CHAPTER

8

Complex Biochemical Processes

Biochemical processes use a number of strategies to achieve kinetic control. Chief among them is the use of enzymes to accelerate and regulate the rates of chemical reactions that, though thermodynamically favorable under intracellular conditions, would be too slow to account for the observed rate of growth of organisms. With the constant development of powerful experimental techniques, biochemists are beginning to decipher the mechanisms of even the most complex biological processes, such as the transport of nutrients across cell membranes and the transfer of electrons between proteins during glucose metabolism and photosynthesis. In this chapter we describe these processes and develop the physical and chemical concepts that will be used throughout the remainder of the text.

Transport across biological membranes

We saw in Chapter 5 that many cellular processes, such as the propagation of impulses in neurons and the synthesis of ATP by ATPases, are controlled by the transport of molecules and ions across biological membranes. **Passive transport** is the spontaneous movement of species down concentration and membrane potential gradients, whereas **active transport** is nonspontaneous movement against these gradients driven by ATP hydrolysis. Here we complement the thermodynamic treatment of Chapter 5 with a kinetic analysis that begins with a consideration of the laws governing the motion of molecules and ions in liquids and then describes modes of transport across cell membranes.

8.1 Molecular motion in liquids

Because the rate at which molecules move in solution may be a controlling factor of the maximum rate of a biochemical reaction in the intracellular medium, we need to describe the factors that limit molecular motion in a liquid.

A molecule in a liquid is surrounded by other molecules and can move only a fraction of a diameter in each step it takes, perhaps because its neighbors move aside momentarily, before colliding. Molecular motion in liquids is a series of short steps, with ever-changing directions, like people in an aimless, milling crowd.

The process of migration by means of a random jostling motion through a liquid is called **diffusion**. We can think of the motion of the molecule as a series of short jumps in random directions, a so-called **random walk** (Fig. 8.1).[1] If there is

[1]In Chapter 12 we develop a statistical view of the random walk and apply it to the molecular description of a number of biological processes.

Table 8.1 Diffusion coefficients in water, $D/(10^{-9}\ m^2\ s^{-1})$

Water, H_2O*	2.26
Glycine, NH_2CH_2COOH*	1.055
Sucrose, $C_{12}H_{22}O_{11}$*	0.522
Lysozyme†	0.112
Serum albumin†	0.0594
Catalase†	0.0410
Fibrinogen†	0.0202
Bushy stunt virus†	0.0115

*Measured at 25°C.
†Measured at 20°C.

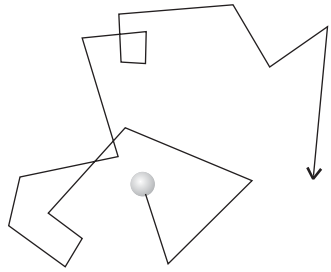

Fig. 8.1 One possible path of a random walk in three dimensions. In this general case, the step length is also a random variable.

an initial concentration gradient in the liquid (for instance, a solution may have a high concentration of solute in one region), then the rate at which the molecules spread out is proportional to the concentration gradient and we write

Rate of diffusion $\propto$ concentration gradient

To express this relation mathematically, we introduce the **flux**, J, which is the number of particles passing through an imaginary window in a given time interval, divided by the area of the window and the duration of the interval:

$$J = \frac{\text{number of particles passing through window}}{\text{area of window} \times \text{time interval}} \qquad \textbf{(8.1a)}$$

Then we write **Fick's first law** of diffusion (see *Further information* 8.1 for a derivation):

$$J = -D\frac{\mathrm{d}c}{\mathrm{d}x} \qquad \textbf{(8.1b)}$$

where $\mathrm{d}c/\mathrm{d}x$ is the gradient of the number concentration c (molecules m^{-3}, for instance) and the coefficient D, which has the dimensions of area divided by time (with units $m^2\ s^{-1}$), is called the **diffusion coefficient** (Table 8.1). Large values of D correspond to rapid diffusion. The negative sign in eqn 8.1b simply means that if the concentration gradient is negative (down from left to right, Fig. 8.2), then the flux is positive (flowing from left to right). To calculate the number of molecules passing through a given window in a given time interval, we multiply the flux by the area of the window and the time interval. If the concentration in eqn 8.1b is a molar concentration, then the flux is expressed in moles rather than number of molecules.

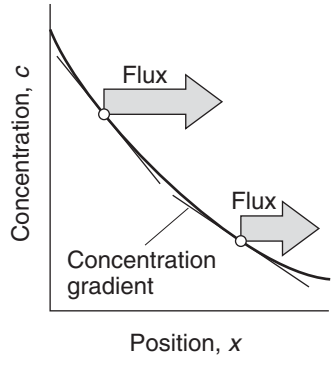

Fig. 8.2 The flux of solute particles is proportional to the concentration gradient. Here we see a solution in which the concentration falls from left to right. The gradient is negative (down from left to right) and the flux is positive (towards the right). The greatest flux is found where the gradient is steepest (at the left).

ILLUSTRATION 8.1 Using Fick's first law of diffusion

Suppose that in a region of an unstirred aqueous solution of sucrose the molar concentration gradient is $-0.10\ mol\ L^{-1}\ cm^{-1}$. Then, because $1\ L = 10^{-3}\ m^3$

COMMENT 8.1 Because the concentration is a function of both time and location, the derivatives are in fact *partial* derivatives, but we are not using that notation in this book. For more details, see our *Physical chemistry* 7e (2002). ■

(so 1 $L^{-1} = 10^3\ m^{-3}$) and 1 cm = 10^{-2} m (so 1 $cm^{-1} = 10^2\ m^{-1}$), the flux arising from this gradient is

$$J = -(0.522 \times 10^{-9}\ m^2\ s^{-1}) \times (-0.10\ mol\ L^{-1}\ cm^{-1})$$
$$= 0.522 \times 0.10 \times 10^{-9}\ m^2\ s^{-1}\ mol \times (10^3\ m^{-3}) \times (10^2\ m^{-1})$$
$$= 5.2 \times 10^{-6}\ mol\ m^{-2}\ s^{-1}$$

The amount of sucrose molecules passing through a 1.0-cm square window in 10 minutes is therefore

$$n = JA\Delta t = (5.2 \times 10^{-6}\ mol\ m^{-2}\ s^{-1}) \times (1.0 \times 10^{-2}\ m)^2 \times (10 \times 60\ s)$$
$$= 3.1 \times 10^{-7}\ mol \blacksquare$$

Diffusion coefficients are of the greatest importance for discussing the spread of pollutants in lakes and through the atmosphere. In both cases, the spread of pollutant may be assisted—and is normally greatly dominated—by bulk motion of the fluid as a whole (as when a wind blows in the atmosphere). This motion is called **convection**. Because diffusion is often a slow process, we speed up the spread of solute molecules by inducing convection by stirring a fluid or turning on an extractor fan.

One of the most important equations in the physical chemistry of fluids is the **diffusion equation**, which enables us to predict the rate at which the concentration of a solute changes in a non-uniform solution. In essence, the diffusion equation expresses the fact that wrinkles in the concentration tend to disperse. The formal statement of the diffusion equation, which is also known as **Fick's second law** of diffusion, is

$$\frac{dc}{dt} = D\frac{d^2c}{dx^2} \qquad \textbf{(8.2)}$$

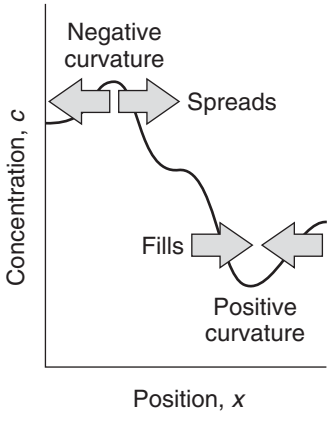

Fig. 8.3 Nature abhors a wrinkle. The diffusion equation tells us that peaks in a distribution (regions of negative curvature) spread and troughs (regions of positive curvature) fill in.

where dc/dt is the rate of change of concentration in a region and d^2c/dx^2 may be thought of as the curvature of the concentration in the region. The "curvature" is a measure of the wrinkliness of the concentration (see below). The derivation of this expression from Fick's first law is given in *Further information* 8.1. The concentrations on the left and right of this equation may be either number concentrations or molar concentrations.

The diffusion equation tells us that a uniform concentration and a concentration with unvarying slope through the region (so $d^2c/dx^2 = 0$ in each case) results in no net change in concentration because the rate of influx through one wall of the region is equal to the rate of efflux through the opposite wall. Only if the slope of the concentration varies through a region—only if the concentration is wrinkled—is there a change in concentration. Where the curvature is positive (a dip, Fig. 8.3), the change in concentration is positive: the dip tends to fill. Where the curvature is negative (a heap), the change in concentration is negative: the heap tends to spread.

We can understand the nature of diffusion more deeply by considering it as the outcome of a random walk. Although a molecule undergoing a random walk may take many steps in a given time, it has only a small probability of being found far from its starting point because some of the steps lead it away from the starting

point, but others lead it back. The net distance traveled in a time t from the starting point is measured by the **root mean square distance**, d, with

$$d = (2Dt)^{1/2} \tag{8.3}$$

Thus, the net distance increases only as the square root of the time, so for a particle to be found twice as far (on average) from its starting point, we must wait four times as long.

SELF-TEST 8.1 The diffusion coefficient of an H_2O molecule in bulk water is 2.26×10^{-9} m^2 s^{-1} at 25°C. How long does it take for an H_2O molecule to travel (a) 1.0 cm, (b) 2.0 cm from its starting point in a sample of unstirred water?

Answer: (a) 6.1 h, (b) 25 h

The relation between the diffusion coefficient and the rate at which the molecule takes its steps and the distance of each step is called the **Einstein-Smoluchowski equation**:

$$D = \frac{\lambda^2}{2\tau} \tag{8.4}$$

where λ (lambda) is the length of each step (which in the model is assumed to be the same for each step) and τ (tau) is the time each step takes. This equation tells us that a molecule that takes rapid, long steps has a high diffusion coefficient. We can interpret τ as the average lifetime of a molecule near another molecule before it makes a sudden jump to its next position.

SELF-TEST 8.2 Suppose an H_2O molecule moves through one molecular diameter (about 200 pm) each time it takes a step in a random walk. What is the time for each step at 25°C?

Answer: 9 ps

The diffusion coefficient increases with temperature because an increase in temperature enables a molecule to escape more easily from the attractive forces exerted by its neighbors. If we suppose that the rate (expressed as $1/\tau$, the inverse of the time constant defined in Section 6.6) of the random walk follows an Arrhenius temperature dependence with an activation energy E_a, then the diffusion coefficient will follow the relation

$$D = D_0 e^{-E_a/RT} \tag{8.5}$$

The rate at which particles diffuse through a liquid is related to the viscosity, and we should expect a high diffusion coefficient to be found for fluids that have a low viscosity. That is, we can suspect that $\eta \propto 1/D$, where η (eta) is the **coefficient of viscosity**. In fact, the **Stokes-Einstein relation** states that

$$D = \frac{kT}{6\pi\eta a} \tag{8.6}$$

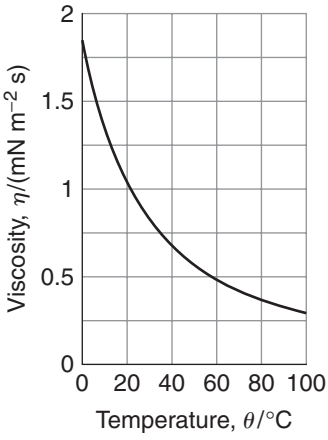

Fig. 8.4 The experimental temperature dependence of the viscosity of water. As the temperature is increased, more molecules are able to escape from the potential wells provided by their neighbors, so the liquid becomes more fluid.

where a is the radius of the molecule. It follows that

$$\eta = \eta_0 e^{E_a/RT} \tag{8.7}$$

Note the positive sign of the exponent, which is consistent with the fact that viscosity decreases as the temperature is raised. We are supposing that the strong temperature dependence of the exponential term dominates the weak linear dependence on T in the numerator of eqn 8.6. The temperature dependence shown in eqn 8.7 is indeed observed, at least over reasonably small temperature ranges (Fig. 8.4).

SELF-TEST 8.3 Estimate the activation energy for the viscosity of water from the graph in Fig. 8.4 by using the viscosities at 40°C and 80°C. *Hint:* Use an equation such as eqn 8.7 to formulate an expression for the logarithm of the ratio of the two viscosities.

Answer: 19 kJ mol^{-1}

8.2 Molecular motion across membranes

A crucial aspect of biochemical change is the rate at which species are transported across a membrane, so we need to understand the kinetic factors that facilitate or impede transport.

Consider the passive transport of an uncharged species A across a lipid bilayer of thickness l. To simplify the problem, we assume that the concentration of A is always maintained at $[A] = [A]_0$ on one surface of the membrane and at $[A] = 0$ on the other surface, perhaps by a perfect balance between the rate of the process that produces A on one side and the rate of another process that consumes A completely on the other side. Then $d[A]/dt = 0$ because the two boundary conditions ensure that the interior of the membrane is maintained at a constant but not necessarily uniform concentration, and eqn 8.2 simplifies to

$$D\frac{d^2[A]}{dx^2} = 0$$

where D is the diffusion coefficient. We use the conditions $[A](0) = [A]_0$ and $[A](l) = 0$ to solve this differential equation above and the result, which may be verified by differentiation, is

$$[A](x) = [A]_0\left(1 - \frac{x}{l}\right) \tag{8.8}$$

which implies that [A] decreases linearly inside the membrane. We now use Fick's first law to calculate the flux J of A through the membrane. From eqn 8.8, it follows that

$$\frac{d[A]}{dx} = -\frac{[A]_0}{l}$$

and from this result and eqn 8.1b obtain

$$J = D\,\frac{[A]_0}{l}$$

We need to modify this equation slightly to account for the fact that the concentration of A on the surface of a membrane is not always equal to the concentration of A measured in the bulk solution, which we assume to be aqueous. This difference arises from the significant difference in the solubility of A in an aqueous environment and in the solution-membrane interface. One way to deal with this problem is to define a *partition coefficient* κ (kappa) as

$$\kappa = \frac{[A]_0}{[A]_s}$$

where $[A]_s$ is the concentration of A in the bulk aqueous solution. It follows that

$$J = \kappa D\,\frac{[A]_s}{l} \qquad \textbf{(8.9)}$$

In spite of the assumptions that led to its final form, eqn 8.9 describes adequately the passive transport of many nonelectrolytes through membranes of blood cells.

In many cases the flux is underestimated by eqn 8.9, which suggests that the membrane is more permeable than expected. However, because the permeability increases only for certain species, we can infer that in these cases, transport is mediated by carrier molecules. One example is the transporter protein that carries glucose into cells.

A characteristic of a carrier C is that it binds to the transported species A and the dissociation of the AC complex is described by

$$AC \rightleftharpoons A + C \qquad K = \frac{[A][C]}{[AC]}$$

where we have used concentrations instead of activities. After writing $[C]_0 = [C] + [AC]$, where $[C]_0$ is the total concentration of carrier, it follows that

$$[AC] = \frac{[A][C]_0}{[A] + K}$$

We can now use eqn 8.9 to write an expression for the flux of the species AC through the membrane:

$$J = \kappa_{AC} D_{AC} \frac{[AC]}{l} = \frac{\kappa_{AC} D_{AC} [C]_0}{l} \frac{[A]}{[A] + K} = J_{max} \frac{[A]}{[A] + K}$$

where κ_{AC} and D_{AC} are the partition coefficient and diffusion coefficient of the species AC, respectively. We see from Fig. 8.5 that when $[A] << K$, the flux varies linearly with [A] and that the flux reaches a maximum value of $J_{max} = \kappa_{AC} D_{AC} [C]_0 / l$ when $[A] >> K$. This behavior is characteristic of mediated transport.

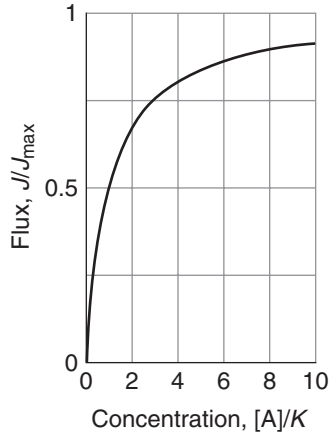

Fig. 8.5 The flux of the species AC through a membrane varies with the concentration of the species A. The behavior shown in the figure and explained in the text is characteristic of mediated transport of A, with C as a carrier molecule.

8.3 The mobility of ions

Ion transport through membranes is central to the operation of many biological processes, particularly signal transduction in neurons, and we need to be equipped to describe ion migration quantitatively.

COMMENT 8.2 An electric field acts on charged particles, whether stationary or moving, whereas a magnetic field acts only on moving charged particles. ■

An ion in solution responds to the presence of an electric field, migrates through the solution, and carries charge from one location to another. The study of the motion of ions down a potential gradient gives an indication of their size, the effect of solvation, and details of the type of motion they undergo. When an ion is subjected to an electric field $\mathcal{E}$, it accelerates. However, the faster it travels through the solution, the greater the retarding force it experiences from the viscosity of the medium. As a result, it settles down into a limiting velocity called its **drift velocity**, s, which is proportional to the strength of the applied field:

$$s = u\mathcal{E} \tag{8.10}$$

The **mobility**, u, depends on the radius, a, of the ion and the viscosity, η, of the solution:

$$u = \frac{ez}{6\pi\eta a} \tag{8.11}$$

DERIVATION 8.1 The ionic mobility

An *electric field* is an influence that accelerates a charged particle. An ion of charge ze in an electric field $\mathcal{E}$ (typically in volts per meter, V m^{-1}) experiences a force of magnitude $ze\mathcal{E}$, which accelerates it. However, the ion experiences a frictional force due to its motion through the medium, and that retarding force increases the faster the ion travels. The viscous drag on a spherical particle of radius a traveling at a speed s is given by *Stokes' law*:

$$F = 6\pi\eta as$$

When the particle has reached its drift speed, the accelerating and viscous retarding forces are equal, so we can write

$$ez\mathcal{E} = 6\pi\eta as$$

and solve this expression for s:

$$s = \frac{ez\mathcal{E}}{6\pi\eta a}$$

At this point we can compare this expression for the drift speed with eqn 8.10 and hence find the expression for mobility given in eqn 8.11.

Equation 8.11 tells us that the mobility of an ion is high if it is highly charged, is small, and is in a solution with low viscosity. These features appear to contradict the trends in Table 8.2, which lists the mobilities of a number of ions. For instance,

Table 8.2 Ionic mobilities in water at 298 K, $u/(10^{-8}\ m^2\ s^{-1}\ V^{-1})$

Cations		Anions	
H^+ (H_3O^+)	36.23	OH^-	20.64
Li^+	4.01	F^-	5.74
Na^+	5.19	Cl^-	7.92
K^+	7.62	Br^-	8.09
Rb^+	8.06	I^-	7.96
Cs^+	8.00	CO_3^{2-}	7.18
Mg^{2+}	5.50	NO_3^-	7.41
Ca^{2+}	6.17	SO_4^{2-}	8.29
Sr^{2+}	6.16		
NH_4^+	7.62		
$[N(CH_3)_4]^+$	4.65		
$[N(CH_2CH_3)_4]^+$	3.38		

the mobilities of the Group 1 cations *increase* down the group despite their increasing radii (Section 9.14). The explanation is that the radius to use in eqn 8.11 is the **hydrodynamic radius**, the *effective* radius for the migration of the ions taking into account the entire object that moves. When an ion migrates, it carries its hydrating water molecules with it, and as small ions are more extensively hydrated than large ions (because they give rise to a stronger electric field in their vicinity), ions of small radius actually have a large hydrodynamic radius. Thus, hydrodynamic radius *decreases* down Group1 because the extent of hydration decreases with increasing ionic radius.

One significant deviation from this trend is the very high mobility of the proton in water. It is believed that this high mobility reflects an entirely different mechanism for conduction, the **Grotthus mechanism**, in which the proton on one H_2O molecule migrates to its neighbors, the proton on that H_2O molecule migrates to its neighbors, and so on along a chain (Fig. 8.6). The motion is therefore an *effective* motion of a proton, not the actual motion of a single proton.

8.4 Toolbox: Electrophoresis

An important application of the preceding material is to the determination of the molar mass of biological macromolecules.

Electrophoresis is the motion of a charged macromolecule, such as DNA, in response to an electric field. Electrophoretic mobility is a result of a constant drift speed, so the mobility of a macromolecule in an electric field depends on its net charge, size (and hence molar mass), and shape.

Electrophoresis is a very valuable tool for the separation of biopolymers from complex mixtures, such as those resulting from fractionation of biological cells. We shall consider several strategies controlling the drift speeds of biomolecules in order to achieve separation of a mixture into its components.

In **gel electrophoresis**, migration takes place through a slab of a porous gel, a semi-rigid dispersion of a solid in a liquid. Because the molecules must pass through the pores in the gel, the larger the macromolecule, the less mobile it is in the electric field and, conversely, the smaller the macromolecule, the more swiftly it moves

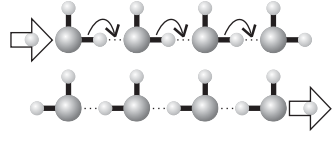

Fig. 8.6 A simplified version of the "Grotthus mechanism" of proton conduction through water. The proton leaving the chain on the right is not the same as the proton entering the chain on the left.

through the pores. In this way, gel electrophoresis allows for the separation of components of a mixture according to their molar masses. Two common gel materials for the study of proteins and nucleic acids are agarose and cross-linked polyacrylamide. Agarose has large pores and is better suited for the study of large macromolecules, such as DNA and enzyme complexes. Polyacrylamide gels with varying pore sizes can be made by changing the concentration of acrylamide in the polymerization solution. In general, smaller pores form as the concentration of acrylamide is increased, making possible the separation of relatively small macromolecules by **polyacrylamide gel electrophoresis** (PAGE).

The separation of very large pieces of DNA, such as chromosomes, by conventional gel electrophoresis is not effective, making the analysis of genomic material rather difficult. Double-stranded DNA molecules are thin enough to pass through gel pores, but long and flexible DNA coils can become trapped in the pores and the result is impaired mobility along the direction of the applied electric field. This problem can be avoided with **pulsed-field electrophoresis**, in which a brief burst of the electric field is applied first along one direction and then along a perpendicular direction. In response to the switching back and forth between field directions, the DNA coils writhe about and eventually pass through the gel pores. In this way, the mobility of the macromolecule can be related to its molar mass.

We have seen that charge also determines the drift speed. For example, proteins of the same size but different net charge travel along the slab at different speeds. One way to avoid this problem and to achieve separation by molar mass is to denature the proteins in a controlled way. Sodium dodecyl sulfate is an anionic detergent that is very useful in this respect: it denatures proteins, whatever their initial shapes, into rods by forming a complex with them. Moreover, most protein molecules bind a constant number of ions, so the net charge per protein is well regulated. Under these conditions, different proteins in a mixture may be separated according to size only. The molar mass of each constituent protein is estimated by comparing its mobility in its rod-like complex form with a standard sample of known molar mass. However, molar masses obtained by this method, often referred to as **SDS-PAGE** when polyacrylamide gels are used, are not as accurate as those obtained by the sophisticated techniques discussed in Chapter 12.

Another technique that deals with the effect of charge on drift speed takes advantage of the fact that the overall charge of proteins and other biopolymers depends on the pH of the medium. For instance, in acidic environments protons attach to basic groups and the net charge is positive; in basic media the net charge is negative as a result of proton loss. At the **isoelectric point**, the pH is such that there is no net charge on the biopolymer. Consequently, the drift speed of a biopolymer depends on the pH of the medium, with $s = 0$ at the isoelectric point (see *Example* 8.1 and Fig. 8.7). **Isoelectric focusing** is an electrophoresis method that exploits the dependence of drift speed on pH. In this technique, a mixture of proteins is dispersed in a medium with a pH gradient along the direction of an applied electric field. Each protein in the mixture will stop moving at a position in the gradient where the pH is equal to the isoelectric point. In this manner, the protein mixture can be separated into its components.

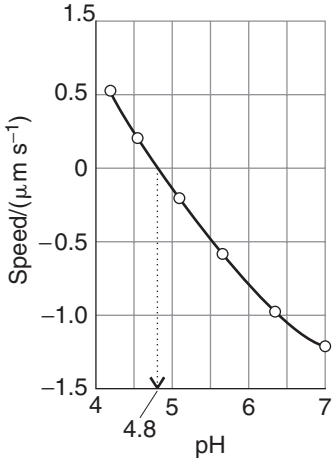

Fig. 8.7 The plot of the speed of a moving macromolecule against pH allows the isoelectric point to be detected as the pH at which the speed is zero. The data are from *Example* 8.1.

EXAMPLE 8.1 The isoelectric point of a protein

The speed with which bovine serum albumin (BSA) moves through water under the influence of an electric field was monitored at several values of pH, and the data are listed below. What is the isoelectric point of the protein?

pH	4.20	4.56	5.20	5.65	6.30	7.00
Velocity/(μm s^{-1})	0.50	0.18	−0.25	−0.65	−0.90	−1.25

Strategy If we plot speed against pH, we can use interpolation to find the pH at which the speed is zero, which is the pH at which the molecule has zero net charge.

Solution The data are plotted in Fig. 8.7. The velocity passes through zero at pH = 4.8; hence pH = 4.8 is the isoelectric point.

SELF-TEST 8.4 The following data were obtained for another protein:

pH	4.5	5.0	5.5	6.0
Velocity/(μm s^{-1})	−0.10	−0.20	−0.30	−0.35

Estimate the pH of the isoelectric point.

Answer: 4.1 ■

The separation of complicated mixtures of macromolecules may be difficult by SDS-PAGE or isoelectric focusing alone. However, the two techniques can be combined in **two-dimensional (2D) electrophoresis**. In a typical experiment, a protein mixture is separated first by isoelectric focusing, yielding a pattern of bands in a gel slab such as the one shown in Fig. 8.8a. To improve the separation of closely spaced bands, the first slab is attached to a second slab and SDS-PAGE is performed with the electric field being applied in a direction that is perpendicular to the direction

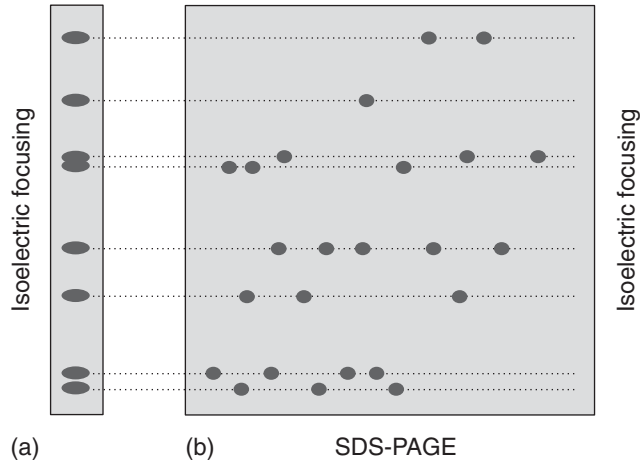

Fig. 8.8 The experimental steps taken during separation of a mixture of biopolymers by two-dimensional electrophoresis. (a) Isoelectric focusing is performed on a thin gel slab, resulting in separation along the vertical direction of the illustration. (b) The first slab is attached to a second, larger slab and SDS-PAGE is performed with the electric field oriented in the horizontal direction of the illustration, resulting in further separation by molar mass. The dashed horizontal lines show how the bands in the two-dimensional gel correspond to the bands in the gel on which isoelectric focusing was performed.

in which isoelectric focusing was performed. The macromolecules separate according to their molar masses along this second dimension of the experiment, and the result is that spots are spread widely over the surface of the slab, leading to enhanced separation of the mixture's components (Fig. 8.8b).

The techniques described so far give good separations, but the drift speeds attained by macromolecules in traditional electrophoresis methods are rather low; as a result, several hours are often necessary to achieve good separation of complex mixtures. According to eqn 8.10, one way to increase the drift speed is to increase the electric field strength. However, there are limits to this strategy because very large electric fields can heat the large surfaces of an electrophoresis apparatus unevenly, leading to a non-uniform distribution of electrophoretic mobilities and poor separation.

In **capillary electrophoresis**, the sample is dispersed in a medium (such as methylcellulose) and held in a thin glass or plastic tube with diameters ranging from 20 to 100 μm. The small size of the apparatus makes it easy to dissipate heat when large electric fields are applied. Excellent separations may be achieved in minutes rather than hours.

8.5 Transport across ion channels and ion pumps

We now have enough background information about ion transport to consider the centrally important processes of ion transport mediated by ion channels and ion pumps, which are involved in the propagation of action potentials and the synthesis of ATP.

The thermodynamic treatment of ion transport in Chapter 5 does not explain the fact that ion channels and pumps discriminate between ions. For example, it is found experimentally that a K^+ ion channel is not permeable to Na^+ ions. We shall see that the key to the selectivity of an ion channel or pump lies in the mechanism of transport and, consequently, in the structure of the protein.

Let's begin by considering some of the experimental approaches used in the study of ion channels. The structures of a number of channel proteins have been obtained by the now traditional X-ray diffraction techniques that will be described in greater detail in Chapter 12. Information about the flow of ions across channels and pumps is supplied by the **patch clamp technique**. One of many possible experimental arrangements is shown in Fig. 8.9. With mild suction, a "patch" of membrane from a whole cell or a small section of a broken cell can be attached tightly

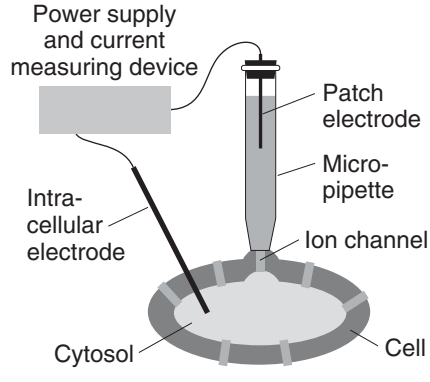

Fig. 8.9 A representation of the patch clamp technique for the measurement of ionic currents through membranes in intact cells. A section of membrane containing an ion channel (shown as a gray rectangle) is in tight contact with the tip of a micropipette containing an electrolyte solution and the patch electrode. An intracellular electronic conductor is inserted into the cytosol of the cell and the two conductors are connected to a power supply and current-measuring device.

to the tip of a micropipet filled with an electrolyte solution and containing an electronic conductor, the so-called *patch electrode*. A potential difference (the "clamp") is applied between the patch electrode and an intra-cellular electronic conductor in contact with the cytosol of the cell. If the membrane is permeable to ions at the applied potential difference, a current flows through the completed circuit. Using narrow micropipette tips with diameters of less than 1 μm, ion currents of a few picoamperes (1 pA = 10^{-12} A) have been measured across sections of membranes containing only one ion channel protein.

A detailed picture of the mechanism of action of ion channels has emerged from analysis of patch clamp data and structural data. Here we focus on the K^+ ion channel protein, which, like all other mediators of ion transport, spans the membrane bilayer (Fig. 8.10). The pore through which ions move has a length of 3.4 nm and is divided into two regions: a wide region with a length of 2.2 nm and diameter of 1.0 nm, and a narrow region with a length of 1.2 nm and diameter of 0.3 nm. The narrow region is called the *selectivity filter* of the K^+ ion channel because it allows only K^+ ions to pass.

Filtering is a subtle process that depends on ionic size and the thermodynamic tendency of an ion to lose its hydrating water molecules. Upon entering the selectivity filter, the K^+ ion is stripped of its hydrating shell and is then gripped by carbonyl groups of the protein. Dehydration of the K^+ ion is endergonic ($\Delta_{dehyd}G^{\ominus}$ = +203 kJ mol^{-1}) but is driven by the energy of interaction between the ion and the protein. The Na^+ ion, though smaller than the K^+ ion, does not pass through the selectivity filter of the K^+ ion channel because interactions with the protein are not sufficient to compensate for the high Gibbs energy of dehydration of Na^+ ($\Delta_{dehyd}G^{\ominus}$ = +301 kJ mol^{-1}). More specifically, a dehydrated Na^+ ion is too small and cannot be held tightly by the protein carbonyl groups, which are positioned for ideal interactions with the larger K^+ ion. In its hydrated form, the Na^+ ion is too large (larger than a dehydrated K^+ ion), does not fit in the selectivity filter, and does not cross the membrane.

Though very selective, a K^+ ion channel can still let other ions pass through. For example, K^+ and Tl^+ ions have similar radii and Gibbs energies of dehydration, so Tl^+ can cross the membrane. As a result, Tl^+ is a neurotoxin because it replaces K^+ in many neuronal functions.

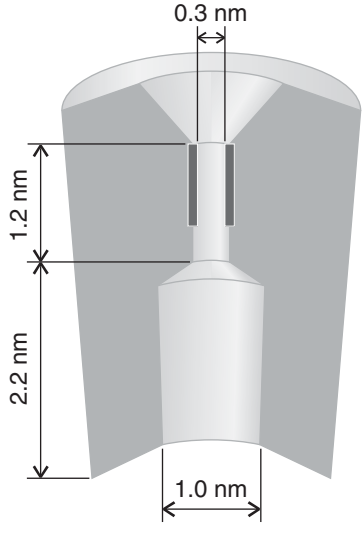

Fig. 8.10 A schematic representation of the cross section of a membrane-spanning K^+ ion channel protein. The bulk of the protein is shown in light gray. The pore through which ions move is divided into two regions: a wide region with a length of 2.2 nm and diameter of 1.0 nm, and a narrow region, the selectivity filter, with a length of 1.2 nm and diameter of 0.3 nm. The selectivity filter has a number of carbonyl groups (shown in darker gray) that grip K^+ ions. As explained in the text, electrostatic repulsions between two bound K^+ ions encourage ionic movement through the selectivity filter and across the membrane.

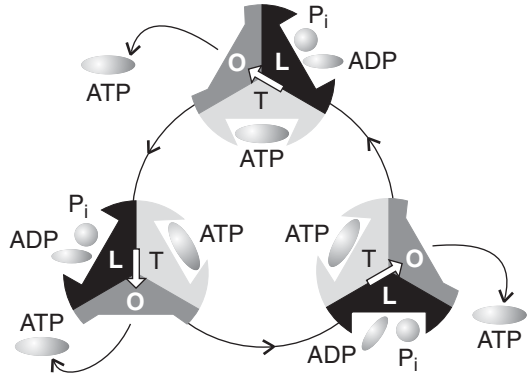

Fig. 8.11 The mechanism of action of H^+-ATPase, a molecular motor that transports protons across the mitochondrial membrane and catalyzes either the formation or hydrolysis of ATP.

The efficiency of transfer of K^+ ions through the channel can also be explained by structural features of the protein. For efficient transport to occur, a K^+ ion must enter the protein but then must not be allowed to remain inside for very long, so that as one K^+ ion enters the channel from one side, another K^+ ion leaves from the opposite side. An ion is lured into the channel by water molecules about halfway through the length of the membrane. Consequently, the thermodynamic cost of moving an ion from an aqueous environment to the less hydrophilic interior of the protein is minimized. The ion is "encouraged" to leave the protein by electrostatic interactions in the selectivity filter, which can bind two K^+ ions simultaneously, usually with a bridging water molecule. Electrostatic repulsion prevents the ions from binding too tightly, minimizing the residence time of an ion in the selectivity filter and maximizing the transport rate.

Now we turn our attention to a very important ion pump, the H^+-ATPase responsible for coupling of proton flow to synthesis of ATP from ADP and P_i (Chapter 4). Structural studies show that the channel through which the protons flow is linked in tandem to a unit composed of six protein molecules arranged in pairs of α and β subunits to form three interlocked $\alpha\beta$ segments (Fig. 8.11). The conformations of the three pairs may be loose, (L), tight (T), or open (O), and one of each type is present at each stage. A protein at the center of the interlocked structure, the subunit shown as a gray arrow, rotates and induces structural changes that cycle each of the three segments between L, T, and O conformations. At the start of a cycle, a T unit holds an ATP molecule. Then ADP and a P_i group migrate into the L site, and as it closes into T, the earlier T site opens into O and releases its ATP. The ADP and P_i in the T site meanwhile condense into ATP, and the new L site is ready for the cycle to begin again. The proton flux drives the rotation of the γ subunit, and hence the conformational changes of the $\alpha\beta$ segments, as well as providing the energy for the condensation reaction itself. Several key aspects of this mechanism have been confirmed experimentally. For example, the rotation of the γ subunit has been portrayed directly by using single-molecule spectroscopy (Chapter 13).

Enzymes

We remarked in *Case study* 6.2 that enzymes are homogeneous biological catalysis that work by lowering the activation energy of a reaction pathway. Enzymes are special biological polymers that contain an **active site**, which is responsible

for binding the **substrates**, the reactants, and processing them into products. As is true of any catalyst, the active site returns to its original state after the products are released. Many enzymes consist primarily of proteins, some featuring organic or inorganic co-factors in their active sites. However, certain ribonucleic acid (RNA) molecules[2] can also be biological catalysts, forming **ribozymes**. A very important example of a ribozyme is the **ribosome**, a large assembly of proteins and catalytically active RNA molecules responsible for the synthesis of proteins in the cell.

The structure of the active site is specific to the reaction that it catalyzes, with groups in the substrate interacting with groups in the active site via intermolecular interactions, such as hydrogen bonding, electrostatic, or van der Waals interactions.[3] Figure 8.12 shows two models that explain the binding of a substrate to the active site of an enzyme. In the **lock-and-key model,** the active site and substrate have complementary three-dimensional structures and dock perfectly without the need for major atomic rearrangements. Experimental evidence favors the **induced fit model**, in which binding of the substrate induces a conformational change in the active site. Only after the change does the substrate fit snugly in the active site.

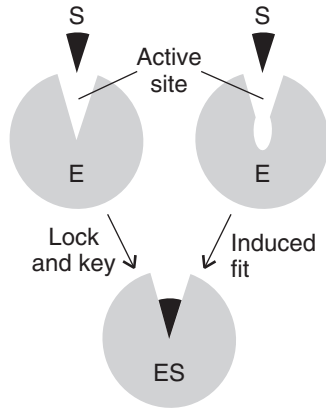

Fig. 8.12 Two models that explain the binding of a substrate to the active site of an enzyme. In the lock-and-key model, the active site and substrate have complementary three-dimensional structures and dock perfectly without the need for major atomic rearrangements. In the induced fit model, binding of the substrate induces a conformational change in the active site. The substrate fits well in the active site after the conformational change has taken place.

Enzyme-catalyzed reactions are prone to inhibition by molecules that interfere with the formation of product. As we remarked in the *Prologue*, many drugs for the treatment of disease inhibit enzymes of infectious agents, such as bacteria and viruses. Here we focus on the kinetic analysis of enzyme inhibition, and in Chapter 10 we shall see how computational methods contribute to the design of efficient inhibitors and potent drugs.

8.6 The Michaelis-Menten mechanism of enzyme catalysis

Because enzyme-controlled reactions are so important in biochemistry, we need to build a model of their mechanism. The simplest approach proposed by Michaelis and Menten is our starting point.

Experimental studies of enzyme kinetics are typically conducted by monitoring the initial rate of product formation in a solution in which the enzyme is present at very low concentration. Indeed, enzymes are such efficient catalysts that significant accelerations may be observed even when their concentrations are more than three orders of magnitude smaller than those of their substrates.

The principal features of many enzyme-catalyzed reactions are as follows (Fig 8.13):

1. For a given initial concentration of substrate, $[S]_0$, the initial rate of product formation is proportional to the total concentration of enzyme, $[E]_0$.
2. For a given $[E]_0$ and low values of $[S]_0$, the rate of product formation is proportional to $[S]_0$.
3. For a given $[E]_0$ and high values of $[S]_0$, the rate of product formation becomes independent of $[S]_0$, reaching a maximum value known as the **maximum velocity**, v_{max}.

[2]The structure of RNA is discussed in Chapter 11.

[3]Intermolecular interactions are discussed in Chapter 11.