2

Water

Water is the most abundant substance in living systems, making up 70% or more of the weight of most organisms. The first living organisms on Earth doubtless arose in an aqueous environment, and the course of evolution has been shaped by the properties of the aqueous medium in which life began.

This chapter begins with descriptions of the physical and chemical properties of water, to which all aspects of cell structure and function are adapted. The attractive forces between water molecules and the slight tendency of water to ionize are of crucial importance to the structure and function of biomolecules. We review the topic of ionization in terms of equilibrium constants, pH, and titration curves, and consider how aqueous solutions of weak acids or bases and their salts act as buffers against pH changes in biological systems. The water molecule and its ionization products, H^+ and OH^-, profoundly influence the structure, self-assembly, and properties of all cellular components, including proteins, nucleic acids, and lipids. The noncovalent interactions responsible for the strength and specificity of "recognition" among biomolecules are decisively influenced by water's properties as a solvent, including its ability to form hydrogen bonds with itself and with solutes.

2.1 Weak Interactions in Aqueous Systems

Hydrogen bonds between water molecules provide the cohesive forces that make water a liquid at room temperature and a crystalline solid (ice) with a highly ordered arrangement of molecules at cold temperatures. Polar biomolecules dissolve readily in water because they can replace water-water interactions with more energetically favorable water-solute interactions. In contrast, nonpolar biomolecules are poorly soluble in water because they interfere with water-water interactions but are unable to form water-solute interactions. In aqueous solutions, nonpolar molecules tend to cluster together. Hydrogen bonds and ionic, hydrophobic (Greek, "water-fearing"), and van der Waals interactions are individually weak, but collectively they have a very significant influence on the three-dimensional structures of proteins, nucleic acids, polysaccharides, and membrane lipids.

Hydrogen Bonding Gives Water Its Unusual Properties

Water has a higher melting point, boiling point, and heat of vaporization than most other common solvents (Table 2–1). These unusual properties are a consequence of attractions between adjacent water molecules that give liquid water great internal cohesion. A look at the electron structure of the H_2O molecule reveals the cause of these intermolecular attractions.

Each hydrogen atom of a water molecule shares an electron pair with the central oxygen atom. The geometry of the molecule is dictated by the shapes of the outer electron orbitals of the oxygen atom, which are similar to the sp^3 bonding orbitals of carbon (see Fig. 1–15). These orbitals describe a rough tetrahedron, with a hydrogen atom at each of two corners and unshared electron pairs at the other two corners **(Fig. 2–1a)**. The H—O—H bond angle is 104.5°, slightly less than the 109.5° of a perfect tetrahedron because of crowding by the nonbonding orbitals of the oxygen atom.

The oxygen nucleus attracts electrons more strongly than does the hydrogen nucleus (a proton); that is, oxygen is more electronegative. This means that the shared electrons are more often in the vicinity of the oxygen atom than of the hydrogen. The result of this unequal electron sharing is two electric dipoles in the water molecule, one along each of the H—O bonds;

TABLE 2–1 Melting Point, Boiling Point, and Heat of Vaporization of Some Common Solvents

	Melting point (°C)	Boiling point (°C)	Heat of vaporization (J/g)*
Water	0	100	2,260
Methanol (CH_3OH)	−98	65	1,100
Ethanol (CH_3CH_2OH)	−117	78	854
Propanol ($CH_3CH_2CH_2OH$)	−127	97	687
Butanol ($CH_3(CH_2)_2CH_2OH$)	−90	117	590
Acetone (CH_3COCH_3)	−95	56	523
Hexane ($CH_3(CH_2)_4CH_3$)	−98	69	423
Benzene (C_6H_6)	6	80	394
Butane ($CH_3(CH_2)_2CH_3$)	−135	−0.5	381
Chloroform ($CHCl_3$)	−63	61	247

*The heat energy required to convert 1.0 g of a liquid at its boiling point and at atmospheric pressure into its gaseous state at the same temperature. It is a direct measure of the energy required to overcome attractive forces between molecules in the liquid phase.

each hydrogen atom bears a partial positive charge ($\delta+$), and the oxygen atom bears a partial negative charge equal in magnitude to the sum of the two partial positives ($2\delta-$). As a result, there is an electrostatic attraction between the oxygen atom of one water molecule and the hydrogen of another (Fig. 2–1b), called a **hydrogen bond**. Throughout this book, we represent hydrogen bonds with three parallel blue lines, as in Figure 2–1b.

Hydrogen bonds are relatively weak. Those in liquid water have a **bond dissociation energy** (the energy required to break a bond) of about 23 kJ/mol, compared with 470 kJ/mol for the covalent O—H bond in water or 348 kJ/mol for a covalent C—C bond. The hydrogen

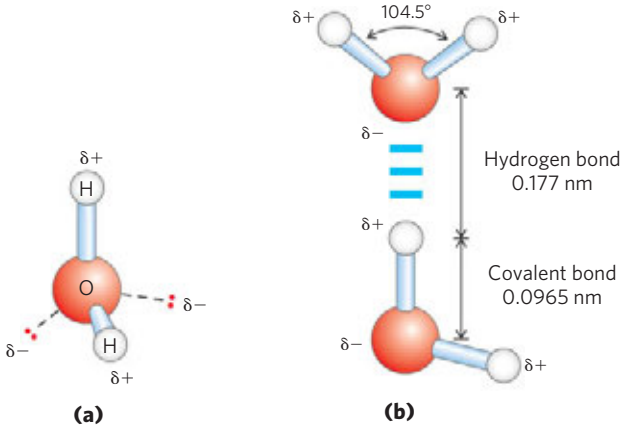


FIGURE 2–1 Structure of the water molecule. (a) The dipolar nature of the H_2O molecule is shown in a ball-and-stick model; the dashed lines represent the nonbonding orbitals. There is a nearly tetrahedral arrangement of the outer-shell electron pairs around the oxygen atom; the two hydrogen atoms have localized partial positive charges ($\delta+$) and the oxygen atom has a partial negative charge ($\delta-$). **(b)** Two H_2O molecules joined by a hydrogen bond (designated here, and throughout this book, by three blue lines) between the oxygen atom of the upper molecule and a hydrogen atom of the lower one. Hydrogen bonds are longer and weaker than covalent O—H bonds.

bond is about 10% covalent, due to overlaps in the bonding orbitals, and about 90% electrostatic. At room temperature, the thermal energy of an aqueous solution (the kinetic energy of motion of the individual atoms and molecules) is of the same order of magnitude as that required to break hydrogen bonds. When water is heated, the increase in temperature reflects the faster motion of individual water molecules. At any given time, most of the molecules in liquid water are hydrogen bonded, but the lifetime of each hydrogen bond is just 1 to 20 picoseconds ($1\ ps = 10^{-12}$ s); when one hydrogen bond breaks, another hydrogen bond forms, with the same partner or a new one, within 0.1 ps. The apt phrase "flickering clusters" has been applied to the short-lived groups of water molecules interlinked by hydrogen bonds in liquid water. The sum of all the hydrogen bonds between H_2O molecules confers great internal cohesion on liquid water. Extended networks of hydrogen-bonded water molecules also form bridges between solutes (proteins and nucleic acids, for example) that allow the larger molecules to interact with each other over distances of several nanometers without physically touching.

The nearly tetrahedral arrangement of the orbitals about the oxygen atom (Fig. 2–1a) allows each water molecule to form hydrogen bonds with as many as four neighboring water molecules. In liquid water at room temperature and atmospheric pressure, however, water molecules are disorganized and in continuous motion, so that each molecule forms hydrogen bonds with an average of only 3.4 other molecules. In ice, on the other hand, each water molecule is fixed in space and forms hydrogen bonds with a full complement of four other water molecules to yield a regular lattice structure **(Fig. 2–2)**. Hydrogen bonds account for the relatively high melting point of water, because much thermal energy is required to break a sufficient proportion of hydrogen bonds to destabilize the crystal lattice of ice

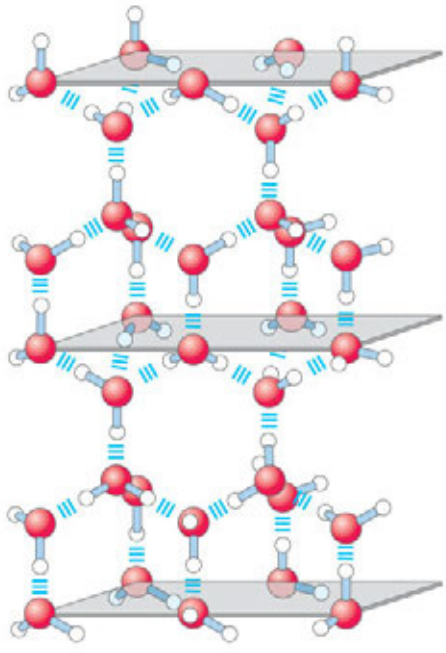


FIGURE 2–2 Hydrogen bonding in ice. In ice, each water molecule forms four hydrogen bonds, the maximum possible for a water molecule, creating a regular crystal lattice. By contrast, in liquid water at room temperature and atmospheric pressure, each water molecule hydrogen-bonds with an average of 3.4 other water molecules. This crystal lattice structure makes ice less dense than liquid water, and thus ice floats on liquid water.

(Table 2–1). When ice melts or water evaporates, heat is taken up by the system:

$$H_2O(\text{solid}) \longrightarrow H_2O(\text{liquid}) \qquad \Delta H = +5.9 \text{ kJ/mol}$$
$$H_2O(\text{liquid}) \longrightarrow H_2O(\text{gas}) \qquad \Delta H = +44.0 \text{ kJ/mol}$$

During melting or evaporation, the entropy of the aqueous system increases as the highly ordered arrays of water molecules in ice relax into the less orderly hydrogen-bonded arrays in liquid water or into the wholly disordered gaseous state. At room temperature, both the melting of ice and the evaporation of water occur spontaneously; the tendency of the water molecules to associate through hydrogen bonds is outweighed by the energetic push toward randomness. Recall that the free-energy change (ΔG) must have a negative value for a process to occur spontaneously: $\Delta G = \Delta H - T\Delta S$, where ΔG represents the driving force, ΔH the enthalpy change from making and breaking bonds, and ΔS the change in randomness. Because ΔH is positive for melting and evaporation, it is clearly the increase in entropy (ΔS) that makes ΔG negative and drives these changes.

Water Forms Hydrogen Bonds with Polar Solutes

Hydrogen bonds are not unique to water. They readily form between an electronegative atom (the hydrogen acceptor, usually oxygen or nitrogen) and a hydrogen atom covalently bonded to another electronegative atom (the hydrogen donor) in the same or another molecule **(Fig. 2–3)**. Hydrogen atoms covalently bonded to carbon atoms do not participate in hydrogen bonding, because carbon is only slightly more electronegative than hydrogen and thus the C—H bond is only very weakly polar. The distinction explains why butanol ($CH_3(CH_2)_2CH_2OH$) has a relatively high boiling point of 117 °C, whereas butane ($CH_3(CH_2)_2CH_3$) has a boiling point of only −0.5 °C. Butanol has a polar hydroxyl group and thus can form intermolecular hydrogen bonds. Uncharged but polar biomolecules such as sugars dissolve readily in water because of the stabilizing effect of hydrogen bonds between the hydroxyl groups or carbonyl oxygen of the sugar and the polar water molecules. Alcohols, aldehydes, ketones, and compounds containing N—H bonds all form hydrogen bonds with water molecules **(Fig. 2–4)** and tend to be soluble in water.

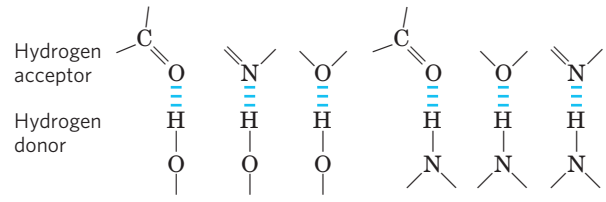


FIGURE 2–3 Common hydrogen bonds in biological systems. The hydrogen acceptor is usually oxygen or nitrogen; the hydrogen donor is another electronegative atom.

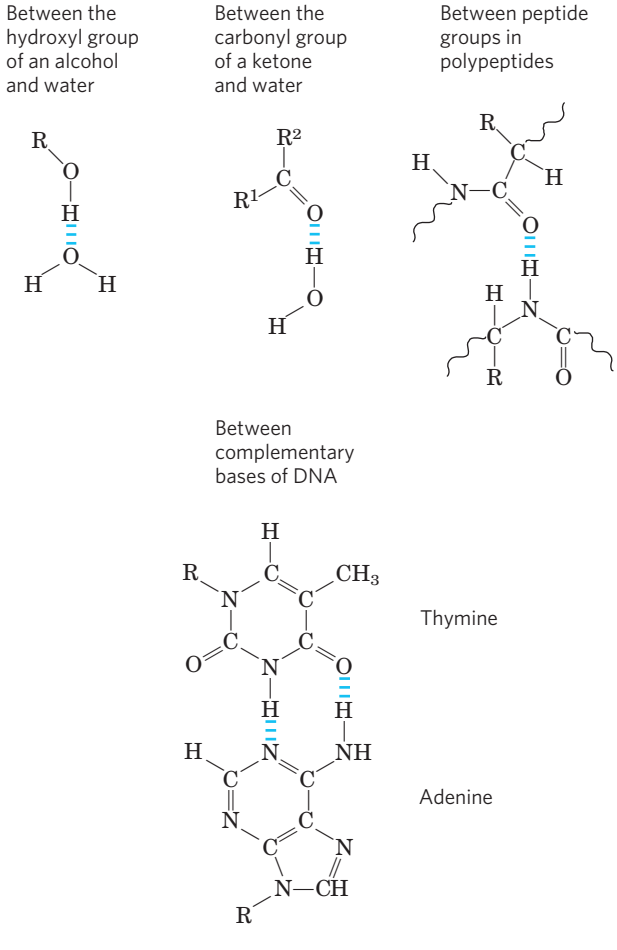


FIGURE 2–4 Some biologically important hydrogen bonds.

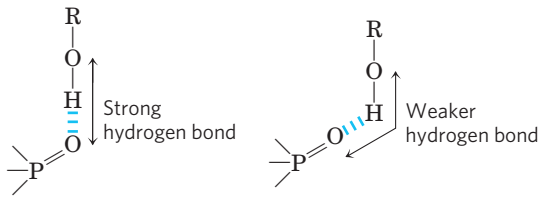


FIGURE 2–5 Directionality of the hydrogen bond. The attraction between the partial electric charges (see Fig. 2–1) is greatest when the three atoms involved in the bond (in this case O, H, and O) lie in a straight line. When the hydrogen-bonded moieties are structurally constrained (when they are parts of a single protein molecule, for example), this ideal geometry may not be possible and the resulting hydrogen bond is weaker.

Hydrogen bonds are strongest when the bonded molecules are oriented to maximize electrostatic interaction, which occurs when the hydrogen atom and the two atoms that share it are in a straight line—that is, when the acceptor atom is in line with the covalent bond between the donor atom and H **(Fig. 2–5)**. This arrangement puts the positive charge of the hydrogen ion directly between the two partial negative charges. Hydrogen bonds are thus highly directional and capable of holding two hydrogen-bonded molecules or groups in a specific geometric arrangement. As we shall see later, this property of hydrogen bonds confers very precise three-dimensional structures on protein and nucleic acid molecules, which have many intramolecular hydrogen bonds.

Water Interacts Electrostatically with Charged Solutes

Water is a polar solvent. It readily dissolves most biomolecules, which are generally charged or polar compounds (Table 2–2); compounds that dissolve easily in water are **hydrophilic** (Greek, "water-loving"). In contrast, nonpolar solvents such as chloroform and benzene are poor solvents for polar biomolecules but easily dissolve those that are **hydrophobic**—nonpolar molecules such as lipids and waxes.

Water dissolves salts such as NaCl by hydrating and stabilizing the Na^+ and Cl^- ions, weakening the electrostatic interactions between them and thus counteracting their tendency to associate in a crystalline lattice **(Fig. 2–6)**. Water also readily dissolves charged biomolecules, including compounds with functional groups such as ionized carboxylic acids (—COO^-), protonated amines ($-NH_3^+$), and phosphate esters or anhydrides. Water replaces the solute-solute hydrogen bonds linking these biomolecules to each other with solute-water hydrogen bonds, thus screening the electrostatic interactions between solute molecules.

Water is effective in screening the electrostatic interactions between dissolved ions because it has a high dielectric constant, a physical property that reflects the number of dipoles in a solvent. The strength, or force (F), of ionic interactions in a solution depends on the magnitude of the charges (Q), the distance between the charged groups (r), and the dielectric constant (ε, which is dimensionless) of the solvent in which the interactions occur:

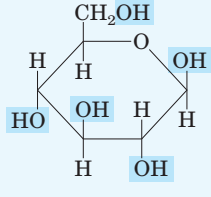
$$F = \frac{Q_1 Q_2}{\varepsilon r^2}$$

For water at 25 °C, ε is 78.5, and for the very nonpolar solvent benzene, ε is 4.6. Thus, ionic interactions between dissolved ions are much stronger in less polar environments. The dependence on r^2 is such that ionic attractions or repulsions operate only over short distances—in the range of 10 to 40 nm (depending on the electrolyte concentration) when the solvent is water.

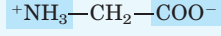
TABLE 2–2 Some Examples of Polar, Nonpolar, and Amphipathic Biomolecules (Shown as Ionic Forms at pH 7)

Polar

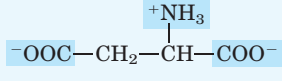
Glucose



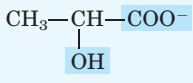
Glycine



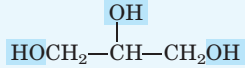
Aspartate



Lactate

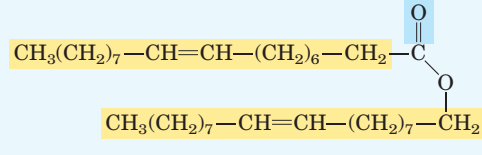


Glycerol



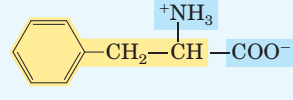
Nonpolar

Typical wax

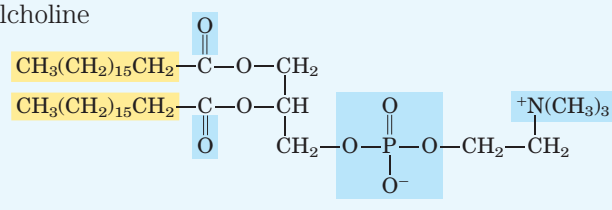


Amphipathic

Phenylalanine



Phosphatidylcholine



Polar groups Nonpolar groups

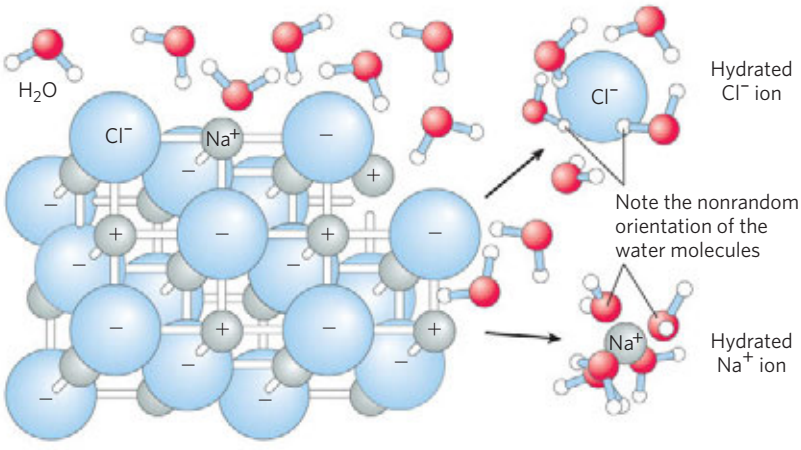


FIGURE 2–6 Water as solvent. Water dissolves many crystalline salts by hydrating their component ions. The NaCl crystal lattice is disrupted as water molecules cluster about the Cl^- and Na^+ ions. The ionic charges are partially neutralized, and the electrostatic attractions necessary for lattice formation are weakened.

Entropy Increases as Crystalline Substances Dissolve

As a salt such as NaCl dissolves, the Na^+ and Cl^- ions leaving the crystal lattice acquire far greater freedom of motion (Fig. 2–6). The resulting increase in entropy (randomness) of the system is largely responsible for the ease of dissolving salts such as NaCl in water. In thermodynamic terms, formation of the solution occurs with a favorable free-energy change: $\Delta G = \Delta H - T\Delta S$, where ΔH has a small positive value and $T\Delta S$ a large positive value; thus ΔG is negative.

Nonpolar Gases Are Poorly Soluble in Water

The molecules of the biologically important gases CO_2, O_2, and N_2 are nonpolar. In O_2 and N_2, electrons are shared equally by both atoms. In CO_2, each C═O bond is polar, but the two dipoles are oppositely directed and cancel each other (Table 2–3). The movement of molecules from the disordered gas phase into aqueous solution constrains their motion and the motion of water molecules and therefore represents a decrease in entropy. The nonpolar nature of these gases and the decrease in entropy when they enter solution combine to make them very poorly soluble in water (Table 2–3). Some organisms have water-soluble "carrier proteins" (hemoglobin and myoglobin, for example) that facilitate the transport of O_2. Carbon dioxide forms carbonic acid (H_2CO_3) in aqueous solution and is transported as the HCO_3^- (bicarbonate) ion, either free—bicarbonate is very soluble in water (~100 g/L at 25 °C)—or bound to hemoglobin. Three other gases, NH_3, NO, and H_2S, also have biological roles in some organisms; these gases are polar, dissolve readily in water, and ionize in aqueous solution.

Nonpolar Compounds Force Energetically Unfavorable Changes in the Structure of Water

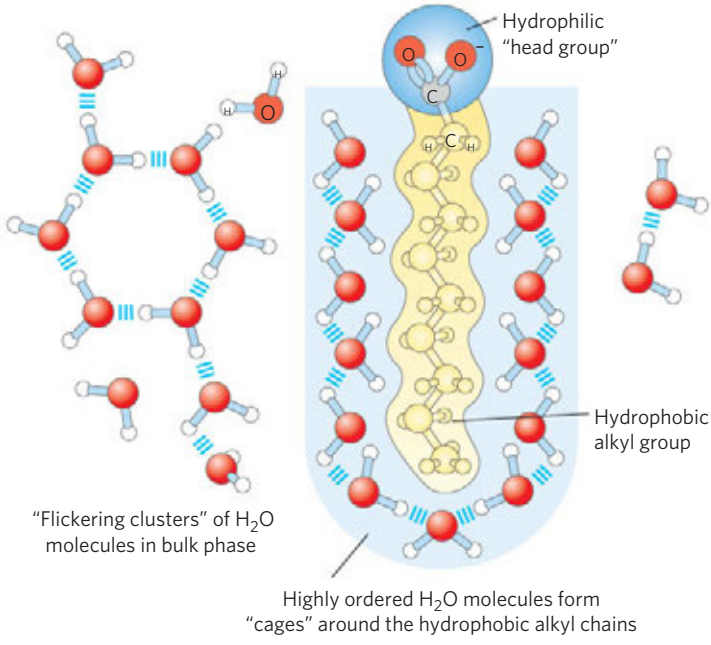
When water is mixed with benzene or hexane, two phases form; neither liquid is soluble in the other. Nonpolar compounds such as benzene and hexane are hydrophobic—they are unable to undergo energetically favorable interactions with water molecules, and they interfere with the hydrogen bonding among water molecules. All molecules or ions in aqueous solution interfere with the hydrogen bonding of some water

TABLE 2–3 Solubilities of Some Gases in Water

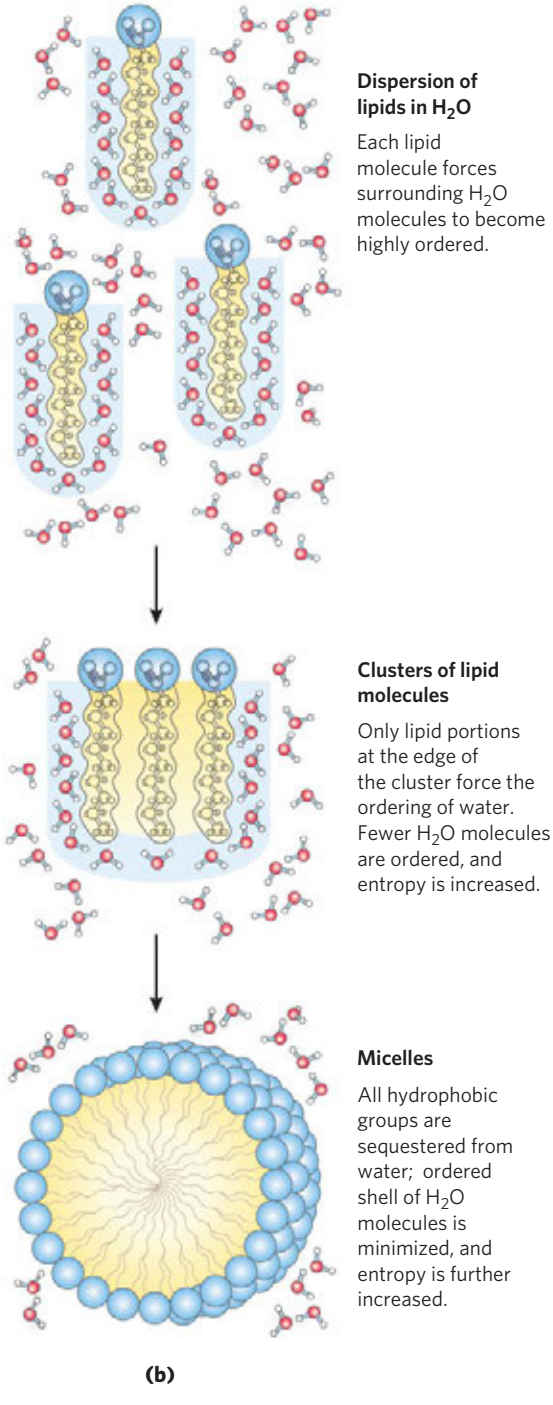
Gas	Structure*	Polarity	Solubility in water (g/L)†
Nitrogen	N≡N	Nonpolar	0.018 (40 °C)
Oxygen	O═O	Nonpolar	0.035 (50 °C)
Carbon dioxide	δ− ← O═C═O → δ−	Nonpolar	0.97 (45 °C)
Ammonia	H, H, H, N, δ−	Polar	900 (10 °C)
Hydrogen sulfide	H, H, S, δ−	Polar	1,860 (40 °C)

*The arrows represent electric dipoles; there is a partial negative charge (δ−) at the head of the arrow, a partial positive charge (δ+ ; not shown here) at the tail.

†Note that polar molecules dissolve far better even at low temperatures than do nonpolar molecules at relatively high temperatures.



(a)



(b)

FIGURE 2–7 Amphipathic compounds in aqueous solution. (a) Long-chain fatty acids have very hydrophobic alkyl chains, each of which is surrounded by a layer of highly ordered water molecules. **(b)** By clustering together in micelles, the fatty acid molecules expose the smallest possible hydrophobic surface area to the water, and fewer water molecules are required in the shell of ordered water. The energy gained by freeing immobilized water molecules stabilizes the micelle.

molecules in their immediate vicinity, but polar or charged solutes (such as NaCl) compensate for lost water-water hydrogen bonds by forming new solute-water interactions. The net change in enthalpy (ΔH) for dissolving these solutes is generally small. Hydrophobic solutes, however, offer no such compensation, and their addition to water may therefore result in a small gain of enthalpy; the breaking of hydrogen bonds between water molecules takes up energy from the system, requiring the input of energy from the surroundings. In addition to requiring this input of energy, dissolving hydrophobic compounds in water produces a measurable decrease in entropy. Water molecules in the immediate vicinity of a nonpolar solute are constrained in their possible orientations as they form a highly ordered cagelike shell around each solute molecule. These water molecules are not as highly oriented as those in **clathrates**, crystalline compounds of nonpolar solutes and water, but the effect is the same in both cases: the ordering of water molecules reduces entropy. The number of ordered water molecules, and therefore the magnitude of the entropy decrease, is proportional to the surface area of the hydrophobic solute enclosed within the cage of water molecules. The free-energy change for dissolving a nonpolar solute in water is thus unfavorable: $\Delta G = \Delta H - T\Delta S$, where ΔH has a positive value, ΔS has a negative value, and ΔG is positive.

Amphipathic compounds contain regions that are polar (or charged) and regions that are nonpolar (Table 2–2). When an amphipathic compound is mixed with water, the polar, hydrophilic region interacts favorably with the water and tends to dissolve, but the nonpolar, hydrophobic region tends to avoid contact with the water **(Fig. 2–7a)**. The nonpolar regions of the molecules cluster together to present the smallest hydrophobic area to the aqueous solvent, and the polar regions are arranged to maximize their interaction with the solvent (Fig. 2–7b). These stable structures of amphipathic compounds in water, called **micelles**, may contain hundreds or thousands of molecules. The forces that

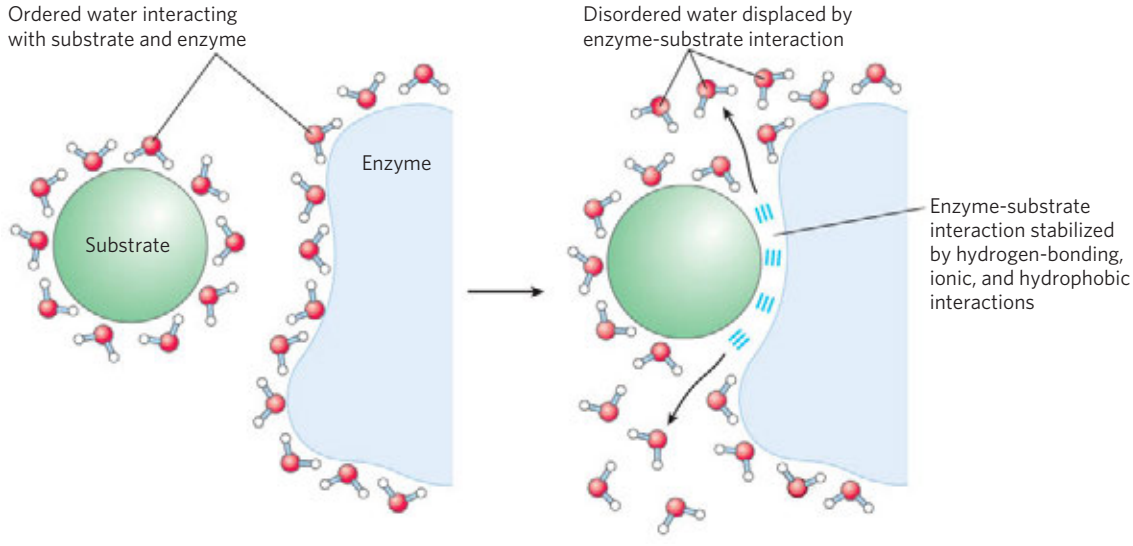


FIGURE 2–8 Release of ordered water favors formation of an enzyme-substrate complex. While separate, both enzyme and substrate force neighboring water molecules into an ordered shell. Binding of substrate to enzyme releases some of the ordered water, and the resulting increase in entropy provides a thermodynamic push toward formation of the enzyme-substrate complex (see p. 198).

hold the nonpolar regions of the molecules together are called **hydrophobic interactions.** The strength of hydrophobic interactions is not due to any intrinsic attraction between nonpolar moieties. Rather, it results from the system's achieving the greatest thermodynamic stability by minimizing the number of ordered water molecules required to surround hydrophobic portions of the solute molecules.

Many biomolecules are amphipathic; proteins, pigments, certain vitamins, and the sterols and phospholipids of membranes all have both polar and nonpolar surface regions. Structures composed of these molecules are stabilized by hydrophobic interactions among the nonpolar regions. Hydrophobic interactions among lipids, and between lipids and proteins, are the most important determinants of structure in biological membranes. Hydrophobic interactions between nonpolar amino acids also stabilize the three-dimensional structures of proteins.

Hydrogen bonding between water and polar solutes also causes an ordering of water molecules, but the energetic effect is less significant than with nonpolar solutes. Disruption of ordered water molecules is part of the driving force for binding of a polar substrate (reactant) to the complementary polar surface of an enzyme: entropy increases as the enzyme displaces ordered water from the substrate, and as the substrate displaces ordered water from the enzyme surface **(Fig. 2–8)**.

van der Waals Interactions Are Weak Interatomic Attractions

When two uncharged atoms are brought very close together, their surrounding electron clouds influence each other. Random variations in the positions of the electrons around one nucleus may create a transient electric dipole, which induces a transient, opposite electric dipole in the nearby atom. The two dipoles weakly attract each other, bringing the two nuclei closer. These weak attractions are called **van der Waals interactions** (also known as London forces). As the two nuclei draw closer together, their electron clouds begin to repel each other. At the point where the net attraction is maximal, the nuclei are said to be in van der Waals contact. Each atom has a characteristic **van der Waals radius**, a measure of how close that atom will allow another to approach (Table 2–4). In the "space-filling" molecular models shown throughout this book, the atoms are depicted in sizes proportional to their van der Waals radii.

TABLE 2–4 van der Waals Radii and Covalent (Single-Bond) Radii of Some Elements

Element	van der Waals radius (nm)	Covalent radius for single bond (nm)
H	0.11	0.030
O	0.15	0.066
N	0.15	0.070
C	0.17	0.077
S	0.18	0.104
P	0.19	0.110
I	0.21	0.133

Sources: For van der Waals radii, Chauvin, R. (1992) Explicit periodic trend of van der Waals radii. *J. Phys. Chem.* 96, 9194–9197. For covalent radii, Pauling, L. (1960) *Nature of the Chemical Bond,* 3rd edn, Cornell University Press, Ithaca, NY.

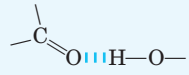
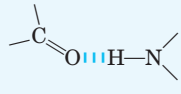
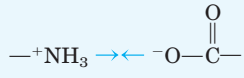
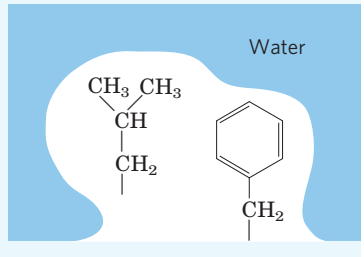
Note: van der Waals radii describe the space-filling dimensions of atoms. When two atoms are joined covalently, the atomic radii at the point of bonding are less than the van der Waals radii, because the joined atoms are pulled together by the shared electron pair. The distance between nuclei in a van der Waals interaction or a covalent bond is about equal to the sum of the van der Waals or covalent radii, respectively, for the two atoms. Thus the length of a carbon–carbon single bond is about 0.077 nm + 0.077 nm = 0.154 nm.

Weak Interactions Are Crucial to Macromolecular Structure and Function

> I believe that as the methods of structural chemistry are further applied to physiological problems, it will be found that the significance of the hydrogen bond for physiology is greater than that of any other single structural feature.
>
> —*Linus Pauling,*
> The Nature of the Chemical Bond, *1939*

The noncovalent interactions we have described—hydrogen bonds and ionic, hydrophobic, and van der Waals interactions (Table 2–5)—are much weaker than covalent bonds. An input of about 350 kJ of energy is required to break a mole of (6×10^{23}) C—C single bonds, and about 410 kJ to break a mole of C—H bonds, but as little as 4 kJ is sufficient to disrupt a mole of typical van der Waals interactions. Hydrophobic interactions are also much weaker than covalent bonds, although they are substantially strengthened by a highly polar solvent (a concentrated salt solution, for example). Ionic interactions and hydrogen bonds are variable in strength, depending on the polarity of the solvent and the alignment of the hydrogen-bonded atoms, but they are always significantly weaker than covalent bonds. In aqueous solvent at 25 °C, the available thermal energy can be of the same order of magnitude as the strength of these weak interactions, and the interaction between solute and solvent (water) molecules is nearly as favorable as solute-solute interactions. Consequently, hydrogen bonds and ionic, hydrophobic, and van der Waals interactions are continually forming and breaking.

TABLE 2–5 Four Types of Noncovalent ("Weak") Interactions among Biomolecules in Aqueous Solvent

Hydrogen bonds	
Between neutral groups	
Between peptide bonds	
Ionic interactions	
Attraction	
Repulsion	
Hydrophobic interactions	
van der Waals interactions	Any two atoms in close proximity

Although these four types of interactions are individually weak relative to covalent bonds, the cumulative effect of many such interactions can be very significant. For example, the noncovalent binding of an enzyme to its substrate may involve several hydrogen bonds and one or more ionic interactions, as well as hydrophobic and van der Waals interactions. The formation of each of these weak bonds contributes to a net decrease in the free energy of the system. We can calculate the stability of a noncovalent interaction, such as the hydrogen bonding of a small molecule to its macromolecular partner, from the binding energy, the reduction in the energy of the system when binding occurs. Stability, as measured by the equilibrium constant (see below) of the binding reaction, varies *exponentially* with binding energy. In order to dissociate two biomolecules (such as an enzyme and its bound substrate) that are associated noncovalently through multiple weak interactions, all these interactions must be disrupted at the same time. Because the interactions fluctuate randomly, such simultaneous disruptions are very unlikely. Therefore, 5 or 20 weak interactions bestow much greater molecular stability than would be expected intuitively from a simple summation of small binding energies.

Macromolecules such as proteins, DNA, and RNA contain so many sites of potential hydrogen bonding or ionic, van der Waals, or hydrophobic interactions that the cumulative effect of the many small binding forces can be enormous. For macromolecules, the most stable (that is, the native) structure is usually that in which weak interactions are maximized. The folding of a single polypeptide or polynucleotide chain into its three-dimensional shape is determined by this principle. The binding of an antigen to a specific antibody depends on the cumulative effects of many weak interactions. As noted earlier, the energy released when an enzyme binds noncovalently to its substrate is the main source of the enzyme's catalytic power. The binding of a hormone or a neurotransmitter to its cellular receptor protein is the result of multiple weak interactions. One consequence of the large size of enzymes and receptors (relative to their substrates or ligands) is that their extensive surfaces provide many opportunities for weak interactions. At the molecular level, the complementarity between interacting biomolecules reflects the complementarity and weak interactions between polar, charged, and hydrophobic groups on the surfaces of the molecules.

When the structure of a protein such as hemoglobin **(Fig. 2–9)** is determined by x-ray crystallography (see Box 4–5), water molecules are often found to be bound so tightly that they are part of the crystal structure; the same is true for water in crystals of RNA or DNA. These bound water molecules, which can also be detected in aqueous solutions by nuclear magnetic resonance, have distinctly different properties from

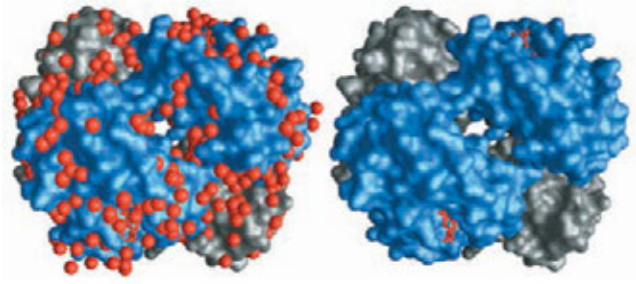


FIGURE 2–9 Water binding in hemoglobin. (PDB ID 1A3N) The crystal structure of hemoglobin, shown **(a)** with bound water molecules (red spheres) and **(b)** without the water molecules. The water molecules are so firmly bound to the protein that they affect the x-ray diffraction pattern as though they were fixed parts of the crystal. The two α subunits of hemoglobin are shown in gray, the two β subunits in blue. Each subunit has a bound heme group (red stick structure), visible only in the β subunits in this view. The structure and function of hemoglobin are discussed in detail in Chapter 5.

those of the "bulk" water of the solvent. They are, for example, not osmotically active (see below). For many proteins, tightly bound water molecules are essential to their function. In a key reaction in photosynthesis, for example, protons flow across a biological membrane as light drives the flow of electrons through a series of electron-carrying proteins (see Fig. 19–62). One of these proteins, cytochrome *f*, has a chain of five bound water molecules **(Fig. 2–10)** that may provide a path for protons to move through the membrane by a process known as "proton hopping" (described below). Another such light-driven proton pump, bacteriorhodopsin, almost certainly uses a chain of precisely oriented bound water molecules in the transmembrane movement of protons (see Fig. 19–69b). Tightly bound water molecules can also form an essential part of the binding site of a protein for its ligand. In a bacterial arabinose-binding protein, for example, five water molecules form hydrogen bonds that provide critical cross-links between the sugar (arabinose) and the amino acid residues in the sugar-binding site **(Fig. 2–11)**.

Solutes Affect the Colligative Properties of Aqueous Solutions

Solutes of all kinds alter certain physical properties of the solvent, water: its vapor pressure, boiling point, melting point (freezing point), and osmotic pressure. These are called **colligative properties** (colligative meaning "tied together"), because the effect of solutes on all four properties has the same basis: the concentration of water is lower in solutions than in pure water. The effect of solute concentration on the colligative properties of water is independent of the chemical properties of the solute; it depends only on the *number* of solute particles (molecules or ions) in a given amount of water. For example, a compound such as NaCl, which dissociates in solution, has an effect on osmotic pressure

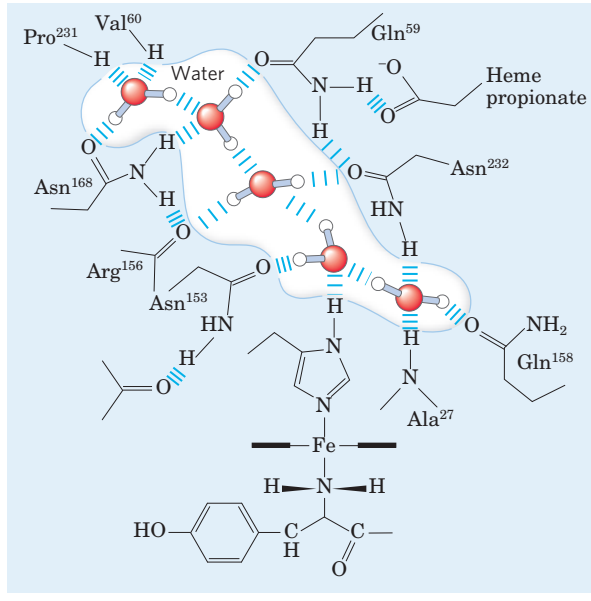


FIGURE 2–10 Water chain in cytochrome *f*. Water is bound in a proton channel of the membrane protein cytochrome *f*, which is part of the energy-trapping machinery of photosynthesis in chloroplasts (see Fig. 19–61). Five water molecules are hydrogen-bonded to each other and to functional groups of the protein: the peptide backbone atoms of valine, proline, arginine, and alanine residues, and the side chains of three asparagine and two glutamine residues. The protein has a bound heme (see Fig. 5–1), its iron ion facilitating electron flow during photosynthesis. Electron flow is coupled to the movement of protons across the membrane, which probably involves "proton hopping" (see Fig. 2–14) through this chain of bound water molecules.

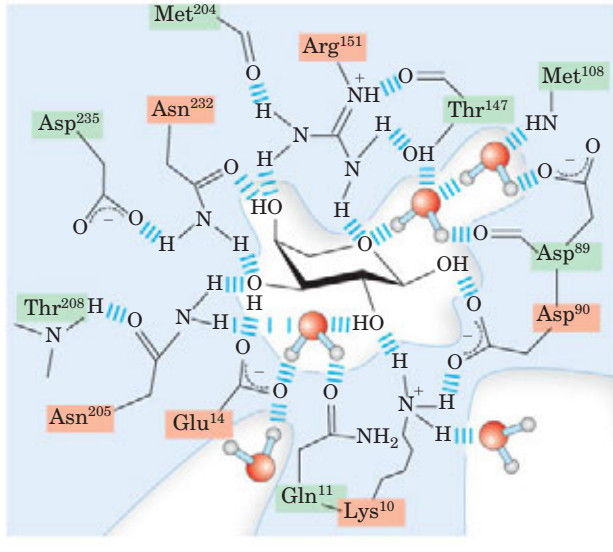


FIGURE 2–11 Hydrogen-bonded water as part of a protein's sugar-binding site. In the L-arabinose-binding protein of the bacterium *E. coli*, five water molecules are essential components of the hydrogen-bonded network of interactions between the sugar arabinose (center) and at least 13 amino acid residues in the sugar-binding site. Viewed in three dimensions, these interacting groups constitute two layers of binding moieties; amino acid residues in the first layer are screened in red, those in the second layer in green. Some of the hydrogen bonds are drawn longer than others for clarity; they are not actually longer than the others.

that is twice that of an equal number of moles of a nondissociating solute such as glucose.

Water molecules tend to move from a region of higher water concentration to one of lower water concentration, in accordance with the tendency in nature for a system to become disordered. When two different aqueous solutions are separated by a semipermeable membrane (one that allows the passage of water but not solute molecules), water molecules diffusing from the region of higher water concentration to the region of lower water concentration produce osmotic pressure **(Fig. 2–12)**. Osmotic pressure, Π, measured as the force necessary to resist water movement (Fig. 2–12c), is approximated by the van't Hoff equation:

$$\Pi = icRT$$

in which R is the gas constant and T is the absolute temperature. The symbol i is the van't Hoff factor, which is a measure of the extent to which the solute dissociates into two or more ionic species. The term ic is the **osmolarity** of the solution, the product of the van't Hoff factor i and the solute's molar concentration c. In dilute NaCl solutions, the solute completely dissociates into Na^+ and Cl^-, doubling the number of solute particles, and thus $i = 2$. For all nonionizing solutes, $i = 1$. For solutions of several (n) solutes, Π is the sum of the contributions of each species:

$$\Pi = RT(i_1c_1 + i_2c_2 + \cdots + i_nc_n)$$

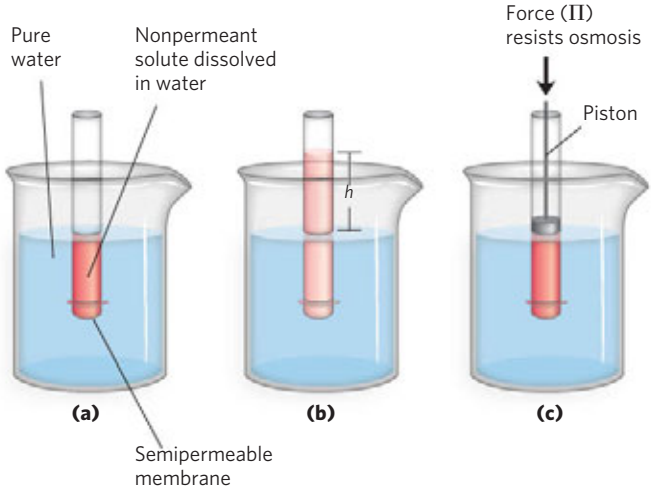


FIGURE 2–12 Osmosis and the measurement of osmotic pressure. **(a)** The initial state. The tube contains an aqueous solution, the beaker contains pure water, and the semipermeable membrane allows the passage of water but not solute. Water flows from the beaker into the tube to equalize its concentration across the membrane. **(b)** The final state. Water has moved into the solution of the nonpermeant compound, diluting it and raising the column of solution within the tube. At equilibrium, the force of gravity operating on the solution in the tube exactly balances the tendency of water to move into the tube, where its concentration is lower. **(c)** Osmotic pressure (Π) is measured as the force that must be applied to return the solution in the tube to the level of the water in the beaker. This force is proportional to the height, *h*, of the column in (b).

Osmosis, water movement across a semipermeable membrane driven by differences in osmotic pressure, is an important factor in the life of most cells. Plasma membranes are more permeable to water than to most other small molecules, ions, and macromolecules because protein channels (aquaporins; see Fig. 11–45) in the membrane selectively permit the passage of water. Solutions of osmolarity equal to that of a cell's cytosol are said to be **isotonic** relative to that cell. Surrounded by an isotonic solution, a cell neither gains nor loses water **(Fig. 2–13)**. In a **hypertonic** solution, one with higher osmolarity than that of the cytosol, the cell shrinks as water moves out. In a **hypotonic** solution, one with a lower osmolarity than the cytosol, the cell swells as water enters. In their natural environments, cells generally contain higher concentrations of biomolecules and ions than their surroundings, so osmotic pressure tends to drive water into cells. If not somehow counterbalanced, this inward movement of water would distend the plasma membrane and eventually cause bursting of the cell (osmotic lysis).

Several mechanisms have evolved to prevent this catastrophe. In bacteria and plants, the plasma membrane is surrounded by a nonexpandable cell wall of sufficient rigidity and strength to resist osmotic pressure

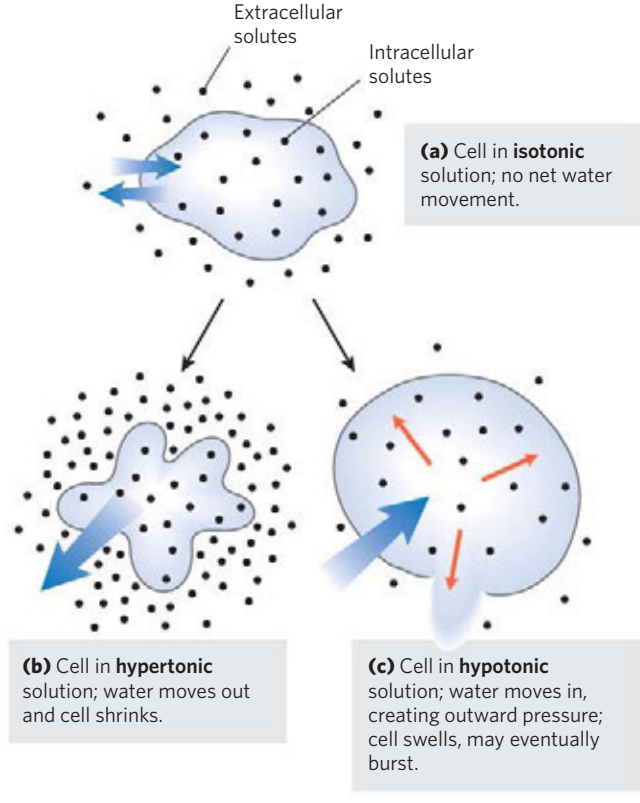


FIGURE 2–13 Effect of extracellular osmolarity on water movement across a plasma membrane. When a cell in osmotic balance with its surrounding medium—that is, a cell in **(a)** an isotonic medium—is transferred into **(b)** a hypertonic solution or **(c)** a hypotonic solution, water moves across the plasma membrane in the direction that tends to equalize osmolarity outside and inside the cell.

and prevent osmotic lysis. Certain freshwater protists that live in a highly hypotonic medium have an organelle (contractile vacuole) that pumps water out of the cell. In multicellular animals, blood plasma and interstitial fluid (the extracellular fluid of tissues) are maintained at an osmolarity close to that of the cytosol. The high concentration of albumin and other proteins in blood plasma contributes to its osmolarity. Cells also actively pump out Na^+ and other ions into the interstitial fluid to stay in osmotic balance with their surroundings.

Because the effect of solutes on osmolarity depends on the *number* of dissolved particles, not their *mass*, macromolecules (proteins, nucleic acids, polysaccharides) have far less effect on the osmolarity of a solution than would an equal mass of their monomeric components. For example, a *gram* of a polysaccharide composed of 1,000 glucose units has the same effect on osmolarity as a *milligram* of glucose. Storing fuel as polysaccharides (starch or glycogen) rather than as glucose or other simple sugars avoids an enormous increase in osmotic pressure in the storage cell.

Plants use osmotic pressure to achieve mechanical rigidity. The very high solute concentration in the plant cell vacuole draws water into the cell (Fig. 2–13), but the nonexpandable cell wall prevents swelling; instead, the pressure exerted against the cell wall (turgor pressure) increases, stiffening the cell, the tissue, and the plant body. When the lettuce in your salad wilts, it is because loss of water has reduced turgor pressure. Osmosis also has consequences for laboratory protocols. Mitochondria, chloroplasts, and lysosomes, for example, are enclosed by semipermeable membranes. In isolating these organelles from broken cells, biochemists must perform the fractionations in isotonic solutions (see Fig. 1–8) to prevent excessive entry of water into the organelles and the swelling and bursting that would follow. Buffers used in cellular fractionations commonly contain sufficient concentrations of sucrose or some other inert solute to protect the organelles from osmotic lysis.

WORKED EXAMPLE 2–1 Osmotic Strength of an Organelle I

Suppose the major solutes in intact lysosomes are KCl (~0.1 M) and NaCl (~0.03 M). When isolating lysosomes, what concentration of sucrose is required in the extracting solution at room temperature (25 °C) to prevent swelling and lysis?

Solution: We want to find a concentration of sucrose that gives an osmotic strength equal to that produced by the KCl and NaCl in the lysosomes. The equation for calculating osmotic strength (the van't Hoff equation) is

$$\Pi = RT(i_1c_1 + i_2c_2 + i_3c_3 + \cdots + i_nc_n)$$

where R is the gas constant 8.315 J/mol · K, T is the absolute temperature (Kelvin), c_1, c_2, and c_3 are the molar concentrations of each solute, and i_1, i_2, and i_3 are the numbers of particles each solute yields in solution ($i = 2$ for KCl and NaCl).

The osmotic strength of the lysosomal contents is

$$\begin{aligned}\Pi_{\text{lysosome}} &= RT(i_{\text{KCl}}c_{\text{KCl}} + i_{\text{NaCl}}c_{\text{NaCl}})\\ &= RT[(2)(0.1\text{ mol/L}) + (2)(0.03\text{ mol/L})]\\ &= RT(0.26\text{ mol/L})\end{aligned}$$

The osmotic strength of a sucrose solution is given by

$$\Pi_{\text{sucrose}} = RT(i_{\text{sucrose}}c_{\text{sucrose}})$$

In this case, $i_{\text{sucrose}} = 1$, because sucrose does not ionize. Thus,

$$\Pi_{\text{sucrose}} = RT(c_{\text{sucrose}})$$

The osmotic strength of the lysosomal contents equals that of the sucrose solution when

$$\begin{aligned}\Pi_{\text{sucrose}} &= \Pi_{\text{lysosome}}\\ RT(c_{\text{sucrose}}) &= RT(0.26\text{ mol/L})\\ c_{\text{sucrose}} &= 0.26\text{ mol/L}\end{aligned}$$

So the required concentration of sucrose (FW 342) is (0.26 mol/L)(342 g/mol) = 88.92 g/L. Because the solute concentrations are only accurate to one significant figure, $c_{\text{sucrose}} = 0.09$ kg/L.

WORKED EXAMPLE 2–2 Osmotic Strength of an Organelle II

Suppose we decided to use a solution of a polysaccharide, say glycogen (p. 255), to balance the osmotic strength of the lysosomes (described in Worked Example 2–1). Assuming a linear polymer of 100 glucose units, calculate the amount of this polymer needed to achieve the same osmotic strength as the sucrose solution in Worked Example 2–1. The M_r of the glucose polymer is ~18,000, and, like sucrose, it does not ionize in solution.

Solution: As derived in Worked Example 2–1,

$$\Pi_{\text{sucrose}} = RT(0.26\text{ mol/L})$$

Similarly,

$$\Pi_{\text{glycogen}} = RT(i_{\text{glycogen}}c_{\text{glycogen}}) = RT(c_{\text{glycogen}})$$

For a glycogen solution with the same osmotic strength as the sucrose solution,

$$\begin{aligned}\Pi_{\text{glycogen}} &= \Pi_{\text{sucrose}}\\ RT(c_{\text{glycogen}}) &= RT(0.26\text{ mol/L})\\ c_{\text{glycogen}} &= 0.26\text{ mol/L} = (0.26\text{ mol/L})(18{,}000\text{ g/mol})\\ &= 4.68\text{ kg/L}\end{aligned}$$

Or, when significant figures are taken into account, $c_{\text{glycogen}} = 5$ kg/L, an absurdly high concentration.

As we'll see later (p. 256), cells of liver and muscle store carbohydrate not as low molecular weight sugars such as glucose or sucrose but as the high molecular weight polymer glycogen. This allows the cell to contain a large mass of glycogen with a minimal effect on the osmolarity of the cytosol.

SUMMARY 2.1 Weak Interactions in Aqueous Systems

- The very different electronegativities of H and O make water a highly polar molecule, capable of forming hydrogen bonds with itself and with solutes. Hydrogen bonds are fleeting, primarily electrostatic, and weaker than covalent bonds. Water is a good solvent for polar (hydrophilic) solutes, with which it forms hydrogen bonds, and for charged solutes, with which it interacts electrostatically.
- Nonpolar (hydrophobic) compounds dissolve poorly in water; they cannot hydrogen-bond with the solvent, and their presence forces an energetically unfavorable ordering of water molecules at their hydrophobic surfaces. To minimize the surface exposed to water, nonpolar compounds such as lipids form aggregates (micelles) in which the hydrophobic moieties are sequestered in the interior, associating through hydrophobic interactions, and only the more polar moieties interact with water.
- Weak, noncovalent interactions, in large numbers, decisively influence the folding of macromolecules such as proteins and nucleic acids. The most stable macromolecular conformations are those in which hydrogen bonding is maximized within the molecule and between the molecule and the solvent, and in which hydrophobic moieties cluster in the interior of the molecule away from the aqueous solvent.
- The physical properties of aqueous solutions are strongly influenced by the concentrations of solutes. When two aqueous compartments are separated by a semipermeable membrane (such as the plasma membrane separating a cell from its surroundings), water moves across that membrane to equalize the osmolarity in the two compartments. This tendency for water to move across a semipermeable membrane produces the osmotic pressure.

2.2 Ionization of Water, Weak Acids, and Weak Bases

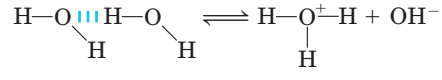
Although many of the solvent properties of water can be explained in terms of the uncharged H_2O molecule, the small degree of ionization of water to hydrogen ions (H^+) and hydroxide ions (OH^-) must also be taken into account. Like all reversible reactions, the ionization of water can be described by an equilibrium constant. When weak acids are dissolved in water, they contribute H^+ by ionizing; weak bases consume H^+ by becoming protonated. These processes are also governed by equilibrium constants. The total hydrogen ion concentration from all sources is experimentally measurable and is expressed as the pH of the solution. To predict the state of ionization of solutes in water, we must take into account the relevant equilibrium constants for each ionization reaction. We therefore turn now to a brief discussion of the ionization of water and of weak acids and bases dissolved in water.

Pure Water Is Slightly Ionized

Water molecules have a slight tendency to undergo reversible ionization to yield a hydrogen ion (a proton) and a hydroxide ion, giving the equilibrium

$$H_2O \rightleftharpoons H^+ + OH^- \qquad (2\text{–}1)$$

Although we commonly show the dissociation product of water as H^+, free protons do not exist in solution; hydrogen ions formed in water are immediately hydrated to form **hydronium ions** (H_3O^+). Hydrogen bonding between water molecules makes the hydration of dissociating protons virtually instantaneous:



The ionization of water can be measured by its electrical conductivity; pure water carries electrical current as H_3O^+ migrates toward the cathode and OH^- toward the anode. The movement of hydronium and hydroxide ions in the electric field is extremely fast compared with that of other ions such as Na^+, K^+, and Cl^-. This high ionic mobility results from the kind of "proton hopping" shown in **Figure 2–14**. No individual proton moves very

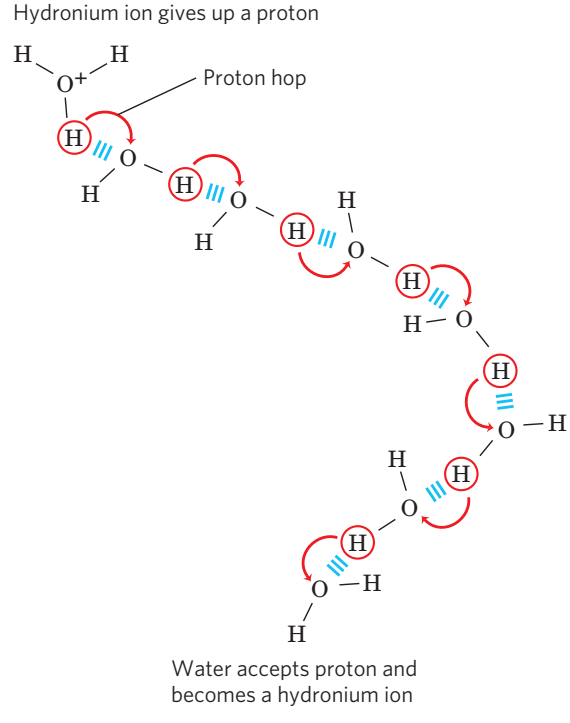


FIGURE 2–14 Proton hopping. Short "hops" of protons between a series of hydrogen-bonded water molecules result in an extremely rapid net movement of a proton over a long distance. As a hydronium ion (upper left) gives up a proton, a water molecule some distance away (bottom) acquires one, becoming a hydronium ion. Proton hopping is much faster than true diffusion and explains the remarkably high ionic mobility of H^+ ions compared with other monovalent cations such as Na^+ and K^+.

far through the bulk solution, but a series of proton hops between hydrogen-bonded water molecules causes the *net* movement of a proton over a long distance in a remarkably short time. (OH^- also moves rapidly by proton hopping, but in the opposite direction.) As a result of the high ionic mobility of H^+, acid-base reactions in aqueous solutions are exceptionally fast. As noted above, proton hopping very likely also plays a role in biological proton-transfer reactions (Fig. 2–10; see also Fig. 19–69b).

Because reversible ionization is crucial to the role of water in cellular function, we must have a means of expressing the extent of ionization of water in quantitative terms. A brief review of some properties of reversible chemical reactions shows how this can be done.

The position of equilibrium of any chemical reaction is given by its **equilibrium constant, K_{eq}** (sometimes expressed simply as K). For the generalized reaction

$$A + B \rightleftharpoons C + D \qquad (2–2)$$

the equilibrium constant K_{eq} can be defined in terms of the concentrations of reactants (A and B) and products (C and D) at equilibrium:

$$K_{eq} = \frac{[C]_{eq}[D]_{eq}}{[A]_{eq}[B]_{eq}}$$

Strictly speaking, the concentration terms should be the *activities*, or effective concentrations in nonideal solutions, of each species. Except in very accurate work, however, the equilibrium constant may be approximated by measuring the *concentrations* at equilibrium. For reasons beyond the scope of this discussion, equilibrium constants are dimensionless. Nonetheless, we have generally retained the concentration units (M) in the equilibrium expressions used in this book to remind you that molarity is the unit of concentration used in calculating K_{eq}.

The equilibrium constant is fixed and characteristic for any given chemical reaction at a specified temperature. It defines the composition of the final equilibrium mixture, regardless of the starting amounts of reactants and products. Conversely, we can calculate the equilibrium constant for a given reaction at a given temperature if the equilibrium concentrations of all its reactants and products are known. As we showed in Chapter 1 (p. 26), the standard free-energy change ($\Delta G°$) is directly related to $\ln K_{eq}$.

The Ionization of Water Is Expressed by an Equilibrium Constant

The degree of ionization of water at equilibrium (Eqn 2–1) is small; at 25 °C only about two of every 10^9 molecules in pure water are ionized at any instant. The equilibrium constant for the reversible ionization of water is

$$K_{eq} = \frac{[H^+][OH^-]}{[H_2O]} \qquad (2–3)$$

In pure water at 25 °C, the concentration of water is 55.5 M—grams of H_2O in 1 L divided by its gram molecular weight: (1,000 g/L)/(18.015 g/mol)—and is essentially constant in relation to the very low concentrations of H^+ and OH^-, namely 1×10^{-7} M. Accordingly, we can substitute 55.5 M in the equilibrium constant expression (Eqn 2–3) to yield

$$K_{eq} = \frac{[H^+][OH^-]}{[55.5\ \text{M}]}$$

On rearranging, this becomes

$$(55.5\ \text{M})(K_{eq}) = [H^+][OH^-] = K_w \qquad (2–4)$$

where K_w designates the product $(55.5\ \text{M})(K_{eq})$, the **ion product of water** at 25 °C.

The value for K_{eq}, determined by electrical-conductivity measurements of pure water, is 1.8×10^{-16} M at 25 °C. Substituting this value for K_{eq} in Equation 2–4 gives the value of the ion product of water:

$$K_w = [H^+][OH^-] = (55.5\ \text{M})(1.8 \times 10^{-16}\ \text{M})$$
$$= 1.0 \times 10^{-14}\ \text{M}^2$$

Thus the product $[H^+][OH^-]$ in aqueous solutions at 25 °C always equals 1×10^{-14} M^2. When there are exactly equal concentrations of H^+ and OH^-, as in pure water, the solution is said to be at **neutral pH**. At this pH, the concentration of H^+ and OH^- can be calculated from the ion product of water as follows:

$$K_w = [H^+][OH^-] = [H^+]^2 = [OH^-]^2$$

Solving for $[H^+]$ gives

$$[H^+] = \sqrt{K_w} = \sqrt{1 \times 10^{-14}\ \text{M}^2}$$
$$[H^+] = [OH^-] = 10^{-7}\ \text{M}$$

As the ion product of water is constant, whenever $[H^+]$ is greater than 1×10^{-7} M, $[OH^-]$ must be less than 1×10^{-7} M, and vice versa. When $[H^+]$ is very high, as in a solution of hydrochloric acid, $[OH^-]$ must be very low. From the ion product of water we can calculate $[H^+]$ if we know $[OH^-]$, and vice versa.

WORKED EXAMPLE 2–3 Calculation of $[H^+]$

What is the concentration of H^+ in a solution of 0.1 M NaOH?

Solution: We begin with the equation for the ion product of water:

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

With $[OH^-] = 0.1$ M, solving for $[H^+]$ gives

$$[H^+] = \frac{K_w}{[OH^-]} = \frac{1 \times 10^{-14}\ \text{M}^2}{0.1\ \text{M}} = \frac{10^{-14}\ \text{M}^2}{10^{-1}\ \text{M}}$$
$$= 10^{-13}\ \text{M}$$

WORKED EXAMPLE 2–4 Calculation of $[OH^-]$

What is the concentration of OH^- in a solution with an H^+ concentration of 1.3×10^{-4} M?

Solution: We begin with the equation for the ion product of water:

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

With $[H^+] = 1.3 \times 10^{-4}$ M, solving for $[OH^-]$ gives

$$[OH^-] = \frac{K_w}{[H^+]} = \frac{1 \times 10^{-14}\ \text{M}^2}{0.00013\ \text{M}} = \frac{10^{-14}\ \text{M}^2}{1.3 \times 10^{-4}\ \text{M}} = 7.7 \times 10^{-11}\ \text{M}$$

In all calculations be sure to round your answer to the correct number of significant figures, as here.

The pH Scale Designates the H^+ and OH^- Concentrations

The ion product of water, K_w, is the basis for the **pH scale** (Table 2–6). It is a convenient means of designating the concentration of H^+ (and thus of OH^-) in any aqueous solution in the range between 1.0 M H^+ and 1.0 M OH^-. The term **pH** is defined by the expression

$$\text{pH} = \log \frac{1}{[H^+]} = -\log [H^+]$$

The symbol p denotes "negative logarithm of." For a precisely neutral solution at 25 °C, in which the concentration of hydrogen ions is 1.0×10^{-7} M, the pH can be calculated as follows:

$$\text{pH} = \log \frac{1}{1.0 \times 10^{-7}} = 7.0$$

Note that the concentration of H^+ must be expressed in molar (M) terms.

The value of 7 for the pH of a precisely neutral solution is not an arbitrarily chosen figure; it is derived from the absolute value of the ion product of water at 25 °C, which by convenient coincidence is a round number. Solutions having a pH greater than 7 are alkaline or basic; the concentration of OH^- is greater than that of H^+. Conversely, solutions having a pH less than 7 are acidic.

Keep in mind that the pH scale is logarithmic, not arithmetic. To say that two solutions differ in pH by 1 pH unit means that one solution has ten times the H^+ concentration of the other, but it does not tell us the absolute magnitude of the difference. **Figure 2–15** gives the pH values of some common aqueous fluids. A cola drink (pH 3.0) or red wine (pH 3.7) has an H^+ concentration approximately 10,000 times that of blood (pH 7.4).

The pH of an aqueous solution can be approximately measured with various indicator dyes, including litmus, phenolphthalein, and phenol red. These dyes undergo color changes as a proton dissociates from the dye

TABLE 2–6 The pH Scale

$[H^+]$ (M)	pH	$[OH^-]$ (M)	pOH*
10^0 (1)	0	10^{-14}	14
10^{-1}	1	10^{-13}	13
10^{-2}	2	10^{-12}	12
10^{-3}	3	10^{-11}	11
10^{-4}	4	10^{-10}	10
10^{-5}	5	10^{-9}	9
10^{-6}	6	10^{-8}	8
10^{-7}	7	10^{-7}	7
10^{-8}	8	10^{-6}	6
10^{-9}	9	10^{-5}	5
10^{-10}	10	10^{-4}	4
10^{-11}	11	10^{-3}	3
10^{-12}	12	10^{-2}	2
10^{-13}	13	10^{-1}	1
10^{-14}	14	10^0 (1)	0

*The expression pOH is sometimes used to describe the basicity, or OH^- concentration, of a solution; pOH is defined by the expression $\text{pOH} = -\log[OH^-]$, which is analogous to the expression for pH. Note that in all cases, pH + pOH = 14.

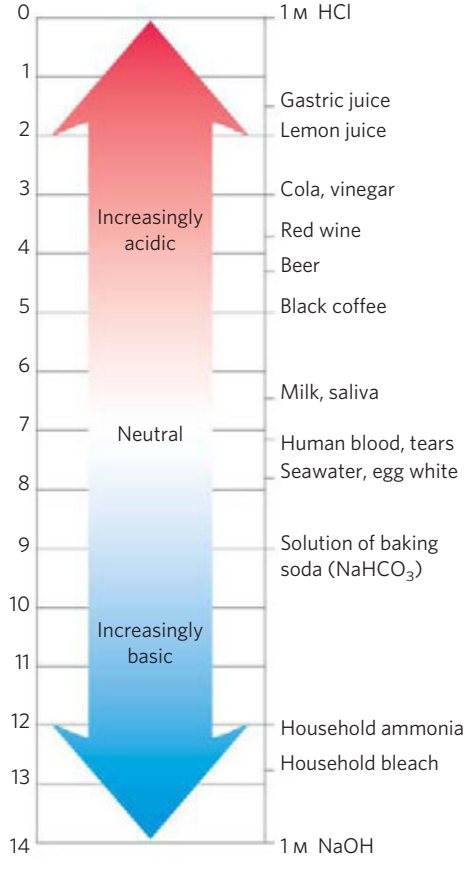


FIGURE 2–15 The pH of some aqueous fluids.

molecule. Accurate determinations of pH in the chemical or clinical laboratory are made with a glass electrode that is selectively sensitive to H^+ concentration but insensitive to Na^+, K^+, and other cations. In a pH meter, the signal from the glass electrode placed in a test solution is amplified and compared with the signal generated by a solution of accurately known pH.

Measurement of pH is one of the most important and frequently used procedures in biochemistry. The pH affects the structure and activity of biological macromolecules; for example, the catalytic activity of enzymes is strongly dependent on pH (see Fig. 2–22). Measurements of the pH of blood and urine are commonly used in medical diagnoses. The pH of the blood plasma of people with severe, uncontrolled diabetes, for example, is often below the normal value of 7.4; this condition is called **acidosis** (described in more detail below). In certain other diseases the pH of the blood is higher than normal, a condition known as **alkalosis**. Extreme acidosis or alkalosis can be life-threatening. ■

Weak Acids and Bases Have Characteristic Acid Dissociation Constants

Hydrochloric, sulfuric, and nitric acids, commonly called strong acids, are completely ionized in dilute aqueous solutions; the strong bases NaOH and KOH are also completely ionized. Of more interest to biochemists is the behavior of weak acids and bases—those not completely ionized when dissolved in water. These are ubiquitous in biological systems and play important roles in metabolism and its regulation. The behavior of aqueous solutions of weak acids and bases is best understood if we first define some terms.

Acids may be defined as proton donors and bases as proton acceptors. When a proton donor such as acetic acid (CH_3COOH) loses a proton, it becomes the corresponding proton acceptor, in this case the acetate anion (CH_3COO^-). A proton donor and its corresponding proton acceptor make up a **conjugate acid-base pair (Fig. 2–16)**, related by the reversible reaction

$$CH_3COOH \rightleftharpoons CH_3COO^- + H^+$$

Each acid has a characteristic tendency to lose its proton in an aqueous solution. The stronger the acid, the greater its tendency to lose its proton. The tendency of any acid (HA) to lose a proton and form its conjugate base (A^-) is defined by the equilibrium constant (K_{eq}) for the reversible reaction

$$HA \rightleftharpoons H^+ + A^-$$

for which

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

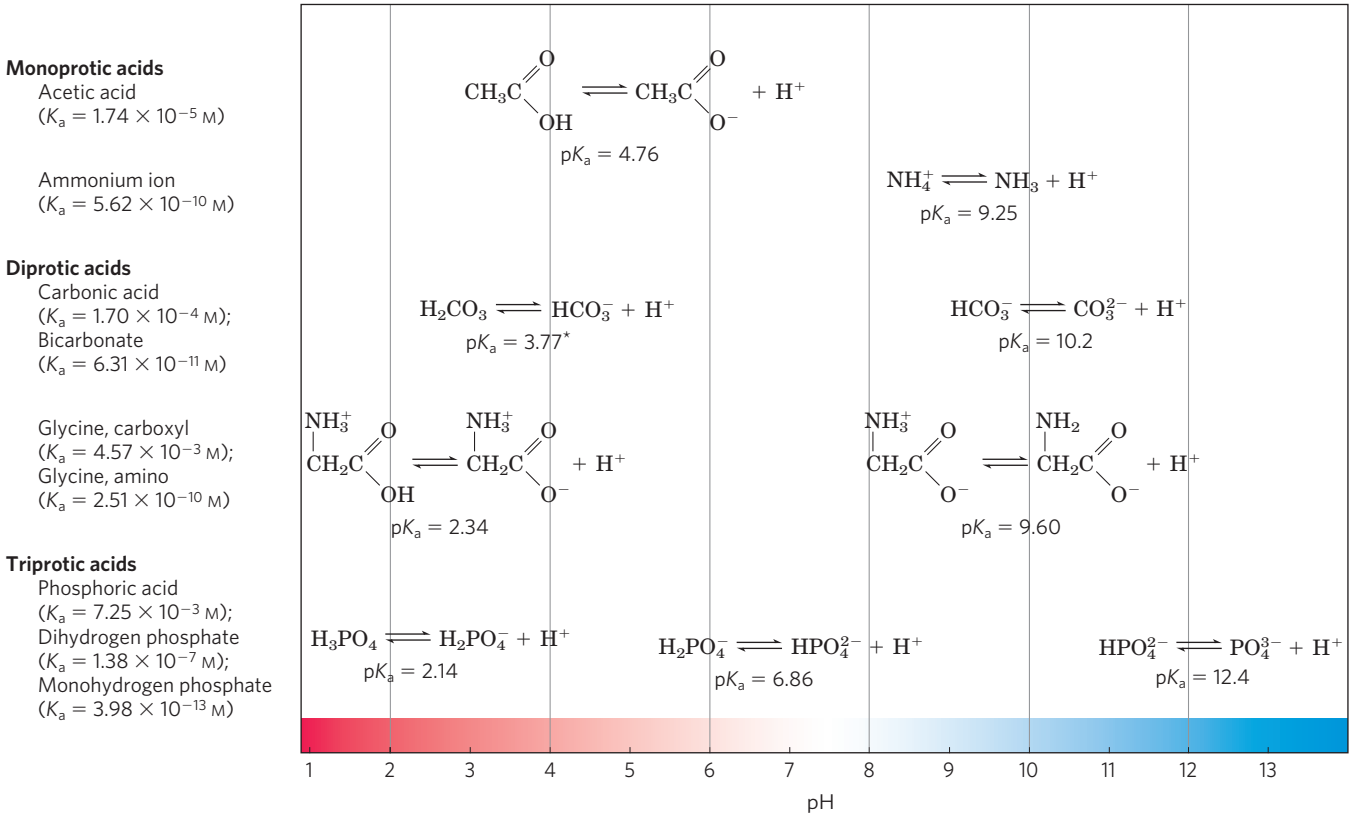


FIGURE 2–16 Conjugate acid-base pairs consist of a proton donor and a proton acceptor. Some compounds, such as acetic acid and ammonium ion, are monoprotic; they can give up only one proton. Others are diprotic (carbonic acid and glycine) or triprotic (phosphoric acid). The dissociation reactions for each pair are shown where they occur along a pH gradient. The equilibrium or dissociation constant (K_a) and its negative logarithm, the pK_a, are shown for each reaction. *For an explanation of apparent discrepancies in pK_a values for carbonic acid (H_2CO_3), see p. 67.

Equilibrium constants for ionization reactions are usually called **ionization constants** or **acid dissociation constants**, often designated K_a. The dissociation constants of some acids are given in Figure 2–16. Stronger acids, such as phosphoric and carbonic acids, have larger ionization constants; weaker acids, such as monohydrogen phosphate (HPO_4^{2-}), have smaller ionization constants.

Also included in Figure 2–16 are values of $\mathbf{pK_a}$, which is analogous to pH and is defined by the equation

$$pK_a = \log \frac{1}{K_a} = -\log K_a$$

The stronger the tendency to dissociate a proton, the stronger is the acid and the lower its pK_a. As we shall now see, the pK_a of any weak acid can be determined quite easily.

Titration Curves Reveal the pK_a of Weak Acids

Titration is used to determine the amount of an acid in a given solution. A measured volume of the acid is titrated with a solution of a strong base, usually sodium hydroxide (NaOH), of known concentration. The NaOH is added in small increments until the acid is consumed (neutralized), as determined with an indicator dye or a pH meter. The concentration of the acid in the original solution can be calculated from the volume and concentration of NaOH added. The amounts of acid and base in titrations are often expressed in terms of equivalents, where one equivalent is the amount of a substance that will react with, or supply, one mole of hydrogen ions in an acid-base reaction.

A plot of pH against the amount of NaOH added (a **titration curve**), reveals the pK_a of the weak acid. Consider the titration of a 0.1 M solution of acetic acid with 0.1 M NaOH at 25 °C **(Fig. 2–17)**. Two reversible equilibria are involved in the process (here, for simplicity, acetic acid is denoted HAc):

$$H_2O \rightleftharpoons H^+ + OH^- \qquad (2–5)$$

$$HAc \rightleftharpoons H^+ + Ac^- \qquad (2–6)$$

The equilibria must simultaneously conform to their characteristic equilibrium constants, which are, respectively,

$$K_w = [H^+][OH^-] = 1 \times 10^{-14}\ M^2 \qquad (2–7)$$

$$K_a = \frac{[H^+][Ac^-]}{[HAc]} = 1.74 \times 10^{-5}\ M \qquad (2–8)$$

At the beginning of the titration, before any NaOH is added, the acetic acid is already slightly ionized, to an extent that can be calculated from its ionization constant (Eqn 2–8).

As NaOH is gradually introduced, the added OH^- combines with the free H^+ in the solution to form H_2O, to an extent that satisfies the equilibrium relationship in Equation 2–7. As free H^+ is removed, HAc dissociates further to satisfy its own equilibrium constant (Eqn 2–8). The net result as the titration proceeds is that more and more HAc ionizes, forming Ac^-, as the NaOH is added. At the midpoint of the titration, at which exactly 0.5 equivalent of NaOH has been added per equivalent of the acid, one-half of the original acetic acid has undergone dissociation, so that the concentration of the proton donor, [HAc], now equals that of the proton acceptor, [Ac^-]. At this midpoint a very important relationship holds: the pH of the equimolar solution of acetic acid and acetate is exactly equal to the pK_a of acetic acid (pK_a = 4.76; Figs 2–16, 2–17). The basis for this relationship, which holds for all weak acids, will soon become clear.

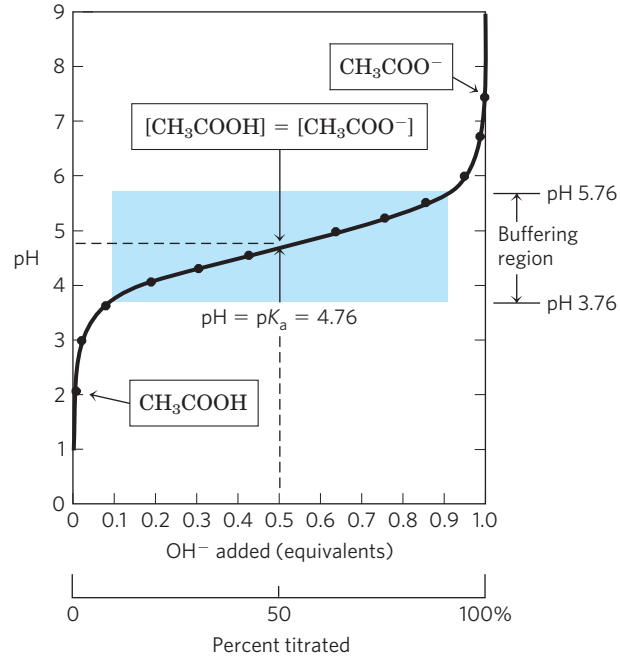


FIGURE 2–17 The titration curve of acetic acid. After addition of each increment of NaOH to the acetic acid solution, the pH of the mixture is measured. This value is plotted against the amount of NaOH added, expressed as a fraction of the total NaOH required to convert all the acetic acid (CH_3COOH) to its deprotonated form, acetate (CH_3COO^-). The points so obtained yield the titration curve. Shown in the boxes are the predominant ionic forms at the points designated. At the midpoint of the titration, the concentrations of the proton donor and proton acceptor are equal, and the pH is numerically equal to the pK_a. The shaded zone is the useful region of buffering power, generally between 10% and 90% titration of the weak acid.

As the titration is continued by adding further increments of NaOH, the remaining nondissociated acetic acid is gradually converted into acetate. The end point of the titration occurs at about pH 7.0: all the acetic acid has lost its protons to OH^-, to form H_2O and acetate. Throughout the titration the two equilibria (Eqns 2–5, 2–6) coexist, each always conforming to its equilibrium constant.

Figure 2–18 compares the titration curves of three weak acids with very different ionization constants: acetic acid (pK_a = 4.76); dihydrogen phosphate,

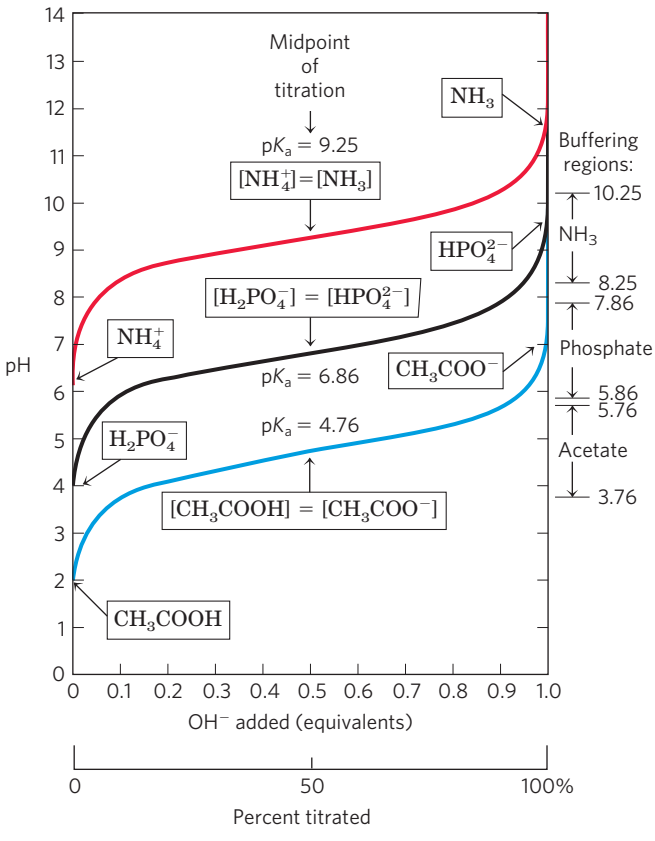


FIGURE 2–18 Comparison of the titration curves of three weak acids. Shown here are the titration curves for CH_3COOH, $H_2PO_4^-$, and NH_4^+. The predominant ionic forms at designated points in the titration are given in boxes. The regions of buffering capacity are indicated at the right. Conjugate acid-base pairs are effective buffers between approximately 10% and 90% neutralization of the proton-donor species.

$H_2PO_4^-$ ($pK_a = 6.86$); and ammonium ion, NH_4^+ ($pK_a = 9.25$). Although the titration curves of these acids have the same shape, they are displaced along the pH axis because the three acids have different strengths. Acetic acid, with the highest K_a (lowest pK_a) of the three, is the strongest of the three weak acids (loses its proton most readily); it is already half dissociated at pH 4.76. Dihydrogen phosphate loses a proton less readily, being half dissociated at pH 6.86. Ammonium ion is the weakest acid of the three and does not become half dissociated until pH 9.25.

The titration curve of a weak acid shows graphically that a weak acid and its anion—a conjugate acid-base pair—can act as a buffer, as we describe in the next section.

SUMMARY 2.2 Ionization of Water, Weak Acids, and Weak Bases

- Pure water ionizes slightly, forming equal numbers of hydrogen ions (hydronium ions, H_3O^+) and hydroxide ions. The extent of ionization is described by an equilibrium constant, $K_{eq} = \dfrac{[H^+][OH^-]}{[H_2O]}$, from which the ion product of water, K_w, is derived. At 25 °C, $K_w = [H^+][OH^-] = (55.5\ \text{M})(K_{eq}) = 10^{-14}\ \text{M}^2$.
- The pH of an aqueous solution reflects, on a logarithmic scale, the concentration of hydrogen ions:

$$pH = \log \frac{1}{[H^+]} = -\log [H^+].$$

- The greater the acidity of a solution, the lower its pH. Weak acids partially ionize to release a hydrogen ion, thus lowering the pH of the aqueous solution. Weak bases accept a hydrogen ion, increasing the pH. The extent of these processes is characteristic of each particular weak acid or base and is expressed as an acid dissociation constant:

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

- The pK_a expresses, on a logarithmic scale, the relative strength of a weak acid or base:

$$pK_a = \log \frac{1}{K_a} = -\log K_a.$$

- The stronger the acid, the smaller its pK_a; the stronger the base, the larger its pK_a. The pK_a can be determined experimentally; it is the pH at the midpoint of the titration curve for the acid or base.

2.3 Buffering against pH Changes in Biological Systems

Almost every biological process is pH-dependent; a small change in pH produces a large change in the rate of the process. This is true not only for the many reactions in which the H^+ ion is a direct participant, but also for those reactions in which there is no apparent role for H^+ ions. The enzymes that catalyze cellular reactions, and many of the molecules on which they act, contain ionizable groups with characteristic pK_a values. The protonated amino and carboxyl groups of amino acids and the phosphate groups of nucleotides, for example, function as weak acids; their ionic state is determined by the pH of the surrounding medium. (When an ionizable group is sequestered in the middle of a protein, away from the aqueous solvent, its pK_a, or apparent pK_a, can be significantly different from its pK_a in water.) As we noted above, ionic interactions are among the forces that stabilize a protein molecule and allow an enzyme to recognize and bind its substrate.

Cells and organisms maintain a specific and constant cytosolic pH, usually near pH 7, keeping biomolecules in their optimal ionic state. In multicellular organisms, the pH of extracellular fluids is also tightly regulated. Constancy of pH is achieved primarily by biological buffers: mixtures of weak acids and their conjugate bases.

Buffers Are Mixtures of Weak Acids and Their Conjugate Bases

Buffers are aqueous systems that tend to resist changes in pH when small amounts of acid (H^+) or base (OH^-) are added. A buffer system consists of a weak acid (the proton donor) and its conjugate base (the proton acceptor). As an example, a mixture of equal concentrations of acetic acid and acetate ion, found at the midpoint of the titration curve in Figure 2–17, is a buffer system. Notice that the titration curve of acetic acid has a relatively flat zone extending about 1 pH unit on either side of its midpoint pH of 4.76. In this zone, a given amount of H^+ or OH^- added to the system has much less effect on pH than the same amount added outside the zone. This relatively flat zone is the **buffering region** of the acetic acid–acetate buffer pair. At the midpoint of the buffering region, where the concentration of the proton donor (acetic acid) exactly equals that of the proton acceptor (acetate), the buffering power of the system is maximal; that is, its pH changes least on addition of H^+ or OH^-. The pH at this point in the titration curve of acetic acid is equal to its pK_a. The pH of the acetate buffer system does change slightly when a small amount of H^+ or OH^- is added, but this change is very small compared with the pH change that would result if the same amount of H^+ or OH^- were added to pure water or to a solution of the salt of a strong acid and strong base, such as NaCl, which has no buffering power.

Buffering results from two reversible reaction equilibria occurring in a solution of nearly equal concentrations of a proton donor and its conjugate proton acceptor. **Figure 2–19** explains how a buffer system works. Whenever H^+ or OH^- is added to a buffer, the result is a small change in the ratio of the relative concentrations of the weak acid and its anion and thus a small change in pH. The decrease in concentration of one component of the system is balanced exactly by an increase in the other. The sum of the buffer components does not change, only their ratio.

Each conjugate acid-base pair has a characteristic pH zone in which it is an effective buffer (Fig. 2–18). The $H_2PO_4^-/HPO_4^{2-}$ pair has a pK_a of 6.86 and thus can serve as an effective buffer system between approximately pH 5.9 and pH 7.9; the NH_4^+/NH_3 pair, with a pK_a of 9.25, can act as a buffer between approximately pH 8.3 and pH 10.3.

The Henderson-Hasselbalch Equation Relates pH, pK_a, and Buffer Concentration

The titration curves of acetic acid, $H_2PO_4^-$, and NH_4^+ (Fig. 2–18) have nearly identical shapes, suggesting that these curves reflect a fundamental law or relationship. This is indeed the case. The shape of the titration curve of any weak acid is described by the Henderson-Hasselbalch equation, which is important for understanding buffer action and acid-base balance in the blood and tissues of vertebrates. This equation is simply a useful way of restating the expression for the ionization constant of an acid. For the ionization of a weak acid HA, the Henderson-Hasselbalch equation can be derived as follows:

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

First solve for $[H^+]$:

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

Then take the negative logarithm of both sides:

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

Substitute pH for $-\log [H^+]$ and pK_a for $-\log K_a$:

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

Now invert $-\log [HA]/[A^-]$, which involves changing its sign, to obtain the **Henderson-Hasselbalch equation**:

$$pH = pK_a + \log \frac{[A^-]}{[HA]} \qquad (2\text{–}9)$$

This equation fits the titration curve of all weak acids and enables us to deduce some important quantitative relationships. For example, it shows why the pK_a of a

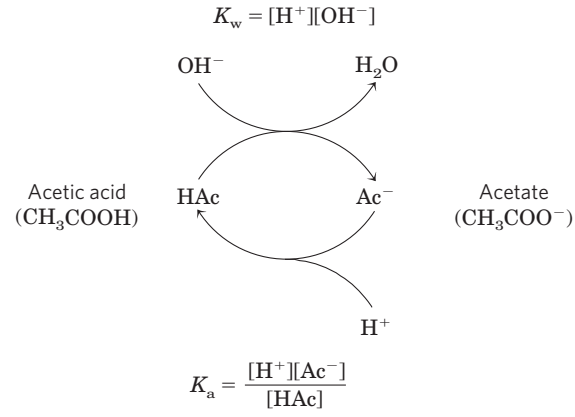


FIGURE 2–19 The acetic acid–acetate pair as a buffer system. The system is capable of absorbing either H^+ or OH^- through the reversibility of the dissociation of acetic acid. The proton donor, acetic acid (HAc), contains a reserve of bound H^+, which can be released to neutralize an addition of OH^- to the system, forming H_2O. This happens because the product $[H^+][OH^-]$ transiently exceeds K_w (1×10^{-14} M^2). The equilibrium quickly adjusts to restore the product to 1×10^{-14} M^2 (at 25 °C), thus transiently reducing the concentration of H^+. But now the quotient $[H^+][Ac^-]/[HAc]$ is less than K_a, so HAc dissociates further to restore equilibrium. Similarly, the conjugate base, Ac^-, can react with H^+ ions added to the system; again, the two ionization reactions simultaneously come to equilibrium. Thus a conjugate acid-base pair, such as acetic acid and acetate ion, tends to resist a change in pH when small amounts of acid or base are added. Buffering action is simply the consequence of two reversible reactions taking place simultaneously and reaching their points of equilibrium as governed by their equilibrium constants, K_w and K_a.

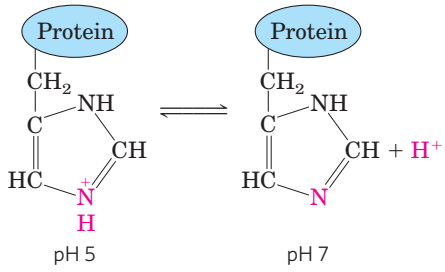


FIGURE 2–20 Ionization of histidine. The amino acid histidine, a component of proteins, is a weak acid. The pK_a of the protonated nitrogen of the side chain is 6.0.

weak acid is equal to the pH of the solution at the midpoint of its titration. At that point, [HA] = [A^-], and

$$\text{pH} = \text{p}K_a + \log 1 = \text{p}K_a + 0 = \text{p}K_a$$

The Henderson-Hasselbalch equation also allows us to (1) calculate pK_a, given pH and the molar ratio of proton donor and acceptor; (2) calculate pH, given pK_a and the molar ratio of proton donor and acceptor; and (3) calculate the molar ratio of proton donor and acceptor, given pH and pK_a.

Weak Acids or Bases Buffer Cells and Tissues against pH Changes

The intracellular and extracellular fluids of multicellular organisms have a characteristic and nearly constant pH. The organism's first line of defense against changes in internal pH is provided by buffer systems. The cytoplasm of most cells contains high concentrations of proteins, and these proteins contain many amino acids with functional groups that are weak acids or weak bases. For example, the side chain of histidine **(Fig. 2–20)** has a pK_a of 6.0 and thus can exist in either the protonated or unprotonated form near neutral pH. Proteins containing histidine residues therefore buffer effectively near neutral pH.

WORKED EXAMPLE 2–5 Ionization of Histidine

Calculate the fraction of histidine that has its imidazole side chain protonated at pH 7.3. The pK_a values for histidine are pK_1 = 1.8, pK_2 (imidazole) = 6.0, and pK_3 = 9.2 (see Fig. 3–12b).

Solution: The three ionizable groups in histidine have sufficiently different pK_a values that the first acid (—COOH) is completely ionized before the second (protonated imidazole) begins to dissociate a proton, and the second ionizes completely before the third ($—NH_3^+$) begins to dissociate its proton. (With the Henderson-Hasselbalch equation, we can easily show that a weak acid goes from 1% ionized at 2 pH units below its pK_a to 99% ionized at 2 pH units above its pK_a; see also Fig. 3–12b.) At pH 7.3, the carboxyl group of histidine is entirely deprotonated ($—COO^-$) and the α-amino group is fully protonated ($—NH_3^+$). We can therefore assume that at pH 7.3, the only group that is partially dissociated is the imidazole group, which can be protonated (we'll abbreviate as $HisH^+$) or not (His).

We use the Henderson-Hasselbalch equation:

$$\text{pH} = \text{p}K_a + \log \frac{[A^-]}{[HA]}$$

Substituting pK_2 = 6.0 and pH = 7.3:

$$7.3 = 6.0 + \log \frac{[\text{His}]}{[\text{HisH}^+]}$$

$$1.3 = \log \frac{[\text{His}]}{[\text{HisH}^+]}$$

$$\text{antilog } 1.3 = \frac{[\text{His}]}{[\text{HisH}^+]} = 2.0 \times 10^1$$

This gives us the *ratio* of [His] to [$HisH^+$] (20 to 1 in this case). We want to convert this ratio to the *fraction* of total histidine that is in the unprotonated form His at pH 7.3. That fraction is 20/21 (20 parts His per 1 part $HisH^+$, *in a total of 21 parts histidine* in either form), or about 95.2%; the remainder (100% minus 95.2%) is protonated—about 5%.

Nucleotides such as ATP, as well as many metabolites of low molecular weight, contain ionizable groups that can contribute buffering power to the cytoplasm. Some highly specialized organelles and extracellular compartments have high concentrations of compounds that contribute buffering capacity: organic acids buffer the vacuoles of plant cells; ammonia buffers urine.

Two especially important biological buffers are the phosphate and bicarbonate systems. The phosphate buffer system, which acts in the cytoplasm of all cells, consists of $H_2PO_4^-$ as proton donor and HPO_4^{2-} as proton acceptor:

$$H_2PO_4^- \rightleftharpoons H^+ + HPO_4^{2-}$$

The phosphate buffer system is maximally effective at a pH close to its pK_a of 6.86 (Figs 2–16, 2–18) and thus tends to resist pH changes in the range between about 5.9 and 7.9. It is therefore an effective buffer in biological fluids; in mammals, for example, extracellular fluids and most cytoplasmic compartments have a pH in the range of 6.9 to 7.4.

WORKED EXAMPLE 2–6 Phosphate Buffers

(a) What is the pH of a mixture of 0.042 M NaH_2PO_4 and 0.058 M Na_2HPO_4?

Solution: We use the Henderson-Hasselbalch equation, which we'll express here as

$$\text{pH} = \text{p}K_a + \log \frac{[\text{conjugate base}]}{[\text{acid}]}$$

In this case, the acid (the species that gives up a proton) is $H_2PO_4^-$, and the conjugate base (the species that gains a proton) is HPO_4^{2-}. Substituting the given concentrations of acid and conjugate base and the pK_a (6.86),

$$\text{pH} = 6.86 + \log \frac{0.058}{0.042} = 6.86 + 0.14 = 7.0$$

We can roughly check this answer. When more conjugate base than acid is present, the acid is more than 50% titrated and thus the pH is above the pK_a (6.86), where the acid is exactly 50% titrated.

(b) If 1.0 mL of 10.0 M NaOH is added to a liter of the buffer prepared in (a), how much will the pH change?

Solution: A liter of the buffer contains 0.042 mol of NaH_2PO_4. Adding 1.0 mL of 10.0 M NaOH (0.010 mol) would titrate an equivalent amount (0.010 mol) of NaH_2PO_4 to Na_2HPO_4, resulting in 0.032 mol of NaH_2PO_4 and 0.068 mol of Na_2HPO_4. The new pH is

$$\text{pH} = \text{p}K_a + \log\frac{[HPO_4^{2-}]}{[H_2PO_4^-]}$$
$$= 6.86 + \log\frac{0.068}{0.032} = 6.86 + 0.33 = 7.2$$

(c) If 1.0 mL of 10.0 M NaOH is added to a liter of pure water at pH 7.0, what is the final pH? Compare this with the answer in (b).

Solution: The NaOH dissociates completely into Na^+ and OH^-, giving $[OH^-] = 0.010$ mol/L $= 1.0 \times 10^{-2}$ M. The pOH is the negative logarithm of $[OH^-]$, so pOH = 2.0. Given that in all solutions, pH + pOH = 14, the pH of the solution is 12.

So, an amount of NaOH that increases the pH of water from 7 to 12 increases the pH of a buffered solution, as in (b), from 7.0 to just 7.2. Such is the power of buffering!

Blood plasma is buffered in part by the bicarbonate system, consisting of carbonic acid (H_2CO_3) as proton donor and bicarbonate (HCO_3^-) as proton acceptor (K_1 is the first of several equilibrium constants in the bicarbonate buffering system):

$$H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$
$$K_1 = \frac{[H^+][HCO_3^-]}{[H_2CO_3]}$$

This buffer system is more complex than other conjugate acid-base pairs because one of its components, carbonic acid (H_2CO_3), is formed from dissolved (d) carbon dioxide and water, in a reversible reaction:

$$CO_2(d) + H_2O \rightleftharpoons H_2CO_3$$
$$K_2 = \frac{[H_2CO_3]}{[CO_2(d)][H_2O]}$$

Carbon dioxide is a gas under normal conditions, and CO_2 dissolved in an aqueous solution is in equilibrium with CO_2 in the gas (g) phase:

$$CO_2(g) \rightleftharpoons CO_2(d)$$
$$K_3 = \frac{[CO_2(d)]}{[CO_2(g)]}$$

The pH of a bicarbonate buffer system depends on the concentration of H_2CO_3 and HCO_3^-, the proton donor and acceptor components. The concentration of H_2CO_3 in turn depends on the concentration of dissolved CO_2, which in turn depends on the concentration of CO_2 in the gas phase, or the **partial pressure** of CO_2, denoted pCO_2. Thus the pH of a bicarbonate buffer exposed to a gas phase is ultimately determined by the concentration of HCO_3^- in the aqueous phase and by pCO_2 in the gas phase.

The bicarbonate buffer system is an effective physiological buffer near pH 7.4, because the H_2CO_3 of blood plasma is in equilibrium with a large reserve capacity of CO_2(g) in the air space of the lungs. As noted above, this buffer system involves three reversible equilibria, in this case between gaseous CO_2 in the lungs and bicarbonate (HCO_3^-) in the blood plasma **(Fig. 2–21)**.

Blood can pick up H^+, such as from the lactic acid produced in muscle tissue during vigorous exercise. Alternatively, it can lose H^+, such as by protonation of the NH_3 produced during protein catabolism. When H^+ is added to blood as it passes through the tissues, reaction 1 in Figure 2–21 proceeds toward a new equilibrium, in which $[H_2CO_3]$ is increased. This in turn increases $[CO_2(d)]$ in the blood (reaction 2) and thus increases the partial pressure of CO_2(g) in the air space of the lungs (reaction 3); the extra CO_2 is exhaled. Conversely, when H^+ is lost from the blood, the opposite events occur: more H_2CO_3 dissociates into H^+ and HCO_3^- and thus more CO_2(g) from the lungs dissolves in blood plasma. The rate of respiration—that is, the rate of inhaling and exhaling—can quickly adjust these equilibria to keep the blood pH nearly constant. The rate of respiration is controlled by the brain stem, where detection of an increased blood pCO_2 or decreased blood pH triggers deeper and more frequent breathing.

At the pH of blood plasma (7.4) very little H_2CO_3 is present in comparison with HCO_3^-, and the addition of a small amount of base (NH_3 or OH^-) would titrate this H_2CO_3, exhausting the buffering capacity. The important

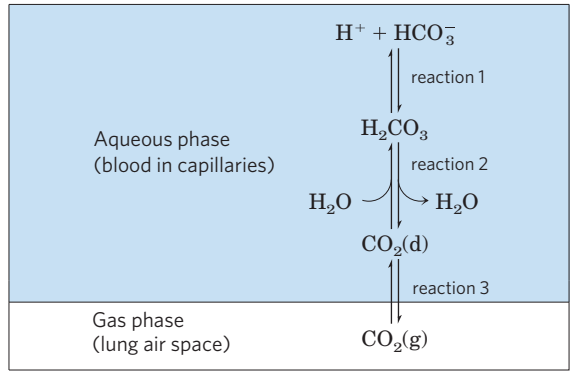


FIGURE 2–21 **The bicarbonate buffer system.** CO_2 in the air space of the lungs is in equilibrium with the bicarbonate buffer in the blood plasma passing through the lung capillaries. Because the concentration of dissolved CO_2 can be adjusted rapidly through changes in the rate of breathing, the bicarbonate buffer system of the blood is in near-equilibrium with a large potential reservoir of CO_2.

role for H_2CO_3 (pK_a = 3.57 at 37 °C) in buffering blood plasma (~pH 7.4) seems inconsistent with our earlier statement that a buffer is most effective in the range of 1 pH unit above and below its pK_a. The explanation for this paradox is the large reservoir of CO_2(d) in blood. Its rapid equilibration with H_2CO_3 results in the formation of additional H_2CO_3:

$$CO_2(d) + H_2O \rightleftharpoons H_2CO_3$$

It is useful in clinical medicine to have a simple expression for blood pH in terms of dissolved CO_2, which is commonly monitored along with other blood gases. We can define a constant, K_h, which is the equilibrium constant for the hydration of CO_2 to form H_2CO_3:

$$K_h = \frac{[H_2CO_3]}{[CO_2(d)]}$$

Then, to take the CO_2(d) reservoir into account, we can express $[H_2CO_3]$ as $K_h[CO_2(d)]$, and substitute this expression for $[H_2CO_3]$ in the equation for the acid dissociation of H_2CO_3:

$$K_a = \frac{[H^+][HCO_3^-]}{[H_2CO_3]} = \frac{[H^+][HCO_3^-]}{K_h[CO_2(d)]}$$

Now, the overall equilibrium for dissociation of H_2CO_3 can be expressed in these terms:

$$K_hK_a = K_{combined} = \frac{[H^+][HCO_3^-]}{[CO_2(d)]}$$

We can calculate the value of the new constant, $K_{combined}$, and the corresponding apparent pK, or $pK_{combined}$, from the experimentally determined values of K_h (3.0×10^{-3} M) and K_a (2.7×10^{-4} M) at 37 °C:

$$K_{combined} = (3.0 \times 10^{-3}\ \text{M})(2.7 \times 10^{-4}\ \text{M})$$
$$= 8.1 \times 10^{-7}\ \text{M}^2$$
$$pK_{combined} = 6.1$$

In clinical medicine, it is common to refer to CO_2(d) as the conjugate acid and to use the apparent, or combined, pK_a of 6.1 to simplify calculation of pH from $[CO_2(d)]$. In this convention,

$$pH = 6.1 + \log \frac{[HCO_3^-]}{(0.23 \times pCO_2)}$$

where pCO_2 is expressed in kilopascals (kPa; typically, pCO_2 is 4.6 to 6.7 kPa) and 0.23 is the corresponding solubility coefficient for CO_2 in water; thus the term $0.23 \times pCO_2 \approx 1.2$ kPa. Plasma $[HCO_3^-]$ is normally about 24 mM. ■

Untreated Diabetes Produces Life-Threatening Acidosis

Human blood plasma normally has a pH between 7.35 and 7.45, and many of the enzymes that function in the blood have evolved to have maximal activity in that pH range. Enzymes typically show maximal catalytic activity at a characteristic pH, called the **pH optimum (Fig. 2–22)**. On either side of this optimum pH, catalytic activity often declines sharply. Thus, a small change in pH can make a large difference in the rate of some crucial enzyme-catalyzed reactions. Biological control of the pH of cells and body fluids is therefore of central importance in all aspects of metabolism and cellular activities, and changes in blood pH have marked physiological consequences (described with gusto in Box 2–1!).

In individuals with untreated diabetes mellitus, the lack of insulin, or insensitivity to insulin (depending on the type of diabetes), disrupts the uptake of glucose from blood into the tissues and forces the tissues to use stored fatty acids as their primary fuel. For reasons we will describe in detail later (see Fig. 24–30), this dependence on fatty acids results in the accumulation of high concentrations of two carboxylic acids, β-hydroxybutyric acid and acetoacetic acid (blood plasma level of 90 mg/100 mL, compared with <3 mg/100 mL in control (healthy) individuals; urinary excretion of 5 g/24 hr, compared with <125 mg/24 hr in controls). Dissociation of these acids lowers the pH of blood plasma to less than 7.35, causing acidosis. Severe acidosis leads to headache, drowsiness, nausea, vomiting, and diarrhea, followed by stupor, coma, and convulsions, presumably because at the lower pH, some enzyme(s) do not function optimally. When a patient is found to have high blood glucose, low plasma pH, and high levels of β-hydroxybutyric acid and acetoacetic acid in blood and urine, diabetes mellitus is the likely diagnosis.

Other conditions can also produce acidosis. Fasting and starvation force the use of stored fatty acids as fuel,

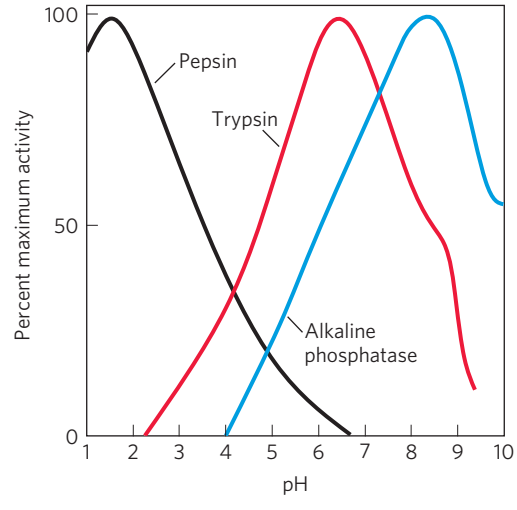


FIGURE 2–22 The pH optima of some enzymes. Pepsin is a digestive enzyme secreted into gastric juice, which has a pH of ~1.5, allowing pepsin to act optimally. Trypsin, a digestive enzyme that acts in the small intestine, has a pH optimum that matches the neutral pH in the lumen of the small intestine. Alkaline phosphatase of bone tissue is a hydrolytic enzyme thought to aid in bone mineralization.