CHAPTER

5

Chemical Forces

In addition to mechanical and thermal forces, proteins are also subject to chemical forces. By **chemical forces**, we mean the forces that arise from the formation of intermolecular bonds. For example, consider what happens when a protein first comes in contact with another molecule such as a small ligand or another protein. As energetically favorable contacts are made, the protein may become stretched or distorted from its equilibrium conformation. If the protein can adopt two different structures, the binding could preferentially stabilize one of these structures. Chemical forces also arise from changes in bound ligands. One example is the *cis*-to-*trans* isomerization of retinal bound to the opsin protein: Following the adsorption of light, the all-*trans*-retinal is initially in a highly strained conformation, and its relaxation drives the slower structural changes of the opsin (Peteanu et al., 1993). Another example is the hydrolysis of the gamma phosphate bond of ATP bound to myosin (Chapter 14). In all the above cases, the chemical change produces a local distortion that in turn pushes the protein into a new low-energy conformation.

To understand how protein machines work, it is essential to understand how proteins move in response to these chemical forces. Just as a chemical force might cause a protein to move in one direction, an external mechanical force might cause the protein to move in the opposite direction. For example, the binding of a ligand might stabilize the closure of a cleft, whereas an external tensile force might stabilize the opening of the cleft; as a result, the mechanical force is expected to oppose the binding of the ligand. Thus mechanical forces can oppose chemical reactions, and, conversely, chemical reactions can oppose mechanical ones. If the chemical force is strong enough, the chemical reaction will proceed even in the presence of a mechanical load. In this case we say that the reaction generates force.

The purpose of this chapter is to elucidate the general principles by which applied forces affect both the rates and equilibria of chemical reactions, and, by extension, how chemical reactions generate force.

Chemical Equilibria

A central question is how force affects the equilibrium between two structural states of a protein

$$E_1 \rightleftharpoons E_2$$

However, we immediately encounter a difficulty: What is a structural state of a protein? The difficulty arises because thermal fluctuations cause a complex molecule like a protein to occupy, sequentially, an enormous number of different **conformational states**, where a conformational state is a set of positions or coordinates of all the atoms. (We ignore the velocities of the atoms, which will average out over the picosecond timescale, as argued in the last chapter.) If the fluctuations in the positions of the atoms are not too large (or if the atoms do not spend too much time at large distortions), then we can think of the different conformations as small deviations about a stable, minimum-energy state. We call the time-average of these conformational states a **structural state**; it will be similar to the minimum-energy state (and the two will be the same if the fluctuations are symmetrical). Structural states can be solved by X-ray crystallography or NMR. According to our definition, a structural state is an ensemble of a large number of the individual conformational states that do not vary too much from the mean. An unfolded protein, by contrast, has such large fluctuations that the distances between atoms in different amino acids vary by more than the size of the amino acids themselves; in this case, we say that the protein is unstructured.

It is possible that the conformational states segregate into two ensembles with different means. In this case we say that the protein adopts two structural states. We denote the states as E_1 and E_2. The different structural states might have different functional properties. An enzyme in one state might be catalytically active whereas in the other state it might be inactive. In this case, the structural states are "on" and "off." An ion channel might be open or closed. Different states might have different affinities for another protein, for DNA, or for a ligand. In the case of a motor protein, the lever might be up or down, corresponding to the pre- and post-working strokes.

To determine the probability of finding the protein in one of the two structural states E_1 and E_2, we would like to apply Boltzmann's law. However, as stated in the last chapter, Boltzmann's law applies only to individual conformational states and not to ensembles of states. There is, however, a way around this. For a structural state, E, which is really an ensemble of states with energies $\{U_i\}$ (Figure 5.1), we define the **free energy** as

$$G = U - TS \tag{5.1}$$

where U is the average potential energy, T is the temperature, and S is the entropy (Appendix 5.1). U comprises the internal energy—the energy associ-

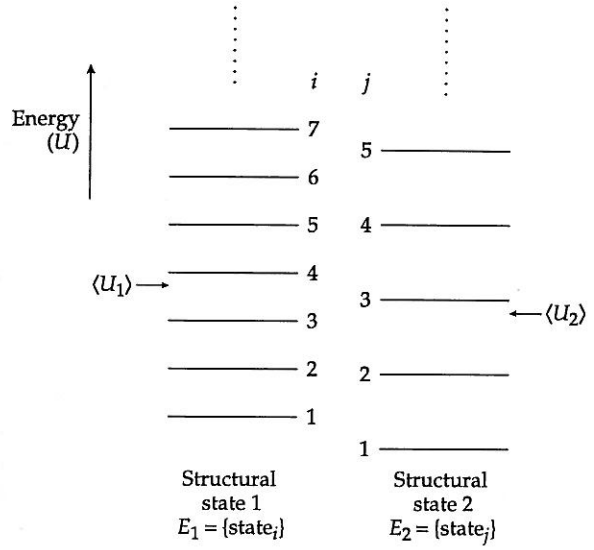


Figure 5.1 Ensembles of states
Suppose a molecule can be in one of two structural states E_1 and E_2, each comprising an ensemble of individual conformational states with different potential energies. Each conformational state has energy U_i or U_j and, at equilibrium, the probability of finding the molecule in conformational state i, p_i, is proportional to $\exp[-U_i/kT]$. The probability of finding the molecule in structural state E_1 is the sum of all the probabilities p_i. Likewise, the probability of finding the channel in structural state E_2 is the sum of all the probabilities p_j. The result of this calculation, given in Appendix 5.1, is a generalized form of Boltzmann's law that relates the probabilities to the free energies (see Equation 5.2).

ated with all the bonds (covalent, electrostatic, van der Waals)—plus other terms corresponding to potential energies arising from external variables such as pressure, force, electrical fields, or gravity. The entropy is a measure of disorder: The larger the number of conformations in an ensemble, the greater the entropy. It is shown in the Appendix that Boltzmann's law holds for ensembles of conformational states, E_1 and E_2, if the energies are replaced by the free energies, G_1 and G_2. In other words,

$$\frac{[E_2]}{[E_1]} = \frac{p_2}{p_1} = \exp\left[-\frac{\Delta G}{kT}\right] = \text{constant} \equiv K_{eq} \qquad \Delta G = G_2 - G_1 \qquad (5.2)$$

This equation is important because it shows that there is such a thing as an **equilibrium constant**, K_{eq}. It is a constant in the sense that it does not depend on the concentrations of E_1 and E_2, though in general it will depend on the temperature, ionic strength, and other variables. Equation 5.2 is known as the **Law of Mass Action** because if E_1 and E_2 are in equilibrium and more protein in the E_1 form is added, then the amount of E_2 will increase as the system returns to the equilibrium ratio. Likewise, adding E_2 pushes the reaction back toward E_1. The law applies equally well to the equilibrium between small molecules such as substrate and product as between protein conformations E_1 and E_2.

If we ignore the external potential energy terms, G is the **Helmholtz free energy**. If the only potential energy corresponds to pressure–volume work, PV, then G is the **Gibbs free energy** and U is called the **enthalpy**. If there are other potential energy terms arising from mechanical forces or electrical potentials, then we simply call G the free energy. Usually, the difference between the

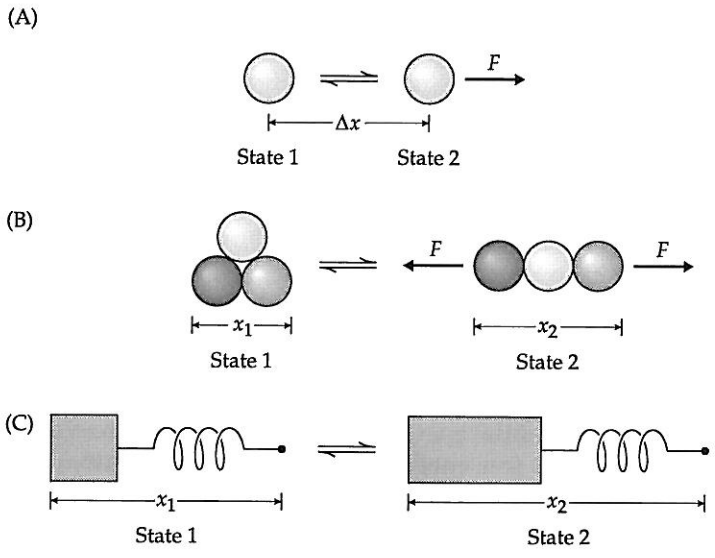
Helmholtz and Gibbs free energies can be ignored because the volume changes associated with structural changes in proteins are quite small, no more than a few percent of the total volume. Because the total volume of proteins is small anyway, on the order of 120 nm^3 for a 100 kDa protein, the volume changes are only on the order of 1 nm^3. At the standard pressure of 1 atmosphere (~100 kPa), the $P\Delta V$ work is only ~0.1×10^{-21} J, which, because it is much less than kT (4×10^{-21} J), will have very little effect on the equilibrium. Volume changes are expected to be important only in the depths of the ocean where pressures may exceed 100 atmospheres. On the other hand, as we shall see, the potential energy due to mechanical external forces can be very significant.

The Effect of Force on Chemical Equilibria

Boltzmann's law allows us to calculate how a force influences the equilibrium between two (or more) structural states. If the difference between two structural states is purely translational—that is, if state E_2 corresponds to a movement through a distance Δx with respect to state E_1, as occurs when a motor moves along a filament against a constant force—then the difference in free energy is $\Delta G = -F \cdot \Delta x$, where F is the magnitude of the force *in the direction* of the translation (Figure 5.2A). If the length of a molecule changes by a distance Δx as a result of a conformational change (Figure 5.2B), then the difference in free energy is

$$\Delta G \cong \Delta G^0 - F\Delta x$$

Figure 5.2 Displacements associated with structural changes
(A) Translation. (B) Lengthening. (C) Lengthening with constant stiffness.



where F is the tension across the molecule and ΔG^0 is the free energy difference in the absence of tension. The equality is exact if the molecule is composed of rigid domains that undergo relative translation as shown in Figure 5.2B, or if the two structural states have equal stiffness (Figure 5.2C) (see below). At equilibrium

$$\frac{[E_2]}{[E_1]} = \exp\left[-\frac{\Delta G}{kT}\right] \cong \exp\left[-\frac{\Delta G^0 - F\Delta x}{kT}\right] = K_{eq}^0 \exp\left[\frac{F\Delta x}{kT}\right] \qquad (5.3)$$

where K_{eq}^0 is the equilibrium constant in the absence of the force. The crucial point is that an external force will couple to a structural change if it is associated with a length change in the direction of the force. If the change in length of the molecule is 4 nm, then a force of 1 pN will change the free energy by 4 pN·nm $\cong kT$. According to Equation 5.3, this will lead to an e-fold change in the ratio of the concentrations. Because protein conformational changes are measured in nanometers, and energies range from 1 kT (thermal energy) to 25 kT (ATP hydrolysis) (see Table 4.2), it is expected that relevant biological forces will be on the scale of **piconewtons**.

An example of how forces modulate the state of a protein—in this case a mechanically sensitive ion channel—is explored in Example 5.1.

An expression analogous to Equation 5.3 holds for voltage-gated ion channels (Hille, 1992). In this case, the structural change associated with the opening of such a channel is coupled to movement of charge, Δq, across the electric field caused by the transmembrane potential, V. Mutagenesis studies indicate that the moving charges include positively charged arginine residues in the S4 transmembrane helix (Hille, 1992). From a physical viewpoint, charge (Δq) is analogous to displacement (Δx), and potential (V) is analogous to force (F). The energy difference between the open and closed states therefore includes a term $V\Delta q$, and this makes the opening sensitive to the voltage. The openings of the voltage-dependent Na and K channels that underlie the action potential are strongly voltage dependent: Classic experiments by Hodgkin and Huxley showed that the ratio of the open probability to the closed probability increased approximately e-fold per 4 mV (Hodgkin, 1964). This indicates that the opening of each channel is associated with the movement of about six electronic charges across the membrane ($\Delta q = kT/V \cong 6e$, where e is the charge on the electron). The predicted movement of these electronic charges has been directly measured as a nonlinear capacitance of the membrane (Armstrong and Bezanilla, 1974) that is analogous to the nonlinear stiffness of the hair bundle in Example 5.1.

Protein conformational changes are sensitive to many other "generalized" forces including membrane tension, osmotic pressure, hydrostatic pressure, and temperature. Sensitivity to these forces requires that conjugate structural changes occur in the protein (Howard et al., 1988). In the case of membrane tension, σ, the conjugate variable is area, Δa, and the energy difference equals $\sigma\Delta a$: The sensitivity of stretch-activated ion channels to membrane tension (Guharay and Sachs, 1984; Chang et al., 1998; Batiza et al., 1999) suggests that the conformational change associated with channel opening leads to an increase

Example 5.1 Mechanically sensitive ion channels in hair cells

The sensory hair cells of the inner ear underlie the perception of sound, linear acceleration, angular acceleration, and gravity. When the hair bundle is deflected by an external force, there is shear between adjacent stereocilia within the bundle (see Figure 8.2). This shear in turn tenses elastic tip links that pull on and open ion channels at the tips of the stereocilia (Figures A and B). Because the opening of a channel shortens the tip link, the open state is stabilized by deflections that increase the tension in the tip link. As a result, the open probability increases as the hair bundle is displaced to the right, defined as the positive direction (Figure B). Flow of potassium ions through the channels alters the electrical potential across the membrane, producing the receptor potential that can be measured by inserting a glass microelectrode into the cell (Figure C). The receptor potential triggers synaptic release and eventually leads to perception by the central nervous system. Due to the extra degree of freedom corresponding to the opening and closing of the channels, the stiffness of the hair bundle is not constant, but depends on the displacement. The stiffness can be measured by displacing the bundle with a flexible glass fiber (see Figure 15.3) and measuring the flexion in the fiber using a photodiode detector (see Figure 15.5). As seen in Figure D, the stiffness has a minimum when the channels are open 50% of the time. The displacement dependence of the stiffness indicates that the "swing" of the gate (Δx) is 2 to 4 nm and that there are 1 or 2 channels per stereocilium (Howard and Hudspeth, 1988; Hudspeth et al., 1990; Denk et al., 1995).

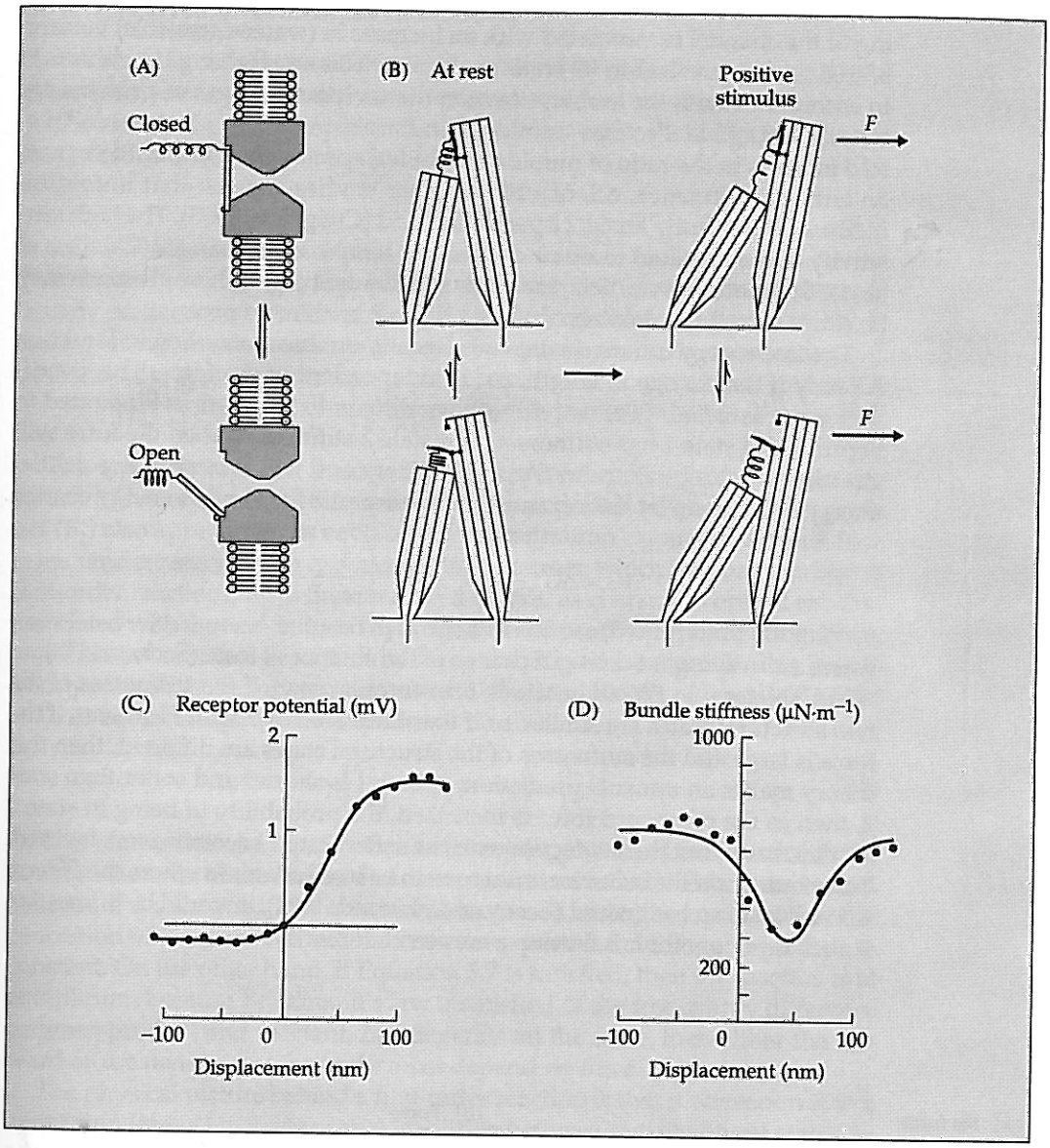
The open probability is

$$p_{\text{open}} = \frac{1}{1+\exp\left[-\dfrac{F\Delta x}{kT}\right]} \qquad F = a\kappa(X - X_0)$$

where a ($\cong 0.14$) is a geometric factor that relates the displacement of the bundle to the shear between stereocilia, κ is the stiffness of the tip link, X is the average displacement of the hair bundle, and X_0 is the displacement at which the channels are open 50% of the time. This equation follows from Equation 5.3, and provides a good fit to the receptor potential (Figure C). The stiffness of the hair bundle is

$$K = \frac{d^2G}{dX^2} = K_S + N\kappa a^2 - N\frac{(a\kappa\Delta x)^2}{kT}p(1-p)$$

where the free energy of the bundle, G, includes an entropy term corresponding to the opening and closing of the channels (see Appendix 5.1) (Hudspeth et al., 1990; Markin and Hudspeth, 1995). K_S is an additional constant term corresponding to stiffness arising from the bending of the bases of the stereocilia, and N is the number of channels. This equation provides a good fit to the stiffness data, with $\Delta x = 4$ nm and $\kappa = 0.5$ pN/nm.



in membrane area of 4 to 8 nm^2. Sensitivity of channels to changes in osmotic pressure, π, induced by addition of sucrose or sorbitol, suggests that their opening is associated with a change in "solute accessible volume": ΔV is ~1 nm^3 for the potassium channel from nerve (Zimmerberg et al., 1990) and ~30 nm^3 for the large anion channel from mitochondria (Zimmerberg and Parsegian, 1986). These volumes correspond to 30 to 1000 water molecules. The sensitivity of the acetylcholine receptor channel to pressure (Heinemann et al., 1987), suggests that the binding of acetylcholine and subsequent open-

ing of the channel is associated with an increase in (water-accessible) volume of 0.08 nm^3, equivalent to ~3 water molecules. This small change in volume is in accordance with the earlier assertion that volume changes of proteins are usually negligible. Proteins unfold when the temperature is increased: An *e*-fold increase in the ratio of unfolded to folded species per 3°C would suggest an entropy difference, ΔS, of ~100 *k*. Other studies suggest that unfolding increases the entropy about 2 *k* per amino acid (Creighton, 1993). The high sensitivity of the vanilloid receptor channels to temperature changes (Caterina et al., 1997; Caterina et al., 1999) indicates that the opening of these channels may be coupled to the unfolding of a large domain within the protein.

The free energy difference depends linearly on force according to Equation 5.3 only if the change in length, Δx, is independent of the force. This condition is not satisfied if the two states have different stiffnesses as illustrated in Figure 5.3. If state 1 has stiffness κ_1 and state 2 stiffness κ_2, then the force will stretch the states by F/κ_1 and F/κ_2, respectively, and will increase the potential energy of the states by $\frac{1}{2}F^2/\kappa_1$ and $\frac{1}{2}F^2/\kappa_2$, respectively. The free energy change will then be

$$\Delta G = \Delta G^0 - F\Delta x^0 - \tfrac{1}{2}F^2\left[\frac{1}{\kappa_2} - \frac{1}{\kappa_1}\right] \tag{5.4}$$

where $\Delta x^0 = x_2 - x_1$ is the length change in the absence of force (Sachs and Lecar, 1991). The term in F^2 will be small if the force is small, if the stiffnesses of the two structural states are similar, or if the states are very rigid. However, if the force is large and the stiffnesses of the structural states are different, then this theory makes an unusual prediction: If state 1 is shorter and softer than state 2, then as the rightward force is increased, the probability of being in state 2 first increases, but then it decreases as the softer state 1 becomes more favored. This nonmonotonic behavior is not seen in hair cell channels where the F^2 term is small and can be ignored (Corey and Howard, 1994); it would be interesting if such nonmonotonic behavior were seen in other systems.

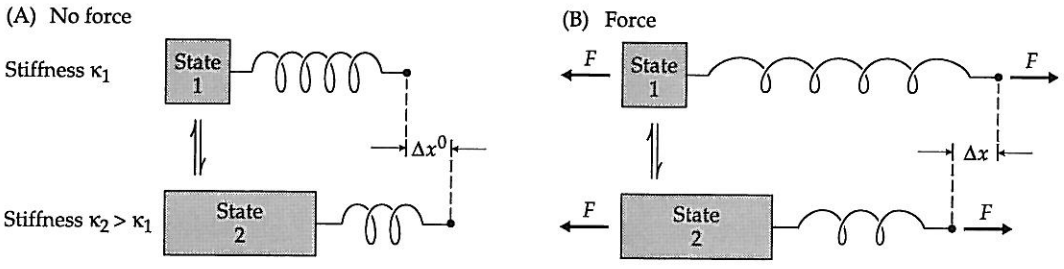


Figure 5.3 Differences in compliance between structural states
(A) No force. (B) Force. The effect of force on the free energy is calculated in detail in Appendix 5.1 and summarized in Equation 5.4.

Rate Theories of Chemical Reactions

Forces also affect the rates of chemical reactions. In order to understand how this occurs, we must first discuss theories of chemical reactions.

The simplest chemical reaction is the interconversion between two species that satisfies

$$E_1 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E_2 \qquad \frac{d[E_1]}{dt} = -k_1[E_1] + k_{-1}[E_2] \tag{5.5}$$

This reaction is said to obey first-order kinetics because the rate of change depends linearly on the concentration of the species. The constants of proportionality, k_1 and k_{-1} are called **rate constants** and they have units of s^{-1}. The solution is

$$\frac{[E_1](t)}{[E_1]+[E_2]} = [E_1](0) + \left\{\frac{k_{-1}}{k_1 + k_{-1}} - [E_1](0)\right\}\left\{1 - \exp[-(k_1 + k_{-1})t]\right\} \tag{5.6}$$

This equation says that the reactant (E_1) approaches its equilibrium concentration with an exponential time course. The time constant is $(k_1 + k_{-1})^{-1}$. The product (E_2) also approaches its equilibrium concentration exponentially (with the same time constant). Such exponential time courses, which are characteristic of first-order reactions, were first measured in 1850 as changes in optical rotation associated with the acid-induced hydrolysis of sucrose (Eyring and Eyring, 1963).

When the reaction described by Equation 5.5 reaches equilibrium, it is at steady state with the forward and reverse reactions exactly balanced ($d[E_1]/dt = 0$). At equilibrium, we therefore have

$$\frac{k_1}{k_{-1}} = \frac{[E_2]}{[E_1]} = K_{eq} = \exp\left[-\frac{\Delta G}{kT}\right] \tag{5.7}$$

This shows that the equilibrium constant (K_{eq}) defined in Equation 5.2 is equal to the ratio of the forward and reverse rate constants. This makes sense, because if the forward rate constant is increased, then the equilibrium concentration of product will also increase as expected for a larger equilibrium constant. On the other hand, if Equation 5.7 is satisfied, then the reaction is at equilibrium because Boltzmann's law is satisfied. If the free energy difference between product and reactant, ΔG, depends on the force, then either the forward or the reverse rate (or both) must depend on force.

The physical picture behind a first-order reaction is that it corresponds to a very rapid (almost instantaneous) transition between two structural states. In other words, the duration of the transition is very much shorter than the average lifetimes of the states ($1/k_1$ for E_1 and $1/k_{-1}$ for E_2). How fast might the transition be? Covalent chemical changes occur very rapidly, on the 0.1 picosecond timescale of molecular vibrations (corresponding to optical wavenumbers of ~1000 cm^{-1}). But global protein conformational changes occur much more slowly. The speed is ultimately limited by the speed of sound (Appendix 5.2), so the fastest relaxations of very rigid proteins have time constants of ~10 ps (see Problem 3.5). For more typical, softer proteins such as motor proteins, the relaxations are even slower, with time constants on the order of 10 ns (see Example 2.5 and Figure 2.4D). Nevertheless, even a transition lasting 10 ns is

a very short time compared to the lifetimes of structural states, which are typically 1 ms or longer.

Several properties of first-order reactions can be understood using the idea that the reaction proceeds via a high-energy **activated state**, or **transition state** (Figure 5.4). Because the activated state has a free energy, G_a, much greater than that of the initial or final states, the probability that the protein will be in the activated state is very low. The short-lived activated state accords with the transition itself being very rapid. Because the activated state occurs at an energy maximum, it differs from a structural state in that it is not stable: We do not expect to be able to crystallize a protein in one of its transition states.

The activated-state concept leads naturally to the idea that chemical reactions can indeed be described using rate constants. To make this connection requires two additional assumptions. First, it is assumed that the reactant (E_1) is in equilibrium with the activated state (E_a). And second, it is assumed that the activated state is equally likely to break down to reactant or product with some rate A. By Boltzmann's law, the probability of being in the activated state is $[E_a]/[E_1] = \exp[-(G_a - G_1)/kT]$ and so the rate of formation of product is $A[E_a] = A[E_1]\exp[-(G_a - G_1)/kT]$. Thus the rate is linearly proportional to reagent concentration, $[E_1]$, as expected for a first-order reaction. The rate constant is

$$k_1 = A\exp\left[-\frac{\Delta G_{a1}}{kT}\right] \qquad \Delta G_{a1} = G_a - G_1 \tag{5.8}$$

A similar expression holds for the reverse reaction; the ratio, $k_1/k_{-1} = K_{eq}$, accords with the Law of Mass Action. Equation 5.8 is called the **Arrhenius equation** and the constant A is called the **frequency factor**, or **pre-exponential factor**.

The activated-state concept also accounts for the strong temperature dependence of chemical reactions. Because the frequency factor, A, is not expected to depend strongly on temperature, the Arrhenius equation predicts that the rate constant depends on temperature according to $\sim\exp(-\Delta G_{a1}/kT)$. This agrees with the experimental results that biochemical reactions have strong temper-

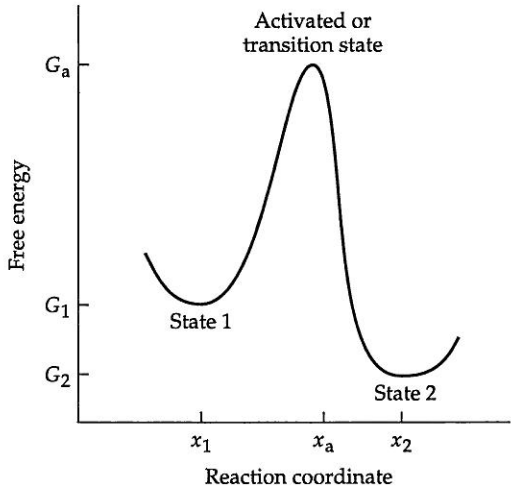


Figure 5.4 The activated state
The activated state corresponds to a position (x_a) along the reaction coordinate, intermediate between the initial (x_1) and final (x_2) positions.

ature dependencies. Typically, the rates of biochemical reactions double to quadruple for every 10°C increase in temperature: We say that the Q_{10} is between 2 and 4. Such Q_{10}s imply that the enthalpy, ΔU_a, of the transition state is some 20 to 40 kT above the initial state (Appendix 5.2). Thus, as argued by Arrhenius, the strong temperature dependence of chemical reactions supports the concept of a high-energy activated state.

To predict the absolute rate of a biochemical reaction, a more detailed theory is needed because the Arrhenius theory provides no information about the frequency factor, A. Two such detailed theories are the Eyring rate theory and the Kramers rate theory. Both require that the **reaction coordinate**, the parameter that measures the progression of the reaction, be specified. If a protein changes overall length as a result of the $E_1 \rightarrow E_2$ transition, then we could make length the reaction coordinate, though many other reaction coordinates are possible; indeed, the distance between any two atoms that move relative to one another during the reaction could be used as a reaction coordinate. *If the protein is subject to a force, then a natural reaction coordinate is the length of the protein in the direction of the force.*

In the **Eyring rate theory**, the reaction is assumed to correspond to the breakdown of a single quantum-mechanical vibration of the protein. In this case the frequency factor is $\sim kT/h \cong 6 \times 10^{12}\ s^{-1}$, where h is the Planck constant (Atkins, 1986). The absolute rate is then the frequency factor reduced by the exponential term. For example, a reaction with a rate constant of $10^3\ s^{-1}$ would have an activation free energy (ΔG_{a1}) of 22 kT. The Eyring theory is expected to apply to covalent changes of proteins and their ligands. However, it is not expected to apply to global conformational changes of proteins in which a large number of bonds are made and broken, because in this case the reaction does not correspond to a single mode of vibration of the protein.

In the case of global protein conformational changes, a more physically realistic model is the **Kramers rate theory**. According to this model, the protein diffuses into the transition state with a rate that is the reciprocal of the diffusion time

$$k_1 = \frac{\varepsilon_1}{\pi}\frac{1}{\tau_1}\sqrt{\frac{\Delta G_{a1}}{kT}}\exp\left[-\frac{\Delta G_{a1}}{kT}\right] \qquad \Delta G_{a1} = G_a - G_1 \qquad (5.9)$$

ε_1 is an "efficiency factor" equal to the probability of making the transition when at the transition state (Appendix 5.2). According to Kramers rate theory, the frequency factor is approximately equal to the inverse of the relaxation time, $\tau^{-1} = \kappa/\gamma$ (the other pre-exponential terms are close to unity). This makes intuitive sense: We can think of the protein sampling a different energy level every τ seconds, because τ is the time over which the protein's shape becomes statistically uncorrelated. The protein can react only when it attains the energy of the transition state, and the probability of this occurring is proportional to $\exp(-\Delta G_{a1}/kT)$.

The Eyring and Kramers rate theories represent two extreme views of the mechanism of global conformational changes of proteins. In the Eyring model, the transition state is like the initial state (Figure 5.5). A sudden, local chemical change (such as the binding of a ligand or the chemical change in a bound ligand) creates a highly strained protein that then relaxes into a new stable conformation. The relaxation is along the quadratic energy curve and has time

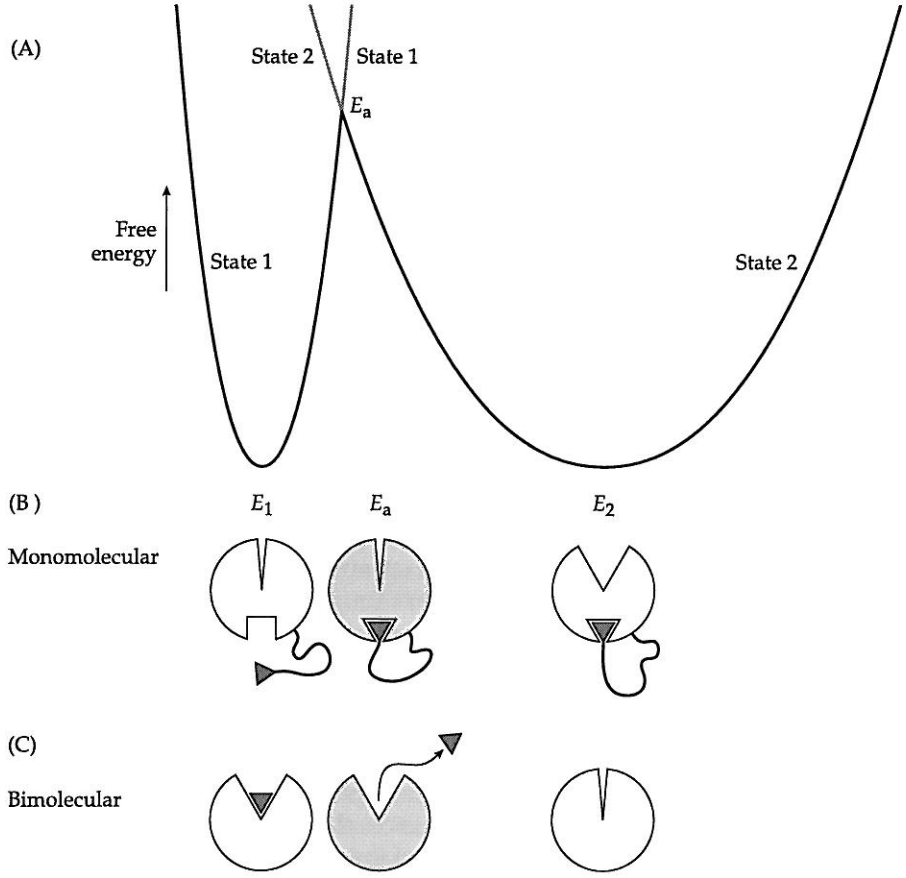


Figure 5.5 Eyring-like mechanism
(A) Energy diagram. (B) Monomolecular model. (C) Bimolecular model. The reaction coordinate is the extent of opening or closing of the cleft. The shaded structures are the strained transition states.

constant $\tau = \gamma/\kappa$. This formulation has been used to model the working stroke of myosin (Eisenberg and Hill, 1978; see Figure 16.3): After myosin has bound to actin in its pre-powerstroke state, the phosphate rapidly dissociates, leaving the protein in a highly strained post-powerstroke state. The relaxation of this highly strained state drives the sliding of the filaments and the shortening of the muscle. If the filaments are prevented from sliding, the strained state will maintain the tension in the muscle.

The energy profile associated with an Eyring-like protein conformational change is analogous to that associated with a spectroscopic change. When a small molecule absorbs light and undergoes an electronic change, the transition is drawn as a vertical line in accordance with the Franck–Condon principle, which states that the more massive nuclei take much longer to move than the lighter electrons. Consequently, the newly formed state is strained and

relaxes slowly (though still on the subpicosecond timescale) as the nuclei move into their new stable positions. The analogous principle for proteins is that global structural or "physical" changes of proteins are much slower than local chemical changes, because structural changes are slowed by protein and solvent viscosity. The fast local changes leave the protein in an unstable global conformation which then relaxes more slowly into a new stable state. (These ideas are expanded in Figure 16.2.) The transition is not really vertical: it is actually a very steep parabola, with curvature appropriate for the high rigidity of the local bonds.

In the Kramers view, the protein undergoes a global diffusion into the activated state. When a sufficiently large conformational change has been achieved, the protein converts to the final state (Figure 5.6). This is reminiscent of the mechanism postulated by Marcus to explain electrochemical reactions in solution (Marcus, 1996). In the extreme, the protein diffuses all the way to the final state, which is then locked in by a subsequent chemical change (Figure 5.7). This extreme case has been called a **thermal ratchet** mechanism on account of the prominent role played by diffusion in reaching the transition state (Hunt et al., 1994; Peskin and Oster, 1995). The application of these ideas to motor proteins is discussed in Example 5.2. Of course, if the forward process is purely diffusive, then the reverse is Eyring-like. However, it should be pointed out that even in the Eyring-like mechanism, the activated state is also reached by a ther-

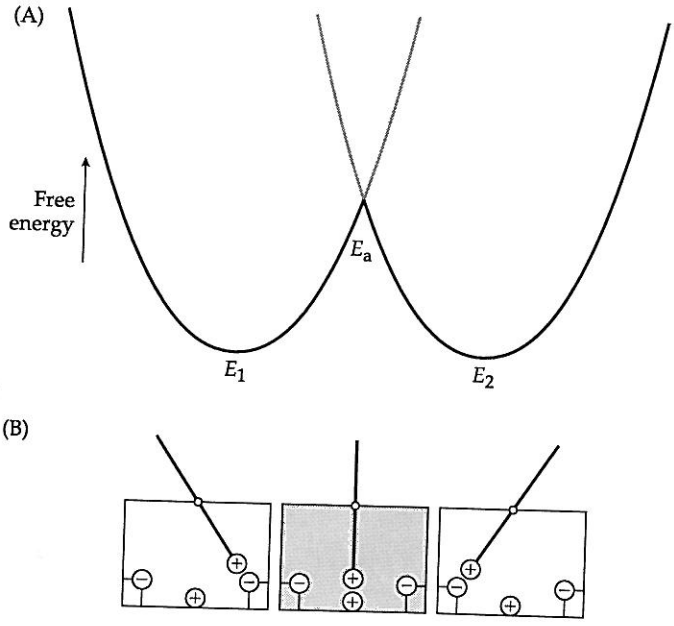


Figure 5.6 Marcus mechanism
(A) Energy diagram. (B) Physical model that gives the reaction profile. The reaction coordinate is the position of the end of the lever. The shaded structure is the high-energy transition state.

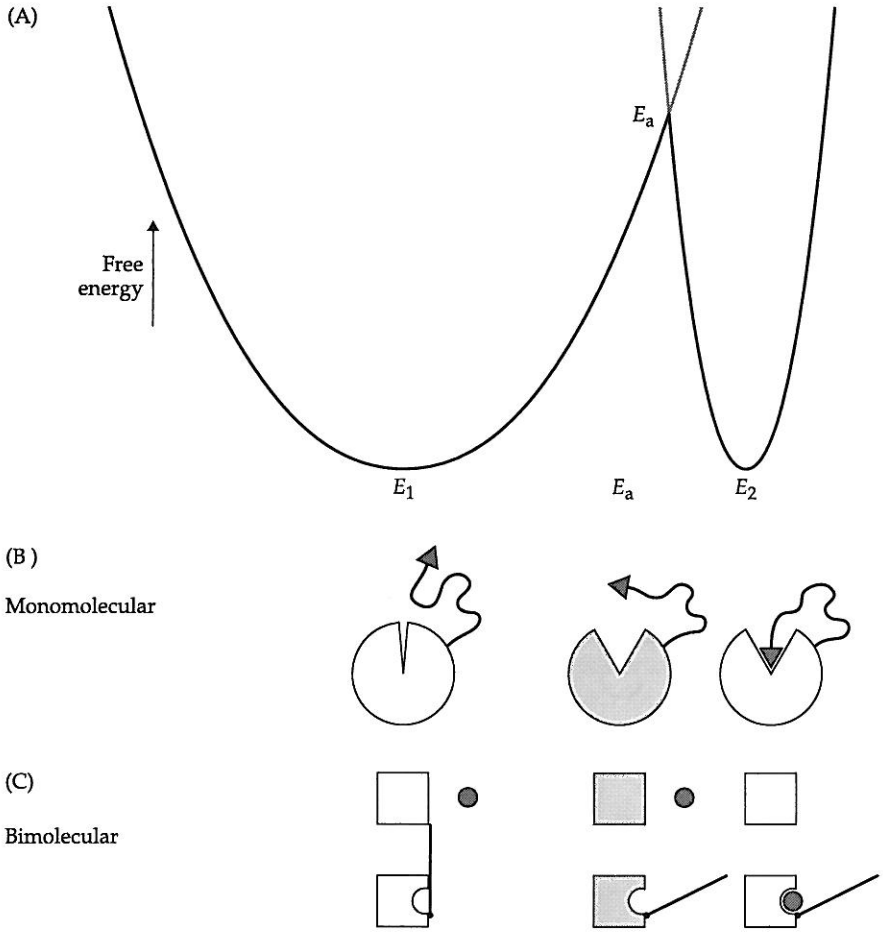


Figure 5.7 Kramers-like mechanism (thermal ratchet)
(A) Energy diagram. (B) Monomolecular model (C) Bimolecular model. The physical picture is of a "foot-in-the-door" mechanism such that the global conformational change must be completed before the structural change can occur. The reaction coordinate is the extent of opening of the cleft or the gate. The shaded structures are the strained transition states.

mal fluctuation, just one that is more localized. The role of these different mechanisms in force generation by motor proteins is discussed in Chapter 16.

Effect of Force on Chemical Rate Constants

The activated-state concept makes specific predictions of how rate constants depend on external force. If the protein structures are very rigid and the transitions $E_1 \rightarrow E_a \rightarrow E_2$ are associated with displacements x_1, x_a, and x_2 *in the direc-*

tion of the force, F, then the energies of the states will be decreased by Fx_1, Fx_a, and Fx_2, respectively. This implies that

$$k_1 = A\exp\left[-\frac{\Delta G_{a1} - F\Delta x_{a1}}{kT}\right] = k_1^0 \exp\left[\frac{F\Delta x_{a1}}{kT}\right] \tag{5.10}$$

where $\Delta G_{a1} = G_a - G_1$ and $\Delta x_{a1} = x_a - x_1$. An analogous expression holds for k_{-1}. Another way of writing this is to let $\Delta x_{a1} = \theta(x_2 - x_1)$, where θ is the fraction of the distance of the transition state toward the final state (Hille, 1992). Note that the ratio of the forward and reverse rate constants must give the correct force dependence for the equilibrium (Equations 5.3 and 5.7).

A useful way of thinking about the effect of force on the reaction rates is that it tilts the free energy diagram of the reaction (Figure 5.8). If the displacement of the activated state is intermediate between the initial and final states ($x_1 < x_a < x_2$), then a negative external force (a load) will slow the reaction, whereas a positive external force (a push) will accelerate the reaction. However, if $x_a = x_1$—that is, if the transition state is reactant-like—then force will have little effect on the forward rate constant. On the other hand, if $x_a = x_2$—that is, if the transition state is product-like—then the force will have little effect on the reverse rate constant. If the displacement of the activated state is not intermediate, it is even possible that a load could actually increase the forward rate constant (if $x_a < x_1$), though in this case the backward rate would be increased even more.

Example 5.2 Thermal ratchet models for motor proteins

Consider a hypothetical motor protein with $\kappa = 4$ pN/nm and radius 3 nm (Example 2.4) and where the total free energy available from ATP hydrolysis at physiological ATP, ADP, and P_i concentrations is 25 *kT*. The drag coefficient, γ, is 60 pN·s·m^{-1} (see Table 2.2), and the relaxation time is ~15 ns. According to the Kramers theory (Equation 5.9), it would take about 10 s to pick up 20 *kT* of energy by a purely diffusive process. But for myosin, the complete ATP hydrolysis reaction only takes about 0.05 s (Chapter 14). Therefore, if the ATP hydrolysis reaction has an efficiency of 80% (20 *kT*/25 *kT*), such a diffusive step could not be on myosin's reaction pathway. This was the argument used by Eisenberg and Hill (1978) to rule out a Kramers-like mechanism postulated by A. F. Huxley (1957). However, a more reasonable efficiency for myosin is 50% (Chapter 16), corresponding to an energy of 12.5 *kT*. To pick up this amount of energy would take only 7 ms, less than the time that myosin spends detached from the actin filament. Thus the kinetics of myosin is not inconsistent with a Kramers-like mechanism after all.

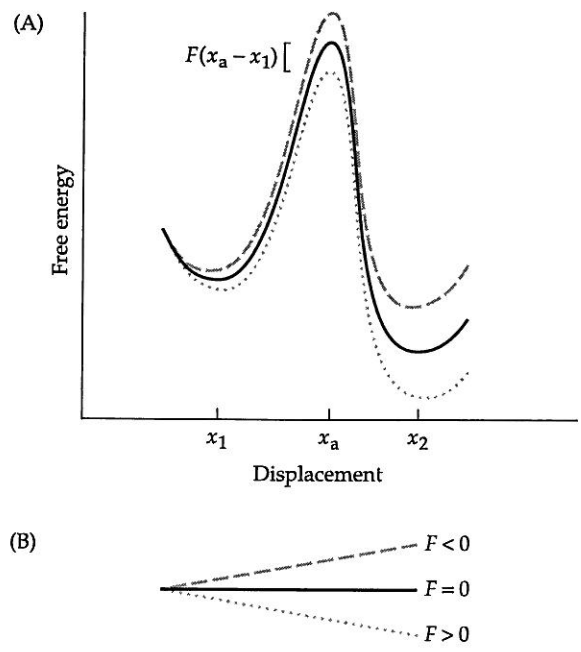
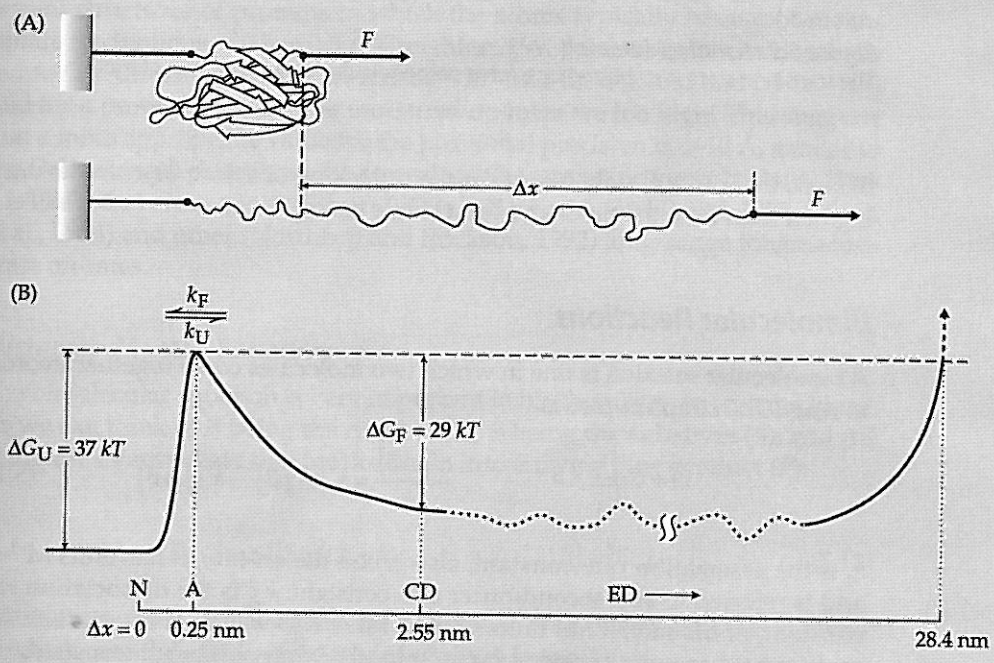


Figure 5.8 Force tilts the energy profile
(A) The energy profile in the presence of negative (dashed) and positive (dotted) external forces. The external force is equal to the slope of the tilt shown in (B). A negative external force is a load that slows down the reaction. A positive external force is a push that accelerates the reaction.

The activated-state concept makes several predictions for how forces affect the rates of chemical reactions. It is a challenge for experimentalists to test these predictions. For example, the rate constant for the opening of the transduction channels in hair cells is strongly force dependent, but that for closing is not (Corey and Hudspeth, 1983). This suggests that the transition state is more like the open state: The gate has to move almost all the way towards its open position before the conductance becomes significant (Howard et al., 1988). The rate constant for the opening of voltage-dependent ion channels is also more voltage dependent than the closing (Hille, 1992). Another example of force-dependent rate constants is the detachment of myosin from actin. Negative force (load) slows down the detachment rate (Finer et al., 1994), suggesting that the transition state is associated with the movement towards the post-powerstroke position. Another example is the unfolding and folding of titin (Erickson, 1994; Kellermayer et al., 1997; Rief et al., 1997; Tskhovrebova et al., 1997), illustrated in Example 5.3.

In general, the transition state could correspond to a distortion of the initial state in any of three directions, not just in the direction corresponding to the final state (Lecar and Morris, 1993). In this general case, the rate constant will be affected by any force that has a component in the direction of the transition state. This can be appreciated by making an analogy to a door handle or a latch, where the transition state (handle down) corresponds to movement in a direction perpendicular to the direction of opening of the door (Figure 5.9). An example of this occurs with motor proteins: Under certain circumstances, the speed of movement of kinesin along a microtubule can be accelerated by

Example 5.3 Unfolding titin using an atomic force microscope An atomic force microscope can be used to reversibly unfold immunoglobulin modules, ~100 amino acid domains found in a variety of proteins including the muscle protein titin. This is shown in Figure A, where the one end of the protein is attached to a solid support, and the other end is attached to the tip of an AFM and pulled. The unfolding rate depends only weakly on force (*e*-fold increase per 16 pN), indicating that the transition state for unfolding is of similar length to the folded state ($\Delta x = kT/F = 0.25$ nm). On the other hand, the folding is strongly dependent on force (*e*-fold slowing per 1.6 pN). These results support the energy profile shown in Figure B: A small strain of 0.25 nm, about 5% of the length of the folded protein (5.1 nm), is enough to completely destabilize the structure and lead to unfolding. But the folding of the protein requires the formation of a nearly fully folded transition state. The free energies in Figure B are calculated from the rate constants using Eyring rate theory (though this may not be valid, as argued above). The abbreviations in the figure are: N = native state, A = activated state, CD = compact disordered state, and ED = extended state (Carrion-Vazquez et al., 1999).



(After Carrion-Vazquez et al., 1999.)

forces perpendicular to the direction of motion (Gittes et al., 1996). Thus it is important to realize that force is a vector quantity and that force will couple to a reaction whenever it has a component that is in the direction of the reaction pathway. In the most general case, the reaction pathway and its force dependence could be very complicated. It could involve a highly convoluted sequence

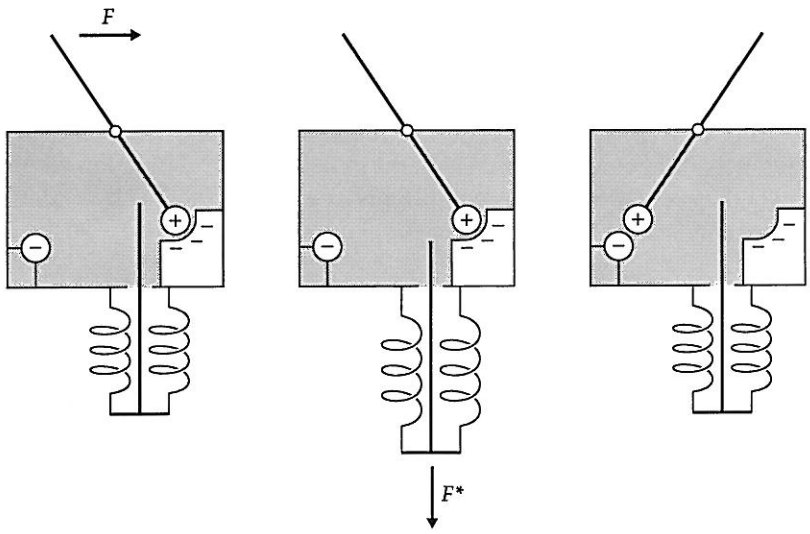


Figure 5.9 A molecular latch
The transition state is stabilized by a force perpendicular to the direction of motion.

of steps that more closely resembles the unlocking of a door or even the untying of a knot than the passage over a single energy barrier.

Bimolecular Reactions

A bimolecular reaction is one in which two molecules come together in order to react. The simplest case is

$$A + B \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} AB \qquad \frac{d[AB]}{dt} = k_1[A][B] - k_{-1}[AB] \tag{5.11}$$

k_1 is the **association rate constant**, also called the **on-rate**; it has units $M^{-1}{\cdot}s^{-1}$ and is referred to as a second-order rate constant. k_{-1} is the **dissociation rate constant**, or **off-rate**; it has units s^{-1}. The ratio, $K_d = k_{-1}/k_1$, is the **dissociation constant**; it has units M and is the reciprocal of the equilibrium constant.

Association Rates

Diffusion sets an upper limit on the association rate constant. By solving the diffusion equation in three dimensions, it can be shown that the rate constant for **diffusion-limited** collisions between spheres of equal diameter is ~8×10^9 $M^{-1}{\cdot}s^{-1}$ (Appendix 5.3). If one of the spheres is larger than the other, the rate constant will be even larger. If one of the spheres, the target, is thought of as a fixed point, then the collision rate constant is ~2×10^9 $M^{-1}{\cdot}s^{-1}$.

Only the very fastest atomic and diatomic reactions have on-rates that approach these diffusion-limited collision rate constants. Typical ligand–protein on-rates are in the range of 10^6 to 10^8 $M^{-1}{\cdot}s^{-1}$ (e.g., pyruvate, H_2O_2, CO_2, ACh, GTP, tRNA; Fersht, 1985; ATP, Chapter 14). The fastest protein–protein on-rates are in the range 10^6 to 10^7 $M^{-1}{\cdot}s^{-1}$: Examples include the polymerization of actin and tubulin (Chapter 10) and the binding of motors to their filaments (Chapter 14).

It is not surprising that the measured on-rates for protein reactions are less than the diffusion-limited rate constants that describe the collision of spheres. The reason is that the binding of ligands to proteins and proteins to proteins is **stereospecific**, meaning that it depends on the precise position and orientation of the reacting species. These steric considerations lead to a reduction in the diffusion-limited collision rate. The reduction is by a factor of $\sim(s/2R)^4$ (Doi, 1975; Bell, 1978; Berg and von Hippel, 1985; Appendix 5.3), where s is the positional precision and $2R$ is the diameter of the binding species (~1 nm for a ligand, ~6 nm for a protein). If the positional precision is 1Å (as judged from the crystal structures of proteins in which the atoms typically have root-mean-square fluctuations of 0.3 to 0.5 Å; Creighton, 1993), these steric considerations reduce the diffusion-limited on-rate by $\sim 10^4$-fold for a ligand and up to $\sim 10^7$-fold for a protein. But now the measured on-rates are too high! This suggests that a more appropriate value for the positional precision is 5–10 Å, similar to the Debye length describing the screening of electrostatic forces by ions. Thus it is likely that electrostatic (Schurr, 1970a,b; Berg and von Hippel, 1985; Gilson et al., 1994) and other (Northrup and Erickson, 1992) long-range forces accelerate on-rates.

Michaelis–Menten Equation

The bimolecular equation is very important in biochemistry. In enzyme kinetics we can think of A being the enzyme (E), B being the **substrate** (S), and AB being the intermediate that breaks down into enzyme plus **product** (P):

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P \qquad [E] + [ES] = [E_t] \tag{5.12}$$

Often, the substrate, also called the **reagent**, is well in excess of the enzyme, in which case the steady-state rate of product formation is

$$\text{Rate} = \frac{d[P]/dt}{[E_t]} = k_{cat}\frac{S}{K_M + S} \qquad k_{cat} = k_2 \qquad K_M = \frac{k_{-1} + k_2}{k_1} \tag{5.13}$$

(Appendix 5.4). This is the **Michaelis–Menten equation**, and K_M is the Michaelis–Menten constant. k_{cat} is the maximum rate per enzyme molecule and K_M is the concentration of substrate for which the rate is half maximal. If ES is in equilibrium with E and S ($k_{-1} >> k_2$), then the reaction is said to follow a Michaelis–Menten kinetic mechanism. In this case, the Michaelis–Menten con-

stant is equal to the dissociation constant $K_d = k_{-1}/k_1$, and the half-maximal rate occurs when the enzyme is 50% occupied by substrate. If $k_{-1} << k_2$, the reaction is said to follow a Briggs–Haldane mechanism. In this case, the K_M is the substrate concentration at which the enzyme spends half its time waiting for the substrate to bind.

Protein Complexes

Another important application of the bimolecular equation (Equation 5.12) is the case where the complex AB is an active species that catalyzes another reaction

$$A + B \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} AB \downarrow^{C}_{D} r \tag{5.14}$$

For example, AB might be a motor (A) walking down a filament (B) catalyzing the hydrolysis of ATP (C) to products (D) with some rate constant r. Or AB might be an active signaling complex that in turn activates another molecule or catalyzes the formation of a messenger molecule. Or it might be an active transcription factor. The difference between this scheme and the Michaelis–Menten scheme is that the complex can catalyze multiple additional reactions that do not necessarily lead to the dissociation of the complex AB. The solution is given in the Appendix.

Cyclic Reactions and Free Energy Transduction

If we permit the reverse reaction to occur, then the Michaelis–Menten scheme becomes a cyclic reaction

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P \tag{5.15}$$

A general cyclic reaction (an ***n*-cycle**) is

$$E_1 \underset{k_{-1}}{\overset{k_1^*}{\rightleftharpoons}} E_2 \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \ldots E_n \underset{k_{-n}^*}{\overset{k_n}{\rightleftharpoons}} E_1 \tag{5.16}$$

where we have absorbed the concentration of $[S]$ into the rate constant so k_1^* is now a first-order rate constant. At steady state, the average rate of flow of substrate through the reaction, the **flux**, is given by

$$\text{Flux} = \frac{k_i[E_i] - k_{-i}[E_{i+1}]}{[E_t]} \tag{5.17}$$

Note that the flux is the same at each step; otherwise, there would be build-up of one of the species, contradicting the steady-state assumption. If we define

the transition $E_n \rightarrow E_1$ as the completion of a cycle, then the flux is equal to the net number of complete cycles per second.

If the reaction is at equilibrium, then the average flux equals zero (though there will always be fluctuations in the flux just as there are thermal fluctuations in the position of a particle). This is a consequence of the **Principle of Detailed Balancing**: If a system in which several chemical reactions take place is at equilibrium, then each of the individual reactions is separately at equilibrium. The principle follows from Boltzmann's law because, at equilibrium, the ratio of the concentrations of the consecutive states, $[E_{i+1}]/[E_i]$, depends only on the relative energies of the two states and not on the absolute reaction rates. Therefore, the ratio remains unchanged if we "freeze out" all the other transitions except those between the two states in question. In this case the steady-state condition implies that $k_i[E_i] = k_{-i}[E_{i+1}]$, and so the flux is zero. This corollary of Boltzmann's law is also called the **Principle of Microscopic Reversibility** because at equilibrium a reaction is equally likely to be going in the forward or reverse direction. Zero flux at equilibrium means that there can be no perpetual motion machines. Conversely, if the average flux is zero, then each step is at equilibrium (because it satisfies Boltzmann's law, Equation 5.7) and thus the entire system is in equilibrium.

Another consequence of equilibrium is that the ratio of the products of the forward and reverse rate constants is unity

$$1 \equiv \frac{[E_1]}{[E_2]}\frac{[E_2]}{[E_3]}\cdots\frac{[E_n]}{[E_1]} = \frac{k_1[S]}{k_{-1}}\frac{k_2}{k_{-2}}\cdots\frac{k_n}{k_{-n}[P]} = \frac{k_1[S]k_2k_3\ldots k_n}{k_{-1}k_{-2}k_{-3}\ldots k_{-n}[P]}$$

Conversely, if this ratio is unity, then the reaction is in equilibrium (this follows by writing $k_i/k_{-i} = [E_{i+1}]/[E_i]$ + flux·($[E_t]/[E_i]$)). Thus there are several necessary and sufficient conditions for a cyclic reaction to be in equilibrium.

The steady-state flux through a cycle can be solved in terms of the rate constants using analytical methods (Hill, 1989) or matrix-inversion numerical methods (Press, 1997). If there are many steps, then the general solution is a complicated function of the rate constants. However, if each step is irreversible, then there is a particularly simple formula for the steady-state flux: The average duration of one cycle (1/flux) is the sum of the average durations of each of the steps: $(\text{rate})^{-1} = (k_1[S])^{-1} + (k_2)^{-1} + \cdots + (k_n)^{-1}$ or

$$\text{Flux} = k_{\text{cat}}\frac{[S]}{K_M + [S]} \qquad \frac{1}{k_{\text{cat}}} = \frac{1}{k_2} + \cdots + \frac{1}{k_n} \qquad K_M = \frac{k_{\text{cat}}}{k_1}$$

A cyclic reaction that has a nonzero flux is an example of a process that is at steady state but not at equilibrium. A specific example is a 2-cycle with $k_1 = 2 \times 10^6\ M^{-1}\cdot s^{-1}$, $k_{-1} = k_2 = 1\ s^{-1}$, $k_{-2} = 1 \times 10^6\ M^{-1}\cdot s^{-1}$, $[S] = [P] = 1\ \mu M$. The flux is equal to $0.2\ s^{-1}$. Evidently, the steady state is maintained by the replenishment

of substrate molecules, which provides an energy source to maintain the system away from equilibrium.

The energy to drive a cyclic chemical reaction comes from the change in free energy associated with converting one molecule of S to a molecule of P

$$\Delta G^0_{S\to P} = \Delta G^0 + kT\ln\frac{[P]}{[S]} = kT\ln\frac{K}{K_{eq}} = kT\ln\frac{[P]}{[P_{eq}]}\frac{[S_{eq}]}{[S]} \tag{5.18}$$

where ΔG^0 is defined as the **standard free energy**, the difference in free energy when the substrate (or substrates) and product (or products) are both in their standard states (Appendix 5.5). Because the standard state has both the substrates and the products at 1 M concentration, another way to view the standard free energy is that it is the free energy associated with the reaction when all the reactants are at 1 M concentration. Note that the free energy of a reaction is not really dependent on the standard free energy, but rather, it depends on the extent to which the substrates and products are out of equilibrium. For example, the standard free energy for the hydrolysis of ATP is -54×10^{-21} J, but at typical cellular concentrations of ATP, ADP, and P_i (1 mM, 10 μM, and 1 mM, respectively) the free energy is even more negative, -101×10^{-21} J (Chapter 14). However, if the ATP concentration were 1 aM (10^{-18} M) and the ADP and P_i concentrations were each 100 mM, then the free energy would have a similar magnitude but would be of opposite sign ($\sim+100\times10^{-21}$ J). In this case we could view ADP, rather than ATP, as the energy source.

If a reaction is coupled to movement, such as a motor moving along a filament, then the free energy of the reaction will be affected by an external force. If one cycle results in a net displacement through distance Δx, then

$$\Delta G_{S\to P} = \Delta G^0 - kT\ln\frac{[S]}{[P]} - F\cdot\Delta x \tag{5.19}$$

Because the substrate and product molecules are free in solution and so are not affected by a force, ΔG^0 is independent of force. The force at which the free energy change is zero is the **equilibrium force**, $F_{eq} = (\Delta G^0 - kT\ln[S]/[P])/\Delta x$. When $F = F_{eq}$, the free energy change is zero and the reaction is at equilibrium. The work done by the reaction is $w = F\Delta x$, which has a maximum value at equilibrium equal to $F_{eq}\Delta x = \Delta G^0 - kT\ln[S]/[P]$. At equilibrium the flux is zero and so the velocity of movement, v = flux·Δx, is also zero. The equilibrium force is also the **reversal force**. It is the force about which the velocity changes sign; when $F < F_{eq}$, the velocity is positive, whereas for $F > F_{eq}$, the velocity is negative.

For small changes in force about the equilibrium force, $|(F - F_{eq})\Delta x| << kT$, the velocity will depend linearly on $(F - F_{eq})$. But for larger forces, the more common case, the effect of force on the velocity is more complicated. To determine how the velocity depends on force, the force dependencies of all the rate constants need to be specified, and the steady-state solution for the flux solved

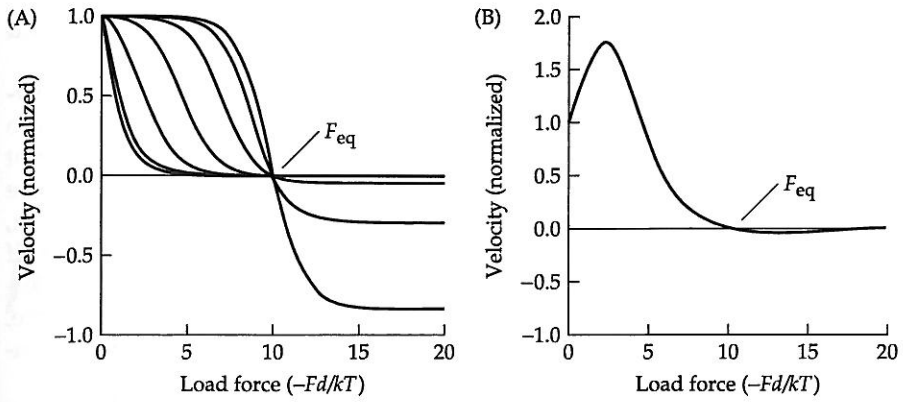


Figure 5.10 Force–velocity curves
(A) Families of velocity curves, $v(F)$, obtained by varying a single force-sensitive rate constant. (B) Nonmonotonic velocity curve obtained if the cycle has a small force-sensitive backward step followed by a large force-sensitive forward step.

in terms of these rate constants. Even for a 2-cycle with only one force-dependent rate constant, the velocity can depend on the force in a number of qualitatively different ways (Figure 5.10A). If there are two force-dependent steps, then the force–velocity curve can be even more interesting. Indeed, the speed need not even be monotonic: If there is a small backward step followed by a large forward step, then the speed can be nonmonotonic with a small load increasing the speed and a large load eventually decreasing it (Figure 5.10B).

Summary

Forces can influence the rates and equilibria of chemical reactions. If a protein has two different structural states, then at equilibrium the probability of finding it in one of these states is related to the difference in free energy according to Boltzmann's law. If work is done on the molecule as a result of the interconversion between the states, then the difference in free energy is altered and the equilibrium is shifted toward the state most stabilized by the force. The change in equilibrium could be due to a change in the forward rate constant, to a change in the backward rate constant, or both.

Different kinetic models make different predictions of how force affects rates of reactions. In Eyring-like models, highly localized conformational changes of the initial state occur prior to and drive slower global conformational changes into the final state. Because there is little distortion of the protein in the transition state, little work will be done on it by an external force, so the rate constant will not depend strongly on force. On the other hand, in Kramers-like models, global conformational changes of the initial state occur prior to more localized changes that lock the protein into the final state. In this case,

the transition state is highly distorted, and the rate constant will depend strongly on force.

At equilibrium the forward and reverse reaction rates are the same. However, if the interconversion between different states of a protein is coupled to a source of chemical energy (the breakdown of a chemical substrate that is not at equilibrium with its product), then it is possible that the interconversion rate in one direction is greater than that in the other. In this case there is a flux, which, if coupled to a displacement, will lead to movement of the enzyme. If the chemical free energy associated with the conversion of a substrate molecule to product is greater than the mechanical work done by an external force, then the flux will continue even against the mechanical load. In this way, chemical reactions can generate force.

Problems

5.1 Suppose that one structural state, the T state ("tense"), is 1% denser than the other state, the R state ("relaxed"). How could the different densities be measured? If the molecular mass is 100 kDa, what is the volume difference? At what pressure would you expect the equilibrium between the states to be affected?

5.2 Suppose that one could pull directly on the gate of an ion channel, and that the gate swings through 2 nm as it goes from the closed to the open position. If, in the absence of force, the channel spends half its time open and half its time closed, how much force is needed to increase the open probability to 0.9?

5.3 Suppose that a protein has a stiffness of 2 pN/nm in state 1 and a stiffness of 1 pN/nm in state 2, but that the two states have the same resting length (the length in the absence of a force). If there is initially a very low probability of being in state 2, how much force is needed to increase the open probability *e*-fold?

5.4* Suppose that the ratio of substrate to product in a mixture is ten times greater than the ratio at equilibrium. How much mechanical work could be obtained by converting one molecule of substrate to one molecule of product? Suppose that you have a total of N substrate plus product molecules and that the equilibrium ratio is 1. What is the total amount of mechanical work that could be done with the mixture before it becomes completely spent?

*More difficult

CHAPTER

6

Polymer Mechanics

This chapter is about the mechanical properties of slender rods—that is, rods whose lengths are much greater than their diameters. Slender rods are good models for biological polymers such as DNA and the protein filaments that make up the cytoskeleton. I ask: How do slender rods bend in response to mechanical and hydrodynamic forces? How much force is needed to buckle a slender rod? How quickly will it bend or buckle? And how much does the shape of a slender rod fluctuate in response to thermal forces?

Because the cytoskeletal filaments are the most important structural elements within cells, knowing the forces required to bend and buckle slender rods should allow one to model the mechanical properties of cells. Such cellular models are the starting point for modeling the mechanical properties of tissues and organs. In this way, the theory of slender rods developed here allows us to relate the mechanical properties of individual molecules to the mechanical properties of cells and tissues.

After discussing the statics and dynamics of more rigid polymers such as actin filaments and microtubules, I turn to the mechanics of flexible polymers such as DNA and unfolded protein chains. We arrive at the interesting notion of an entropic spring: Even a completely unstructured polymer chain resists being straightened because of its tendency to return to its more disordered configurations. Thus a very flexible polymer behaves like a spring and we say it has entropic, or rubber-like, stiffness. The entropic stiffness of segmented proteins or even random polypeptides is remarkably large. We arrive at the counterintuitive result that the entropic stiffness due to stretching a disordered domain may be as high as the enthalpic stiffness due to bending a well-ordered domain; This finding must be incorporated into our thinking about the molecular mechanics of motors and other proteins.