Biochemistry & Medicine

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INTRODUCTION

Biochemistry can be defined as *the science concerned with the chemical basis of life* (Gk *bios* "life"). The **cell** is the structural unit of living systems. Thus, biochemistry can also be described as *the science concerned with the chemical constituents of living cells and with the reactions and processes they undergo.* By this definition, biochemistry encompasses large areas of **cell biology,** of **molecular biology,** and of **molecular genetics.**

The Aim of Biochemistry Is to Describe & Explain, in Molecular Terms, All Chemical Processes of Living Cells

The major objective of biochemistry is the complete understanding, at the molecular level, of all of the chemical processes associated with living cells. To achieve this objective, biochemists have sought to isolate the numerous molecules found in cells, determine their structures, and analyze how they function. Many techniques have been used for these purposes; some of them are summarized in Table 1–1.

A Knowledge of Biochemistry Is Essential to All Life Sciences

The biochemistry of the nucleic acids lies at the heart of genetics; in turn, the use of genetic approaches has been critical for elucidating many areas of biochemistry. Physiology, the study of body function, overlaps with biochemistry almost completely. Immunology employs numerous biochemical techniques, and many immunologic approaches have found wide use by biochemists. Pharmacology and pharmacy rest on a sound knowledge of biochemistry and physiology; in particular, most drugs are metabolized by enzyme-catalyzed reactions. Poisons act on biochemical reactions or processes; this is the subject matter of toxicology. Biochemical approaches are being used increasingly to study basic aspects of pathology (the study of disease), such as inflammation, cell injury, and cancer. Many workers in microbiology, zoology, and botany employ biochemical approaches almost exclusively. These relationships are not surprising, because life as we know it depends on biochemical reactions and processes. In fact, the old barriers among the life sciences are breaking down, and biochemistry is increasingly becoming their common language.

A Reciprocal Relationship Between Biochemistry & Medicine Has Stimulated Mutual Advances

The two major concerns for workers in the health sciences—and particularly physicians—are the understanding and maintenance of health and the understanding and effective treatment of diseases. Biochemistry impacts enormously on both of these fundamental concerns of medicine. In fact, the interrelationship of biochemistry and medicine is a wide, two-way street. Biochemical studies have illuminated many aspects of health and disease, and conversely, the study of various aspects of health and disease has opened up new areas of biochemistry. Some examples of this two-way street are shown in Figure 1–1. For instance, a knowledge of protein structure and function was necessary to elucidate the single biochemical difference between normal hemoglobin and sickle cell hemoglobin. On the other hand, analysis of sickle cell hemoglobin has contributed significantly to our understanding of the structure and function of both normal hemoglobin and other proteins. Analogous examples of reciprocal benefit between biochemistry and medicine could be cited for the other paired items shown in Figure 1–1. Another example is the pioneering work of Archibald Garrod, a physician in England during the early 1900s. He studied patients with a number of relatively rare disorders (alkaptonuria, albinism, cystinuria, and pentosuria; these are described in later chapters) and established that these conditions were genetically determined. Garrod designated these conditions as **inborn errors of metabolism.** His insights provided a major foundation for the development of the field of human biochemical genetics. More recent efforts to understand the basis of the genetic disease known as **familial hypercholesterolemia,** which results in severe atherosclerosis at an early age, have led to dramatic progress in understanding of cell receptors and of mechanisms of uptake of cholesterol into cells. Studies of **oncogenes** in cancer cells have directed attention to the molecular mechanisms involved in the control of normal cell growth. These and many other examples emphasize how the study of

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Table 1–1. The principal methods and preparations used in biochemical laboratories.

Methods for Separating and Purifying Biomolecules[1]
  Salt fractionation (eg, precipitation of proteins with ammonium sulfate)
  Chromatography: Paper; ion exchange; affinity; thin-layer; gas-liquid; high-pressure liquid; gel filtration
  Electrophoresis: Paper; high-voltage; agarose; cellulose acetate; starch gel; polyacrylamide gel; SDS-polyacrylamide gel
  Ultracentrifugation
Methods for Determining Biomolecular Structures
  Elemental analysis
  UV, visible, infrared, and NMR spectroscopy
  Use of acid or alkaline hydrolysis to degrade the biomolecule under study into its basic constituents
  Use of a battery of enzymes of known specificity to degrade the biomolecule under study (eg, proteases, nucleases, glycosidases)
  Mass spectrometry
  Specific sequencing methods (eg, for proteins and nucleic acids)
  X-ray crystallography
Preparations for Studying Biochemical Processes
  Whole animal (includes transgenic animals and animals with gene knockouts)
  Isolated perfused organ
  Tissue slice
  Whole cells
  Homogenate
  Isolated cell organelles
  Subfractionation of organelles
  Purified metabolites and enzymes
  Isolated genes (including polymerase chain reaction and site-directed mutagenesis)

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[1]Most of these methods are suitable for analyzing the components present in cell homogenates and other biochemical preparations. The sequential use of several techniques will generally permit purification of most biomolecules. The reader is referred to texts on methods of biochemical research for details.

disease can open up areas of cell function for basic biochemical research.

The relationship between medicine and biochemistry has important implications for the former. As long as medical treatment is firmly grounded in a knowledge of biochemistry and other basic sciences, the practice of medicine will have a rational basis that can be adapted to accommodate new knowledge. This contrasts with unorthodox health cults and at least some "alternative medicine" practices, which are often founded on little more than myth and wishful thinking and generally lack any intellectual basis.

NORMAL BIOCHEMICAL PROCESSES ARE THE BASIS OF HEALTH

The World Health Organization (WHO) defines health as a state of "complete physical, mental and social well-being and not merely the absence of disease and infirmity." From a strictly biochemical viewpoint, health may be considered that situation in which all of the many thousands of intra- and extracellular reactions that occur in the body are proceeding at rates commensurate with the organism's maximal survival in the physiologic state. However, this is an extremely reductionist view, and it should be apparent that caring for the health of patients requires not only a wide knowledge of biologic principles but also of psychologic and social principles.

Biochemical Research Has Impact on Nutrition & Preventive Medicine

One major prerequisite for the maintenance of health is that there be optimal dietary intake of a number of chemicals; the chief of these are **vitamins,** certain **amino acids,** certain **fatty acids,** various **minerals,** and **water.** Because much of the subject matter of both biochemistry and nutrition is concerned with the study of various aspects of these chemicals, there is a close relationship between these two sciences. Moreover, more emphasis is being placed on systematic attempts to maintain health and forestall disease, ie, on **preventive medicine.** Thus, nutritional approaches to—for example—the prevention of atherosclerosis and cancer are receiving increased emphasis. Understanding nutrition depends to a great extent on a knowledge of biochemistry.

Most & Perhaps All Disease Has a Biochemical Basis

We believe that most if not all diseases are manifestations of abnormalities of molecules, chemical reactions, or biochemical processes. The major factors responsible for causing diseases in animals and humans are listed in Table 1–2. All of them affect one or more critical chemical reactions or molecules in the body. Numerous examples of the biochemical bases of diseases will be encountered in this text; the majority of them are due to causes 5, 7, and 8. In most of these conditions, biochemical studies contribute to both the diagnosis and treatment. Some major uses of biochemical investigations and of laboratory tests in relation to diseases are summarized in Table 1–3.

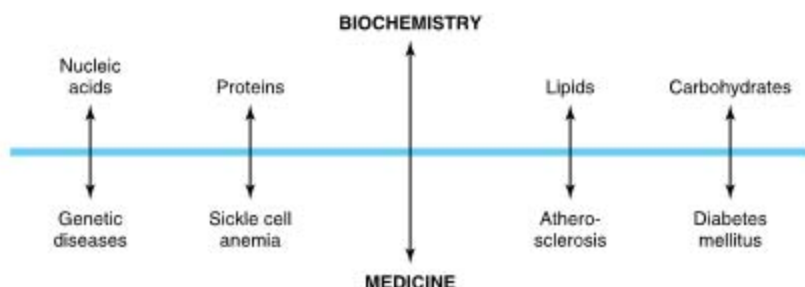Additional examples of many of these uses are presented in various sections of this text.

Figure 1–1. Examples of the two-way street connecting biochemistry and medicine. Knowledge of the biochemical molecules shown in the top part of the diagram has clarified our understanding of the diseases shown in the bottom half—and conversely, analyses of the diseases shown below have cast light on many areas of biochemistry. Note that sickle cell anemia is a genetic disease and that both atherosclerosis and diabetes mellitus have genetic components.

Impact of the Human Genome Project (HGP) on Biochemistry & Medicine

Remarkable progress was made in the late 1990s in sequencing the human genome. This culminated in July 2000, when leaders of the two groups involved in this effort (the International Human Genome Sequencing Consortium and Celera Genomics, a private company) announced that over 90% of the genome had been sequenced. Draft versions of the sequence were published

Table 1–2. The major causes of diseases. All of the causes listed act by influencing the various biochemical mechanisms in the cell or in the body.[1]

1. Physical agents: Mechanical trauma, extremes of temperature, sudden changes in atmospheric pressure, radiation, electric shock.
2. Chemical agents, including drugs: Certain toxic compounds, therapeutic drugs, etc.
3. Biologic agents: Viruses, bacteria, fungi, higher forms of parasites.
4. Oxygen lack: Loss of blood supply, depletion of the oxygen-carrying capacity of the blood, poisoning of the oxidative enzymes.
5. Genetic disorders: Congenital, molecular.
6. Immunologic reactions: Anaphylaxis, autoimmune disease.
7. Nutritional imbalances: Deficiencies, excesses.
8. Endocrine imbalances: Hormonal deficiencies, excesses.

[1]Adapted, with permission, from Robbins SL, Cotram RS, Kumar V: *The Pathologic Basis of Disease*, 3rd ed. Saunders, 1984.

Table 1–3. Some uses of biochemical investigations and laboratory tests in relation to diseases.

Use	Example
1. To reveal the fundamental causes and mechanisms of diseases	Demonstration of the nature of the genetic defects in cystic fibrosis.
2. To suggest rational treatments of diseases based on (1) above	A diet low in phenylalanine for treatment of phenylketonuria.
3. To assist in the diagnosis of specific diseases	Use of the plasma enzyme creatine kinase MB (CK-MB) in the diagnosis of myocardial infarction.
4. To act as screening tests for the early diagnosis of certain diseases	Use of measurement of blood thyroxine or thyroid-stimulating hormone (TSH) in the neonatal diagnosis of congenital hypothyroidism.
5. To assist in monitoring the progress (eg, recovery, worsening, remission, or relapse) of certain diseases	Use of the plasma enzyme alanine aminotransferase (ALT) in monitoring the progress of infectious hepatitis.
6. To assist in assessing the response of diseases to therapy	Use of measurement of blood carcinoembryonic antigen (CEA) in certain patients who have been treated for cancer of the colon.

in early 2001. It is anticipated that the entire sequence will be completed by 2003. The implications of this work for biochemistry, all of biology, and for medicine are tremendous, and only a few points are mentioned here. Many previously unknown genes have been revealed; their protein products await characterization. New light has been thrown on human evolution, and procedures for tracking disease genes have been greatly refined. The results are having major effects on areas such as proteomics, bioinformatics, biotechnology, and pharmacogenomics. Reference to the human genome will be made in various sections of this text. The Human Genome Project is discussed in more detail in Chapter 54.

SUMMARY

- Biochemistry is the science concerned with studying the various molecules that occur in living cells and organisms and with their chemical reactions. Because life depends on biochemical reactions, biochemistry has become the basic language of all biologic sciences.

- Biochemistry is concerned with the entire spectrum of life forms, from relatively simple viruses and bacteria to complex human beings.

- Biochemistry and medicine are intimately related. Health depends on a harmonious balance of biochemical reactions occurring in the body, and disease reflects abnormalities in biomolecules, biochemical reactions, or biochemical processes.

- Advances in biochemical knowledge have illuminated many areas of medicine. Conversely, the study of diseases has often revealed previously unsuspected aspects of biochemistry. The determination of the sequence of the human genome, nearly complete, will have a great impact on all areas of biology, including biochemistry, bioinformatics, and biotechnology.

- Biochemical approaches are often fundamental in illuminating the causes of diseases and in designing appropriate therapies.

- The judicious use of various biochemical laboratory tests is an integral component of diagnosis and monitoring of treatment.

- A sound knowledge of biochemistry and of other related basic disciplines is essential for the rational practice of medical and related health sciences.

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(The numbers assigned to the entries in MIM and OMIM will be cited in selected chapters of this work. Consulting this extensive collection of diseases and other relevant entries—specific proteins, enzymes, etc—will greatly expand the reader's knowledge and understanding of various topics referred to and discussed in this text. The online version is updated almost daily.)

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Water & pH

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BIOMEDICAL IMPORTANCE

Water is the predominant chemical component of living organisms. Its unique physical properties, which include the ability to solvate a wide range of organic and inorganic molecules, derive from water's dipolar structure and exceptional capacity for forming hydrogen bonds. The manner in which water interacts with a solvated biomolecule influences the structure of each. An excellent nucleophile, water is a reactant or product in many metabolic reactions. Water has a slight propensity to dissociate into hydroxide ions and protons. The acidity of aqueous solutions is generally reported using the logarithmic pH scale. Bicarbonate and other buffers normally maintain the pH of extracellular fluid between 7.35 and 7.45. Suspected disturbances of acid-base balance are verified by measuring the pH of arterial blood and the CO_2 content of venous blood. Causes of acidosis (blood pH < 7.35) include diabetic ketosis and lactic acidosis. Alkalosis (pH > 7.45) may, for example, follow vomiting of acidic gastric contents. Regulation of water balance depends upon hypothalamic mechanisms that control thirst, on antidiuretic hormone (ADH), on retention or excretion of water by the kidneys, and on evaporative loss. Nephrogenic diabetes insipidus, which involves the inability to concentrate urine or adjust to subtle changes in extracellular fluid osmolarity, results from the unresponsiveness of renal tubular osmoreceptors to ADH.

WATER IS AN IDEAL BIOLOGIC SOLVENT

Water Molecules Form Dipoles

A water molecule is an irregular, slightly skewed tetrahedron with oxygen at its center (Figure 2–1). The two hydrogens and the unshared electrons of the remaining two sp^3-hybridized orbitals occupy the corners of the tetrahedron. The 105-degree angle between the hydrogens differs slightly from the ideal tetrahedral angle, 109.5 degrees. Ammonia is also tetrahedral, with a 107-degree angle between its hydrogens. Water is a **dipole**, a molecule with electrical charge distributed asymmetrically about its structure. The strongly electronegative oxygen atom pulls electrons away from the hydrogen nuclei, leaving them with a partial positive charge, while its two unshared electron pairs constitute a region of local negative charge.

Water, a strong dipole, has a high **dielectric constant.** As described quantitatively by Coulomb's law, the strength of interaction F between oppositely charged particles is inversely proportionate to the dielectric constant ε of the surrounding medium. The dielectric constant for a vacuum is unity; for hexane it is 1.9; for ethanol it is 24.3; and for water it is 78.5. Water therefore greatly decreases the force of attraction between charged and polar species relative to water-free environments with lower dielectric constants. Its strong dipole and high dielectric constant enable water to dissolve large quantities of charged compounds such as salts.

Water Molecules Form Hydrogen Bonds

An unshielded hydrogen nucleus covalently bound to an electron-withdrawing oxygen or nitrogen atom can interact with an unshared electron pair on another oxygen or nitrogen atom to form a **hydrogen bond.** Since water molecules contain both of these features, hydrogen bonding favors the self-association of water molecules into ordered arrays (Figure 2–2). Hydrogen bonding profoundly influences the physical properties of water and accounts for its exceptionally high viscosity, surface tension, and boiling point. On average, each molecule in liquid water associates through hydrogen bonds with 3.5 others. These bonds are both relatively weak and transient, with a half-life of about one microsecond. Rupture of a hydrogen bond in liquid water requires only about 4.5 kcal/mol, less than 5% of the energy required to rupture a covalent O—H bond.

Hydrogen bonding enables water to dissolve many organic biomolecules that contain functional groups which can participate in hydrogen bonding. The oxygen atoms of aldehydes, ketones, and amides provide pairs of electrons that can serve as hydrogen acceptors. Alcohols and amines can serve both as hydrogen acceptors and as donors of unshielded hydrogen atoms for formation of hydrogen bonds (Figure 2–3).
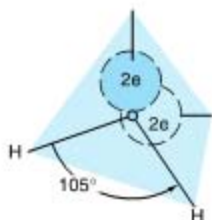
Figure 2–1. The water molecule has tetrahedral geometry.

INTERACTION WITH WATER INFLUENCES THE STRUCTURE OF BIOMOLECULES

Covalent & Noncovalent Bonds Stabilize Biologic Molecules

The covalent bond is the strongest force that holds molecules together (Table 2–1). Noncovalent forces, while of lesser magnitude, make significant contributions to the structure, stability, and functional competence of macromolecules in living cells. These forces, which can be either attractive or repulsive, involve interactions both within the biomolecule and between it and the water that forms the principal component of the surrounding environment.

Biomolecules Fold to Position Polar & Charged Groups on Their Surfaces

Most biomolecules are **amphipathic;** that is, they possess regions rich in charged or polar functional groups as well as regions with hydrophobic character. Proteins tend to fold with the R-groups of amino acids with hydrophobic side chains in the interior. Amino acids with charged or polar amino acid side chains (eg, arginine, glutamate, serine) generally are present on the surface in contact with water. A similar pattern prevails in a phospholipid bilayer, where the charged head groups of
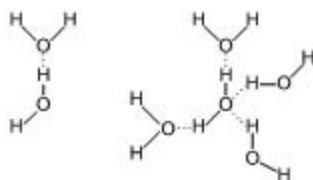

Figure 2–2. **Left:** Association of two dipolar water molecules by a hydrogen bond (dotted line). **Right:** Hydrogen-bonded cluster of four water molecules. Note that water can serve simultaneously both as a hydrogen donor and as a hydrogen acceptor.
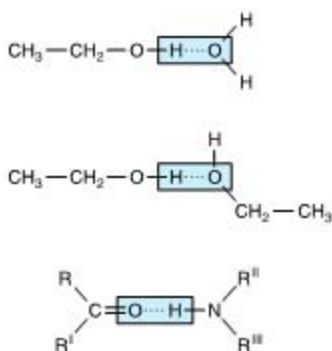

Figure 2–3. Additional polar groups participate in hydrogen bonding. Shown are hydrogen bonds formed between an alcohol and water, between two molecules of ethanol, and between the peptide carbonyl oxygen and the peptide nitrogen hydrogen of an adjacent amino acid.

phosphatidyl serine or phosphatidyl ethanolamine contact water while their hydrophobic fatty acyl side chains cluster together, excluding water. This pattern maximizes the opportunities for the formation of energetically favorable charge-dipole, dipole-dipole, and hydrogen bonding interactions between polar groups on the biomolecule and water. It also minimizes energetically unfavorable contact between water and hydrophobic groups.

Hydrophobic Interactions

Hydrophobic interaction refers to the tendency of nonpolar compounds to self-associate in an aqueous environment. This self-association is driven neither by mutual attraction nor by what are sometimes incorrectly referred to as "hydrophobic bonds." Self-association arises from the need to minimize energetically unfavorable interactions between nonpolar groups and water.

Table 2–1. Bond energies for atoms of biologic significance.

Bond Type	Energy (kcal/mol)	Bond Type	Energy (kcal/mol)
O—O	34	O=O	96
S—S	51	C—H	99
C—N	70	C=S	108
S—H	81	O—H	110
C—C	82	C=C	147
C—O	84	C=N	147
N—H	94	C=O	164

While the hydrogens of nonpolar groups such as the methylene groups of hydrocarbons do not form hydrogen bonds, they do affect the structure of the water that surrounds them. Water molecules adjacent to a hydrophobic group are restricted in the number of orientations (degrees of freedom) that permit them to participate in the maximum number of energetically favorable hydrogen bonds. Maximal formation of multiple hydrogen bonds can be maintained only by increasing the order of the adjacent water molecules, with a corresponding decrease in entropy.

It follows from the second law of thermodynamics that the optimal free energy of a hydrocarbon-water mixture is a function of both maximal enthalpy (from hydrogen bonding) and minimum entropy (maximum degrees of freedom). Thus, nonpolar molecules tend to form droplets with minimal exposed surface area, reducing the number of water molecules affected. For the same reason, in the aqueous environment of the living cell the hydrophobic portions of biopolymers tend to be buried inside the structure of the molecule, or within a lipid bilayer, minimizing contact with water.

Electrostatic Interactions

Interactions between charged groups shape biomolecular structure. Electrostatic interactions between oppositely charged groups within or between biomolecules are termed **salt bridges.** Salt bridges are comparable in strength to hydrogen bonds but act over larger distances. They thus often facilitate the binding of charged molecules and ions to proteins and nucleic acids.

Van der Waals Forces

Van der Waals forces arise from attractions between transient dipoles generated by the rapid movement of electrons on all neutral atoms. Significantly weaker than hydrogen bonds but potentially extremely numerous, van der Waals forces decrease as the sixth power of the distance separating atoms. Thus, they act over very short distances, typically 2–4 Å.

Multiple Forces Stabilize Biomolecules

The DNA double helix illustrates the contribution of multiple forces to the structure of biomolecules. While each individual DNA strand is held together by covalent bonds, the two strands of the helix are held together exclusively by noncovalent interactions. These noncovalent interactions include hydrogen bonds between nucleotide bases (Watson-Crick base pairing) and van der Waals interactions between the stacked purine and pyrimidine bases. The helix presents the charged phosphate groups and polar ribose sugars of

the backbone to water while burying the relatively hydrophobic nucleotide bases inside. The extended backbone maximizes the distance between negatively charged backbone phosphates, minimizing unfavorable electrostatic interactions.

WATER IS AN EXCELLENT NUCLEOPHILE

Metabolic reactions often involve the attack by lone pairs of electrons on electron-rich molecules termed **nucleophiles** on electron-poor atoms called **electrophiles.** Nucleophiles and electrophiles do not necessarily possess a formal negative or positive charge. Water, whose two lone pairs of sp^3 electrons bear a partial negative charge, is an excellent nucleophile. Other nucleophiles of biologic importance include the oxygen atoms of phosphates, alcohols, and carboxylic acids; the sulfur of thiols; the nitrogen of amines; and the imidazole ring of histidine. Common electrophiles include the carbonyl carbons in amides, esters, aldehydes, and ketones and the phosphorus atoms of phosphoesters.

Nucleophilic attack by water generally results in the cleavage of the amide, glycoside, or ester bonds that hold biopolymers together. This process is termed **hydrolysis.** Conversely, when monomer units are joined together to form biopolymers such as proteins or glycogen, water is a product, as shown below for the formation of a peptide bond between two amino acids.
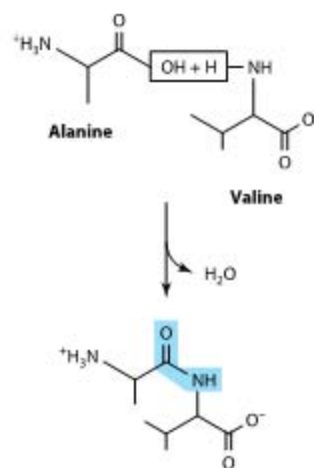
While hydrolysis is a thermodynamically favored reaction, the amide and phosphoester bonds of polypeptides and oligonucleotides are stable in the aqueous environment of the cell. This seemingly paradoxic behavior reflects the fact that the thermodynamics governing the equilibrium of a reaction do not determine the rate at which it will take place. In the cell, protein catalysts called **enzymes** are used to accelerate the rate

of hydrolytic reactions when needed. **Proteases** catalyze the hydrolysis of proteins into their component amino acids, while **nucleases** catalyze the hydrolysis of the phosphoester bonds in DNA and RNA. Careful control of the activities of these enzymes is required to ensure that they act only on appropriate target molecules.

Many Metabolic Reactions Involve Group Transfer

In group transfer reactions, a group G is transferred from a donor D to an acceptor A, forming an acceptor group complex A–G:

$$D-G + A = A-G + D$$

The hydrolysis and phosphorolysis of glycogen represent group transfer reactions in which glucosyl groups are transferred to water or to orthophosphate. The equilibrium constant for the hydrolysis of covalent bonds strongly favors the formation of split products. The biosynthesis of macromolecules also involves group transfer reactions in which the thermodynamically unfavored synthesis of covalent bonds is coupled to favored reactions so that the overall change in free energy favors biopolymer synthesis. Given the nucleophilic character of water and its high concentration in cells, why are biopolymers such as proteins and DNA relatively stable? And how can synthesis of biopolymers occur in an apparently aqueous environment? Central to both questions are the properties of enzymes. In the absence of enzymic catalysis, even thermodynamically highly favored reactions do not necessarily take place rapidly. Precise and differential control of enzyme activity and the sequestration of enzymes in specific organelles determine under what physiologic conditions a given biopolymer will be synthesized or degraded. Newly synthesized polymers are not immediately hydrolyzed, in part because the active sites of biosynthetic enzymes sequester substrates in an environment from which water can be excluded.

Water Molecules Exhibit a Slight but Important Tendency to Dissociate

The ability of water to ionize, while slight, is of central importance for life. Since water can act both as an acid and as a base, its ionization may be represented as an intermolecular proton transfer that forms a hydronium ion (H_3O^+) and a hydroxide ion (OH^-):

$$H_2O + H_2O \rightleftharpoons H_3O^+ + OH^-$$
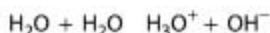
The transferred proton is actually associated with a cluster of water molecules. Protons exist in solution not only as H_3O^+, but also as multimers such as $H_5O_2^+$ and $H_7O_3^+$. The proton is nevertheless routinely represented as H^+, even though it is in fact highly hydrated.

Since hydronium and hydroxide ions continuously recombine to form water molecules, an *individual* hydrogen or oxygen cannot be stated to be present as an ion or as part of a water molecule. At one instant it is an ion. An instant later it is part of a molecule. Individual ions or molecules are therefore not considered. We refer instead to the *probability* that at any instant in time a hydrogen will be present as an ion or as part of a water molecule. Since 1 g of water contains 3.46×10^{22} molecules, the ionization of water can be described statistically. To state that the probability that a hydrogen exists as an ion is 0.01 means that a hydrogen atom has one chance in 100 of being an ion and 99 chances out of 100 of being part of a water molecule. The actual probability of a hydrogen atom in pure water existing as a hydrogen ion is approximately 1.8×10^{-9}. The probability of its being part of a molecule thus is almost unity. Stated another way, for every hydrogen ion and hydroxyl ion in pure water there are 1.8 billion or 1.8×10^9 water molecules. Hydrogen ions and hydroxyl ions nevertheless contribute significantly to the properties of water.

For dissociation of water,

$$K = \frac{[H^+][OH^-]}{[H_2O]}$$

where brackets represent molar concentrations (strictly speaking, molar activities) and K is the **dissociation constant.** Since one mole (mol) of water weighs 18 g, one liter (L) (1000 g) of water contains $1000 \times 18 = 55.56$ mol. Pure water thus is 55.56 molar. Since the probability that a hydrogen in pure water will exist as a hydrogen ion is 1.8×10^{-9}, the molar concentration of H^+ ions (or of OH^- ions) in pure water is the product of the probability, 1.8×10^{-9}, times the molar concentration of water, 55.56 mol/L. The result is 1.0×10^{-7} mol/L.

We can now calculate K for water:

$$K = \frac{[H^+][OH^-]}{[H_2O]} = \frac{[10^{-7}][10^{-7}]}{[55.56]}$$
$$= 0.018 \times 10^{-14} = 1.8 \times 10^{-16} \, mol/L$$

The molar concentration of water, 55.56 mol/L, is too great to be significantly affected by dissociation. It therefore is considered to be essentially constant. This constant may then be incorporated into the dissociation constant K to provide a useful new constant K_w termed the **ion product** for water. The relationship between K_w and K is shown below:

$$K = \frac{[H^+][OH^-]}{[H_2O]} = 1.8 \times 10^{-16} \text{ mol/L}$$

$$K_w = (K)[H_2O] = [H^+][OH^-]$$

$$= (1.8 \times 10^{-16} \text{ mol/L}) (55.56 \text{ mol/L})$$

$$= 1.00 \times 10^{-14} \text{ (mol/L)}^2$$

Note that the dimensions of K are moles per liter and those of K_w are moles^2 per liter^2. As its name suggests, the ion product K_w is numerically equal to the product of the molar concentrations of H^+ and OH^-:

$$K_w = [H^+][OH^-]$$

At 25 °C, $K_w = (10^{-7})^2$, or 10^{-14} (mol/L)^2. At temperatures below 25 °C, K_w is somewhat less than 10^{-14}; and at temperatures above 25 °C it is somewhat greater than 10^{-14}. Within the stated limitations of the effect of temperature, **K_w equals 10^{-14} (mol/L)^2 for all aqueous solutions, even solutions of acids or bases.** We shall use K_w to calculate the pH of acidic and basic solutions.

pH IS THE NEGATIVE LOG OF THE HYDROGEN ION CONCENTRATION

The term **pH** was introduced in 1909 by Sörensen, who defined pH as the negative log of the hydrogen ion concentration:

$$pH = -\log [H^+]$$

This definition, while not rigorous, suffices for many biochemical purposes. To calculate the pH of a solution:

1. Calculate hydrogen ion concentration $[H^+]$.
2. Calculate the base 10 logarithm of $[H^+]$.
3. pH is the negative of the value found in step 2.

For example, for pure water at 25°C,

$$pH = -\log [H^+] = -\log 10^{-7} = -(-7) = 7.0$$

Low pH values correspond to high concentrations of H^+ and high pH values correspond to low concentrations of H^+.

Acids are **proton donors** and bases are **proton acceptors. Strong acids** (eg, HCl or H_2SO_4) completely dissociate into anions and cations even in strongly acidic solutions (low pH). **Weak acids** dissociate only partially in acidic solutions. Similarly, **strong bases** (eg, KOH or NaOH)—but not **weak bases** (eg, Ca[OH]$_2$)—are completely dissociated at high pH. Many biochemicals are weak acids. Exceptions include phosphorylated in-termediates, whose phosphoryl group contains two dissociable protons, the first of which is strongly acidic.

The following examples illustrate how to calculate the pH of acidic and basic solutions.

Example 1: What is the pH of a solution whose hydrogen ion concentration is 3.2×10^{-4} mol/L?

$$pH = -\log [H^+]$$

$$= -\log (3.2 \times 10^{-4})$$

$$= -\log (3.2) - \log (10^{-4})$$

$$= -0.5 + 4.0$$

$$= 3.5$$

Example 2: What is the pH of a solution whose hydroxide ion concentration is 4.0×10^{-4} mol/L? We first define a quantity **pOH** that is equal to $-\log [OH^-]$ and that may be derived from the definition of K_w:

$$K_w = [H^+][OH^-] = 10^{-14}$$

Therefore:

$$\log [H^+] + \log [OH^-] = \log 10^{-14}$$

or

$$pH + pOH = 14$$

To solve the problem by this approach:

$$[OH^-] = 4.0 \times 10^{-4}$$

$$pOH = -\log [OH^-]$$

$$= -\log (4.0 \times 10^{-4})$$

$$= -\log (4.0) - \log (10^{-4})$$

$$= -0.60 + 4.0$$

$$= 3.4$$

Now:

$$pH = 14 - pOH = 14 - 3.4$$

$$= 10.6$$

Example 3: What are the pH values of (a) 2.0×10^{-2} mol/L KOH and of (b) 2.0×10^{-6} mol/L KOH? The OH^- arises from two sources, KOH and water. Since pH is determined by the total $[H^+]$ (and pOH by the total $[OH^-]$), both sources must be considered. In the first case (a), the contribution of water to the total $[OH^-]$ is negligible. The same cannot be said for the second case (b):

	Concentration (mol/L)	
	(a)	(b)
Molarity of KOH	2.0×10^{-2}	2.0×10^{-6}
[OH⁻] from KOH	2.0×10^{-2}	2.0×10^{-6}
[OH⁻] from water	1.0×10^{-7}	1.0×10^{-7}
Total [OH⁻]	2.00001×10^{-2}	2.1×10^{-6}

Once a decision has been reached about the significance of the contribution by water, pH may be calculated as above.

The above examples assume that the strong base KOH is completely dissociated in solution and that the concentration of OH⁻ ions was thus equal to that of the KOH. This assumption is valid for dilute solutions of strong bases or acids but not for weak bases or acids. Since weak electrolytes dissociate only slightly in solution, we must use the **dissociation constant** to calculate the concentration of [H⁺] (or [OH⁻]) produced by a given molarity of a weak acid (or base) before calculating total [H⁺] (or total [OH⁻]) and subsequently pH.

Functional Groups That Are Weak Acids Have Great Physiologic Significance

Many biochemicals possess functional groups that are weak acids or bases. Carboxyl groups, amino groups, and the second phosphate dissociation of phosphate esters are present in proteins and nucleic acids, most coenzymes, and most intermediary metabolites. Knowledge of the dissociation of weak acids and bases thus is basic to understanding the influence of intracellular pH on structure and biologic activity. Charge-based separations such as electrophoresis and ion exchange chromatography also are best understood in terms of the dissociation behavior of functional groups.

We term the protonated species (eg, HA or R—NH₃⁺) the **acid** and the unprotonated species (eg, A⁻ or R—NH₂) its **conjugate base.** Similarly, we may refer to a **base** (eg, A⁻ or R—NH₂) and its **conjugate acid** (eg, HA or R—NH₃⁺). Representative weak acids (left), their conjugate bases (center), and the pK_a values (right) include the following:

$$R—CH_2—COOH \quad R—CH_2—COO^- \quad pK_a = 4-5$$
$$R—CH_2—NH_3^+ \quad R—CH_2—NH_2 \quad pK_a = 9-10$$
$$H_2CO_3 \quad HCO_3^- \quad pK_a = 6.4$$
$$H_2PO_4^- \quad HPO_4^{-2} \quad pK_a = 7.2$$

We express the relative strengths of weak acids and bases in terms of their dissociation constants. Shown below are the expressions for the dissociation constant (K_a) for two representative weak acids, R—COOH and R—NH₃⁺.

$$R—COOH \quad R—COO^- + H^+$$
$$K_a = \frac{[R—COO^-][H^+]}{[R—COOH]}$$
$$R—NH_3^+ \quad R—NH_2 + H^+$$
$$K_a = \frac{[R—NH_2][H^+]}{[R—NH_3^+]}$$

Since the numeric values of K_a for weak acids are negative exponential numbers, we express K_a as pK_a, where

$$pK_a = -\log K$$

Note that pK_a is related to K_a as pH is to [H⁺]. The stronger the acid, the lower its pK_a value.

pK_a is used to express the relative strengths of both acids and bases. For any weak acid, its conjugate is a strong base. Similarly, the conjugate of a strong base is a weak acid. The relative strengths of bases are expressed in terms of the pK_a of their conjugate acids. For polyproteic compounds containing more than one dissociable proton, a numerical subscript is assigned to each in order of relative acidity. For a dissociation of the type

$$R—NH_3^+ \rightarrow R—NH_2$$
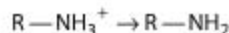
the pK_a is the pH at which the concentration of the acid R—NH₃⁺ equals that of the base R—NH₂.

From the above equations that relate K_a to [H⁺] and to the concentrations of undissociated acid and its conjugate base, when

$$[R—COO^-] = [R—COOH]$$

or when

$$[R—NH_2] = [R—NH_3^+]$$

then

$$K_a = [H^+]$$

Thus, when the associated (protonated) and dissociated (conjugate base) species are present at equal concentrations, the prevailing hydrogen ion concentration [H⁺] is numerically equal to the dissociation constant, K_a. If the logarithms of both sides of the above equation are

taken and both sides are multiplied by −1, the expressions would be as follows:

$$K_a = [H^+]$$
$$-\log K_a = -\log [H^+]$$

Since −log K_a is defined as pK_a, and −log [H⁺] defines pH, the equation may be rewritten as

$$pK_a = pH$$

ie, the pK_a of an acid group is the pH at which the protonated and unprotonated species are present at equal concentrations. The pK_a for an acid may be determined by adding 0.5 equivalent of alkali per equivalent of acid. The resulting pH will be the pK_a of the acid.

The Henderson-Hasselbalch Equation Describes the Behavior of Weak Acids & Buffers

The Henderson-Hasselbalch equation is derived below. A weak acid, HA, ionizes as follows:
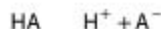
$$HA \quad H^+ + A^-$$

The equilibrium constant for this dissociation is

$$K_a = \frac{[H^+][A^-]}{[HA]}$$

Cross-multiplication gives

$$[H^+][A^-] = K_a[HA]$$

Divide both sides by [A⁻]:

$$[H^+] = K_a \frac{[HA]}{[A^-]}$$

Take the log of both sides:

$$\log [H^+] = \log \left(K_a \frac{[HA]}{[A^-]} \right)$$
$$= \log K_a + \log \frac{[HA]}{[A^-]}$$

Multiply through by −1:

$$-\log [H^+] = -\log K_a - \log \frac{[HA]}{[A^-]}$$

Substitute pH and pK_a for −log [H⁺] and −log K_a, respectively; then:

$$pH = pK_a - \log \frac{[HA]}{[A^-]}$$

Inversion of the last term removes the minus sign and gives the Henderson-Hasselbalch equation:

$$pH = pK_a + \log \frac{[A^-]}{[HA]}$$

The Henderson-Hasselbalch equation has great predictive value in protonic equilibria. For example,

(1) When an acid is exactly half-neutralized, [A⁻] = [HA]. Under these conditions,

$$pH = pK_a + \log \frac{[A^-]}{[HA]} = pK_a + \log \frac{1}{1} = pK_a + 0$$

Therefore, at half-neutralization, pH = pK_a.

(2) When the ratio [A⁻]/[HA] = 100:1,

$$pH = pK_a + \log \frac{[A^-]}{[HA]}$$
$$pH = pK_a + \log 100/1 = pK_a + 2$$

(3) When the ratio [A⁻]/[HA] = 1:10,

$$pH = pK_a + \log 1/10 = pK_a + (-1)$$

If the equation is evaluated at ratios of [A⁻]/[HA] ranging from 10^3 to 10^{-3} and the calculated pH values are plotted, the resulting graph describes the titration curve for a weak acid (Figure 2–4).

Solutions of Weak Acids & Their Salts Buffer Changes in pH

Solutions of weak acids or bases and their conjugates exhibit buffering, the ability to resist a change in pH following addition of strong acid or base. Since many metabolic reactions are accompanied by the release or uptake of protons, most intracellular reactions are buffered. Oxidative metabolism produces CO_2, the anhydride of carbonic acid, which if not buffered would produce severe acidosis. Maintenance of a constant pH involves buffering by phosphate, bicarbonate, and proteins, which accept or release protons to resist a change
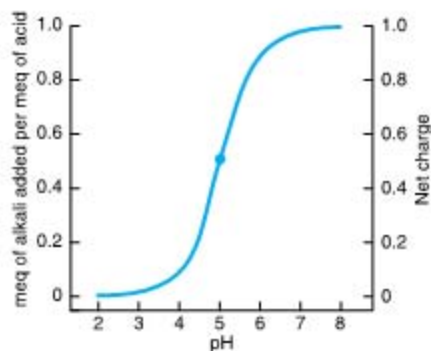
Figure 2–4. Titration curve for an acid of the type HA. The heavy dot in the center of the curve indicates the pK_a 5.0.

in pH. For experiments using tissue extracts or enzymes, constant pH is maintained by the addition of buffers such as MES ([2-N-morpholino]ethanesulfonic acid, pK_a 6.1), inorganic orthophosphate (pK_{a2} 7.2), HEPES (N-hydroxyethylpiperazine-N'-2-ethanesulfonic acid, pK_a 6.8), or Tris (tris[hydroxymethyl] aminomethane, pK_a 8.3). The value of pK_a relative to the desired pH is the major determinant of which buffer is selected.

Buffering can be observed by using a pH meter while titrating a weak acid or base (Figure 2–4). We can also calculate the pH shift that accompanies addition of acid or base to a buffered solution. In the example, the buffered solution (a weak acid, pK_a = 5.0, and its conjugate base) is initially at one of four pH values. We will calculate the pH shift that results when 0.1 meq of KOH is added to 1 meq of each solution:

Initial pH	5.00	5.37	5.60	5.86
$[A^-]_{initial}$	0.50	0.70	0.80	0.88
$[HA]_{initial}$	0.50	0.30	0.20	0.12
$([A^-]/[HA])_{initial}$	1.00	2.33	4.00	7.33
Addition of 0.1 meq of KOH produces				
$[A^-]_{final}$	0.60	0.80	0.90	0.98
$[HA]_{final}$	0.40	0.20	0.10	0.02
$([A^-]/[HA])_{final}$	1.50	4.00	9.00	49.0
log $([A^-]/[HA])_{final}$	0.176	0.602	0.95	1.69
Final pH	5.18	5.60	5.95	6.69
ΔpH	0.18	0.60	0.95	1.69

Notice that the change in pH per milliequivalent of OH^- added depends on the initial pH. The solution resists changes in pH most effectively at pH values close

to the pK_a. A solution of a weak acid and its conjugate base buffers most effectively in the pH range pK_a ± 1.0 pH unit.

Figure 2–4 also illustrates the net charge on one molecule of the acid as a function of pH. A fractional charge of −0.5 does not mean that an individual molecule bears a fractional charge, but the *probability* that a given molecule has a unit negative charge is 0.5. Consideration of the net charge on macromolecules as a function of pH provides the basis for separatory techniques such as ion exchange chromatography and electrophoresis.

Acid Strength Depends on Molecular Structure

Many acids of biologic interest possess more than one dissociating group. The presence of adjacent negative charge hinders the release of a proton from a nearby group, raising its pK_a. This is apparent from the pK_a values for the three dissociating groups of phosphoric acid and citric acid (Table 2–2). The effect of adjacent charge decreases with distance. The second pK_a for succinic acid, which has two methylene groups between its carboxyl groups, is 5.6, whereas the second pK_a for glu-

Table 2–2. Relative strengths of selected acids of biologic significance. Tabulated values are the pK_a values (−log of the dissociation constant) of selected monoprotic, diprotic, and triprotic acids.

Monoprotic Acids		
Formic	pK	3.75
Lactic	pK	3.86
Acetic	pK	4.76
Ammonium ion	pK	9.25
Diprotic Acids		
Carbonic	pK_1	6.37
	pK_2	10.25
Succinic	pK_1	4.21
	pK_2	5.64
Glutaric	pK_1	4.34
	pK_2	5.41
Triprotic Acids		
Phosphoric	pK_1	2.15
	pK_2	6.82
	pK_3	12.38
Citric	pK_1	3.08
	pK_2	4.74
	pK_3	5.40

taric acid, which has one additional methylene group, is 5.4.

pK_a Values Depend on the Properties of the Medium

The pK_a of a functional group is also profoundly influenced by the surrounding medium. The medium may either raise or lower the pK_a depending on whether the undissociated acid or its conjugate base is the charged species. The effect of dielectric constant on pK_a may be observed by adding ethanol to water. The pK_a of a carboxylic acid *increases*, whereas that of an amine *decreases* because ethanol decreases the ability of water to solvate a charged species. The pK_a values of dissociating groups in the interiors of proteins thus are profoundly affected by their local environment, including the presence or absence of water.

SUMMARY

- Water forms hydrogen-bonded clusters with itself and with other proton donors or acceptors. Hydrogen bonds account for the surface tension, viscosity, liquid state at room temperature, and solvent power of water.
- Compounds that contain O, N, or S can serve as hydrogen bond donors or acceptors.
- Macromolecules exchange internal surface hydrogen bonds for hydrogen bonds to water. Entropic forces dictate that macromolecules expose polar regions to an aqueous interface and bury nonpolar regions.
- Salt bonds, hydrophobic interactions, and van der Waals forces participate in maintaining molecular structure.
- pH is the negative log of [H^+]. A low pH characterizes an acidic solution, and a high pH denotes a basic solution.
- The strength of weak acids is expressed by pK_a, the negative log of the acid dissociation constant. Strong acids have low pK_a values and weak acids have high pK_a values.
- Buffers resist a change in pH when protons are produced or consumed. Maximum buffering capacity occurs ± 1 pH unit on either side of pK_a. Physiologic buffers include bicarbonate, orthophosphate, and proteins.

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