overview of one carbon metabolism (THF-Tetrahydrofolate).

deficiency. The folic acid deficiency may be due to (one or more causes) ***inadequate dietary intake, defective absorption, use of anticonvulsant drugs*** (phenobarbitone, dilantin, phenyltoin), ***and increased demand.***

In folic acid deficiency, decreased production of purines and dTMP is observed which impairs DNA synthesis. Due to a block in DNA synthesis, the maturation of erythrocytes is slowed down leading to macrocytic RBC. The rapidly dividing cells of bone marrow are seriously affected. The ***macrocytic anemia*** (abnormally large RBC) associated with megaloblastic changes in bone marrow is a characteristic feature of folate deficiency.

Folic acid deficiency in pregnant women may cause neural defects in the fetus. Hence high doses of folic acid are recommended in pregnancy to prevent birth defects.

Folic acid is associated with the metabolism of histidine. Formiminoglutamate (FIGLU), formed in histidine metabolism transfers the one carbon fragment, formimino group (−CH=NH) to tetrahydrofolate to produce N^5-formimino THF. In case of folic acid deficiency, ***FIGLU accumulates and is excreted in urine.*** Histidine load test utilizing the excretion of FIGLU in urine is used to assess folic acid deficiency.

Folic acid and hyperhomocysteinemia

Elevated plasma levels of homocysteine are associated with increased risk of atherosclerosis, thrombosis and hypertension. Hyperhomocysteinemia is mostly due to functional folate deficiency caused by impairment to form methyltetrahydrofolate by the enzyme methylene tetrahydrofolate reductase (***See Fig.7.39***). This results in a failure to convert homocysteine to methionine. Folic acid supplementation reduces hyperhomocysteinemia, and thereby the risk for various health complications.

Folic acid antagonists

Aminopterin and amethopterin (also called as methotrexate) are structural analogues of folic acid. They competitively ***inhibit dihydrofolate reductase*** and block the formation of THF. The biosynthesis of purines, thymine nucleotides and hence DNA is impaired. This results in the blockage of cell proliferation. ***Aminopterin and methotrexate are successfully used in the treatment of many cancers, including leukemia.***

Trimethoprim (a component of the drug septran or bactrim) and pyrimethamine (antimalarial drug) are structurally related to folic acid. They inhibit dihydrofolate reductase, and the formation of THF.

Sulfonamides : Folic acid, as such, cannot enter bacterial cells. However, bacteria can synthesize folic acid from pteridine, PABA and glutamate. Sulfonamides are structural analogues of PABA. They competitively inhibit the enzyme (dihydropteroate synthase) responsible for the incorporation of PABA into pteridine to produce folic acid. For this reason, sulfonamides are used as antibacterial drugs. Sulfonamides, have no effect on human body, since folic acid is not synthesized and supplied through the diet.

COBALAMIN (VITAMIN B_{12})

Vitamin B_{12} is also known as ***anti-pernicious anemia vitamin***. It is a unique vitamin, synthesized by only microorganisms and not by animals and plants. It was the last vitamin to be discovered.

Chemistry

Vitamin B_{12} is the only vitamin with a complex structure. The empirical formula of vitamin B_{12} (cyanocobalamin) is $C_{63}H_{90}N_{14}O_{14}PCo$. The structure of vitamin B_{12} consists of a corrin ring with a ***central cobalt atom***. The corrin ring is almost similar to the tetrapyrrole ring structure found in other porphyrin compounds e.g. heme (with Fe) and chlorophyll (with Mg).

The corrin ring has four pyrrole units, just like a porphyrin. Two of the pyrrole units (A and D) are directly bound to each other whereas the other two (B and C) are held by methene bridges. The groups namely methyl, acetamide and

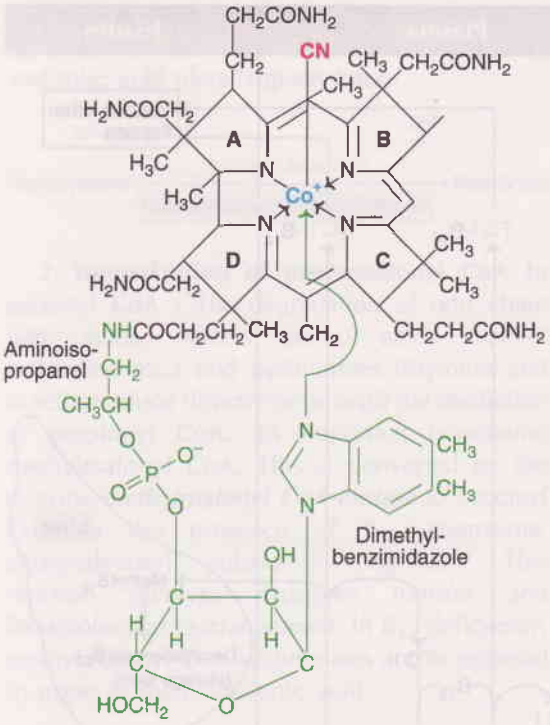


Fig. 7.35 : Structure of vitamin B_{12} (cyanocobalamin).

propionamide are the substituents on the pyrrole rings. Vitamin B_{12} has cobalt atom in a coordination state of six. Cobalt present at the centre of the corrin ring is bonded to the four pyrrole nitrogens. Cobalt also holds (below the corrin plane) dimethylbenzimidazole (DMB) containing ribose 5-phosphate and amino-isopropanol. A nitrogen atom of dimethyl-benzimidazole is linked to cobalt. The amide group of aminoisopropanol binds with D ring of corrin. The cobalt atom also possesses a sixth substituent group located above the plane of corrin ring (***Fig.7.35***). The substituent group may be one of the following

1. Cyanide (predominant) in cyanocobalamin (B_{12a})
2. Hydroxyl in hydroxycobalamin (B_{12b})
3. Nitrite in nitrocobalamin (B_{12c}).

There are two coenzyme forms of vitamin B_{12} (***Fig.7.36***).

(a) **5′-Deoxyadenosyl cobalamin**, cyanide is replaced by 5′ deoxyadenosine forming an unusal carbon cobalt bond.

(b) **Methylcobalamin** in which cyanide is replaced by methyl group.

Absorption, transport and storage

The vitamin B_{12} is present in the diet in a bound form to proteins. B_{12} is liberated by the enzymes (acid hydrolases) in the stomach. The dietary source of B_{12} is known as ***extrinsic factor of Castle***. The stomach secretes a special protein called ***intrinsic factor (IF)***. It is a glycoprotein (8-15% carbohydrate) with a molecular weight

*Fig. 7.36 : Coenzyme derivatives of vitamin B_{12} (**Note** : Corrin ring represented diagrammatically is identical in all; DMB-Dimethylbenzimidazole).*

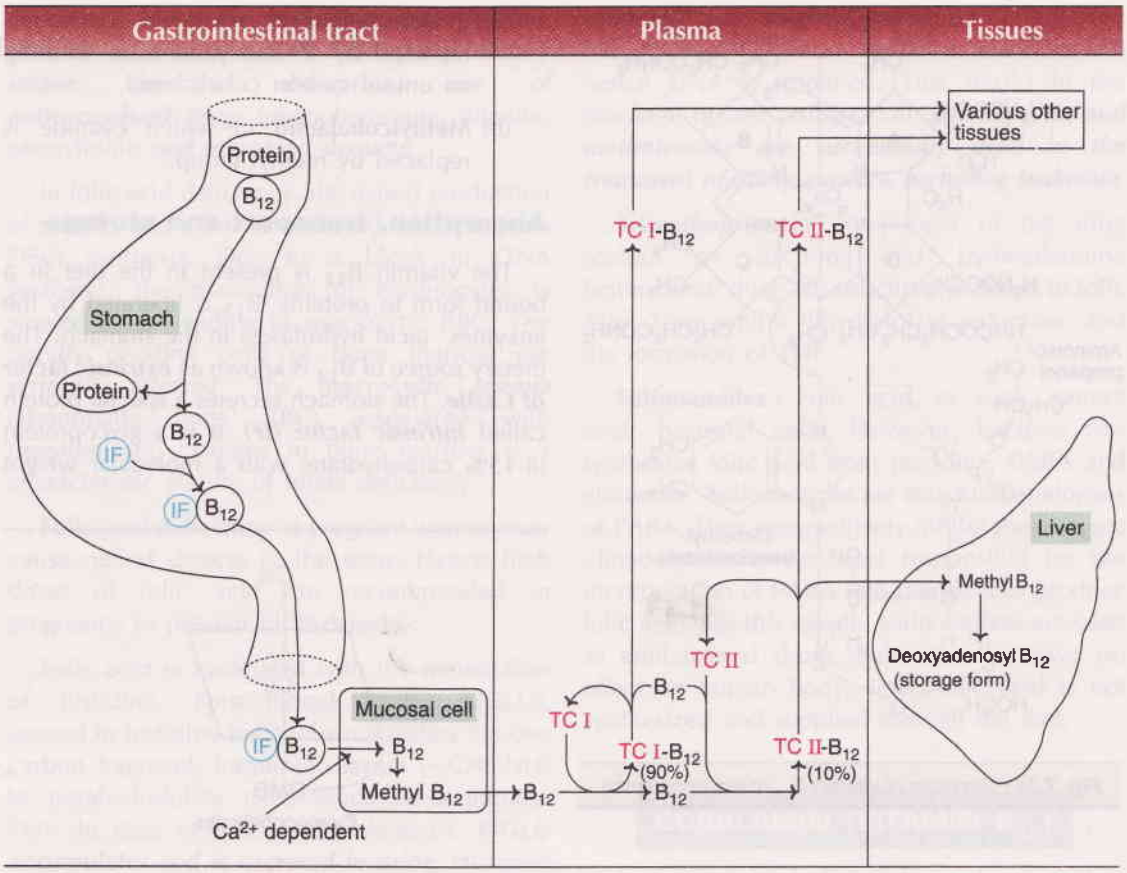


***Fig. 7.37** : Absorption, transport and storage of vitamin B_{12} (IF-Intrinsic factor; TC-Transcobalamins (TC–I, TC–II).*

around 50,000. Intrinsic factor is resistant to proteolytic digestive enzymes. IF generally forms a dimer, binds strongly with 1 or 2 moles of vitamin B_{12}. This binding protects vitamin B_{12} against its uptake and use by bacteria.

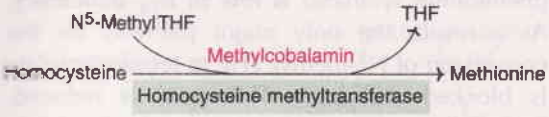
The cobalamin–IF complex travels through the gut. The complex binds to specific receptors on the surface of the mucosal cells of the ileum. The binding of the complex and entry of B_{12} into the mucosal cells is mediated by Ca^{2+} ions. In the mucosal cells, B_{12} is converted to methylcobalamin (***Fig.7.37***). It is then transported in the circulation in a bound form to proteins namely ***transcobalamins*** (TC-I, TC-II). Methylcobalamin is mostly bound to TC-I (90%) and to a lesser degree to TC-II (10%). It is believed that TC-I acts as a repository of B_{12}, while TC-II mediates the tissue uptake of B_{12}. Methylcobalamin which is in excess is taken up by the liver, converted to deoxyadenosyl B_{12} and stored in this form. It is believed that liver can store about 4-5 mg, an amount sufficient to meet the body requirements of B_{12} for 4-6 years.

Biochemical functions

About ten enzymes requiring vitamin B_{12} have been identified. Most of them are found in bacteria (glutamate mutase, ribonucleotide reductase etc.). There are only two reactions in mammals that are dependent on vitamin B_{12}.

1. **Synthesis of methionine from homocysteine :** Vitamin B_{12}, as methylcobalamin is used in this reaction. This is an important reaction involving N^5-methyl tetrahydrofolate from which tetrahydrofolate is liberated (enzyme-homocysteine methyltransferase or

methionine synthase). This metabolic step signifies the interrelation between vitamin B_{12} and folic acid (details given later)



2. **Isomerization of methymalonyl CoA to succinyl CoA :** The degradation of odd chain fatty acids, certain amino acids (valine, isoleucine etc.) and pyrimidines (thymine and uracil) produce directly or through the mediation of propionyl CoA, an important compound methylmalonyl CoA. This is converted by the enzyme ***methylmalonyl CoA mutase*** to succinyl CoA in the presence of B_{12} coenzyme, deoxyadenosyl cobalamin (***Fig.7.38***). This reaction involves hydrogen transfer and intramolecular rearrangement. In B_{12} deficiency, methylmalonyl CoA accumulates and is excreted in urine as methylmalonic acid.

Recommended dietary allowance (RDA)

A daily intake of about ***3 μg*** of vitamin B_{12} is adequate to meet the adult requirements. For children, ***0.5-1.5 μg/day*** is recommended. During pregnancy and lactation, the requirement is ***4 μg/day***.

Dietary sources

Foods of animal origin are the only sources for vitamin B_{12}. The rich sources are liver, kidney, milk, curd, eggs, fish, pork and chicken. Curd is a better source than milk, due to the synthesis of B_{12} by *Lactobacillus*.

Vitamin B_{12} is synthesized only by micro-organisms (anaerobic bacteria). Plants cannot synthesize, hence B_{12} is never found in plant foods. Animals obtain B_{12} either by eating foods, derived from other animals or from the intestinal bacterial synthesis.

Deficiency symptoms

The most important disease associated with vitamin B_{12} deficiency is ***pernicious anemia***. It is characterized by low hemoglobin levels, decreased number of erythrocytes and neurological manifestations. One or more of the following causes are attributed to the occurrence of pernicious anemia.

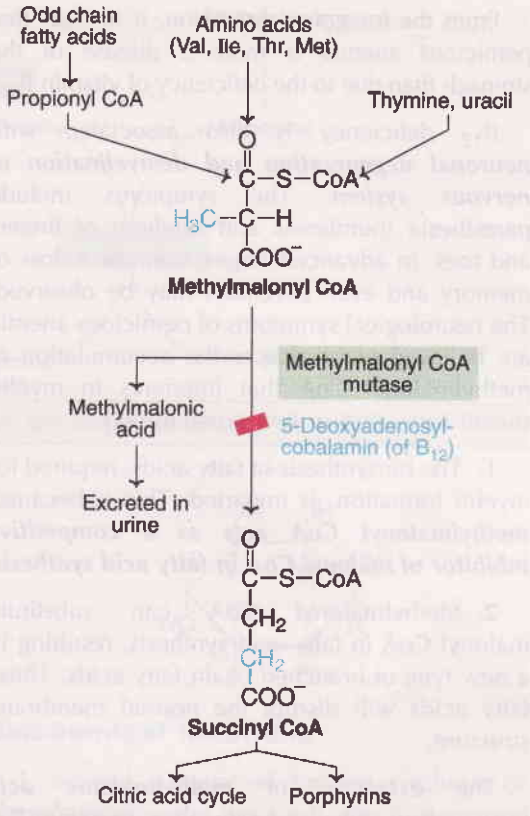


Fig. 7.38 : Role of vitamin B_{12} in isomerization of methylmalonyl CoA to succinyl CoA (■-Blockade in B_{12} deficiency).

1. Autoimmune destruction of gastric parietal cells that secrete intrinsic factor. In the absence of IF, vitamin B_{12} cannot be absorbed.

2. Hereditary malabsorption of vitamin B_{12}.

3. Partial or total gastrectomy – these individuals become intrinsic factor deficient.

4. Insufficient production of IF and/or gastric HCl, occasionally seen in older people.

5. Dietary deficiency of B_{12} is seen among the strict vegetarians of low socioeconomic group in the developing countries (India, Srilanka etc.).

From the foregoing discussion, it is clear that pernicious anemia is more a disease of the stomach than due to the deficiency of vitamin B_{12}.

B_{12} deficiency is also associated with ***neuronal degeneration and demyelination of nervous system.*** The symptoms include paresthesia (numbness and tingling) of fingers and toes. In advanced stages, confusion, loss of memory and even psychosis may be observed. The neurological symptoms of pernicious anemia are believed to be due to the accumulation of methylmalonyl CoA that interferes in myelin sheath formation in two possible ways.

1. The biosynthesis of fatty acids, required for myelin formation, is imparied. This is because, ***methylmalonyl CoA acts as a competitive inhibitor of malonyl CoA in fatty acid synthesis.***

2. Methylmalonyl CoA can substitute malonyl CoA in fatty acid synthesis, resulting in a new type of branched chain fatty acids. These fatty acids will disrupt the normal membrane structure.

The excretion of ***methylmalonic acid*** (elevated) in urine and estimation of serum B_{12} level are used to assess B_{12} deficiency.

Treatment

Vitamin B_{12} is administered in therapeutic doses (100-1000 µg) intramuscularly. Folic acid administration can also reverse hematological abnormalities observed in B_{12} deficiency. However, the neurological symptoms persist. Therefore, a combined supplementation of B_{12} and folate is employed to treat the patients with megaloblastic anemias.

INTERRELATION BETWEEN FOLIC ACID AND VITAMIN B_{12} —FOLATE TRAP OR METHYL TRAP HYPOTHESIS

The deficiency of either folate or vitamin B_{12} results in a similar type of anemia. This suggests a probable biochemical interrelation between these two vitamins. There is only one metabolic reaction known, common to folate and vitamin B_{12} (***Fig.7.39***).

In vitamin B_{12} deficiency, increased folate levels are observed in plasma. The activity of the enzyme ***homocysteine methyltransferase*** (methionine synthase) ***is low in B_{12} deficiency.*** As a result, the only major pathway for the conversion of N^5-methyl THF to tetrahydrofolate is blocked and body THF pool is reduced. Essentially, almost the entire body folate becomes trapped as N^5-methyl THF. This is known as ***folate trap or methyl trap.*** In this manner, B_{12} deficiency results in decreased folate coenzymes that leads to reduced nucleotide and DNA synthesis.

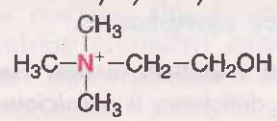
Although the tissue folate levels are adequate or high, there is a ***functional folate deficiency*** due to the lack of THF pool. The outcome is the development of ***megaloblastic anemia.*** Administration of the amino acid methionine has been shown to partially correct the symptoms of B_{12} deficiency. This is due to the fact that the formation of N^5-methyl THF is inhibited by S-adenosylmethionine. A combined therapy of vitamin B_{12} and folic acid is generally employed to treat the patients with megaloblastic anemia.

VITAMIN LIKE COMPOUNDS

Besides the vitamins described above, there are many other compounds present in foods as accessory factors. Earlier workers have described these factors sometime or the other, as essential to higher animals. However, their essential nature and requirement in humans has not been established. Although not essential in the diet, they perform many important functions in the body. Selected examples of such substances which may be regarded as vitamin like compounds are described here.

CHOLINE

Choline is ***trimethylhydroxy ethanolamine.***



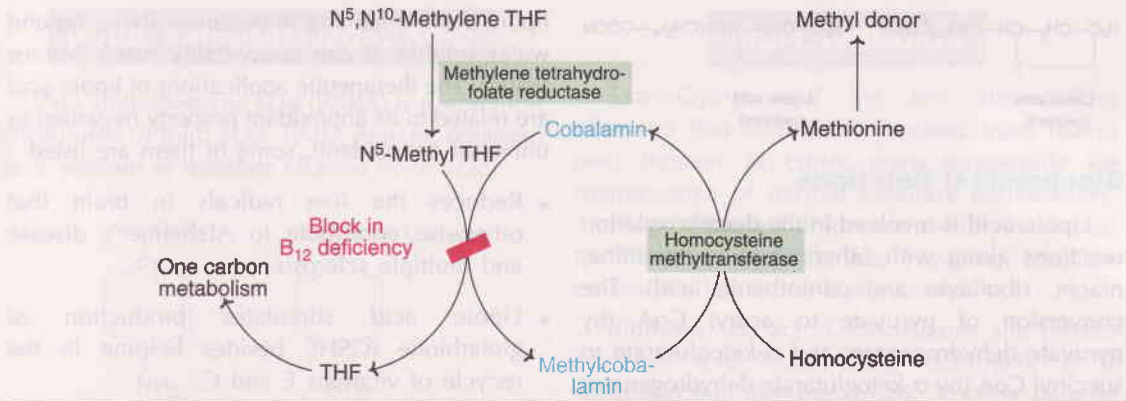


Fig. 7.39 : Interrelationship between folic acid and vitamin B_{12}

It can be synthesized in the body (from serine). It is also available from many dietary sources (e.g. milk, eggs, liver, cereals etc.).

Biochemical functions

1. Choline, as a component of ***phospholipids*** (lecithins), is involved in membrane structure and lipid transport.

2. Choline prevents the accumulation of fat in liver (as ***lipotropic factor***). It promotes the synthesis of phospholipids and lipoproteins and the disposal of ***triacylglycerols*** from liver.

3. Due to the presence of three methyl groups (one carbon fragments), choline is actively involved in ***one carbon metabolism***.

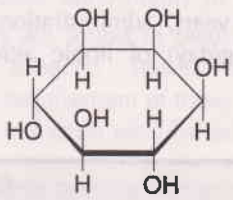
4. Choline is a precursor for the synthesis of acetylcholine which is required for transmission of nerve impulse.

Choline—an essential nutrient?

As such, choline can be synthesized and reutilized in humans. This may however, be insufficient to meet the body needs. Some workers label choline as an essential dietary nutrient with RDA in the range of 400–500 mg/day.

INOSITOL

Inositol is ***hexahydroxy-cyclohexane***. It is also known as *myo*-inositol or *meso*-inositol.



Biochemical functions

1. Inositol is required for the synthesis of ***phosphatidylinositol*** (lipositol) which is a constituent of cell membrane.

2. It acts as a ***lipotropic factor*** (along with choline) and prevents the accumulation of fat in liver.

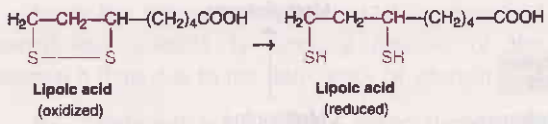
3. For some hormones, inositol acts as a ***second messenger*** at the membrane level for the release of Ca^{2+} ions.

4. Inositol concentration in the heart muscle in high, the significance of which however, is not known.

5. Phytin is hexaphosphate of inositol found is plants. It prevents the absorption of iron and calcium from the intestine.

LIPOIC ACID

Lipoic acid (thioctic acid) is a sulfur containing fatty acid (6,8-dithiooctanoic acid). It exists in an oxidized and reduced form. Lipoic acid is fat as well as water soluble.



Biochemical functions

Lipoic acid is involved in the decarboxylation reactions along with other vitamins (thiamine, niacin, riboflavin and pantothenic acid). The conversion of pyruvate to acetyl CoA (by pyruvate dehydrogenase) and α-ketoglutarate to succinyl CoA (by α-ketoglutarate dehydrogenase) requires lipoic acid.

Therapeutic uses of lipoic acid

In recent years, administration of high doses (100–600 mg/day) of lipoic acid (or dihydrolipoic acid) is gaining importance. Being fat and water soluble, it can comfortably reach various tissues. The therapeutic applications of lipoic acid are related to its antioxidant property (regarded as universal antioxidant), some of them are listed

- Reduces the free radicals in brain that otherwise contribute to Alzheimer's disease and multiple sclerosis.
- Lipoic acid stimulates production of glutathione (GSH), besides helping in the recycle of vitamins E and C.
- Reduces insulin resistance, and brings down plasma low density lipoproteins.
- May be useful in the prevention of stroke and myocardial infarction.

BIOMEDICAL / CLINICAL CONCEPTS

☞ *Distinct deficiency conditions of certain B-complex vitamins are known*

Thiamine — Beri-beri	*Riboflavin — Cheilosis, glossitis*
Niacin — Pellagra	*Pyridoxine — Peripheral neuropathy*
Folic acid — Macrocytic anemia	*Cobalamin — Pernicious anemia*

☞ *B-complex vitamin deficiencies are usually multiple rather than individual with overlapping symptoms.*

☞ *A combined therapy of vitamin B_{12} and folic acid is commonly employed to treat the patients of megaloblastic anemias.*

☞ *Megadoses of niacin are useful in the treatment of hyperlipidemia.*

☞ *Long term use of isoniazid for the treatment of tuberculosis causes B_6 deficiency.*

☞ *Folic acid supplementation reduces elevated plasma homocysteine level which is associated with atherosclerosis and thrombosis.*

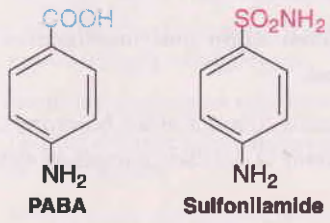
☞ *Sulfonamides serve as antibacterial drugs by inhibiting the incorporation of PABA to produce folic acid.*

☞ *Aminopterin and amethopterin, the structural analogues of folic acid, are employed in the treatment of cancers.*

☞ *Lipoic acid is therapeutically useful as an antioxidant to prevent stroke, myocardial infarction, etc.*

PARA AMINOBENZOIC ACID

Para aminobenzoic acid (PABA) is a structural constituent of folic acid. PABA may be regarded as a ***vitamin in another vitamin*** (folic acid)



The deficiency of PABA was first found to be associated with failure of lactation and greying of black hair in rats. The specific functions of PABA in humans, except that it is a component of folic acid, have not been identified.

PABA is synthesized by the bacteria and is essential for their growth. The sulfa drug sulfonilamide (p-amino benzene sulfanilamide) is a structural analogue of PABA. Sulfonilamide competes with PABA and acts as a bacteriostatic agent. Ingestion of large doses of PABA will compete with the action of drugs and therefore should be avoided during sulfonilamide therapy (trade name—sulfonamides).

BIOFLAVONOIDS

Szent-Gyorgi and his associates (1936) observed that flavonoids, isolated from lemon peel (known as citrin) were responsible for maintenance of normal capillary permeability. The term ***vitamin P*** (P for permeability) was used to this group of substances. However, they are commonly known as bioflavonoids.

Bioflavonoids act as antioxidants and protect ascorbic acid from being destroyed. It is suggested that this antioxidant property may be responsible for maintenance of capillary permeability. Bioflavonoids have been used to correct the vascular abnormality in humans.

Bioflavonoids are found in peel and pulp of citrus fruits, tobacco leaves and many vegetables. The requirement of these compounds in humans has not been established.

ANTIVITAMINS

Antivitamins are ***antagonistic to*** (oppose and block) the ***action of vitamins***. They usually have structural similarities with vitamins. Administration of antivitamins causes vitamin deficiencies. The common antivitamins are discussed as antagonists for each vitamin.

SUMMARY

1. *Vitamins are accessory food factors required in the diet. They are classified as fat soluble (A, D, E and K) and water soluble (B-complex and C).*
2. *Vitamin A is involved in vision, proper growth, differentiation and maintenance of epithelial cells. Its deficiency results in night blindness.*
3. *The active form of vitamin D is calcitriol which functions like a steroid hormone and regulates plasma levels of calcium and phosphate. Vitamin D deficiency leads to rickets in children and osteomalacia in adults.*
4. *Vitamin E is a natural antioxidant necessary for normal reproduction in many animals.*
5. *Vitamin K has a specific coenzyme function. It catalyses the carboxylation of glutamic acid residues in blood clotting factors (II, VII, IX and X) and converts them to active form.*
6. *Thiamine (B_1), as a cocarboxylase (TPP) is involved in energy releasing reactions. Its deficiency leads to beri-beri.*
7. *The coenzymes of riboflavin (FAD and FMN) and niacin (NAD^+ and $NADP^+$) take part in a variety of oxidation-reduction reactions connected with energy generation. Riboflavin deficiency results in cheilosis and glossitis whereas niacin deficiency leads to pellagra.*
8. *Pyridoxal phosphate (PLP), the coenzyme of vitamin B_6, is mostly associated with amino acid metabolism. PLP participates in transamination, decarboxylation, deamination and condensation reactions.*
9. *Biotin (anti-egg white injury factor) participates as a coenzyme in carboxylation reactions of gluconeogenesis, fatty acid synthesis etc.*
10. *Coenzyme A (of pantothenic acid) is involved in the metabolism of carbohydrates, lipids and amino acids, and their integration.*
11. *Tetrahydrofolate (THF), the coenzyme of folic acid participates in the transfer of one carbon units (formyl, methyl etc.) in amino acid and nucleotide metabolism. Megaloblastic anemia is caused by folic acid deficiency.*
12. *Vitamin B_{12} has two coenzymes, deoxyadenosylcobalamin and methylcobalamin. B_{12} deficiency results in pernicious anemia.*
13. *Vitamin C (ascorbic acid) is involved in the hydroxylation of proline and lysine in the formation of collagen. Scurvy is caused by ascorbic acid deficiency. Therapeutic use of megadoses of vitamin C, to cure everything from common cold to cancer, has become controversial.*
14. *Certain vitamin like compounds (choline, inositol, PABA, lipoic acid) participate in many biochemical reactions.*

SELF-ASSESSMENT EXERCISES

I. Essay questions

1. Classify vitamins and briefly discuss their functions and deficiency disorders.
2. Describe the chemistry, biochemical functions, daily requirements, sources and deficiency manifestations of vitamin A.
3. Write an account of folic acid involvement in one carbon metabolism.
4. Discuss the biochemical functions of vitamin C. Add a note on the therapeutic use of megadoses of this vitamin.
5. Write briefly about the coenzymes involved in oxidation-reduction reactions.

II. Short notes

(a) Vitamin D is a hormone-justify, (b) Thiamine pyrophosphate, (c) Coenzymes of niacin, (d) Pyridoxal phosphate in transamination, (e) Folate trap, (f) Tocopherol, (g) Vitamin K in carboxylation, (h) Biocytin, (i) Choline, (j) Pernicious anemia.

III. Fill in the blanks

1. The A in coenzyme A stands for_____.
2. The vitamin containing isoalloxazine ring_____.
3. The vitamin that is regarded as a vitamin in search of a disease_____.
4. Anti-tuberculosis drug, isonicotinic acid hydrazide (INH) leads to the deficiency of vitamin_____.
5. The egg injury factor present in raw egg white_____.
6. The 'burning feet syndrome' in man is associated with the deficiency of_____.
7. The vitamin that is synthesized by only microorganisms_____.
8. The three Ds in pellagra stand for, _____, _____ and _____.
9. The fat soluble vitamin required for carboxylation reaction_____.
10. FIGLU (formimino glutamic acid) is excreted in urine in the deficiency of vitamin_____.

IV. Multiple choice questions

11. Which one of the vitamin A functions as a steroid hormone

    (a) Retinal (b) Retinol (c) Provitamin A (d) β-Carotene.

12. The functionally active form of vitamin D is

    (a) Cholecalciferol (b) Ergocalciferol (c) Dehydrocholesterol (d) Calcitriol.

13. The metabolite excreted in urine in thiamine deficiency

    (a) Pyruvate (b) Glucose (c) Xanthurenic acid (d) FIGLU.

14. The coenzyme directly concerned with the synthesis of biogenic amines

    (a) TPP (b) $NADP^+$ (c) Biotin (d) Pyridoxal phosphate.

15. Folic acid antagonist(s) used in the treatment of cancer

    (a) Methotrexate (b) Trimethoprim (c) Sulfonamide (d) All the three.