C1 Introduction to enzymes

Key Notes

Enzymes as catalysts

Enzymes are catalysts that change the rate of a reaction without being changed themselves. Enzymes are highly specific and their activity can be regulated. Virtually all enzymes are proteins, although some catalytically active RNAs have been identified.

Active site

The active site is the region of the enzyme that binds the substrate, to form an enzyme–substrate complex, and transforms it into product. The active site is a three-dimensional entity, often a cleft or crevice on the surface of the protein, in which the substrate is bound by multiple weak interactions. Two models have been proposed to explain how an enzyme binds its substrate: the lock-and-key model and the induced-fit model.

Substrate specificity

The substrate specificity of an enzyme is determined by the properties and spatial arrangement of the amino acid residues forming the active site. The serine proteases trypsin, chymotrypsin and elastase cleave peptide bonds in protein substrates on the carboxyl side of positively charged, aromatic and small side-chain amino acid residues, respectively, due to complementary residues in their active sites.

Enzyme classification

Enzymes are classified into six major groups on the basis of the type of reaction that they catalyze. Each enzyme has a unique four-digit classification number.

Enzyme assays

An enzyme assay measures the conversion of substrate to product, under conditions of cofactors, pH and temperature at which the enzyme is optimally active. High substrate concentrations are used so that the initial reaction rate is proportional to the enzyme concentration. Either the rate of appearance of product or the rate of disappearance of substrate is measured, often by following the change in absorbance using a spectrophotometer. Reduced nicotinamide adenine dinucleotide (NADH) and reduced nicotinamide adenine dinucleotide phosphate (NADPH), which absorb light at 340 nm, are often used to monitor the progress of an enzyme reaction.

Linked enzyme assays

If neither the substrates nor products of an enzyme-catalyzed reaction absorb light at an appropriate wavelength, the enzyme can be assayed by linking it to another enzyme-catalyzed reaction that does involve a change in absorbance. The second enzyme must be in excess, so that the rate-limiting step in the linked assay is the action of the first enzyme.

Coenzymes and prosthetic groups

Some enzymes require the presence of cofactors, small nonprotein units, to function. Cofactors may be inorganic ions or complex organic molecules called coenzymes. A cofactor that is covalently attached to the enzyme is called a prosthetic group. A holoenzyme is the catalytically active form of the enzyme with its cofactor, whereas an apoenzyme is the

protein part on its own. Many coenzymes are derived from dietary vitamin precursors, and deficiencies in them lead to certain diseases. Nicotinamide adenine dinucleotide (NAD^+), nicotinamide adenine dinucleotide phosphate ($NADP^+$), flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN) are widely occurring coenzymes involved in oxidation–reduction reactions.

Isoenzymes

Isoenzymes are different forms of an enzyme which catalyze the same reaction, but which exhibit different physical or kinetic properties. The isoenzymes of lactate dehydrogenase (LDH) can be separated electrophoretically and can be used clinically to diagnose a myocardial infarction.

Related topics

Bioimaging (A4)
Protein structure (B3)
Protein purification (B6)
Electrophoresis of proteins (B7)
Thermodynamics (C2)
Enzyme kinetics (C3)
Enzyme inhibition (C4)
Regulation of enzyme activity (C5)

Enzymes as catalysts

Enzymes are **catalysts** that increase the rate of a chemical reaction without being changed themselves in the process. In the absence of an enzyme, the reaction may hardly proceed at all, whereas in its presence the rate can be increased up to 10^7-fold. Enzyme catalyzed reactions usually take place under relatively mild conditions (temperatures well below 100°C, atmospheric pressure and neutral pH) as compared with the corresponding chemical reactions. Enzymes are also **highly specific** with respect to the substrates on which they act and the products that they form. In addition, enzyme activity can be **regulated**, varying in response to the concentration of substrates or other molecules (see Topic C5). Nearly all enzymes are **proteins**, although a few catalytically active **RNA molecules** have been identified.

Active site

The **active site** of an enzyme is the region that **binds the substrate** and converts it into product. It is usually a relatively small part of the whole enzyme molecule and is a **three-dimensional entity** formed by amino acid residues that can lie far apart in the linear polypeptide chain (see Topic B3). The active site is often a cleft or crevice on the surface of the enzyme that forms a predominantly nonpolar environment which enhances the binding of the substrate. The substrate(s) is bound in the active site by **multiple weak forces** (electrostatic interactions, hydrogen bonds, van der Waals bonds, hydrophobic interactions; see Topic B3) and in some cases by reversible covalent bonds. Having bound the substrate molecule, and formed an **enzyme–substrate complex**, catalytically active residues within the active site of the enzyme act on the substrate molecule to transform it first into the transition state complex (see Topic C2) and then into product, which is released into solution. The enzyme is now free to bind another molecule of substrate and begin its catalytic cycle again.

Originally two models were proposed to explain how an enzyme binds its substrate. In the **lock-and-key** model proposed by Emil Fischer in 1894, the shape of the substrate and the active site of the enzyme are thought to fit together like a key into its lock (*Fig. 1a*). The two shapes are considered as rigid and fixed, and perfectly complement each other when brought together in the

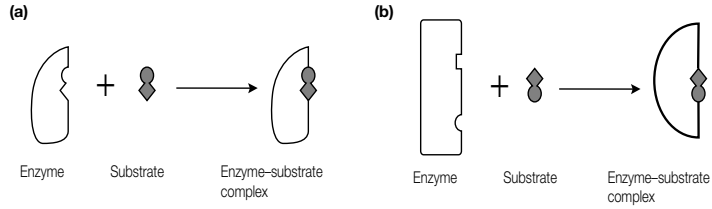

Fig. 1. Binding of a substrate to an enzyme. (a) Lock-and-key model; (b) induced-fit model.

right alignment. In the **induced-fit model** proposed in 1958 by Daniel E. Koshland, Jr., the binding of substrate **induces a conformational change** in the active site of the enzyme (*Fig. 1b*). In addition, the enzyme may distort the substrate, forcing it into a conformation similar to that of the transition state (see Topic C2). For example, the binding of **glucose** to **hexokinase** induces a conformational change in the structure of the enzyme such that the active site assumes a shape that is complementary to the substrate (glucose) only after it has bound to the enzyme. The reality is that different enzymes show features of both models, with some complementarity and some conformational change.

Substrate specificity

The properties and spatial arrangement of the amino acid residues forming the active site of an enzyme will determine which molecules can bind and be substrates for that enzyme. **Substrate specificity** is often determined by changes in relatively few amino acids in the active site. This is clearly seen in the three digestive enzymes **trypsin**, **chymotrypsin** and **elastase** (see Topic C5). These three enzymes belong to a family of enzymes called the **serine proteases** – 'serine' because they have a serine residue in the active site that is critically involved in catalysis and 'proteases' because they catalyze the hydrolysis of peptide bonds in proteins. The three enzymes cleave peptide bonds in protein substrates on the carboxyl side of certain amino acid residues.

Trypsin cleaves on the carboxyl side of positively charged Lys or Arg residues, chymotrypsin cleaves on the carboxyl side of bulky aromatic and hydrophobic amino acid residues, and elastase cleaves on the carboxyl side of residues with small uncharged side-chains. Their differing specificities are determined by the nature of the amino acid groups in their substrate binding sites which are complementary to the substrates upon which they act. Thus trypsin has a negatively charged Asp residue in its substrate binding site which interacts with the positive charge on the Lys and Arg side-chains of the substrate (*Fig. 2a*). Chymotrypsin has amino acid residues with small side-chains, such as Gly and Ser, in its substrate binding site that allow access of the bulky side-chain of the substrate (*Fig. 2b*). In contrast, elastase has the relatively large uncharged amino acid side-chains of Val and Thr protruding into its substrate binding site, preventing access of all but the small side-chains on Gly and Ala (*Fig. 2c*).

Enzyme classification

Many enzymes are named by adding the suffix '-ase' to the name of their substrate. Thus **urease** is the enzyme that catalyzes the hydrolysis of urea, and **fructose-1,6-bisphosphatase** hydrolyzes fructose-1,6-bisphosphate. However, other enzymes, such as trypsin and chymotrypsin, have names that do not denote their substrate. Some enzymes have several alternative names. To

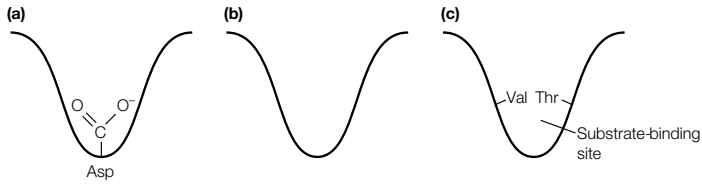

Fig. 2. Schematic representation of the substrate-binding sites in the serine proteases (a) trypsin, (b) chymotrypsin and (c) elastase.

rationalize enzyme names, a system of **enzyme nomenclature** has been internationally agreed. This system places all enzymes into one of **six major classes** based on the type of reaction catalyzed (*Table 1*). Each enzyme is then uniquely identified with a four-digit classification number. Thus trypsin has the Enzyme Commission (EC) number 3.4.21.4, where the first number (3) denotes that it is a hydrolase, the second number (4) that it is a protease that hydrolyzes peptide bonds, the third number (21) that it is a serine protease with a critical serine residue at the active site, and the fourth number (4) indicates that it was the fourth enzyme to be assigned to this class. For comparison, chymotrypsin has the EC number 3.4.21.1, and elastase 3.4.21.36.

Enzyme assays

The amount of enzyme protein present can be determined (**assayed**) in terms of the catalytic effect it produces, that is the conversion of substrate to product (see also Topic B6). In order to **assay** (monitor the activity of) an enzyme, the overall equation of the reaction being catalyzed must be known, and an analytical procedure must be available for determining either the disappearance of substrate or the appearance of product. In addition, one must take into account whether the enzyme requires any **cofactors**, and the **pH** and **temperature** at which the enzyme is optimally active (see Topic C3). For mammalian enzymes, this is usually in the range 25–37°C. Finally, it is essential that the rate of the reaction being assayed is a measure of the enzyme activity present and is not limited by an insufficient supply of substrate. Therefore, very high substrate concentrations are generally required so that the **initial reaction rate**, which is determined experimentally, is proportional to the enzyme concentration (see Topic C3).

Table 1. International classification of enzymes

Class	Name	Type of reaction catalyzed		Example
1	Oxidoreductases	Transfer of electrons	$A^- + B \rightarrow A + B^-$	Alcohol dehydrogenase
2	Transferases	Transfer of functional groups	$A–B + C \rightarrow A + B–C$	Hexokinase
3	Hydrolases	Hydrolysis reactions	$A–B + H_2O \rightarrow A–H + B–OH$	Trypsin
4	Lyases	Cleavage of C–C, C–O, C–N and other bonds, often forming a double bond	$A–B \rightarrow A{=}B + X–Y$ (X, Y)	Pyruvate decarboxylase
5	Isomerases	Transfer of groups within a molecule	$A–B \rightarrow A–B$ (X, Y; Y, X)	Maleate isomerase
6	Ligases (or synthases)	Bond formation coupled to ATP hydrolysis	$A + B \rightarrow A–B$	Pyruvate carboxylase

An enzyme is most conveniently assayed by measuring the **rate of appearance of product** or the **rate of disappearance of substrate**. If the substrate (or product) **absorbs light** at a specific wavelength, then changes in the concentration of these molecules can be measured by following the **change of absorbance** at this wavelength using a **spectrophotometer**. If the substrate (or product) **fluoresces** (see Topic A4), then changes in the concentration can be measured by following the **change in fluorescence** using a **fluorimeter**. Since absorbance (or fluorescence) is proportional to concentration, the rate of change in absorbance (or fluorescence) is proportional to the rate of enzyme activity in moles of substrate used (or product formed) per unit time.

Two of the most common molecules used for absorbance measurement in enzyme assays are the coenzymes **reduced nicotinamide adenine dinucleotide (NADH)** and **reduced nicotinamide adenine dinucleotide phosphate (NADPH)** (see below) which each absorb in the ultraviolet (UV) region at 340 nm. Thus, if NADH or NADPH is produced during the course of the reaction there will be a corresponding increase in absorbance at 340 nm, whilst if the reaction involves the oxidation of NADH or NADPH to NAD^+ or $NADP^+$, respectively, there will be a corresponding decrease in absorbance, since these oxidized forms do not absorb at 340 nm. One example is that the activity of **lactate dehydrogenase** with lactate as substrate can be assayed by following the increase in absorbance at 340 nm, according to the following equation:

$$\underset{\text{lactate}}{CH_3CH(OH)COO^-} + NAD^+ \rightleftharpoons \underset{\text{pyruvate}}{CH_3COCOO^-} + NADH + H^+$$

Linked enzyme assays

Numerous reactions do not involve substrates or products that absorb light at a suitable wavelength. In this case it is often possible to assay the enzyme that catalyzes this reaction by **linking** (or **coupling**) it to a second enzyme reaction that does involve a characteristic absorbance change. For example, the action of the enzyme **glucose oxidase**, which is often used to measure the concentration of glucose in the blood of diabetic patients, does not result in a change in absorbance upon conversion of substrates to products (*Fig. 3*). However, the hydrogen peroxide produced in this reaction can be acted on by a second enzyme, peroxidase, which simultaneously converts a colorless compound into a colored one (**chromogen**) whose absorbance can be easily measured (*Fig. 3*).

If the activity of the first enzyme (glucose oxidase) is to be measured accurately, the second enzyme (peroxidase) and its cosubstrates or coenzymes must be **in excess** so as not to be the **rate-limiting step** of the linked assay. This will ensure that the rate of production of the colored chromogen is proportional to

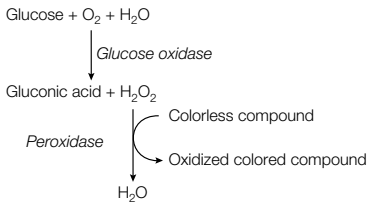

Fig. 3. A linked enzyme assay with glucose oxidase and peroxidase can be used to measure the amount of glucose in a blood sample.

the rate of production of H_2O_2, whose production in turn is proportional to the activity of glucose oxidase.

Coenzymes and prosthetic groups

Many enzymes require the presence of small, nonprotein units or **cofactors** to carry out their particular reaction. Cofactors may be either one or more **inorganic ions**, such as Zn^{2+} or Fe^{2+}, or a complex organic molecule called a **coenzyme**. A metal or coenzyme that is covalently attached to the enzyme is called a **prosthetic group** (cf. heme in hemoglobin; see Topic B4). A complete catalytically-active enzyme together with its coenzyme or metal ion is called a **holoenzyme**. The protein part of the enzyme on its own without its cofactor is termed an **apoenzyme**. Some coenzymes, such as NAD^+, are bound and released by the enzyme during its catalytic cycle and in effect function as cosubstrates. Many coenzymes are derived from **vitamin precursors** (*Table 2*) which are often essential components of the organism's diet, thus giving rise to **deficiency diseases** when in inadequate supply.

Nicotinamide adenine dinucleotide (**NAD^+**) and **nicotinamide adenine dinucleotide phosphate** (**$NADP^+$**) coenzymes are based on a common structure consisting of the base adenine, two ribose sugars linked by phosphate groups and a nicotinamide ring (*Fig. 4*). $NADP^+$ differs from NAD^+ in having an additional phosphate group attached to one of the ribose sugars (*Fig. 4*). These two coenzymes share a common function as they both act as carriers of electrons and are involved in **oxidation–reduction reactions**. NAD^+ is more commonly used in **catabolic** (breakdown) reactions, whilst $NADP^+$ is used in **anabolic** (biosynthetic) reactions. The reactive part of both molecules is the **nicotinamide ring** which exists in either a reduced or an oxidized form, and so acts to accept or donate electrons in an enzymic reaction. The reaction also involves the transfer of protons, according to the equation:

$$NAD^+ + H^+ + 2e^- \rightleftharpoons NADH$$

Flavin adenine dinucleotide (**FAD**) and **flavin mononucleotide** (**FMN**) are also carriers of electrons and have related chemical structures (*Fig. 5*). Both of these coenzymes consist of a **flavine mononucleotide unit** which contains the reactive site. FAD has an additional sugar group and an adenine base which complete its structure. FAD and FMN react with two protons, as well as two electrons, in alternating between the reduced and oxidized state:

$$FAD + 2H^+ + 2e^- \rightleftharpoons FADH_2$$

Table 2. Some common coenzymes, their vitamin precursors and deficiency diseases

Coenzyme	Precursor	Deficiency disease
Coenzyme A	Pantothenic acid	Dermatitis
FAD, FMN	Riboflavin (vitamin B_2)	Growth retardation
NAD^+, $NADP^+$	Niacin	Pellagra
Thiamine pyrophosphate	Thiamine (vitamin B_1)	Beriberi
Tetrahydrofolate	Folic acid	Anemia
Deoxyadenosyl cobalamin	Cobalamin (vitamin B_{12})	Pernicious anemia
Cosubstrate in the hydroxylation of proline in collagen	Vitamin C (ascorbic acid)	Scurvy
Pyridoxal phosphate	Pyridoxine (vitamin B_6)	Dermatitis

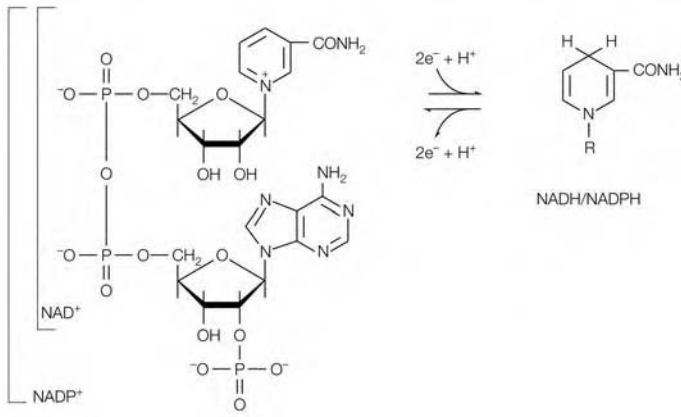

Fig. 4. The structures of the coenzymes NAD⁺ and NADP⁺.

Isoenzymes

Isoenzymes (isozymes) are different forms of an enzyme which **catalyze the same reaction**, but which exhibit **different physical or kinetic properties**, such as isoelectric point, pH optimum, substrate affinity or effect of inhibitors. Different isoenzyme forms of a given enzyme are usually derived from different genes and often occur in different tissues of the body.

An example of an enzyme which has different isoenzyme forms is **lactate dehydrogenase** (LDH) which catalyzes the reversible conversion of pyruvate into lactate in the presence of the coenzyme NADH (see above). LDH is a tetramer of two different types of **subunits**, called H and M, which have small

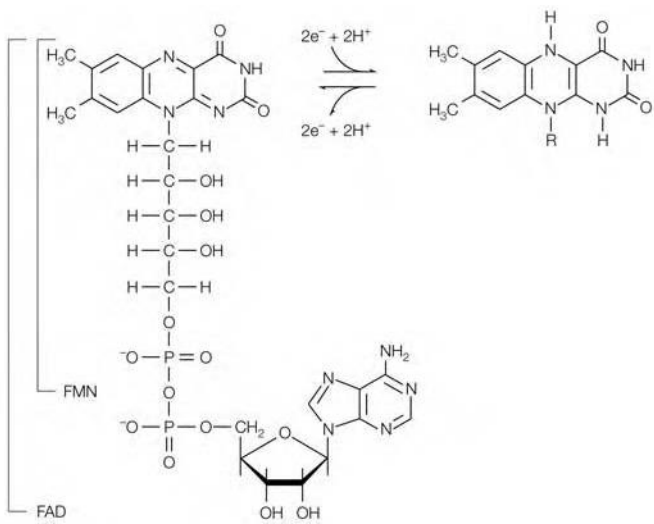

Fig. 5. The structures of the coenzymes FAD and FMN.

differences in amino acid sequence. The two subunits can combine randomly with each other, forming five isoenzymes that have the compositions H_4, H_3M, H_2M_2, HM_3 and M_4. The five isoenzymes can be resolved electrophoretically (see Topic B7). M subunits predominate in skeletal muscle and liver, whereas H subunits predominate in the heart. H_4 and H_3M isoenzymes are found predominantly in the heart and red blood cells; H_2M_2 is found predominantly in the brain; while HM_3 and M_4 are found predominantly in the liver and skeletal muscle. Thus, the isoenzyme pattern is characteristic of a particular tissue, a factor which is of immense diagnostic importance in medicine. Myocardial infarction, **infectious hepatitis** and **muscle diseases** involve cell death of the affected tissue, with release of the cell contents into the blood. As LDH is a soluble, cytosolic protein it is readily released in these conditions. Under normal circumstances there is little LDH in the blood. Therefore the pattern of LDH isoenzymes in the blood is indicative of the tissue that released the isoenzymes and so can be used to diagnose a condition and to monitor the progress of treatment. For the clinical diagnosis of a **myocardial infarction**, in addition to the pattern of LDH isoenzymes, other enzymes, including **creatine kinase** and **aspartate aminotransferase** are routinely measured, along with an electrocardiogram (ECG).

C2 THERMODYNAMICS

Key Notes

Thermodynamics

A knowledge of thermodynamics, which is the description of the relationships among the various forms of energy and how energy affects matter, enables one to determine whether a physical process is possible. The first and second laws of thermodynamics are combined in the thermodynamic function, free energy (*G*). The unit of energy is the Joule (J) or the calorie (cal).

Activation energy and transition state

For a biochemical reaction to proceed, the energy barrier needed to transform the substrate molecules into the transition state has to be overcome. The transition state has the highest free energy in the reaction pathway. The difference in free energy between the substrate and the transition state is termed the Gibbs free energy of activation ($\Delta G‡$). An enzyme stabilizes the transition state and lowers $\Delta G‡$, thus increasing the rate at which the reaction occurs.

Free energy change

The difference in energy level between the substrates and products is termed the change in Gibbs free energy (ΔG). A negative ΔG indicates that the reaction is thermodynamically favorable in the direction indicated, whereas a positive ΔG indicates that the reaction is not thermodynamically favorable and requires an input of energy to proceed in the direction indicated. An energetically unfavorable reaction is often driven by linking it to an energetically favorable reaction, such as the hydrolysis of ATP.

Chemical equilibria

A chemical reaction often exists in a state of dynamic equilibrium. The equilibrium constant (*K*) defines the ratio of the concentrations of substrates and products at equilibrium. Enzymes do not alter the equilibrium position, but do accelerate the attainment of the equilibrium position by speeding up the forward and reverse reactions.

Related topics

Introduction to enzymes (C1)
Enzyme kinetics (C3)
Enzyme inhibition (C4)
Regulation of enzyme activity (C5)

Thermodynamics

A knowledge of thermodynamics enables one to determine whether a physical process is possible, and is required for understanding why proteins fold to their native conformation, why some enzyme-catalyzed reactions require an input of energy, how muscles generate mechanical force, etc. **Thermodynamics** (Greek: *therme*, heat; *dynamis*, power) is the description of the relationships among the various forms of energy and how energy affects matter on the macroscopic level. As it applies to biochemistry, thermodynamics is most often concerned with describing the conditions under which processes occur spontaneously (by themselves).

In thermodynamics, a **system** is the matter within a defined region. The matter in the rest of the universe is called the **surroundings**. **The first law of thermodynamics**, a mathematical statement of the law of conservation of energy, states that the total energy of a system and its surroundings is a constant:

$$\Delta E = E_B - E_A = Q - W$$

in which E_A is the energy of the system at the start of a process and E_B at the end of the process. Q is the heat absorbed by the system and W is the work done by the system. The change in energy of a system depends only on the initial and final states and not on how it reached that state. Processes in which the system releases heat (i.e. have a negative Q) are known as **exothermic** processes; those in which the system gains heat (i.e. have a positive Q) are known as **endothermic**. The SI **unit of energy** is the Joule (J), although the calorie (cal) is still often used (1 kcal = 4.184 kJ).

The first law of thermodynamics cannot be used to predict whether a reaction can occur spontaneously, as some spontaneous reactions have a positive ΔE. Therefore a function different from ΔE is required. One such function is **entropy** (S), which is a measure of the degree of randomness or disorder of a system. The entropy of a system increases (ΔS is positive) when the system becomes more disordered. **The second law of thermodynamics** states that a process can occur spontaneously only if the sum of the entropies of the system and its surroundings increases (or that the universe tends towards maximum disorder), that is:

$$(\Delta S_{\text{system}} + \Delta S_{\text{surroundings}}) > 0 \text{ for a spontaneous process.}$$

However, using entropy as a criterion of whether a biochemical process can occur spontaneously is difficult, as the entropy changes of chemical reactions are not readily measured, and the entropy change of both the system and its surroundings must be known. These difficulties are overcome by using a different thermodynamic function, **free energy** (G), proposed by Josiah Willard Gibbs which combines the first and second laws of thermodynamics:

$$\Delta G = \Delta H - T\Delta S$$

in which ΔG is the free energy of a system undergoing a transformation at constant pressure (P) and temperature (T), ΔH is the change in **enthalpy** (heat content) of this system, and ΔS is the change in the entropy of this system. The enthalpy change is given by:

$$\Delta H = \Delta E + P\,\Delta V.$$

The volume change (ΔV) is small for nearly all biochemical reactions, and so ΔH is nearly equal to ΔE. Therefore

$$\Delta G = \Delta E - T\Delta S.$$

Thus, the ΔG of a reaction depends both on the change in internal energy and on the change in entropy of the system. The change in free energy ΔG of a reaction is a valuable criterion of whether that reaction can occur spontaneously:

- a reaction can occur spontaneously only if ΔG is negative;
- a system is at equilibrium if ΔG is zero;
- a reaction cannot occur spontaneously if ΔG is positive. An input of energy is required to drive such a reaction;

- the ΔG of a reaction is independent of the path of the transformation;
- ΔG provides no information about the rate of a reaction.

Activation energy and transition state

The energy changes that take place during the course of a particular biochemical reaction are shown in *Fig. 1*. In all reactions there is an **energy barrier** that has to be overcome in order for the reaction to proceed. This is the energy needed to transform the substrate molecules into the **transition state** – an unstable chemical form part-way between the substrates and the products. The transition state has the **highest free energy** of any component in the reaction pathway. The **Gibbs free energy of activation** ($\Delta G‡$) is equal to the difference in free energy between the transition state and the substrate (*Fig. 1*). An enzyme works by **stabilizing the transition state** of a chemical reaction and decreasing $\Delta G‡$ (*Fig. 1)*. The enzyme does not alter the energy levels of the substrates or the products. Thus an enzyme increases the rate at which the reaction occurs, but has no effect on the overall change in energy of the reaction.

Free energy change

The change in **Gibbs free energy** (ΔG) dictates whether a reaction will be energetically favorable or not. *Figure 1* shows an example where the overall energy change of the reaction makes it energetically favorable (i.e. the products are at a lower energy level than the substrates and ΔG is negative). It should be noted that ΔG is unrelated to $\Delta G‡$. The ΔG of a reaction is independent of the path of the reaction, and it provides no information about the rate of a reaction since the rate of the reaction is governed by $\Delta G‡$. A negative ΔG indicates that the reaction is thermodynamically favorable in the direction indicated (i.e. that it is likely to occur without an input of energy), whereas a positive ΔG indicates that the reaction is not thermodynamically favorable and requires an **input of energy** to proceed in the direction indicated. In biochemical systems, this input of energy is often achieved by **coupling** the energetically unfavorable reaction with a more energetically favorable one (**coupled reactions**).

It is often convenient to refer to ΔG under a standard set of conditions, defined as when the substrates and products of a reaction are all present at concentrations of 1.0 M and the reaction is taking place at a constant pH of 7.0. Under these conditions a slightly different value for ΔG is found, and this is called $\Delta G^{o\prime}$. An example of an energetically favorable reaction which has a large negative $\Delta G^{o\prime}$ and is commonly used to drive less energetically favorable reactions is the

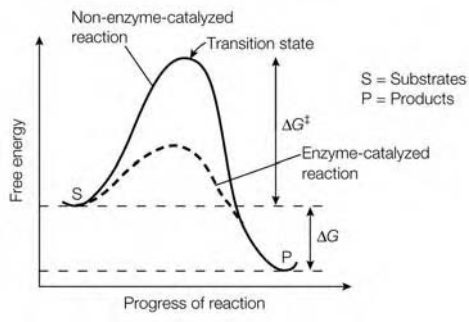

Fig. 1. The energy changes taking place during the course of a biochemical reaction.

hydrolysis of adenosine triphosphate (ATP; *Fig. 2*) to form adenosine diphosphate (ADP) and free inorganic phosphate (P_i):

$$ATP + H_2O \rightarrow ADP + P_i \qquad \Delta G^{o\prime} = \begin{matrix} -30.5 \text{ kJ mol}^{-1} \\ -7.3 \text{ kcal mol}^{-1} \end{matrix}$$

Chemical equilibria

A chemical reaction usually exists in a state of **dynamic equilibrium,** where although new molecules of substrate and product are continually being transformed and formed, the ratio of substrate to product remains at a constant value.

Consider the reaction:

$$A \underset{10^{-6}\ s^{-1}}{\overset{10^{-4}\ s^{-1}}{\rightleftharpoons}} B$$

where the rate of the forward reaction is 10^{-4} per second (s^{-1}) and the rate of the reverse reaction is 10^{-6} s^{-1}. At equilibrium the ratio of the concentrations of the substrate and product gives a constant value, known as the **equilibrium constant** (***K***). The equilibrium constant for a given reaction is defined as:

$$K = \frac{[\text{products}]_{eq}}{[\text{reactants}]_{eq}} = \frac{[B]_{eq}}{[A]_{eq}}$$

where square brackets indicate concentration. The equilibrium constant is also given by the ratio of the forward reaction rate (k_f) and the reverse reaction rate (k_b):

$$K = \frac{k_f}{k_b} = \frac{10^{-4}}{10^{-6}} = 100$$

Thus, for the above reaction at equilibrium, there is 100 times more of product B than there is of substrate A, regardless of whether there is enzyme present or not. This is because enzymes do not alter the equilibrium position of a reaction,

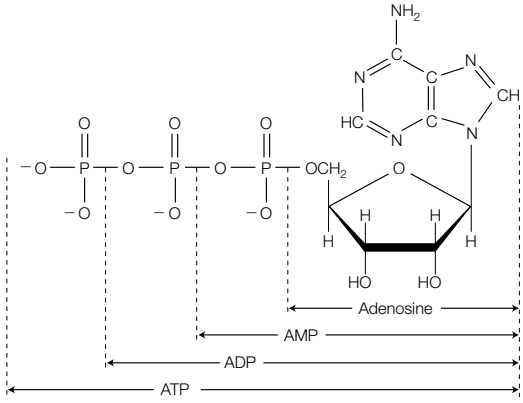

Fig. 2. Structure of adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP) and adenosine.

but accelerate the forward and reverse reactions to the same extent. In other words, **enzymes accelerate the attainment of the equilibrium position** but do not shift its position. For the hypothetical reaction shown above, in the absence of added enzyme the reaction may take over an hour to reach the equilibrium position, whereas in the presence of enzyme the equilibrium position may be reached in less than 1 s.

C3 Enzyme kinetics

Key Notes

Enzyme velocity

Enzyme activity is commonly expressed by the initial rate (V_0) of the reaction being catalyzed. The units of V_0 are μmol min^{-1}, which can also be represented by the enzyme unit (U) or the katal (kat), where 1 μmol min^{-1} = 1 U = 16.67 nanokat. The term activity (or total activity) refers to the total units of enzyme in a sample, whereas specific activity is the number of units per milligram of protein (units mg^{-1}).

Substrate and enzyme concentration

At low substrate concentrations ([S]) a doubling of [S] leads to a doubling of V_0, whereas at higher [S] the enzyme becomes saturated and there is no further increase in V_0. A graph of V_0 against [S] will give a hyperbolic curve. When [S] is saturating, a doubling of the enzyme concentration leads to a doubling of V_0.

Temperature

Temperature affects the rate of an enzyme-catalyzed reaction by increasing the thermal energy of the substrate molecules. This increases the proportion of molecules with sufficient energy to overcome the activation barrier and hence increases the rate of the reaction. In addition, the thermal energy of the component molecules of the enzyme is increased, which leads to an increased rate of denaturation of the enzyme protein due to the disruption of the noncovalent interactions holding the structure together.

pH

Each enzyme has an optimum pH at which the rate of the reaction that it catalyzes is at its maximum. Slight deviations in the pH from the optimum lead to a decrease in the reaction rate. Larger deviations in pH lead to denaturation of the enzyme due to changes in the ionization of amino acid residues and the disruption of noncovalent interactions.

Michaelis–Menten model

The Michaelis–Menten model uses the following concept of enzyme catalysis:

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

where the rate constants k_1, k_2 and k_3 describe the rates associated with each step of the catalytic process. At low [S], V_0 is directly proportional to [S], while at high [S] the velocity tends towards a maximum velocity (V_{max}). The Michaelis–Menten equation:

$$V_0 = \frac{V_{max} \cdot [S]}{K_m + [S]}$$

describes these observations and predicts a hyperbolic curve of V_0 against [S]. The Michaelis constant, K_m, is equal to the sum of the rates of breakdown of the enzyme–substrate complex over its rate of formation, and is a measure of the affinity of an enzyme for its substrate.

Lineweaver-Burk plot V_{max} and K_m can be determined experimentally by measuring V_0 at different substrate concentrations, and then plotting $1/V_0$ against 1/[S] in a double reciprocal or Lineweaver–Burk plot. The intercept on the *y*-axis is equal to $1/V_{max}$, the intercept on the *x*-axis is equal to $-1/K_m$ and the slope of the line is equal to K_m / V_{max}.

Related topics

- Protein structure (B3)
- Introduction to enzymes (C1)
- Thermodynamics (C2)
- Enzyme inhibition (C4)
- Regulation of enzyme activity (C5)
- Polymerase chain reaction (I6)

Enzyme velocity

The rate of an enzyme-catalyzed reaction is often called its velocity. Enzyme velocities are normally reported as values at time zero (**initial velocity**, symbol V_0; μmol min^{-1}), since the rate is fastest at the point where no product is yet present. This is because the substrate concentration is greatest before any substrate has been transformed to product, because enzymes may be subject to **feedback inhibition** by their own products and/or because with a reversible reaction the products will fuel the reverse reaction. Experimentally V_0 is measured before more than approximately 10% of the substrate has been converted to product in order to minimize such complicating factors. A typical **plot of product formed against time** for an enzyme-catalyzed reaction shows an initial period of rapid product formation which gives the linear portion of the plot (*Fig. 1*). This is followed by a slowing down of the enzyme rate as substrate is used up and/or as the enzyme loses activity. V_0 is obtained by drawing a straight line through the linear part of the curve, starting at the zero time-point (*Fig. 1*). The slope of this straight line is equal to V_0.

Enzyme units

Enzyme activity may be expressed in a number of ways. The commonest is by the initial rate (V_0) of the reaction being catalyzed (e.g. μmol of substrate transformed per minute; μmol min^{-1}). There are also two standard units of enzyme activity, the **enzyme unit** (**U**) and the **katal** (**kat**). An enzyme unit is that amount of enzyme which will catalyze the transformation of 1 μmol of substrate per

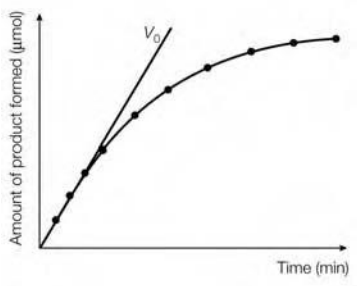

Fig. 1. The relationship between product formation and time for an enzyme-catalyzed reaction.

minute at 25°C under optimal conditions for that enzyme. The katal is the accepted SI unit of enzyme activity and is defined as that catalytic activity which will raise the rate of a reaction by one mole per second in a specified system. It is possible to convert between these different units of activity using 1 μmol min^{-1} = 1 U = 16.67 nanokat. The term **activity** (or **total activity**) refers to the total units of enzyme in the sample, whereas the **specific activity** is the number of enzyme units per milligram of protein (units mg^{-1}). The specific activity is a measure of the purity of an enzyme; during the purification of the enzyme its specific activity increases and becomes maximal and constant when the enzyme is pure.

Substrate and enzyme concentration

The normal pattern of dependence of enzyme rate on **substrate concentration** ([S]) is that at low substrate concentrations a doubling of [S] will lead to a doubling of the initial velocity (V_0). However, at higher substrate concentrations the enzyme becomes **saturated**, and further increases in [S] lead to very small changes in V_0. This occurs because at saturating substrate concentrations effectively all of the enzyme molecules have bound substrate. The overall enzyme rate is now dependent on the rate at which the product can dissociate from the enzyme, and adding further substrate will not affect this. The shape of the resulting graph when V_0 is plotted against [S] is called a **hyperbolic curve** (*Fig. 2*).

In situations where the substrate concentration is saturating (i.e. all the enzyme molecules are bound to substrate), a doubling of the **enzyme concentration** will lead to a doubling of V_0. This gives a straight line graph when V_0 is plotted against enzyme concentration.

Temperature

Temperature affects the rate of enzyme-catalyzed reactions in two ways. First, a rise in temperature increases the **thermal energy** of the substrate molecules. This raises the proportion of substrate molecules with sufficient energy to overcome the Gibbs free energy of activation ($\Delta G‡$) (see Topic C2), and hence increases the rate of the reaction. However, a second effect comes into play at higher temperatures. Increasing the thermal energy of the molecules which make up the protein structure of the enzyme itself will increase the chances of breaking the multiple weak, noncovalent interactions (hydrogen bonds, van der Waals forces, etc.) which hold the three-dimensional structure of the enzyme together (see Topic B3). Ultimately this will lead to the **denaturation** (unfolding) of the enzyme, but

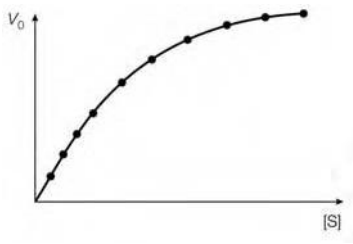

Fig. 2. The relationship between substrate concentration [S] and initial reaction velocity (V_0).

even small changes in the three-dimensional shape of the enzyme can alter the structure of the active site and lead to a decrease in catalytic activity. The overall effect of a rise in temperature on the reaction rate of the enzyme is a balance between these two opposing effects. A graph of temperature plotted against V_0 will therefore show a curve, with a well-defined temperature optimum (*Fig. 3a*). For many mammalian enzymes this is around 37°C, but there are also organisms which have enzymes adapted to working at considerably higher or lower temperatures. For example, ***Taq* polymerase** that is used in the polymerase chain reaction (see Topic I6), is found in a bacterium that lives at high temperatures in hot springs, and thus is adapted to work optimally at high temperatures.

pH

Each enzyme has an **optimum pH** at which the rate of the reaction that it catalyzes is at its maximum. Small deviations in pH from the optimum value lead to decreased activity due to changes in the ionization of groups at the active site of the enzyme. Larger deviations in pH lead to the **denaturation** of the enzyme protein itself, due to interference with the many weak noncovalent bonds maintaining its three-dimensional structure. A graph of V_0 plotted against pH will usually give a bell-shaped curve (*Fig. 3b*). Many enzymes have a pH optimum of around 6.8, but there is great diversity in the pH optima of enzymes, due to the different environments in which they are adapted to work. For example, the digestive enzyme **pepsin** is adapted to work at the acidic pH of the stomach (around pH 2.0).

Michaelis–Menten model

The **Michaelis–Menten model** uses the following concept of enzyme catalysis:

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

The enzyme (E), combines with its substrate (S) to form an **enzyme–substrate complex** (ES). The ES complex can dissociate again to form E + S, or can proceed chemically to form E and the product P. The **rate constants** k_1, k_2 and k_3 describe the rates associated with each step of the catalytic process. It is assumed that there is no significant rate for the backward reaction of enzyme and product (E + P) being converted to ES complex. [ES] remains approximately constant

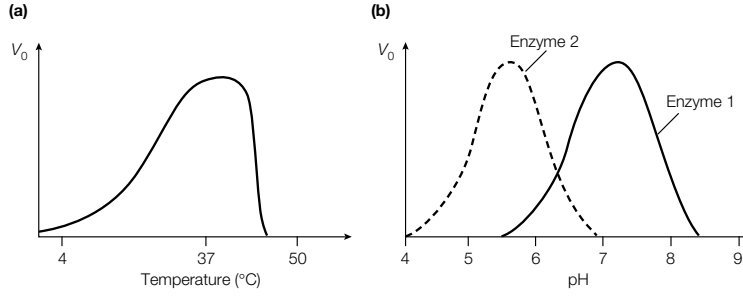

Fig. 3. The effect of (a) temperature and (b) pH on enzyme activity.

until nearly all the substrate is used, hence the rate of synthesis of ES equals its rate of consumption over most of the course of the reaction; that is, [ES] maintains a **steady state**. From the observation of the properties of many enzymes it was known that the initial velocity (V_0) at low substrate concentrations is directly proportional to [S], while at high substrate concentrations the velocity tends towards a maximum value, that is the rate becomes independent of [S] (*Fig. 4a*). This maximum velocity is called V_{max} (units of μmol min^{-1}). The **initial velocity** (V_0) is the velocity measured experimentally before more than approximately 10% of the substrate has been converted to product in order to minimize such complicating factors as the effects of reversible reactions, inhibition of the enzyme by product, and progressive inactivation of the enzyme (see above).

Michaelis and Menten derived an equation to describe these observations, the **Michaelis–Menten equation**:

$$V_0 = \frac{V_{max} \cdot [S]}{K_m + [S]}$$

The equation describes a **hyperbolic curve** of the type shown for the experimental data in *Fig. 1a*. In deriving the equation, Michaelis and Menten defined a new constant, K_m, the **Michaelis constant** [units: Molar (i.e. per mole), M]:

$$K_m = \frac{k_2 + k_3}{k_1}$$

K_m is a measure of the **stability of the ES complex**, being equal to the sum of the rates of breakdown of ES over its rate of formation. For many enzymes k_2 is much greater than k_3. Under these circumstances K_m becomes a measure of the **affinity** of an enzyme for its substrate since its value depends on the relative values of k_1 and k_2 for ES formation and dissociation, respectively. A high K_m indicates weak substrate binding (k_2 predominant over k_1), a low K_m indicates strong substrate binding (k_1 predominant over k_2). K_m can be determined experimentally by the fact that its value is equivalent to the substrate concentration at which the velocity is equal to half of V_{max}.

Lineweaver–Burk plot

Because V_{max} is achieved at infinite substrate concentration, it is impossible to estimate V_{max} (and hence K_m) from a hyperbolic plot as shown in *Fig. 4a*. However, V_{max} and K_m can be determined experimentally by measuring V_0 at

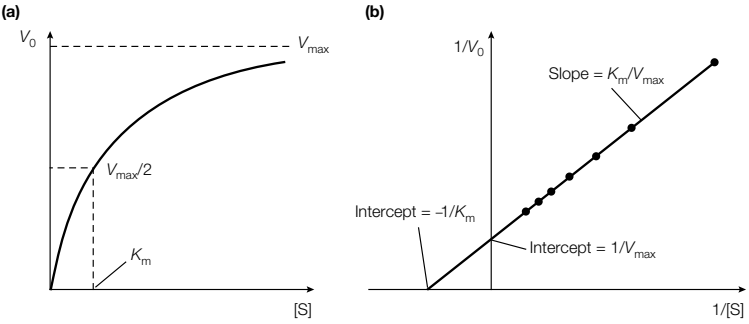

Fig. 4. The relationship between substrate concentration [S] and initial reaction velocity (V_0). (a) A direct plot, (b) a Lineweaver-Burk double-reciprocal plot.

different substrate concentrations (see *Fig. 1*). Then a **double reciprocal** or **Lineweaver–Burk** plot of $1/V_0$ against $1/[S]$ is made (*Fig. 4b*). This plot is a derivation of the Michaelis–Menten equation:

$$\frac{1}{V_0} = \frac{1}{V_{max}} + \frac{K_m}{V_{max}} \cdot \frac{1}{[S]}$$

which gives a straight line, with the **intercept on the *y*-axis equal to $1/V_{max}$**, and the **intercept on the x-axis equal to $-1/K_m$**. The slope of the line is equal to K_m/V_{max} (*Fig. 4b*). The Lineweaver–Burk plot is also a useful way of determining how an inhibitor binds to an enzyme (see Topic C4). The K_m and V_{max} can also be determined from an **Eadie-Hofstee plot** of $V_0/[S]$ against V_0, where the intercept on the x-axis equals V_{max} and the slope of the line is equal to $-1/K_m$.

Although the Michaelis–Menten model provides a very good model of the experimental data for many enzymes, a few enzymes do not conform to Michaelis–Menten kinetics. These enzymes, such as aspartate transcarbamoylase (ATCase), are called **allosteric enzymes** (see Topic C5).

C4 ENZYME INHIBITION

Key Notes

Enzyme inhibition

The catalytic rate of an enzyme can be lowered by inhibitor molecules. Many inhibitors exist, including normal body metabolites, foreign drugs and toxins. Enzyme inhibition can be of two main types: irreversible or reversible. Reversible inhibition can be subdivided into competitive and noncompetitive.

Irreversible inhibition

An irreversible inhibitor binds tightly, often covalently, to amino acid residues at the active site of the enzyme, permanently inactivating the enzyme. Examples of irreversible inhibitors are diisopropylphosphofluoridate (DIPF), iodoacetamide and penicillin.

Reversible competitive inhibition

A competitive inhibitor competes with the substrate molecules for binding to the active site of the enzyme. At high substrate concentration, the effect of a competitive inhibitor can be overcome. On a Lineweaver–Burk plot a competitive inhibitor can be seen to increase the K_m but leave V_{max} unchanged.

Reversible noncompetitive inhibition

A noncompetitive inhibitor binds at a site other than the active site of the enzyme and decreases its catalytic rate by causing a conformational change in the three-dimensional shape of the enzyme. The effect of a noncompetitive inhibitor cannot be overcome at high substrate concentrations. On a Lineweaver–Burk plot a noncompetitive inhibitor can be seen to decrease V_{max} but leave K_m unchanged.

Related topics

Prokaryote cell structure (A1)
Protein purification (B6)
Introduction to enzymes (C1)
Enzyme kinetics (C3)
Regulation of enzyme activity (C5)
Nerve function (E6)

Enzyme inhibition

Many types of molecule exist which are capable of interfering with the activity of an individual enzyme. Any molecule which acts directly on an enzyme to lower its catalytic rate is called an **inhibitor**. Some enzyme inhibitors are normal body metabolites that inhibit a particular enzyme as part of the normal metabolic control of a pathway. Other inhibitors may be foreign substances, such as drugs or toxins, where the effect of enzyme inhibition could be either therapeutic or, at the other extreme, lethal. Enzyme inhibition may be of two main types: **irreversible** or **reversible**, with reversible inhibition itself being subdivided into **competitive** and **noncompetitive** inhibition. Reversible inhibition can be overcome by removing the inhibitor from the enzyme, for example by dialysis (see Topic B6), but this is not possible for irreversible inhibition, by definition.

Irreversible inhibition

Inhibitors which bind **irreversibly** to an enzyme often form a **covalent bond** to an amino acid residue at or near the active site, and permanently inactivate the

enzyme. Susceptible amino acid residues include Ser and Cys residues which have reactive –OH and –SH groups, respectively. The compound **diisopropylphosphofluoridate** (DIPF), a component of nerve gases, reacts with a Ser residue in the active site of the enzyme acetylcholinesterase, irreversibly inhibiting the enzyme and preventing the transmission of nerve impulses (*Fig. 1a*) (see Topic E6). **Iodoacetamide** modifies Cys residues and hence may be used as a diagnostic tool in determining whether one or more Cys residues are required for enzyme activity (*Fig. 1b*). The **antibiotic penicillin** irreversibly inhibits the glycopeptide transpeptidase enzyme that forms the cross-links in the bacterial cell wall by covalently attaching to a Ser residue in the active site of the enzyme (see Topic A1).

Reversible competitive inhibition

A **competitive inhibitor** typically has close structural similarities to the normal substrate for the enzyme. Thus it competes with substrate molecules to bind to the active site (*Fig. 2a*). The enzyme may bind either a substrate molecule or an inhibitor molecule, but not both at the same time (*Fig. 2b*). The competitive inhibitor binds **reversibly to the active site**. At **high substrate concentrations** the action of a competitive inhibitor is overcome because a sufficiently high substrate concentration will successfully compete out the inhibitor molecule in

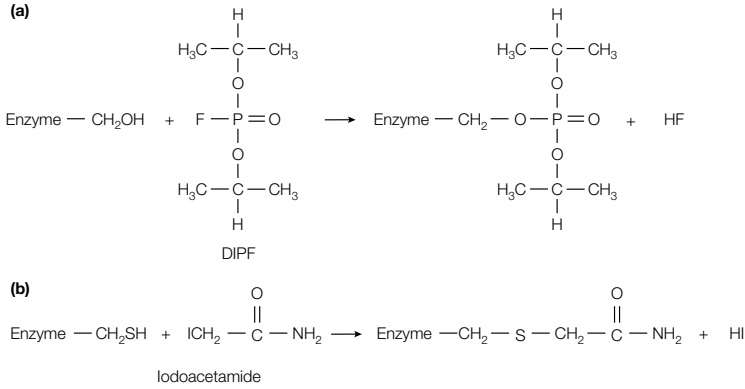

Fig. 1. Structure and mechanism of action of (a) diisopropylphosphofluoridate (DIPF) and (b) iodoacetamide.

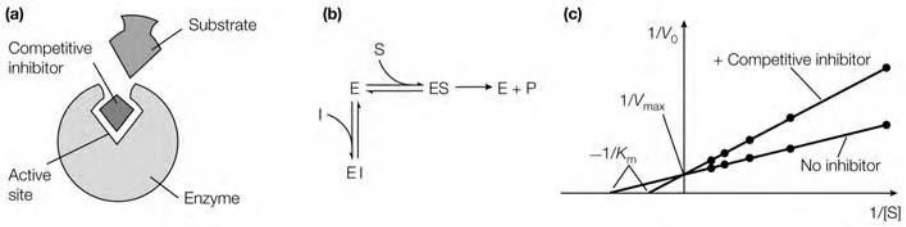

Fig. 2. The characteristics of competitive inhibition. (a) A competitive inhibitor competes with the substrate for binding at the active site of the enzyme; (b) the enzyme can bind either substrate or the competitive inhibitor but not both; (c) Lineweaver-Burk plot showing the effect of a competitive inhibitor on K_m and V_{max}.

binding to the active site. Thus there is no change in the V_{max} of the enzyme but the apparent affinity of the enzyme for its substrate decreases in the presence of the competitive inhibitor, and hence K_m increases.

A good example of competitive inhibition is provided by **succinate dehydrogenase**. This enzyme uses **succinate** as its substrate and is competitively inhibited by **malonate** which differs from succinate in having one rather than two methylene groups (*Fig. 3*). Many drugs work by mimicking the structure of the substrate of a target enzyme, and hence act as competitive inhibitors of the enzyme. Competitive inhibition can be recognized by using a Lineweaver–Burk plot. V_0 is measured at different substrate concentrations in the presence of a fixed concentration of inhibitor. A competitive inhibitor increases the slope of the line on the Lineweaver–Burk plot, and alters the intercept on the *x*-axis (since **K_m is increased**), but leaves the intercept on the *y*-axis unchanged (since **V_{max} remains constant**; *Fig. 2c*).

Reversible noncompetitive inhibition

A **noncompetitive inhibitor** binds **reversibly at a site other than the active site** (*Fig. 4a*) and causes a change in the overall three-dimensional shape of the enzyme that leads to a decrease in catalytic activity. Since the inhibitor binds at a different site from the substrate, the enzyme may bind the inhibitor, the substrate or both the inhibitor and substrate together (*Fig. 4b*). The effects of a noncompetitive inhibitor cannot be overcome by increasing the substrate concentration, so there is a decrease in V_{max}. In noncompetitive inhibition the affinity of the enzyme for the substrate is unchanged and so K_m remains the same. An example of noncompetitive inhibition is the action of **pepstatin** on the enzyme **renin**.

Noncompetitive inhibition can be recognized on a Lineweaver–Burk plot, since it increases the slope of the experimental line, and alters the intercept on the *y*-axis (since **V_{max} is decreased**), but leaves the intercept on the *x*-axis unchanged (since **K_m remains constant**; *Fig. 4c*).

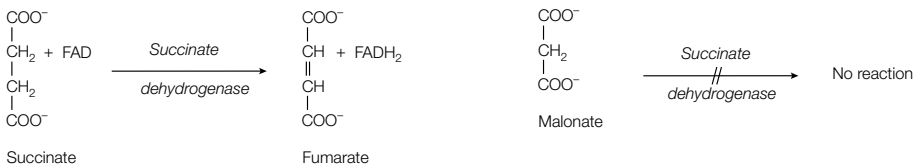

Fig. 3. Inhibition of succinate dehydrogenase by malonate.

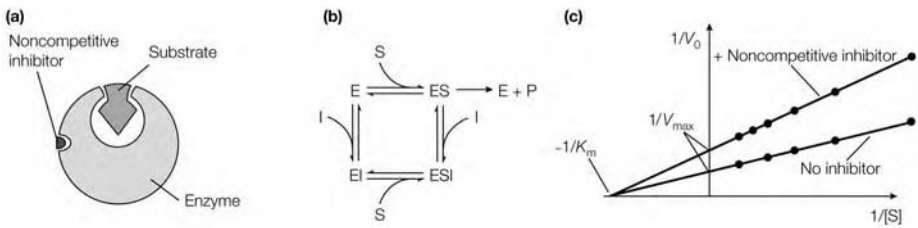

Fig. 4. The characteristics of noncompetitive inhibition. (a) A noncompetitive inhibitor binds at a site distinct from the active site; (b) the enzyme can bind either substrate or the noncompetitive inhibitor or both; (c) Lineweaver-Burk plot showing the effect of a noncompetitive inhibitor on K_m and V_{max}.

C5 Regulation of enzyme activity

Key Notes

Feedback regulation

The rates of enzyme-catalyzed reactions in biological systems are altered by activators and inhibitors, collectively known as effector molecules. In metabolic pathways, the end-product often feedback-inhibits the committed step earlier on in the same pathway to prevent the build up of intermediates and the unnecessary use of metabolites and energy. For branched metabolic pathways a process of sequential feedback inhibition often operates.

Allosteric enzymes

A plot of V_0 against [S] for an allosteric enzyme gives a sigmoidal-shaped curve. Allosteric enzymes usually have more than one active site which cooperatively bind substrate molecules, such that the binding of substrate at one active site induces a conformational change in the enzyme which alters the affinity of the other active sites for substrate. Allosteric enzymes are often multisubunit proteins, with an active site on each subunit. Two models have been proposed to explain the allosteric behavior of enzymes, the concerted or symmetry model and the sequential model. Allosteric enzymes may be controlled by effector molecules (activators or inhibitors) that bind to a site other than the active site and alter the rate of enzyme activity. Aspartate transcarbamoylase is an allosteric enzyme which catalyzes the committed step in pyrimidine biosynthesis. This enzyme consists of six catalytic subunits each with an active site and six regulatory subunits to which the allosteric effectors cytosine triphosphate (CTP) and ATP bind. Aspartate transcarbamoylase is feedback-inhibited by the end-product of the pathway, CTP, which acts as an allosteric inhibitor. In contrast, ATP an intermediate earlier in the pathway, acts as an allosteric activator.

Reversible covalent modification

The activity of many enzymes is altered by the reversible making and breaking of a covalent bond between the enzyme and a small nonprotein group. The most common such modification is the addition and removal of a phosphate group; phosphorylation and dephosphorylation, respectively. Phosphorylation is catalyzed by protein kinases, often using ATP as the phosphate donor, whereas dephosphorylation is catalyzed by protein phosphatases.

Proteolytic activation

Some enzymes are synthesized as larger inactive precursors called proenzymes or zymogens. These are activated by the irreversible hydrolysis of one or more peptide bonds. The pancreatic proteases trypsin, chymotrypsin and elastase are all derived from zymogen precursors (trypsinogen, chymotrypsinogen and proelastase, respectively) by proteolytic activation. Premature activation of these zymogens leads to the condition of acute pancreatitis. The blood clotting cascade and apoptosis (programmed cell death) also involve a series of zymogen activations that brings about a large amplification of the original signal.

Regulation of enzyme synthesis and breakdown

The amount of enzyme present is a balance between the rates of its synthesis and degradation. The level of induction or repression of the gene encoding the enzyme, and the rate of degradation of its mRNA, will alter the rate of synthesis of the enzyme protein. Once the enzyme protein has been synthesized, the rate of its breakdown (half-life) can also be altered as a means of regulating enzyme activity.

Related topics

- Myoglobin and hemoglobin (B4)
- Introduction to enzymes (C1)
- Enzyme kinetics (C3)
- Enzyme inhibition (C4)
- DNA structure (F1)
- Operons (G3)
- Transcription in eukaryotes: an overview (G4)
- Control of glycogen metabolism (J7)

Feedback regulation

In biological systems the rates of many enzymes are altered by the presence of other molecules such as activators and inhibitors (collectively known as **effectors**). A common theme in the control of metabolic pathways is when an enzyme early on in the pathway is inhibited by an end-product of the metabolic pathway in which it is involved. This is called **feedback inhibition** and often takes place at the **committed step** in the pathway (conversion of A to B in *Fig. 1a*). The committed step is the first step to produce an intermediate which is unique to the pathway in question, and therefore normally commits the metabolite to further metabolism along that pathway. Control of the enzyme which carries out the committed step of a metabolic pathway conserves the metabolic energy supply of the organism, and prevents the build up of large quantities of unwanted metabolic intermediates further along the pathway.

As many metabolic pathways are **branched**, feedback inhibition must allow the synthesis of one product of a branched pathway to proceed even when another is present in excess. Here a process of **sequential feedback inhibition** may operate where the end-product of one branch of a pathway will inhibit the first enzyme after the branchpoint (the conversion of C to D or C to E in *Fig. 1b*). When this branchpoint intermediate builds up, it in turn inhibits the first committed step of the whole pathway (conversion of A to B in *Fig. 1b*). Since the end-product of a metabolic pathway involving multiple enzyme reactions is unlikely to resemble the starting compound structurally, the end-product will bind to the enzyme at the control point at a site other than the active site. Such enzymes are always **allosteric enzymes**.

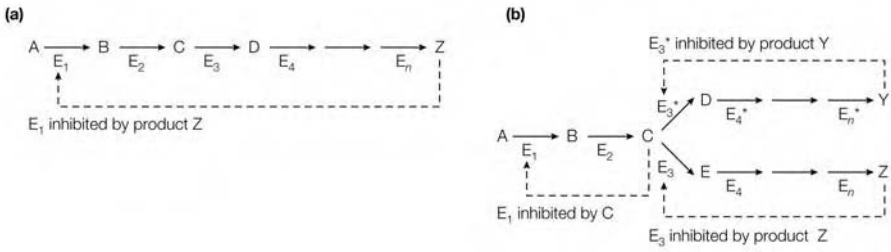

Fig. 1. Feedback inhibition (a) and sequential feedback inhibition (b) in metabolic pathways.

Allosteric enzymes

A plot of V_0 against [S] for an **allosteric enzyme** gives a **sigmoidal curve** rather than the hyperbolic plots predicted by the Michaelis–Menten equation (see Topic C3 and *Fig. 4*). The curve has a steep section in the middle of the substrate concentration range, reflecting the rapid increase in enzyme velocity which occurs over a narrow range of substrate concentrations. This allows allosteric enzymes to be particularly sensitive to small changes in substrate concentration within the physiological range. In allosteric enzymes, the binding of a substrate molecule to one active site affects the binding of substrate molecules to other active sites in the enzyme; the different active sites are said to behave **cooperatively** in binding and acting on substrate molecules (cf. the binding of O_2 to the four subunits of hemoglobin; Topic B4). Thus allosteric enzymes are often multisubunit proteins, with one or more active sites on each subunit. The binding of substrate at one active site **induces a conformational change** in the protein that is conveyed to the other active sites, altering their affinity for substrate molecules.

Two models have been put forward to account for the allosteric effects observed in proteins. In the **symmetry** or **concerted model**, first proposed by Jacques Monod, Jeffries Wyman and Jean-Pierre Changeaux (sometimes referred to as the **Monod-Wyman-Changeaux (MWC) model**) the subunits of an allosteric enzyme can exist in one of only two states, T and R (*Fig. 2a*). T-state subunits are in a tense state that is compact and relatively inactive, while R-state subunits are in a relaxed, expanded, active state with higher affinity for the substrate; no intermediate states are allowed. In the absence of bound substrate, the equilibrium favors the T-state. As substrate binds to each active site in the T-state, the equilibrium shifts towards the R-state. All of the subunits change conformation in a concerted manner, which implies that the conformation of each subunit is constrained by its association with the other subunits; in other

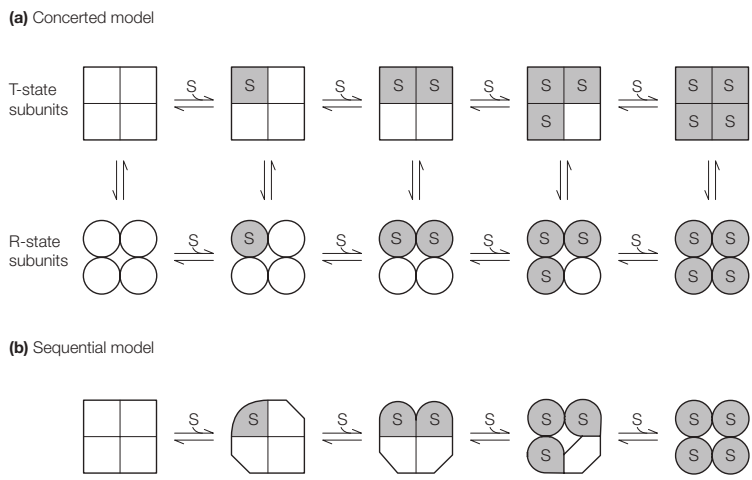

Fig. 2. Models of allosterism. (a) The concerted or symmetry model; the squares and circles represent the T- and R-states, respectively. (b) The sequential model; substrate binding progressively induces conformational changes in the subunits.

words, there are no oligomers that simultaneously contain R- and T-state subunits and the molecular symmetry of the protein is conserved during the conformational change (*Fig. 2a*).

In the alternative **sequential model**, first proposed by Daniel Koshland, sequential changes in structure take place within an oligomeric enzyme as the individual active sites are occupied (*Fig. 2b*). The binding of substrate to one site influences the substrate affinity of neighboring active sites without necessarily inducing a transition encompassing the whole enzyme, such that the molecular symmetry of the whole protein is not necessarily conserved (*Fig. 2b*). The sequential model builds upon the induced-fit hypothesis of enzyme–substrate interaction, whereas the concerted model implicitly assumes the lock-and-key model of substrate binding to the enzyme's active site (see Topic C1). In the sequential model, substrate binding induces a conformational change in a subunit and cooperative interactions arise through the influence that these conformational changes have on neighboring subunits. The strengths of these interactions depend on the degree of mechanical coupling between subunits. In the sequential model the enzyme–substrate binding affinity varies with the number of bound substrate molecules, whereas in the concerted model this affinity depends only on the quaternary state of the enzyme. The results of studies of a number of allosteric proteins suggest that most behave according to a combination of the concerted and sequential models.

Allosteric enzymes may be controlled by **effector** molecules (activators and inhibitors) that bind to the enzyme at a site other than the active site (either on the same subunit or on a different subunit), thereby causing a change in the conformation of the active site which alters the rate of enzyme activity (cf. the binding of CO_2, H^+ and 2,3-bisphosphoglycerate to hemoglobin; see Topic B4). An **allosteric activator** increases the rate of enzyme activity, while an **allosteric inhibitor** decreases the activity of the enzyme.

Aspartate transcarbamoylase

Aspartate transcarbamoylase (aspartate carbamoyltransferase; **ATCase**), a key enzyme in **pyrimidine biosynthesis** (see Topic F1), provides a good example of allosteric regulation. ATCase catalyzes the formation of ***N*-carbamoylaspartate** from aspartate and carbamoyl phosphate, and is the committed step in pyrimidine biosynthesis (*Fig. 3*). The binding of the two substrates aspartate and carbamoyl phosphate is cooperative, as shown by the **sigmoidal curve** of V_0 against substrate concentration (*Fig. 4*).

ATCase consists of six **catalytic subunits** and six **regulatory subunits**. The enzyme is **feedback-inhibited** by the end-product of the pathway, **cytosine triphosphate** (**CTP**; see Topic F1) which acts as an **allosteric inhibitor** (*Fig. 3*). This molecule binds to the regulatory subunits and causes a decrease in the catalytic activity of ATCase by decreasing the affinity of the catalytic subunits for substrate molecules. In contrast, **ATP**, one of the intermediates earlier on in the pathway, acts as an **allosteric activator**, enhancing the affinity of ATCase for its substrates and leading to an increase in activity (*Fig. 3*). ATP competes with the same binding site on the regulatory subunit as CTP. High levels of ATP signal to the cell that energy is available for DNA replication, and so ATCase is activated, resulting in the synthesis of the required pyrimidine nucleotides. When pyrimidines are abundant, the high levels of CTP inhibit ATCase, preventing needless synthesis of *N*-carbamoylaspartate and subsequent intermediates in the pathway.

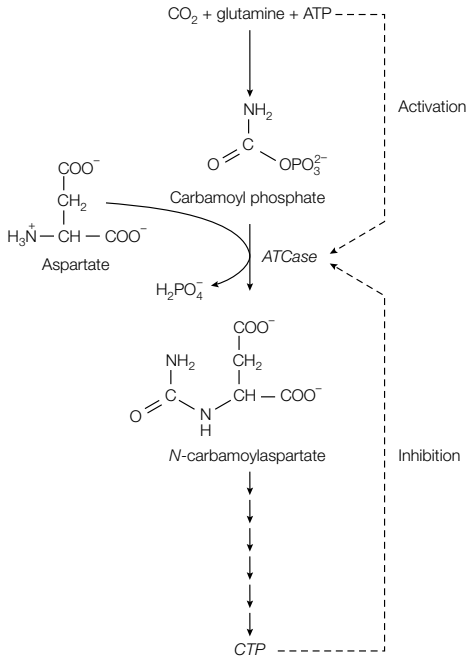

Fig. 3. Formation of N-carbamoylaspartate by aspartate transcarbamoylase (ATCase) is the committed step in pyrimidine biosynthesis and a key control point.

Reversible covalent modification

Reversible covalent modification is the making and breaking of a covalent bond between a nonprotein group and an enzyme molecule. Although a range of nonprotein groups may be reversibly attached to enzymes which affect their activity, the most common modification is the addition and removal of a **phosphate group** (**phosphorylation** and **dephosphorylation**, respectively). Phosphorylation is catalyzed by **protein kinases**, often using ATP as the

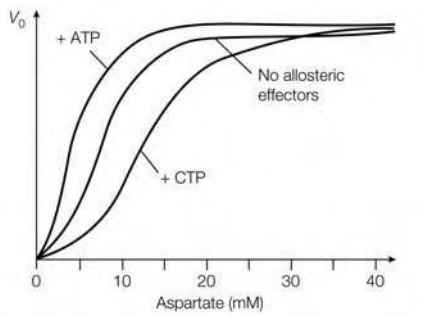

Fig. 4. Plot of initial velocity (V_o) against substrate concentration for the allosteric enzyme aspartate transcarbamoylase.

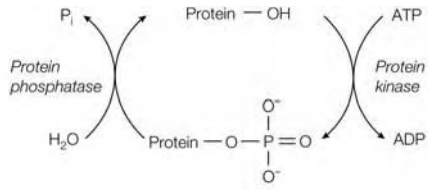

Fig. 5. The reversible phosphorylation and dephosphorylation of an enzyme.

phosphate donor, and dephosphorylation is catalyzed by **protein phosphatases** (*Fig. 5*). The addition and removal of the phosphate group causes changes in the **tertiary structure** of the enzyme that alter its catalytic activity. One class of protein kinases transfers the phosphate specifically on to the hydroxyl group of Ser or Thr residues on the target enzyme [serine/threonine protein kinases, typified by 3′,5′-cyclic adenosine monophosphate (cAMP)-dependent protein kinase], while a second class transfers the phosphate on to the hydroxyl group of Tyr residues (tyrosine kinases). Protein phosphatases catalyze the hydrolysis of phosphate groups from proteins to regenerate the unmodified hydroxyl group of the amino acid and release P_i (*Fig. 5*).

A phosphorylated enzyme may be either more or less active than its dephosphorylated form. Thus phosphorylation/dephosphorylation may be used as a rapid, reversible switch to turn a metabolic pathway on or off according to the needs of the cell. For example, **glycogen phosphorylase**, an enzyme involved in glycogen breakdown, is active in its phosphorylated form, and **glycogen synthase**, involved in glycogen synthesis, is most active in its unphosphorylated form (see Topic J7).

Other types of reversible covalent modification that are used to regulate the activity of certain enzymes include **adenylylation** (the transfer of adenylate from ATP) and **ADP-ribosylation** [the transfer of an adenosine diphosphate (ADP)-ribosyl moiety from NAD^+].

Proteolytic activation

Several enzymes are synthesized as larger inactive precursor forms called proenzymes or **zymogens**. Activation of zymogens involves **irreversible hydrolysis** of one or more **peptide bonds**.

Pancreatic proteases

The digestive enzymes **trypsin**, **chymotrypsin** and **elastase** (see Topic C1) are produced as zymogens in the **pancreas**. They are then transported to the small intestine as their zymogen forms and activated there by cleavage of specific peptide bonds. Trypsin is synthesized initially as the zymogen **trypsinogen**. It is cleaved (and hence activated) in the intestine by the enzyme **enteropeptidase** which is only produced in the intestine. Once activated, trypsin can cleave and activate further trypsinogen molecules as well as other zymogens, such as **chymotrypsinogen** and **proelastase** (*Fig. 6*).

As well as synthesizing and secreting the zymogens, the pancreas also synthesizes a small **trypsin-inhibitor protein**. This inhibitor protein binds very tightly to the active site of trypsin, preventing the pancreas from being destroyed by prematurely activated trypsin molecules. If this safety mechanism fails, for example because of a blocked pancreatic duct, the zymogens can become activated and literally digest the pancreas, a condition known as **acute pancreatitis**.

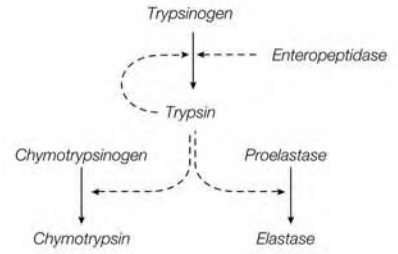

Fig. 6. The central role of trypsin in activating the pancreatic zymogens.

Blood clotting cascade

Another example of the occurrence of inactive zymogens is found in the enzymes involved in the **blood clotting cascade**. Here the whole process of blood clotting is brought about by a **series of zymogen activations**.

Apoptosis

Apoptosis, or **programmed cell death**, is executed by a group of proteases called **caspases**. In response to specific pro-apoptotic signals the inactive procaspases are proteolytically activated to their active form. These activated caspases then act on other procaspases, as well as on other cellular proteins, to bring about cell death.

Zymogen activation may produce a **large amplification** of the initial signal, as a single activated enzyme may act on many thousands of substrate molecules to bring about further activation. Since proteolytic cleavage does not require ATP, zymogen cleavage is a particularly appropriate mechanism for activation of proteins outside cells. However, unlike the covalent modification of an enzyme (see above), zymogen activation is not reversible. Once activated, the enzyme stays active.

Regulation of enzyme synthesis and breakdown

The amount of a particular enzyme present in a cell or tissue changes according to the rates of its **synthesis** and **degradation**.

Factors affecting the rate of synthesis include the level of **induction or repression of the gene** encoding the enzyme (see Topics G3 and G4) and also the **rate of degradation of the mRNA** produced from that gene. Many key enzymes at control points in metabolic pathways have particularly short-lived mRNAs and the rate of enzyme synthesis is thus readily controlled by factors that affect the rate of gene transcription.

The rate of degradation of an enzyme is reflected in its **half-life** – the time taken for 50% of the protein to be degraded. Most enzymes that are important in metabolic regulation have short half-lives, and are termed **labile** enzymes.