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31ST EDITION

HARPER'S
ILLUSTRATED
BIOCHEMISTRY

Harper's Illustrated Biochemistry

THIRTY-FIRST EDITION

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Preface

The authors and publishers are pleased to present the thirty-first edition of *Harper's Illustrated Biochemistry*. The first edition, entitled Harper's Biochemistry, was published in 1939 under the sole authorship of Dr Harold Harper at the University of California School of Medicine, San Francisco, California. Presently entitled *Harper's Illustrated Biochemistry*, the book continues, as originally intended, to provide a concise survey of aspects of biochemistry most relevant to the study of medicine. Various authors have contributed to subsequent editions of this medically oriented biochemistry text, which is now observing its 79th year.

Cover Illustration for the Thirty-first Edition

The illustration on the cover of the thirty-first edition, the structure of Zika virus protein determined at 3.8 Å resolution, was generously prepared and provided by Lei Sun. The supporting data appeared in: Sirohi D, Chen Z, Sun L, Klose T, Pierson TC, Rossmann MG, Kuhn RJ: "The 3.8 Å resolution cryo-EM structure of Zika virus protein", Science 2016;352:497-470. Together with the Zika virus, first recovered in the Zika valley of Uganda, the viruses responsible for yellow fever, West Nile fever, and dengue fever are members of the *Flaviviridae* family of positive-strand DNA viruses. The cover illustration indicates the resolving power of cryo-electron microscopy (cryo-EM). More importantly, it recognizes the medical significance of infection by the Zika virus, which in pregnant women can result in a significant risk of congenital microcephaly and associated severe mental impairment. While Zika virus typically is transmitted by the bite of an infected mosquito, emerging evidence suggests that under certain conditions the Zika virus may also be transmitted between human subjects.

Changes in the Thirty-first Edition

As always, Harper's Illustrated Biochemistry continues to emphasize the close relationship of biochemistry to the understanding of diseases, their pathology and the practice of medicine. The contents of most chapters have been updated and provide to the reader the most current and pertinent information. Toward that end, we have replaced Chapter 10 "Bioinformatics and Computational Biology," most of whose programs and topics (for example protein and nucleotide sequence comparisons and *in silico* approaches in drug design) are available on line or are now common knowledge. Its replacement, new Chapter 10 "Biochemistry of Transition Metals," incorporates material from several chapters, notably

those of blood cells and plasma, which contained extensive content on metal ion adsorption and trafficking, especially of iron and copper. Since approximately a third of all proteins are metalloproteins, new Chapter 10 explicitly addresses the importance and overall pervasiveness of transition metals. Given the overlap with the topics of protein structure and of enzyme reaction mechanisms, new Chapter 10 now follows the three chapters on enzymes as the final chapter in Section II, now renamed Enzymes: Kinetics, Mechanism, Regulation, & Role of Transition Metals.

Organization of the Book

All 58 chapters of the thirty-first edition place major emphasis on the medical relevance of biochemistry. Topics are organized under eleven major headings. Both to assist study and to facilitate retention of the contained information, Questions follow each Section. An Answer Bankk follows the Appendix.

Section I includes a brief history of biochemistry, and emphasizes the interrelationships between biochemistry and medicine. Water, the importance of homeostasis of intracellular pH are reviewed, and the various orders of protein structure are addressed.

Section II begins with a chapter on hemoglobin. Four chapters next address the kinetics, mechanism of action, and metabolic regulation of enzymes, and the role of metal ions in multiple aspects of intermediary metabolism.

Section III addresses bioenergetics and the role of high energy phosphates in energy capture and transfer, the oxidation–reduction reactions involved in biologic oxidation, and metabolic details of energy capture via the respiratory chain and oxidative phosphorylation.

Section IV considers the metabolism of carbohydrates via glycolysis, the citric acid cycle, the pentose phosphate pathway, glycogen metabolism, gluconeogenesis, and the control of blood glucose.

Section V outlines the nature of simple and complex lipids, lipid transport and storage, the biosynthesis and degradation of fatty acids and more complex lipids, and the reactions and metabolic regulation of cholesterol biosynthesis and transport in human subjects.

Section VI discusses protein catabolism, urea biosynthesis, and the catabolism of amino acids and stresses the medically significant metabolic disorders associated with their incomplete catabolism. The final chapter considers the biochemistry of the porphyrins and bile pigments.

Section VII first outlines the structure and function of nucleotides and nucleic acids, then details DNA replication and repair, RNA synthesis and modification, protein synthesis, the principles of recombinant DNA technology, and the regulation of gene expression.

Section VIII considers aspects of extracellular and intracellular communication. Specific topics include membrane structure and function, the molecular bases of the actions of hormones, and signal transduction.

Sections IX, X, & XI address fourteen topics of significant medical importance.

Section IX discusses nutrition, digestion, and absorption, micronutrients including vitamins free radicals and antioxidants, glycoproteins, the metabolism of xenobiotics, and clinical biochemistry.

Section X addresses intracellular traffic and the sorting of proteins, the extracellular matrix, muscle and the cytoskeleton, plasma proteins and immunoglobulins, and the biochemistry of red cells and of white cells.

Section XI includes hemostasis and thrombosis, an overview of cancer, the biochemistry of aging, and a selection of case histories.

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Structures & Functions of Proteins & Enzymes

Biochemistry & Medicine

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OBJECTIVES

After studying this chapter, you should be able to:

- Understand the importance of the ability of cell-free extracts of yeast to ferment sugars, an observation that enabled discovery of the intermediates of fermentation, glycolysis, and other metabolic pathways.

- Appreciate the scope of biochemistry and its central role in the life sciences, and that biochemistry and medicine are intimately related disciplines.

- Appreciate that biochemistry integrates knowledge of the chemical processes in living cells with strategies to maintain health, understand disease, identify potential therapies, and enhance our understanding of the origins of life on earth.

- Describe how genetic approaches have been critical for elucidating many areas of biochemistry, and how the Human Genome Project has furthered advances in numerous aspects of biology and medicine.

BIOMEDICAL IMPORTANCE

Biochemistry and medicine enjoy a mutually cooperative relationship. Biochemical studies have illuminated many aspects of health and disease, and the study of various aspects of health and disease has opened up new areas of biochemistry. The medical relevance of biochemistry both in normal and abnormal situations is emphasized throughout this book. Biochemistry makes significant contributions to the fields of cell biology, physiology, immunology, microbiology, pharmacology, toxicology, and epidemiology, as well as the fields of inflammation, cell injury, and cancer. These close relationships emphasize that life, as we know it, depends on biochemical reactions and processes.

DISCOVERY THAT A CELL-FREE EXTRACT OF YEAST CAN FERMENT SUGAR

Although the ability of yeast to "ferment" various sugars to ethyl alcohol has been known for millennia, only comparatively recently did this process initiate the science of biochemistry. The great French microbiologist Louis Pasteur maintained that fermentation could only occur in intact cells. However, in 1899, the brothers Büchner discovered that fermentation could occur in the *absence* of intact cells when they stored a yeast extract in a crock of concentrated sugar solution, added as a preservative. Overnight, the contents of the crock fermented, spilled over the laboratory bench and floor,

and dramatically demonstrated that fermentation can proceed in the absence of an intact cell. This discovery unleashed an avalanche of research that initiated the science of biochemistry. Investigations revealed the vital roles of inorganic phosphate, ADP, ATP, and NAD(H), and ultimately identified the phosphorylated sugars and the chemical reactions and enzymes that convert glucose to pyruvate (glycolysis) or to ethanol and CO_2 (fermentation). Research beginning in the 1930s identified the intermediates of the citric acid cycle and of urea biosynthesis, and revealed the essential roles of certain vitamin-derived cofactors or "coenzymes" such as thiamin pyrophosphate, riboflavin, and ultimately coenzyme A, coenzyme Q, and cobamide coenzyme. The 1950s revealed how complex carbohydrates are synthesized from, and broken down into simple sugars, and the pathways for biosynthesis of pentoses, and the catabolism of amino acids and fatty acids.

Investigators employed animal models, perfused intact organs, tissue slices, cell homogenates and their subfractions, and subsequently purified enzymes. Advances were enhanced by the development of analytical ultracentrifugation, paper and other forms of chromatography, and the post-World War II availability of radioisotopes, principally ^{14}C, ^{3}H, and ^{32}P, as "tracers" to identify the intermediates in complex pathways such as that of cholesterol biosynthesis. X-ray crystallography was then used to solve the three-dimensional structures of numerous proteins, polynucleotides, enzymes, and viruses. Genetic advances that followed the realization that DNA was a double helix include the polymerase chain reaction, and transgenic animals or those with gene knockouts. The methods used to prepare, analyze, purify, and identify metabolites and the activities of natural and recombinant enzymes and their three-dimensional structures are discussed in the following chapters.

BIOCHEMISTRY & MEDICINE HAVE PROVIDED MUTUAL ADVANCES

The two major concerns for workers in the health sciences—and particularly physicians—are the understanding and maintenance of health and effective treatment of disease. Biochemistry impacts both of these fundamental concerns, and

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 (**Figure 1–1**). An early example of how investigation of protein structure and function revealed the single difference in amino acid sequence between normal hemoglobin and sickle cell hemoglobin. Subsequent analysis of numerous variant sickle cell and other hemoglobins has contributed significantly to our understanding of the structure and function both of hemoglobin and of other proteins. During the early 1900s the English physician Archibald Garrod studied patients with the relatively rare disorders of alkaptonuria, albinism, cystinuria, and pentosuria, and established that these conditions were genetically determined. Garrod designated these conditions as **inborn errors of metabolism**. His insights provided a foundation for the development of the field of human biochemical genetics. A more recent example was investigation of the genetic and molecular basis of familial hypercholesterolemia, a disease that results in early-onset atherosclerosis. In addition to clarifying different genetic mutations responsible for this disease, this provided a deeper understanding of cell receptors and mechanisms of uptake, not only of cholesterol but also of how other molecules cross cell membranes. Studies of **oncogenes** and **tumor suppressor genes** in cancer cells have directed attention to the molecular mechanisms involved in the control of normal cell growth. These examples illustrate how the study of disease can open up areas of basic biochemical research. Science provides physicians and other workers in health care and biology with a foundation that impacts practice, stimulates curiosity, and promotes the adoption of scientific approaches for continued learning.

BIOCHEMICAL PROCESSES UNDERLIE HUMAN HEALTH

Biochemical Research Impacts Nutrition & Preventive Medicine

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 biochemical
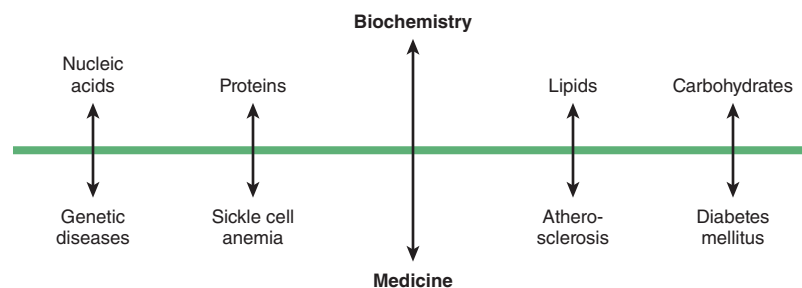
FIGURE 1–1 **A two-way street connects biochemistry and medicine.** Knowledge of the biochemical topics listed above the green line of the diagram has clarified our understanding of the diseases shown below the green line. Conversely, analyses of the diseases have cast light on many areas of biochemistry. Note that sickle cell anemia is a genetic disease, and that both atherosclerosis and diabetes mellitus have genetic components.

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 survival under pressure from both internal and external challenges. The maintenance of health requires optimal dietary intake of **vitamins**, certain **amino acids** and **fatty acids**, various **minerals**, and **water**. Understanding nutrition depends to a great extent on knowledge of biochemistry, and the sciences of biochemistry and nutrition share a focus on these chemicals. Recent increasing emphasis on systematic attempts to maintain health and forestall disease, or **preventive medicine**, includes nutritional approaches to the prevention of diseases such as atherosclerosis and cancer.

Most Diseases Have a Biochemical Basis

Apart from infectious organisms and environmental pollutants, many diseases are manifestations of abnormalities in genes, proteins, chemical reactions, or biochemical processes, each of which can adversely affect one or more critical biochemical functions. Examples of disturbances in human biochemistry responsible for diseases or other debilitating conditions include electrolyte imbalance, defective nutrient ingestion or absorption, hormonal imbalances, toxic chemicals or biologic agents, and DNA-based genetic disorders. To address these challenges, biochemical research continues to be interwoven with studies in disciplines such as genetics, cell biology, immunology, nutrition, pathology, and pharmacology. In addition, many biochemists are vitally interested in contributing to solutions to key issues such as the ultimate survival of mankind, and educating the public to support use of the scientific method in solving environmental and other major problems that confront our civilization.

Impact of the Human Genome Project on Biochemistry, Biology, & Medicine

Initially unanticipated rapid progress in the late 1990s in sequencing the human genome led in the mid-2000s to the announcement that over 90% of the genome had been sequenced. This effort was headed by the International Human Genome Sequencing Consortium and by Celera Genomics. Except for a few gaps, the sequence of the entire human genome was completed in 2003, just 50 years after the description of the double-helical nature of DNA by Watson and Crick. The implications for biochemistry, medicine, and indeed for all of biology, are virtually unlimited. For example, the ability to isolate and sequence a gene and to investigate its structure and function by sequencing and "gene knockout" experiments have revealed previously unknown genes and their products, and new insights have been gained concerning human evolution and procedures for identifying disease-related genes.

Major advances in biochemistry and understanding human health and disease continue to be made by mutation of the genomes of model organisms such as yeast, the fruit fly *Drosophila melanogaster*, the roundworm *Caenorhabditis elegans*, and the zebra fish, all organisms that can be genetically manipulated to provide insight into the functions of individual genes. These advances can potentially provide clues to curing human diseases such as cancer and Alzheimer disease. **Figure 1–2** highlights areas that have developed or accelerated as a direct result of progress made in the Human Genome Project (HGP). New "**-omics**" fields focus on comprehensive study of the structures and functions of the molecules with which each is concerned. The products of genes (RNA molecules and proteins) are being studied using the techniques of **transcriptomics** and **proteomics**. A spectacular example of the speed of progress in transcriptomics is the explosion of knowledge about small RNA molecules as regulators of gene activity. Other -omics fields include **glycomics**, **lipidomics**, **metabolomics**, **nutrigenomics**, and **pharmacogenomics**. To keep pace with the information generated, **bioinformatics** has received much attention. Other related fields to which the impetus from the HGP has carried over are **biotechnology**, **bioengineering**, **biophysics**, and **bioethics**.
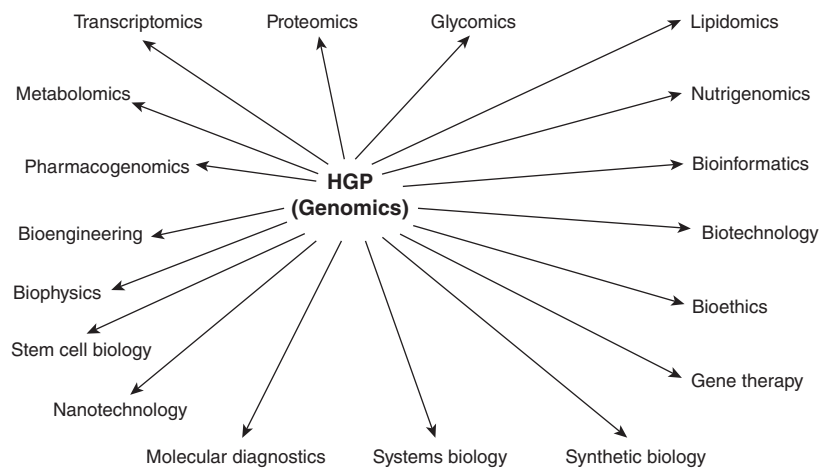
FIGURE 1–2 **The Human Genome Project (HGP) has influenced many disciplines and areas of research.** Biochemistry is not listed since it predates commencement of the HGP, but disciplines such as bioinformatics, genomics, glycomics, lipidomics, metabolomics, molecular diagnostics, proteomics, and transcriptomics are nevertheless active areas of biochemical research.

Definitions of these -omics fields and other terms appear in the Glossary of this chapter. **Nanotechnology** is an active area, which, for example, may provide novel methods of diagnosis and treatment for cancer and other disorders. **Stem cell biology** is at the center of much current research. **Gene therapy** has yet to deliver the promise that it appears to offer, but it seems probable that ultimately will occur. Many new **molecular diagnostic tests** have developed in areas such as genetic, microbiologic, and immunologic testing and diagnosis. **Systems biology** is also burgeoning. The outcomes of research in the various areas mentioned above will impact tremendously the future of biology, medicine, and the health sciences. **Synthetic biology** offers the potential for creating living organisms, initially small bacteria, from genetic material in vitro that might carry out specific tasks such as cleansing petroleum spills. All of the above make the 21st century an exhilarating time to be directly involved in biology and medicine.

SUMMARY

- Biochemistry is the science concerned with the molecules present in living organisms, individual chemical reactions and their enzyme catalysts, and the expression and regulation of each metabolic process. Biochemistry has become the basic language of all biologic sciences.

- Despite the focus on human biochemistry in this text, biochemistry concerns the entire spectrum of life forms, from viruses, bacteria, and plants to complex eukaryotes such as human beings.

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

- Advances in biochemical knowledge have illuminated many areas of medicine, and the study of diseases has often revealed previously unsuspected aspects of biochemistry.

- Biochemical approaches are often fundamental in illuminating the causes of diseases and in designing appropriate therapy. Biochemical laboratory tests also represent 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 medicine and related health sciences.

- Results of the HGP and of research in related areas will have a profound influence on the future of biology, medicine, and other health sciences.

- Genomic research on model organisms such as yeast, the fruit fly *D. melanogaster*, the roundworm *C. elegans*, and the zebra fish provides insight into understanding human diseases.

GLOSSARY

Bioengineering: The application of engineering to biology and medicine.

Bioethics: The area of ethics that is concerned with the application of moral and ethical principles to biology and medicine.

Bioinformatics: The discipline concerned with the collection, storage, and analysis of biologic data, for example, DNA, RNA, and protein sequences.

Biophysics: The application of physics and its techniques to biology and medicine.

Biotechnology: The field in which biochemical, engineering, and other approaches are combined to develop biologic products of use in medicine and industry.

Gene Therapy: Applies to the use of genetically engineered genes to treat various diseases.

Genomics: The genome is the complete set of genes of an organism, and genomics is the in-depth study of the structures and functions of genomes.

Glycomics: The glycome is the total complement of simple and complex carbohydrates in an organism. Glycomics is the systematic study of the structures and functions of glycomes such as the human glycome.

Lipidomics: The lipidome is the complete complement of lipids found in an organism. Lipidomics is the in-depth study of the structures and functions of all members of the lipidome and their interactions, in both health and disease.

Metabolomics: The metabolome is the complete complement of metabolites (small molecules involved in metabolism) present in an organism. Metabolomics is the in-depth study of their structures, functions, and changes in various metabolic states.

Molecular Diagnostics: Refers to the use of molecular approaches such as DNA probes to assist in the diagnosis of various biochemical, genetic, immunologic, microbiologic, and other medical conditions.

Nanotechnology: The development and application to medicine and to other areas of devices such as nanoshells, which are only a few nanometers in size (10^{-9} m = 1 nm).

Nutrigenomics: The systematic study of the effects of nutrients on genetic expression and of the effects of genetic variations on the metabolism of nutrients.

Pharmacogenomics: The use of genomic information and technologies to optimize the discovery and development of new drugs and drug targets.

Proteomics: The proteome is the complete complement of proteins of an organism. Proteomics is the systematic study of the structures and functions of proteomes and their variations in health and disease.

Stem Cell Biology: Stem cells are undifferentiated cells that have the potential to self-renew and to differentiate into any of the adult cells of an organism. Stem cell biology concerns the biology of stem cells and their potential for treating various diseases.

Synthetic Biology: The field that combines biomolecular techniques with engineering approaches to build new biologic functions and systems.

Systems Biology: The field concerns complex biologic systems studied as integrated entities.

Transcriptomics: The comprehensive study of the transcriptome, the complete set of RNA transcripts produced by the genome during a fixed period of time.

APPENDIX

Shown are selected examples of databases that assemble, annotate, and analyze data of biomedical importance.

ENCODE: ENCyclopedia Of DNA Elements. A collaborative effort that combines laboratory and computational approaches to identify every functional element in the human genome.

GenBank: Protein sequence database of the National Institutes of Health (NIH) stores all known biologic nucleotide sequences and their translations in a searchable form.

HapMap: Haplotype **Map**, an international effort to identify single nucleotide polymorphisms (SNPs) associated with common human diseases and differential responses to pharmaceuticals.

ISDB: International **S**equence **D**ata**B**ase that incorporates DNA databases of Japan and of the European Molecular Biology Laboratory (EMBL).

PDB: Protein **D**ata**B**ase. Three-dimensional structures of proteins, polynucleotides, and other macromolecules, including proteins bound to substrates, inhibitors, or other proteins.

Water & pH

Peter J. Kennelly, PhD & Victor W. Rodwell, PhD

- Describe the properties of water that account for its surface tension, viscosity, liquid state at ambient temperature, and solvent power.
- Use structural formulas to represent several organic compounds that can serve as hydrogen bond donors or acceptors.
- Explain the role played by entropy in the orientation, in an aqueous environment, of the polar and nonpolar regions of macromolecules.
- Indicate the quantitative contributions of salt bridges, hydrophobic interactions, and van der Waals forces to the stability of macromolecules.
- Explain the relationship of pH to acidity, alkalinity, and the quantitative determinants that characterize weak and strong acids.
- Calculate the shift in pH that accompanies the addition of a given quantity of acid or base to the pH of a buffered solution.
- Describe what buffers do, how they do it, and the conditions under which a buffer is most effective under physiologic or other conditions.
- Illustrate how the Henderson-Hasselbalch equation can be used to calculate the net charge on a polyelectrolyte at a given pH.

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 both of the biomolecule and of water itself. An excellent nucleophile, water is a reactant or product in many metabolic reactions. 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 has a slight propensity to dissociate into hydroxide ions and protons. The concentration of protons, or **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 follow vomiting of acidic gastric contents.

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° angle between the two hydrogen atoms differs slightly from the ideal tetrahedral angle, 109.5°. Ammonia is also tetrahedral, with a 107° angle between its three hydrogens. The strongly electronegative oxygen atom in a water molecule attracts electrons away from the hydrogen nuclei, leaving them with a partial
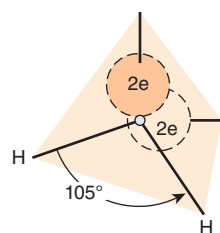
FIGURE 2–1 The water molecule has tetrahedral geometry.

positive charge, while its two unshared electron pairs constitute a region of local negative charge.

A molecule with electrical charge distributed asymmetrically about its structure is referred to as a **dipole**. Water's strong dipole is responsible for its 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 essentially unity; for hexane it is 1.9; for ethanol, 24.3; and for water at 25°C, 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

A partially 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 relatively 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 a few picoseconds. 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
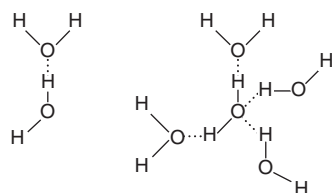
FIGURE 2–2 Water molecules self-associate via hydrogen bonds. Shown are the association of two water molecules (**left**) and a hydrogen-bonded cluster of four water molecules (**right**). Notice that water can serve simultaneously both as a hydrogen donor and as a hydrogen acceptor.
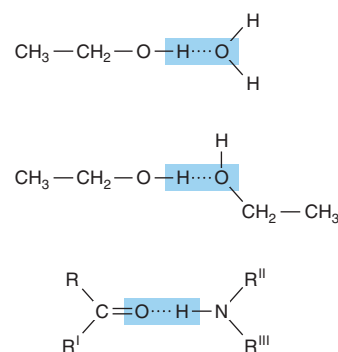
FIGURE 2–3 Additional polar groups participate in hydrogen bonding. Shown are hydrogen bonds formed between alcohol and water, between two molecules of ethanol, and between the peptide carbonyl oxygen and the peptide nitrogen hydrogen of an adjacent amino acid.

can participate in hydrogen bonding. The oxygen atoms of aldehydes, ketones, and amides, for example, provide lone pairs of electrons that can serve as hydrogen acceptors. Alcohols, carboxylic acids, and amines can serve both as hydrogen acceptors and as donors of unshielded hydrogen atoms for formation of hydrogen bonds (**Figure 2–3**).

INTERACTION WITH WATER INFLUENCES THE STRUCTURE OF BIOMOLECULES

Covalent and 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.

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

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, see Table 3–1) generally are present on the surface in contact with water. A similar pattern prevails in a phospholipid bilayer where the charged "head groups" of phosphatidylserine or phosphatidylethanolamine contact water while their hydrophobic fatty acyl side chains cluster together, excluding water (see Figure 40–5). 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 contacts 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 minimizes the disruption of energetically favorable interactions between the surrounding water molecules.

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, which maximizes enthalpy, can be maintained only by increasing the order of the adjacent water molecules, with an accompanying 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 highest entropy (maximum degrees of freedom). Thus, nonpolar molecules tend to form droplets that minimize exposed surface area and reduce the number of water molecules whose motional freedom becomes restricted. Similarly, 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 help 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 therefore often facilitate the binding of charged molecules and ions to proteins and nucleic acids.
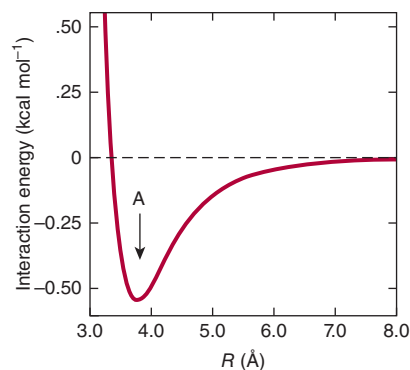
FIGURE 2–4 **The strength of van der Waals interactions varies with the distance, *R*, between interacting species.** The force of interaction between interacting species increases with decreasing distance between them until they are separated by the van der Waals contact distance (see arrow marked A). Repulsion due to interaction between the electron clouds of each atom or molecule then supervenes. While individual van der Waals interactions are extremely weak, their cumulative effect is nevertheless substantial for macromolecules such as DNA and proteins which have many atoms in close contact.

van der Waals Forces

van der Waals forces arise from attractions between transient dipoles generated by the rapid movement of electrons of 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 (**Figure 2–4**). Thus, they act over very short distances, typically 2 to 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 such as hydrogen bonds between nucleotide bases (Watson-Crick base pairing) and van der Waals interactions between the stacked purine and pyrimidine bases. The double helix presents the charged phosphate groups and polar hydroxyl groups from the ribose sugars of the DNA backbone to water while burying the relatively hydrophobic nucleotide bases inside. The extended backbone maximizes the distance between negatively charged phosphates, minimizing unfavorable electrostatic interactions (see Figure 34–2).

WATER IS AN EXCELLENT NUCLEOPHILE

Metabolic reactions often involve the attack by lone pairs of electrons residing on electron-rich molecules termed **nucleophiles** upon 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 (see Figure 2–1), is an excellent nucleophile. Other nucleophiles of biologic importance include the oxygen atoms of phosphates, alcohols, and carboxylic acids; the sulfur of thiols; and the nitrogen atom of amines and of 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 typically 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, for example, during 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 paradoxical behavior reflects the fact that the thermodynamics that govern the equilibrium point of a reaction do not determine the *rate* at which it will proceed toward its equilibrium point. In the cell, protein catalysts called **enzymes** 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 at appropriate times.

Many Metabolic Reactions Involve Group Transfer

Many of the enzymic reactions responsible for synthesis and breakdown of biomolecules involve the transfer of a chemical group G from a donor D to an acceptor A to form an acceptor group complex, A—G:

$$D—G + A \rightleftarrows A—G + D$$
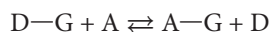
The hydrolysis and phosphorolysis of glycogen, for example, involve the transfer of glucosyl groups to water or to orthophosphate. The equilibrium constant for the hydrolysis of covalent bonds strongly favors the formation of split products. Conversely, many group transfer reactions responsible for the biosynthesis of macromolecules involve the thermodynamically unfavored formation of covalent bonds. Enzyme catalysts play a critical role in surmounting these barriers by virtue of their capacity to directly link two normally separate reactions together. By linking an energetically unfavorable group transfer reaction with a thermodynamically favorable reaction, such as the hydrolysis of ATP, a new coupled reaction can be generated whose net *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 aqueous environment that favors hydrolysis? Central to both questions are the properties of enzymes. In the absence of enzymic catalysis, even reactions that are highly favored thermodynamically do not necessarily take place rapidly. Precise and differential control of enzyme activity and the sequestration of enzymes in specific organelles determine the physiologic circumstances under which a given biopolymer will be synthesized or degraded. The ability of enzyme active sites to sequester substrates in an environment from which water can be excluded facilitates biopolymer synthesis.

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^-):

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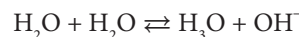
The transferred proton is actually associated with a cluster of water molecules. Protons exist in solution not only as H_3O^+, but also as multimers such as $H_5O_2^+$ and $H_7O_3^+$. The proton is nevertheless routinely represented as H^+, even though it is in fact highly hydrated.

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 water molecule. Individual ions or molecules are therefore not considered. We refer instead to the *probability* that at any instant in time a given 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 at any given moment in time, a hydrogen atom has 1 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 water molecule thus is almost unity. Stated another way, for every hydrogen ion or hydroxide ion in pure water, there are 0.56 billion or 0.56×10^9 water molecules. Hydrogen ions and hydroxide ions nevertheless contribute significantly to the properties of water.

For dissociation of water,

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

where the brackets represent molar concentrations (strictly speaking, molar activities) and K is the **dissociation constant**. Since 1 mole (mol) of water weighs 18 g, 1 liter (L) (1000 g) of water contains $1000 \div 18 = 55.56$ 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