CHAPTER

4

Chemical Equilibrium

Now we arrive at the point where real chemistry begins. Chemical thermodynamics is used to predict whether a mixture of reactants has a spontaneous tendency to change into products, to predict the composition of the reaction mixture at equilibrium, and to predict how that composition will be modified by changing the conditions. In biology, life is the avoidance of equilibrium, and the attainment of equilibrium is death, but knowing whether equilibrium lies in favor of reactants or products under certain conditions is a good indication of the feasibility of a biochemical reaction. Indeed, the material we cover in this chapter is of crucial importance for understanding the mechanisms of oxygen transport in blood, metabolism, and all the processes going on inside organisms.

There is one word of warning that is essential to remember: *thermodynamics is silent about the rates of reaction*. All it can do is to identify whether a particular reaction mixture has a tendency to form products; it cannot say whether that tendency will ever be realized. We explore what determines the rates of chemical reactions in Chapters 6 through 8.

Thermodynamic background

The thermodynamic criterion for spontaneous change at constant temperature and pressure is $\Delta G < 0$. The principal idea behind this chapter, therefore, is that, *at constant temperature and pressure, a reaction mixture tends to adjust its composition until its Gibbs energy is a minimum*. If the Gibbs energy of a mixture varies as shown in Fig. 4.1a, very little of the reactants convert into products before G has reached its minimum value, and the reaction "does not go." If G varies as shown in Fig. 4.1c, then a high proportion of products must form before G reaches its minimum and the reaction "goes." In many cases, the equilibrium mixture contains almost no reactants or almost no products. Many reactions have a Gibbs energy that varies as shown in Fig. 4.1b, and at equilibrium the reaction mixture contains substantial amounts of both reactants and products.

4.1 The reaction Gibbs energy

To explore metabolic processes, we need a measure of the driving power of a chemical reaction, and to understand the chemical composition of cells, we need to know what those compositions would be if the reactions taking place in them had reached equilibrium.

To keep our ideas in focus, we consider two important processes. One is the isomerism of glucose-6-phosphate (**1**, G6P) to fructose-6-phosphate (**2**, F6P), which is an early step in the anaerobic breakdown of glucose (Section 4.8):

$$\mathrm{G6P(aq)} \rightleftharpoons \mathrm{F6P(aq)} \qquad \textbf{(A)}$$

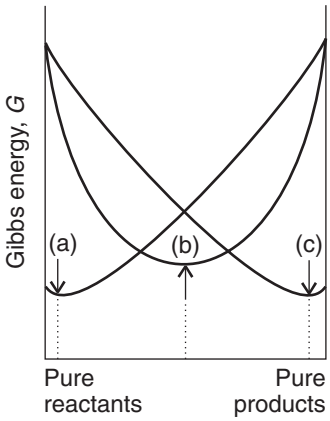
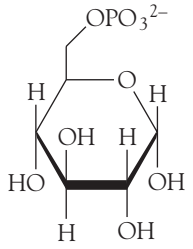
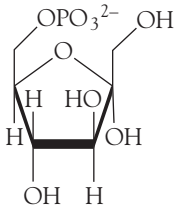


Fig. 4.1 The variation of Gibbs energy of a reaction mixture with progress of the reaction, pure reactants on the left and pure products on the right. (a) This reaction "does not go": the minimum in the Gibbs energy occurs very close to the reactants. (b) This reaction reaches equilibrium with approximately equal amounts of reactants and products present in the mixture. (c) This reaction goes almost to completion, as the minimum in Gibbs energy lies very close to pure products.



1 Glucose-6-phosphate



2 Fructose-6-phosphate

The second is the binding of $O_2(g)$ to the protein hemoglobin, Hb, in blood (*Case study* 4.1):

$$\mathrm{Hb(aq) + 4\,O_2(g) \rightleftharpoons Hb(O_2)_4(aq)} \qquad \textbf{(B)}$$

These two reactions are specific examples of a general reaction of the form

$$a\,\mathrm{A} + b\,\mathrm{B} \rightleftharpoons c\,\mathrm{C} + d\,\mathrm{D} \qquad \textbf{(C)}$$

with arbitrary physical states.

First, consider reaction A. Suppose that in a short interval while the reaction is in progress, the amount of G6P changes infinitesimally by $-\mathrm{d}n$. As a result of this change in amount, the contribution of G6P to the total Gibbs energy of the system changes by $-\mu_{\mathrm{G6P}}\mathrm{d}n$, where μ_{G6P} is the chemical potential (the partial molar Gibbs energy) of G6P in the reaction mixture. In the same interval, the amount of F6P changes by $+\mathrm{d}n$, so its contribution to the total Gibbs energy changes by $+\mu_{\mathrm{F6P}}\mathrm{d}n$, where μ_{F6P} is the chemical potential of F6P. The change in Gibbs energy of the system is

$$\mathrm{d}G = \mu_{\mathrm{F6P}}\mathrm{d}n - \mu_{\mathrm{G6P}}\mathrm{d}n$$

On dividing through by $\mathrm{d}n$, we obtain the **reaction Gibbs energy**, $\Delta_{\mathrm{r}}G$:

$$\frac{\mathrm{d}G}{\mathrm{d}n} = \mu_{\mathrm{F6P}} - \mu_{\mathrm{G6P}} = \Delta_{\mathrm{r}}G \qquad \textbf{(4.1a)}$$

There are two ways to interpret $\Delta_{\mathrm{r}}G$. First, it is the difference of the chemical potentials of the products and reactants *at the composition of the reaction mixture*. Second, we can think of $\Delta_{\mathrm{r}}G$ as the derivative of G with respect to n, or the slope of the graph of G plotted against the changing composition of the system (Fig. 4.2).

The binding of oxygen to hemoglobin provides a slightly more complicated example. If the amount of Hb changes by $-\mathrm{d}n$, then from the reaction stoichiometry we know that the change in the amount of O_2 will be $-4\mathrm{d}n$ and the change in the amount of $Hb(O_2)_4$ will be $+\mathrm{d}n$. Each change contributes to the change in the total Gibbs energy of the mixture, and the overall change is

$$\begin{aligned}\Delta G &= \mu_{\mathrm{Hb(O_2)_4}} \times \mathrm{d}n - \mu_{\mathrm{Hb}} \times \mathrm{d}n - \mu_{\mathrm{O_2}} \times 4\mathrm{d}n \\ &= (\mu_{\mathrm{Hb(O_2)_4}} - \mu_{\mathrm{Hb}} - 4\mu_{\mathrm{O_2}})\mathrm{d}n\end{aligned}$$

where the μ_J are the chemical potentials of the species in the reaction mixture. In this case, therefore, the reaction Gibbs energy is

$$\Delta_r G = \frac{dG}{dn} = \mu_{Hb(O_2)_4} - (\mu_{Hb} + 4\mu_{O_2}) \quad \textbf{(4.1b)}$$

Note that each chemical potential is multiplied by the corresponding stoichiometric coefficient and that reactants are subtracted from products. For the general reaction C,

$$\Delta_r G = (c\mu_C + d\mu_D) - (a\mu_A + b\mu_B) \quad \textbf{(4.1c)}$$

The chemical potential of a substance depends on the composition of the mixture in which it is present and is high when its concentration or partial pressure is high. Therefore, $\Delta_r G$ changes as the composition changes (Fig. 4.3). Remember that $\Delta_r G$ is the *slope* of G plotted against composition. We see that $\Delta_r G < 0$ and the slope of G is negative (down from left to right) when the mixture is rich in the reactants A and B because μ_A and μ_B are then high. Conversely, $\Delta_r G > 0$ and the slope of G is positive (up from left to right) when the mixture is rich in the products C and D because μ_C and μ_D are then high. At compositions corresponding to $\Delta_r G < 0$ the reaction tends to form more products; where $\Delta_r G > 0$, the *reverse* reaction is spontaneous, and the products tend to decompose into reactants. Where $\Delta_r G = 0$ (at the minimum of the graph where the derivative is zero), the reaction has no tendency to form either products or reactants. In other words, the reaction is at equilibrium. That is, *the criterion for chemical equilibrium at constant temperature and pressure* is

$$\Delta_r G = 0 \quad \textbf{(4.2)}$$

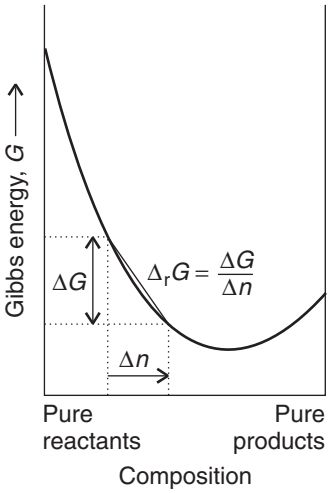


Fig. 4.2 The variation of Gibbs energy with progress of reaction showing how the reaction Gibbs energy, $\Delta_r G$, is related to the slope of the curve at a given composition. When ΔG and Δn are both infinitesimal, the slope is written dG/dn.

4.2 The variation of $\Delta_r G$ with composition

The reactants and products in a biological cell are rarely at equilibrium, so we need to know how the reaction Gibbs energy depends on their concentrations.

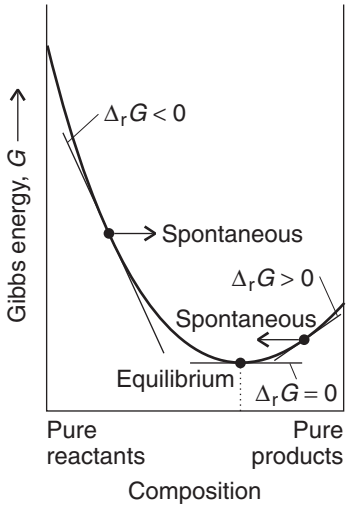


Fig. 4.3 At the minimum of the curve, corresponding to equilibrium, $\Delta_r G = 0$. To the left of the minimum, $\Delta_r G < 0$, and the forward reaction is spontaneous. To the right of the minimum, $\Delta_r G > 0$, and the reverse reaction is spontaneous.

Our starting point is the general expression for the composition dependence of the chemical potential derived in Section 3.11:

$$\mu_J = \mu_J^\ominus + RT \ln a_J \qquad \textbf{(4.3)}$$

where a_J is the activity of the species J. When we are dealing with systems that may be treated as ideal, which will be the case in this chapter, we use the identifications given in Table 3.3:

For solutes in an ideal solution, $a_J = [J]/c^\ominus$, the molar concentration of J relative to the standard value $c^\ominus = 1$ mol L^{-1}.

For perfect gases, $a_J = p_J/p^\ominus$, the partial pressure of J relative to the standard pressure $p^\ominus = 1$ bar.

For pure solids and liquids, $a_J = 1$.

As in Chapter 3, to simplify the appearance of expressions in what follows, we shall not write $c^\ominus$ and $p^\ominus$ explicitly.

Substitution of eqn 4.3 into eqn 4.1c gives

$$\begin{aligned}\Delta_r G &= \{c(\mu_C^\ominus + RT \ln a_C) + d(\mu_D^\ominus + RT \ln a_D)\} \\ &\quad - \{a(\mu_A^\ominus + RT \ln a_A) + b(\mu_B^\ominus + RT \ln a_B)\} \\ &= \{(c\mu_C^\ominus + d\mu_D^\ominus) - (a\mu_A^\ominus + b\mu_B^\ominus)\} \\ &\quad + RT\{c \ln a_C + d \ln a_D - a \ln a_A - b \ln a_B\}\end{aligned}$$

The first term on the right in the second equality is the **standard reaction Gibbs energy**, $\Delta_r G^\ominus$:

$$\Delta_r G^\ominus = \{c\mu_C^\ominus + d\mu_D^\ominus\} - \{a\mu_A^\ominus + b\mu_B^\ominus\} \qquad \textbf{(4.4a)}$$

Because the standard states refer to the pure materials, the standard chemical potentials in this expression are the standard molar Gibbs energies of the (pure) species. Therefore, eqn 4.4a is the same as

$$\Delta_r G^\ominus = \{cG_m^\ominus(C) + dG_m^\ominus(D)\} - \{aG_m^\ominus(A) + bG_m^\ominus(B)\} \qquad \textbf{(4.4b)}$$

We consider this important quantity in more detail shortly. At this stage, therefore, we know that

$$\Delta_r G = \Delta_r G^\ominus + RT\{c \ln a_C + d \ln a_D - a \ln a_A - b \ln a_B\}$$

and the expression for $\Delta_r G$ is beginning to look much simpler.

To make further progress, we rearrange the remaining terms on the right as follows:

$$\begin{aligned}c \ln a_C + d \ln a_D - a \ln a_A - b \ln a_B &\overset{a \ln x = \ln x^a}{=} \ln a_C^c + \ln a_D^d - \ln a_A^a - \ln a_B^b \\ &\overset{\ln x + \ln y = \ln xy}{=} \ln a_C^c a_D^d - \ln a_A^a a_B^b \\ &\overset{\ln x - \ln y = \ln x/y}{=} \ln \frac{a_C^c a_D^d}{a_A^a a_B^b}\end{aligned}$$

At this point, we have deduced that

$$\Delta_r G = \Delta_r G^{\ominus} + RT \ln \frac{a_C^c a_D^d}{a_A^a a_B^b}$$

To simplify the appearance of this expression still further, we introduce the (dimensionless) **reaction quotient**, Q, for reaction C:

$$Q = \frac{a_C^c a_D^d}{a_A^a a_B^b} \qquad \textbf{(4.5)}$$

Note that Q has the form of products divided by reactants, with the activity of each species raised to a power equal to its stoichiometric coefficient in the reaction. We can now write the overall expression for the reaction Gibbs energy at any composition of the reaction mixture as

$$\Delta_r G = \Delta_r G^{\ominus} + RT \ln Q \qquad \textbf{(4.6)}$$

This simple but hugely important equation will occur several times in different disguises.

EXAMPLE 4.1 Formulating a reaction quotient

Formulate the reaction quotients for reactions A (the isomerism of glucose-6-phosphate) and B (the binding of oxygen to hemoglobin).

Strategy Use Table 3.3 to express activities in terms of molar concentrations or pressures. Then use eqn 4.5 to write an expression for the reaction quotient Q. In reactions involving gases and solutes, the expression for Q will contain pressures and molar concentrations.

Solution The reaction quotient for reaction A is

$$Q = \frac{a_{F6P}}{a_{G6P}} = \frac{[F6P]/c^{\ominus}}{[G6P]/c^{\ominus}}$$

However, we are not writing the standard concentration explicitly, so this expression simplifies to

$$Q = \frac{[F6P]}{[G6P]}$$

with [J] the numerical value of the molar concentration of J in moles per liter (so if [F6P] = 2.0 mmol L^{-1}, corresponding to 2.0×10^{-3} mol L^{-1}, we just write [F6P] = 2.0×10^{-3} when using this expression). For reaction B, the binding of oxygen to hemoglobin, the reaction quotient is

$$Q = \frac{[Hb(O_2)_4]/c^{\ominus}}{([Hb]/c^{\ominus})(p_{O_2}/p^{\ominus})^4}$$

Similarly, because we are not writing the standard concentration and pressure explicitly, this expression simplifies to

$$Q = \frac{[Hb(O_2)_4]}{[Hb]p_{O_2}^4}$$

with p_J the numerical value of the partial pressure of J in bar (so if $p_{O_2} = 2.0$ bar, we just write $p_{O_2} = 2.0$ when using this expression).

SELF-TEST 4.1 Write the reaction quotient for the esterification reaction $CH_3COOH + C_2H_5OH \rightleftharpoons CH_3COOC_2H_5 + H_2O$. (All four components are present in the reaction mixture as liquids: the mixture is not an aqueous solution.)

Answer: $Q \approx [CH_3COOC_2H_5][H_2O]/[CH_3COOH][C_2H_5OH]$ ■

4.3 Reactions at equilibrium

We need to be able to identify the equilibrium composition of a reaction so that we can discuss deviations from equilibrium systematically.

At equilibrium, the reaction quotient has a certain value called the **equilibrium constant**, K, of the reaction:

$$K = \left(\frac{a_C^c a_D^d}{a_A^a a_B^b} \right)_{\text{equilibrium}} \quad \textbf{(4.7)}$$

We shall not normally write *equilibrium*; the context will always make it clear that Q refers to an *arbitrary* stage of the reaction, whereas K, the value of Q at equilibrium, is calculated from the equilibrium composition. It now follows from eqn 4.6 that at equilibrium

$$0 = \Delta_r G^\ominus + RT \ln K$$

and therefore that

$$\Delta_r G^\ominus = -RT \ln K \quad \textbf{(4.8)}$$

This is one of the most important equations in the whole of chemical thermodynamics. Its principal use is to predict the value of the equilibrium constant of any reaction from tables of thermodynamic data, like those in the *Data section*. Alternatively, we can use it to determine $\Delta_r G^\ominus$ by measuring the equilibrium constant of a reaction.

ILLUSTRATION 4.1 Calculating the equilibrium constant of a biochemical reaction

The first step in the metabolic breakdown of glucose is its phosphorylation to G6P:

$$\text{glucose(aq)} + P_i\text{(aq)} \longrightarrow \text{G6P(aq)}$$

where P_i denotes an inorganic phosphate group, such as $H_2PO_4^-$. The standard reaction Gibbs energy for the reaction is +14.0 kJ mol^{-1} at 37°C, so it follows from eqn 4.8 that

$$\ln K = -\frac{\Delta_r G^\ominus}{RT} = -\frac{1.40 \times 10^4 \text{ J mol}^{-1}}{(8.314\,47 \text{ J K}^{-1} \text{ mol}^{-1}) \times (310 \text{ K})}$$
$$= -\frac{1.40 \times 10^4}{8.314\,47 \times 310}$$

To calculate the equilibrium constant of the reaction, which (like the reaction quotient) is a dimensionless number, we use the relation $e^{\ln x} = x$ with $x = K$:

$$K = e^{-\frac{1.40 \times 10^4}{8.314\,47 \times 310}} = 4.4 \times 10^{-3}$$

A note on good practice: The exponential function (e^x) is very sensitive to the value of x, so evaluate it only at the end of a numerical calculation. ■

SELF-TEST 4.2 Calculate the equilibrium constant of the reaction $N_2(g) + 3\,H_2(g) \rightleftharpoons 2\,NH_3(g)$ at 25°C, given that $\Delta_r G^\ominus = -32.90$ kJ mol^{-1}.

Answer: 5.8×10^5

An important feature of eqn 4.8 is that it tells us that $K > 1$ if $\Delta_r G^\ominus < 0$. Broadly speaking, $K > 1$ implies that products are dominant at equilibrium, so we can conclude that *a reaction is thermodynamically feasible if* $\Delta_r G^\ominus < 0$ (Fig. 4.4). Conversely, because eqn 4.8 tells us that $K < 1$ if $\Delta_r G^\ominus > 0$, then we know that the reactants will be dominant in a reaction mixture at equilibrium if $\Delta_r G^\ominus > 0$. In other words, *a reaction with* $\Delta_r G^\ominus > 0$ *is not thermodynamically feasible*. Some care must be exercised with these rules, however, because the products will be significantly more abundant than reactants only if $K >> 1$ (more than about 10^3), and even a reaction with $K < 1$ may have a reasonable abundance of products at equilibrium.

Table 4.1 summarizes the conditions under which $\Delta_r G^\ominus < 0$ and $K > 1$. Because $\Delta_r G^\ominus = \Delta_r H^\ominus - T\Delta_r S^\ominus$, the standard reaction Gibbs energy is certainly negative if both $\Delta_r H^\ominus < 0$ (an exothermic reaction) and $\Delta_r S^\ominus > 0$ (a reaction system that becomes more disorderly, such as by forming a gas). The standard reaction Gibbs energy is also negative if the reaction is endothermic ($\Delta_r H^\ominus > 0$) and $T\Delta_r S^\ominus$ is sufficiently large and positive. Note that for an endothermic reaction to have $\Delta_r G^\ominus < 0$, its standard reaction entropy *must* be positive. Moreover, the temperature must be high enough for $T\Delta_r S^\ominus$ to be greater than $\Delta_r H^\ominus$ (Fig. 4.5). The switch of $\Delta_r G^\ominus$ from positive to negative, corresponding to the switch from $K < 1$ (the reaction "does not go") to $K > 1$ (the reaction "goes"), occurs at a temperature given by equating $\Delta_r H^\ominus - T\Delta_r S^\ominus$ to 0, which gives

$$T = \frac{\Delta_r H^\ominus}{\Delta_r S^\ominus} \qquad (4.9)$$

Table 4.1 Thermodynamic criteria of spontaneity

1. If the reaction is exothermic ($\Delta_r H^\ominus < 0$) and $\Delta_r S^\ominus > 0$	$\Delta_r G^\ominus < 0$ and $K > 1$ at all temperatures
2. If the reaction is exothermic ($\Delta_r H^\ominus < 0$) and $\Delta_r S^\ominus < 0$	$\Delta_r G^\ominus < 0$ and $K > 1$ provided that $T < \Delta_r H^\ominus/\Delta_r S^\ominus$
3. If the reaction is endothermic ($\Delta_r H^\ominus > 0$) and $\Delta_r S^\ominus > 0$	$\Delta_r G^\ominus < 0$ and $K > 1$ provided that $T > \Delta_r H^\ominus/\Delta_r S^\ominus$
4. If the reaction is endothermic ($\Delta_r H^\ominus > 0$) and $\Delta_r S^\ominus < 0$	$\Delta_r G^\ominus < 0$ and $K > 1$ at no temperature

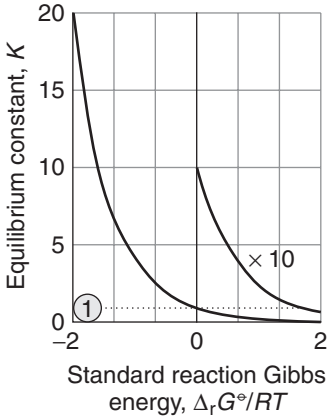


Fig. 4.4 The relation between standard reaction Gibbs energy and the equilibrium constant of the reaction. The curve labeled with "× 10" is magnified by a factor of 10.

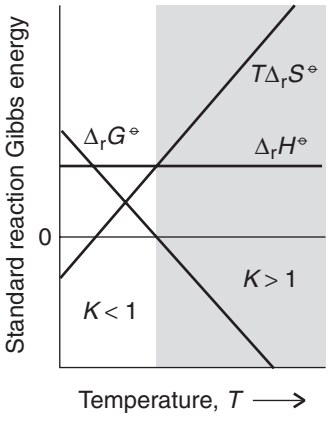


Fig. 4.5 An endothermic reaction may have $K > 1$ provided the temperature is high enough for $T\Delta_r S^\ominus$ to be large enough that, when subtracted from $\Delta_r H^\ominus$, the result is negative.

SELF-TEST 4.3 Calculate the decomposition temperature, the temperature at which the decomposition becomes spontaneous, of calcium carbonate given that $\Delta_r H^\ominus = +178$ kJ mol^{-1} and $\Delta_r S^\ominus = +161$ J K^{-1} mol^{-1} for the reaction $CaCO_3(s) \longrightarrow CaO(s) + CO_2(g)$.

Answer: 1.11×10^3 K

An equilibrium constant expresses the composition of an equilibrium mixture as a ratio of products of activities. Even if we confine our attention to ideal systems, it is still necessary to do some work to extract the actual equilibrium concentrations or partial pressures of the reactants and products given their initial values (see, for example, *Example* 4.5).

EXAMPLE 4.2 Calculating an equilibrium composition

Consider reaction A, for which $\Delta_r G^\ominus = +1.7$ kJ mol^{-1} at 25°C. Estimate the fraction f of F6P in equilibrium with G6P at 25°C, where f is defined as

$$f = \frac{[\text{F6P}]}{[\text{F6P}] + [\text{G6P}]}$$

Strategy Express f in terms of K. To do so, recognize that if the numerator and denominator in the expression for f are both divided by [G6P]; then the ratios [F6P]/[G6P] can be replaced by K. Calculate the value of K by using eqn 4.8.

Solution Division of the numerator and denominator by [G6P] gives

$$f = \frac{[\text{F6P}]/[\text{G6P}]}{([\text{F6P}]/[\text{G6P}]) + 1} = \frac{K}{K + 1}$$

We find the equilibrium constant by using $K = e^{\ln K}$ and rearranging eqn 4.8 into

$$K = e^{-\Delta_r G^\ominus/RT}$$

First, note that because $+1.7$ kJ mol^{-1} is the same as $+1.7 \times 10^3$ J mol^{-1},

$$\frac{\Delta_r G^\ominus}{RT} = \frac{1.7 \times 10^3 \text{ J mol}^{-1}}{(8.3145 \text{ J K}^{-1} \text{ mol}^{-1}) \times (298 \text{ K})} = \frac{1.7 \times 10^3}{8.3145 \times 298}$$

Therefore,

$$K = e^{-\frac{1.7 \times 10^3}{8.3145 \times 298}} = 0.50$$

and

$$f = \frac{0.50}{1 + 0.50} = 0.33$$

That is, at equilibrium, 33% of the solute is F6P and 67% is G6P.

SELF-TEST 4.4 Estimate the composition of a solution in which two isomers A and B are in equilibrium (A $\rightleftharpoons$ B) at 37°C and $\Delta_r G^\ominus = -2.2$ kJ mol^{-1}.

Answer: The fraction of B at equilibrium is $f_{eq} = 0.30$. ■

CASE STUDY 4.1 Binding of oxygen to myoglobin and hemoglobin

Biochemical equilibria can be far more complex than those we have considered so far, but exactly the same principles apply. An example of a complex process is the binding of O_2 by hemoglobin in blood, which is described only approximately by reaction B. The protein myoglobin (Mb) stores O_2 in muscle, and the protein hemoglobin (Hb) transports O_2 in blood. These two proteins are related, for hemoglobin can be regarded, as a first approximation, as a tetramer of myoglobin (Fig. 4.6). There are, in fact, slight differences in the peptide sequence of the myoglobin-like components of hemoglobin, but we can ignore them at this stage. In each protein, the O_2 molecule attaches to an iron ion in a heme group (**3**). For our purposes here, we are concerned with the different equilibrium characteristics for the uptake of O_2 by myoglobin and hemoglobin.

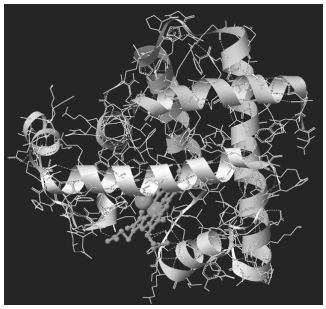


Fig. 4.6 One of the four polypeptide chains that make up the human hemoglobin molecule. The chains, which are similar to the oxygen storage protein myoglobin, consist of helical and sheet-like regions. The heme group is at the lower left.

First, consider the equilibrium between Mb and O_2:

$$\mathrm{Mb(aq) + O_2(g) \rightleftharpoons MbO_2(aq)} \qquad K = \frac{[\mathrm{MbO_2}]}{p[\mathrm{Mb}]}$$

where p is the numerical value of the partial pressure of O_2 gas in bar. It follows that the *fractional saturation*, s, the fraction of Mb molecules that are oxygenated, is

$$s = \frac{[\mathrm{MbO_2}]}{[\mathrm{Mb}]_{\mathrm{total}}} = \frac{[\mathrm{MbO_2}]}{[\mathrm{Mb}] + [\mathrm{MbO_2}]} = \frac{Kp}{1 + Kp}$$

The dependence of s on p is shown in Fig. 4.7.

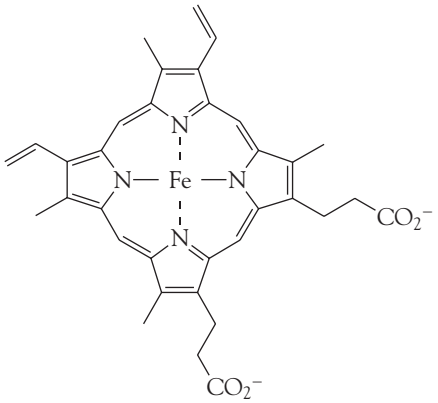
Now consider the equilibrium between Hb and O_2:

$$\mathrm{Hb(aq) + O_2(g) \rightleftharpoons HbO_2(aq)} \qquad K_1 = \frac{[\mathrm{HbO_2}]}{p[\mathrm{Hb}]}$$

$$\mathrm{HbO_2(aq) + O_2(g) \rightleftharpoons Hb(O_2)_2(aq)} \qquad K_2 = \frac{[\mathrm{Hb(O_2)_2}]}{p[\mathrm{HbO_2}]}$$

$$\mathrm{Hb(O_2)_2(aq) + O_2(g) \rightleftharpoons Hb(O_2)_3(aq)} \qquad K_3 = \frac{[\mathrm{Hb(O_2)_3}]}{p[\mathrm{Hb(O_2)_2}]}$$

$$\mathrm{Hb(O_2)_3(aq) + O_2(g) \rightleftharpoons Hb(O_2)_4(aq)} \qquad K_4 = \frac{[\mathrm{Hb(O_2)_4}]}{p[\mathrm{Hb(O_2)_3}]}$$



3 The heme group

To develop an expression for s, we express $[Hb(O_2)_2]$ in terms of $[HbO_2]$ by using K_2, then express $[HbO_2]$ in terms of [Hb] by using K_1, and likewise for all the other concentrations of $Hb(O_2)_3$ and $Hb(O_2)_4$. It follows that

$$[HbO_2] = K_1p[Hb] \qquad [Hb(O_2)_2] = K_1K_2p^2[Hb]$$
$$[Hb(O_2)_3] = K_1K_2K_3p^3[Hb] \qquad [Hb(O_2)_4] = K_1K_2K_3\,K_4p^4[Hb]$$

The total concentration of bound O_2 is

$$\begin{aligned}[O_2]_{bound} &= [HbO_2] + 2[Hb(O_2)_2] + 3[Hb(O_2)_3] + 4[Hb(O_2)_4] \\ &= (1 + 2K_2p + 3K_2K_3p^2 + 4K_2K_3K_4p^3)K_1p[Hb]\end{aligned}$$

where we have used the fact that n O_2 molecules are bound in $Hb(O_2)_n$, so the concentration of bound O_2 in $Hb(O_2)_2$ is $2[Hb(O_2)_2]$, and so on. The total concentration of hemoglobin is

$$[Hb]_{total} = (1 + K_1p + K_1K_2p^2 + K_1K_2K_3p^3 + K_1K_2K_3K_4p^4)[Hb]$$

Because each Hb molecule has four sites at which O_2 can attach, the fractional saturation is

$$s = \frac{[O_2]_{bound}}{4[Hb]_{total}} = \frac{(1 + 2K_2p + 3K_2K_3p^2 + 4K_2K_3K_4p^3)K_1p}{4(1 + K_1p + K_1K_2p^2 + K_1K_2K_3p^3 + K_1K_2K_3K_4p^4)}$$

A reasonable fit of the experimental data can be obtained with $K_1 = 0.01$, $K_2 = 0.02$, $K_3 = 0.04$, and $K_4 = 0.08$ when p is expressed in torr.

The binding of O_2 to hemoglobin is an example of **cooperative binding**, in which the binding of a ligand (in this case O_2) to a biopolymer (in this case Hb) becomes more favorable thermodynamically (that is, the equilibrium constant increases) as the number of bound ligands increases up to the maximum number of binding sites. We see the effect of cooperativity in Fig. 4.7. Unlike the myoglobin saturation curve, the hemoglobin saturation curve is *sigmoidal* (S shaped): the fractional saturation is small at low ligand concentrations, increases sharply at intermediate ligand concentrations, and then levels off at high ligand concentrations. Cooperative binding of O_2 by hemoglobin is explained by an **allosteric effect**, in

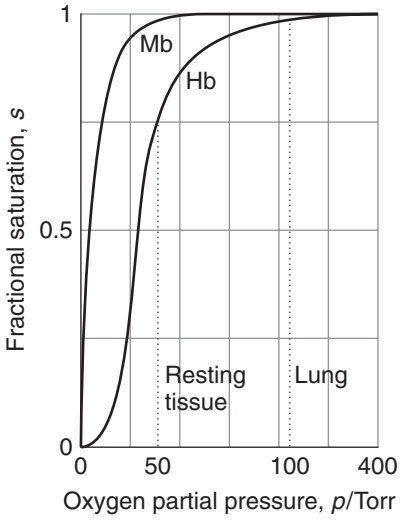


Fig. 4.7 The variation of the fractional saturation of myoglobin and hemoglobin molecules with the partial pressure of oxygen. The different shapes of the curves account for the different biological functions of the two proteins.

which an adjustment of the conformation of a molecule when one substrate binds affects the ease with which a subsequent substrate molecule binds. The details of the allosteric effect in hemoglobin will be explored in *Case study* 10.4.

The differing shapes of the saturation curves for myoglobin and hemoglobin have important consequences for the way O_2 is made available in the body: in particular, the greater sharpness of the Hb saturation curve means that Hb can load O_2 more fully in the lungs and unload it more fully in different regions of the organism. In the lungs, where $p \approx 105$ Torr (14 kPa), $s \approx 0.98$, representing almost complete saturation. In resting muscular tissue, p is equivalent to about 38 Torr (5 kPa), corresponding to $s \approx 0.75$, implying that sufficient O_2 is still available should a sudden surge of activity take place. If the local partial pressure falls to 22 Torr (3 kPa), s falls to about 0.1. Note that the steepest part of the curve falls in the range of typical tissue oxygen partial pressure. Myoglobin, on the other hand, begins to release O_2 only when p has fallen below about 22 Torr, so it acts as a reserve to be drawn on only when the Hb oxygen has been used up. ■

4.4 The standard reaction Gibbs energy

The standard reaction Gibbs energy is central to the discussion of chemical equilibria and the calculation of equilibrium constants. It is also a useful indicator of the energy available from catabolism to drive anabolic processes, such as the synthesis of proteins.

We have seen that standard reaction Gibbs energy, $\Delta_r G^\ominus$, is defined as the difference in standard molar Gibbs energies of the products and the reactants weighted by the stoichiometric coefficients, ν, in the chemical equation

$$\Delta_r G^\ominus = \sum \nu G_m^\ominus(\text{products}) - \sum \nu G_m^\ominus(\text{reactants}) \qquad \textbf{(4.10)}$$

For example, the standard reaction Gibbs energy for reaction A is the difference between the molar Gibbs energies of fructose-6-phosphate and glucose-6-phosphate in solution at 1 mol L^{-1} and 1 bar.

We cannot calculate $\Delta_r G^\ominus$ from the standard molar Gibbs energies themselves, because these quantities are not known. One practical approach is to calculate the standard reaction enthalpy from standard enthalpies of formation (Section 1.14), the standard reaction entropy from Third-Law entropies (Section 2.8), and then to combine the two quantities by using

$$\Delta_r G^\ominus = \Delta_r H^\ominus - T\Delta_r S^\ominus \qquad \textbf{(4.11)}$$

EXAMPLE 4.3 Calculating the standard reaction Gibbs energy of an enzyme-catalyzed reaction

Evaluate the standard reaction Gibbs energy at 25°C for the reaction $CO_2(g) + H_2O(l) \longrightarrow H_2CO_3(aq)$ catalyzed by the enzyme carbonic anhydrase in red blood cells.

Strategy Obtain the relevant standard enthalpies and entropies of formation from the *Data section*. Then calculate the standard reaction enthalpy and the standard reaction entropy from

$$\Delta_r H^\ominus = \sum \nu \Delta_f H^\ominus(\text{products}) - \sum \nu \Delta_f H^\ominus(\text{reactants})$$
$$\Delta_r S^\ominus = \sum \nu S_m^\ominus(\text{products}) - \sum \nu S_m^\ominus(\text{reactants})$$

and the standard reaction Gibbs energy from eqn 4.11.

Solution The standard reaction enthalpy is

$$\begin{aligned}\Delta_r H^\ominus &= \Delta_f H^\ominus(H_2CO_3, aq) - \{\Delta_f H^\ominus(CO_2, g) + \Delta_f H^\ominus(H_2O, l)\} \\ &= -699.65 \text{ kJ mol}^{-1} - \{(-110.53 \text{ kJ mol}^{-1}) + (-285.83 \text{ kJ mol}^{-1})\} \\ &= -303.29 \text{ kJ mol}^{-1}\end{aligned}$$

The standard reaction entropy was calculated in *Illustration* 2.4:

$$\Delta_r S^\ominus = -96.3 \text{ J K}^{-1} \text{ mol}^{-1}$$

which, because 96.3 J is the same as 9.63×10^{-2} kJ, corresponds to -9.63×10^{-2} kJ K^{-1} mol^{-1}. Therefore, from eqn 4.11,

$$\begin{aligned}\Delta_r G^\ominus &= (-303.29 \text{ kJ mol}^{-1}) - (298.15 \text{ K}) \times (-9.63 \times 10^{-2} \text{ kJ K}^{-1} \text{ mol}^{-1}) \\ &= -274.6 \text{ kJ mol}^{-1}\end{aligned}$$

SELF-TEST 4.5 Use the information in the *Data section* to determine the standard reaction Gibbs energy for $3\ O_2(g) \longrightarrow 2\ O_3(g)$ from standard enthalpies of formation and standard entropies.

Answer: $+326.4 \text{ kJ mol}^{-1}$ ■

We saw in Section 1.14 how to use standard enthalpies of formation of substances to calculate standard reaction enthalpies. We can use the same technique for standard reaction Gibbs energies. To do so, we list the **standard Gibbs energy of formation**, $\Delta_f G^\ominus$, of a substance, which is the standard reaction Gibbs energy (per mole of the species) for its formation from the elements in their reference states. The concept of reference state was introduced in Section 1.14; the temperature is arbitrary, but we shall almost always take it to be 25°C (298 K). For example, the standard Gibbs energy of formation of liquid water, $\Delta_f G^\ominus(H_2O, l)$, is the standard reaction Gibbs energy for

$$H_2(g) + \tfrac{1}{2}O_2(g) \longrightarrow H_2O(l)$$

and is -237 kJ mol^{-1} at 298 K. Some standard Gibbs energies of formation are listed in Table 4.2 and more can be found in the *Data section*. It follows from the definition that the standard Gibbs energy of formation of an element in its reference state is zero because reactions such as

$$C(s, \text{graphite}) \longrightarrow C(s, \text{graphite})$$

are null (that is, nothing happens). The standard Gibbs energy of formation of an element in a phase different from its reference state is nonzero:

$$C(s, \text{graphite}) \longrightarrow C(s, \text{diamond}) \qquad \Delta_f G^\ominus(C, \text{diamond}) = +2.90 \text{ kJ mol}^{-1}$$

Many of the values in the tables have been compiled by combining the standard enthalpy of formation of the species with the standard entropies of the compound and the elements, as illustrated above, but there are other sources of data and we encounter some of them later.

Table 4.2 Standard Gibbs energies of formation at 298.15 K*

Substance	$\Delta_f G^\ominus$/(kJ mol^{-1})
Gases	
Carbon dioxide, CO_2	−394.36
Methane, CH_4	−50.72
Nitrogen oxide, NO	+86.55
Water, H_2O	−228.57
Liquids	
Ethanol, CH_3CH_2OH	−174.78
Hydrogen peroxide, H_2O_2	−120.35
Water, H_2O	−237.13
Solids	
α-D-Glucose $C_6H_{12}O_6$	−917.2
Glycine, $CH_2(NH_2)COOH$	−532.9
Sucrose, $C_{12}H_{22}O_{11}$	−1543
Urea, $CO(NH_2)_2$	−197.33
Solutes in aqueous solution	
Carbon dioxide, CO_2	−385.98
Carbonic acid, H_2CO_3	−623.08
Phosphoric acid, H_3PO_4	−1018.7

*Additional values are given in the *Data section*.

Standard Gibbs energies of formation can be combined to obtain the standard Gibbs energy of almost any reaction. We use the now familiar expression

$$\Delta_r G^\ominus = \sum \nu \Delta_f G^\ominus(\text{products}) - \sum \nu \Delta_f G^\ominus(\text{reactants}) \qquad \textbf{(4.12)}$$

ILLUSTRATION 4.2 Calculating a standard reaction Gibbs energy from standard Gibbs energies of formation

To determine the standard reaction Gibbs energy for the complete oxidation of solid sucrose, $C_{12}H_{22}O_{11}(s)$, by oxygen gas to carbon dioxide gas and liquid water,

$$C_{12}H_{22}O_{11}(s) + 12\ O_2(g) \longrightarrow 12\ CO_2(g) + 11\ H_2O(l)$$

we carry out the following calculation:

$$\begin{aligned}\Delta_r G^\ominus &= \{12\Delta_f G^\ominus(CO_2, g) + 11\Delta_f G^\ominus(H_2O, l)\} \\ &\quad - \{\Delta_f G^\ominus(C_{12}H_{22}O_{11}, s) + 12\Delta_f G^\ominus(O_2, g)\} \\ &= \{12 \times (-394\ \text{kJ mol}^{-1}) + 11 \times (-237\ \text{kJ mol}^{-1})\} \\ &\quad - \{-1543\ \text{kJ mol}^{-1} + 0\} \\ &= -5.79 \times 10^3\ \text{kJ mol}^{-1}\ \blacksquare\end{aligned}$$

SELF-TEST 4.6 Calculate the standard reaction Gibbs energy of the oxidation of ammonia to nitric oxide according to the equation $4\ NH_3(g) + 5\ O_2(g) \longrightarrow 4\ NO(g) + 6\ H_2O(g)$.

Answer: $-959.42\ \text{kJ mol}^{-1}$

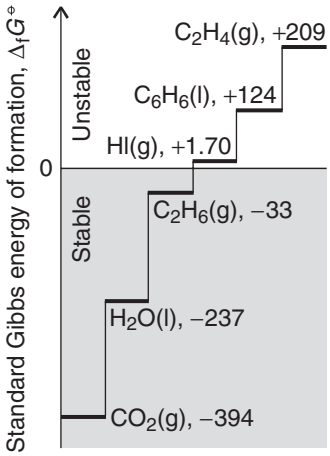


Fig. 4.8 The standard Gibbs energy of formation of a compound is like a measure of the compound's altitude above or below sea level: compounds that lie above sea level have a spontaneous tendency to decompose into the elements (and to revert to sea level). Compounds that lie below sea level are stable with respect to decomposition into the elements.

Standard Gibbs energies of formation of compounds have their own significance as well as being useful in calculations of K. They are a measure of the "thermodynamic altitude" of a compound above or below a "sea level" of stability represented by the elements in their reference states (Fig. 4.8). If the standard Gibbs energy of formation is positive and the compound lies above "sea level," then the compound has a spontaneous tendency to sink toward thermodynamic sea level and decompose into the elements. That is, $K < 1$ for their formation reaction. We say that a compound with $\Delta_f G^\ominus > 0$ is **thermodynamically unstable** with respect to its elements or that it is **endergonic**. Thus, the endergonic substance ozone, for which $\Delta_f G^\ominus = +163\ \text{kJ mol}^{-1}$, has a spontaneous tendency to decompose into oxygen under standard conditions at 25°C. More precisely, the equilibrium constant for the reaction $\frac{3}{2}\,O_2(g) \rightleftharpoons O_3(g)$ is less than 1 (much less, in fact: $K = 2.7 \times 10^{-29}$). However, although ozone is thermodynamically unstable, it can survive if the reactions that convert it into oxygen are slow. That is the case in the upper atmosphere, and the O_3 molecules in the ozone layer survive for long periods. Benzene ($\Delta_f G^\ominus = +124\ \text{kJ mol}^{-1}$) is also thermodynamically unstable with respect to its elements ($K = 1.8 \times 10^{-22}$). However, the fact that bottles of benzene are everyday laboratory commodities also reminds us of the point made at the start of the chapter, that *spontaneity is a thermodynamic tendency that might not be realized at a significant rate in practice*.

Another useful point that can be made about standard Gibbs energies of formation is that there is no point in searching for *direct* syntheses of a thermodynamically unstable compound from its elements (under standard conditions, at the temperature to which the data apply), because the reaction does not occur in the required direction: the *reverse* reaction, decomposition, is spontaneous. Endergonic compounds must be synthesized by alternative routes or under conditions for which their Gibbs energy of formation is negative and they lie beneath thermodynamic sea level.

Compounds with $\Delta_f G^\ominus < 0$ (corresponding to $K > 1$ for their formation reactions) are said to be **thermodynamically stable** with respect to their elements or **exergonic**. Exergonic compounds lie below the thermodynamic sea level of the elements (under standard conditions). An example is the exergonic compound ethane, with $\Delta_f G^\ominus = -33\ \text{kJ mol}^{-1}$: the negative sign shows that the formation of ethane gas is spontaneous in the sense that $K > 1$ (in fact, $K = 7.1 \times 10^5$ at 25°C).

The response of equilibria to the conditions

In introductory chemistry, we meet the empirical rule of thumb known as **Le Chatelier's principle**:

> When a system at equilibrium is subjected to a disturbance, the composition of the system adjusts so as to tend to minimize the effect of the disturbance.

Le Chatelier's principle is only a rule of thumb, and to understand why reactions respond as they do and to calculate the new equilibrium composition, we need to use thermodynamics. We need to keep in mind that some changes in conditions affect the value of $\Delta_r G^\ominus$ and therefore of K (temperature is the only instance), whereas others change the consequences of K having a particular fixed value without changing the value of K (the pressure, for instance).

4.5 The presence of a catalyst

Enzymes are biological versions of catalysts and are so ubiquitous that we need to know how their action affects chemical equilibria.

We study the action of catalysts (a substance that accelerates a reaction without itself appearing in the overall chemical equation) in Chapter 8 and at this stage do not need to know in detail how they work other than that they provide an alternative, faster route from reactants to products. Although the new route from reactants to products is faster, the initial reactants and the final products are the same. The quantity $\Delta_r G^\ominus$ is defined as the difference of the standard molar Gibbs energies of the reactants and products, so it is independent of the path linking the two. It follows that an alternative pathway between reactants and products leaves $\Delta_r G^\ominus$ and therefore K unchanged. That is, *the presence of a catalyst does not change the equilibrium constant of a reaction*.

4.6 The effect of temperature

In organisms, biochemical reactions occur over a very narrow range of temperatures, and changes by only a few degrees can have serious consequences, including death. Therefore, it is important to know how changes in temperature, such as those brought about by infections, affect biological processes.

According to Le Chatelier's principle, we can expect a reaction to respond to a lowering of temperature by releasing heat and to respond to an increase of temperature by absorbing heat. That is:

> When the temperature is raised, the equilibrium composition of an exothermic reaction will tend to shift toward reactants; the equilibrium composition of an endothermic reaction will tend to shift toward products.

In each case, the response tends to minimize the effect of raising the temperature. But *why* do reactions at equilibrium respond in this way? Le Chatelier's principle is only a rule of thumb and gives no clue to the reason for this behavior. As we shall now see, the origin of the effect is the dependence of $\Delta_r G^\ominus$, and therefore of K, on the temperature.

First, we consider the effect of temperature on $\Delta_r G^\ominus$. We use the relation $\Delta_r G^\ominus = \Delta_r H^\ominus - T\Delta_r S^\ominus$ and make the assumption that neither the reaction enthalpy nor the reaction entropy varies much with temperature (over small ranges, at least). It follows that

$$\text{Change in } \Delta_r G^\ominus = -(\text{change in } T) \times \Delta_r S^\ominus \qquad \textbf{(4.13)}$$

This expression is easy to apply when there is a consumption or formation of gas because, as we have seen (Section 2.8), gas formation dominates the sign of the reaction entropy.

Now consider the effect of temperature on K itself. At first, this problem looks troublesome, because both T and $\Delta_r G^\ominus$ appear in the expression for K. However, in fact the effect of temperature can be expressed very simply as the **van 't Hoff equation**.[1]

$$\ln K' - \ln K = \frac{\Delta_r H^\ominus}{R}\left(\frac{1}{T} - \frac{1}{T'}\right) \qquad \textbf{(4.14)}$$

[1]There are several "van 't Hoff equations." To distinguish them, this one is sometimes called the *van 't Hoff isochore*.

where K is the equilibrium constant at the temperature T and K' is its value when the temperature is T'. All we need to know to calculate the temperature dependence of an equilibrium constant, therefore, is the standard reaction enthalpy.

DERIVATION 4.1 The van 't Hoff equation

As before, we use the approximation that the standard reaction enthalpy and entropy are independent of temperature over the range of interest, so the entire temperature dependence of $\Delta_r G^\ominus$ stems from the T in $\Delta_r G^\ominus = \Delta_r H^\ominus - T\Delta_r S^\ominus$. At a temperature T,

$$\ln K = -\frac{\Delta_r G^\ominus}{RT} \overset{\text{Substitute } \Delta_r G^\ominus = \Delta_r H^\ominus - T\Delta_r S^\ominus}{=} -\frac{\Delta_r H^\ominus}{RT} + \frac{\Delta_r S^\ominus}{R}$$

At another temperature T', when $\Delta_r G^{\ominus\prime} = \Delta_r H^\ominus - T'\Delta_r S^\ominus$ and the equilibrium constant is K', a similar expression holds:

$$\ln K' = -\frac{\Delta_r H^\ominus}{RT'} + \frac{\Delta_r S^\ominus}{R}$$

The difference between the two is

$$\ln K' - \ln K = \frac{\Delta_r H^\ominus}{R}\left(\frac{1}{T} - \frac{1}{T'}\right)$$

which is the van 't Hoff equation.

Let's explore the information in the van 't Hoff equation. Consider the case when $T' > T$. Then the term in parentheses in eqn 4.14 is positive. If $\Delta_r H^\ominus > 0$, corresponding to an endothermic reaction, the entire term on the right is positive. In this case, therefore, $\ln K' > \ln K$. That being so, we conclude that $K' > K$ for an endothermic reaction. In general, *the equilibrium constant of an endothermic reaction increases with temperature*. The opposite is true when $\Delta_r H^\ominus < 0$, so we can conclude that *the equilibrium constant of an exothermic reaction decreases with an increase in temperature*.

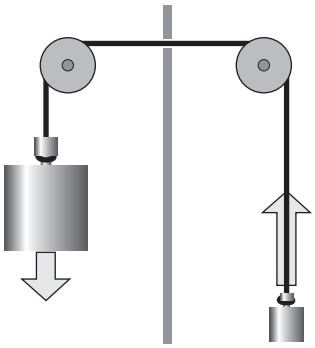
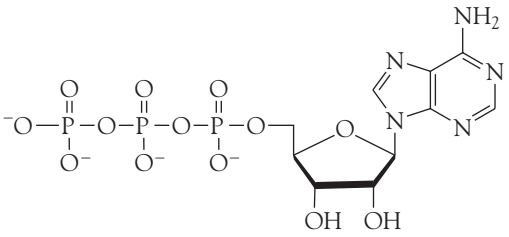


Fig. 4.9 If two weights are coupled as shown here, then the heavier weight will move the lighter weight in its non-spontaneous direction: overall, the process is still spontaneous. The weights are the analogues of two chemical reactions: a reaction with a large negative ΔG can force another reaction with a smaller ΔG to run in its non-spontaneous direction.

Coupled reactions in bioenergetics

A non-spontaneous reaction may be driven by coupling it to a reaction that is spontaneous. A simple mechanical analogy is a pair of weights joined by a string (Fig. 4.9): the lighter of the pair of weights will be pulled up as the heavier weight falls down. Although the lighter weight has a natural tendency to move downward, its coupling to the heavier weight results in it being raised. The thermodynamic analogue is an **endergonic reaction**, a reaction with a positive Gibbs energy, $\Delta_r G$ (the analogue of the lighter weight moving up), being forced to occur by coupling it to an **exergonic reaction**, a reaction with a negative Gibbs energy, $\Delta_r G'$ (the analogue of the heavier weight falling down). The overall reaction is spontaneous because the sum $\Delta_r G + \Delta_r G'$ is negative. The whole of life's activities depend on



4 ATP

couplings of this kind, for the oxidation reactions of food act as the heavy weights that drive other reactions forward and result in the formation of proteins from amino acids, the actions of muscles for propulsion, and even the activities of the brain for reflection, learning, and imagination.

4.7 The function of adenosine triphosphate

The compound adenosine triphosphate is of central importance in bioenergetics, and it is essential to understand its thermodynamic role.

The function of adenosine triphosphate, ATP^{4-}(**4**) or (more succinctly) ATP, is to store the energy made available when food is oxidized and then to supply it on demand to a wide variety of processes, including muscular contraction, reproduction, and vision. We saw in *Case study* 2.2 that the essence of ATP's action is its ability to lose its terminal phosphate group by hydrolysis and to form adenosine diphosphate, ADP^{3-} (**5**):

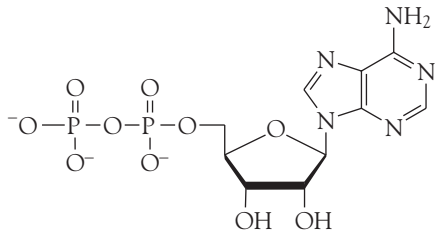
$$ATP^{4-}(aq) + H_2O(l) \longrightarrow ADP^{3-}(aq) + HPO_4^{2-}(aq) + H_3O^+(aq)$$

This reaction is exergonic under the conditions prevailing in cells and can drive an endergonic reaction forward if suitable enzymes are available to couple the reactions.

Before discussing the hydrolysis of ATP quantitatively, we need to note that the conventional standard state of hydrogen ions ($a_{H_3O^+} = 1$, corresponding to pH = 0, a strongly acidic solution) is not appropriate to normal biological conditions inside cells, where the pH is close to 7. Therefore, in biochemistry it is common to adopt the **biological standard state**, in which pH = 7, a neutral solution. We shall adopt this convention in this section and label the corresponding standard quantities as $G^{\oplus}$, $H^{\oplus}$, and $S^{\oplus}$.[2]

COMMENT 4.1 Recall that the hydronium ion concentration is commonly expressed in terms of the pH, which is defined as pH = $-\log a_{H_3O^+}$. In elementary work, we replace the hydronium ion activity by the numerical value of its molar concentration, $[H_3O^+]$. For more details, see Section 4.9. ■

[2]Another convention to denote the biological standard state is to write $X°'$ or $X^{\ominus\prime}$.



5 ADP

EXAMPLE 4.4 Converting between thermodynamic and biological standard states

The standard reaction Gibbs energy for the hydrolysis of ATP is +10 kJ mol^{-1} at 298 K. What is the biological standard state value?

Strategy Because protons occur as products, lowering their concentration (from 1 mol L^{-1} to 10^{-7} mol L^{-1}) suggests that the reaction will have a higher tendency to form products. Therefore, we expect a more negative value of the reaction Gibbs energy for the biological standard than for the thermodynamic standard. The two types of standard are related by eqn 4.6, with the activity of hydrogen ions 10^{-7} in place of 1.

Solution The reaction quotient for the hydrolysis reaction when all the species are in their standard states except the hydrogen ions, which are present at 10^{-7} mol L^{-1}, is

$$Q = \frac{a_{ADP^{3-}} a_{HPO_4^{2-}} a_{H_3O^+}}{a_{ATP^{4-}} a_{H_2O}} = \frac{1 \times 1 \times 10^{-7}}{1 \times 1} = 1 \times 10^{-7}$$

The thermodynamic and biological standard values are therefore related by eqn 4.6 in the form

$$\begin{aligned}\Delta_r G^{\oplus} &= \Delta_r G^{\ominus} + (8.314\ 47 \times 10^{-3}\ \text{J K}^{-1}\ \text{mol}^{-1}) \times (298\ \text{K}) \times \ln(1 \times 10^{-7}) \\ &= 10\ \text{kJ mol}^{-1} - 40\ \text{kJ mol}^{-1} = -30\ \text{kJ mol}^{-1}\end{aligned}$$

Note how the large change in pH changes the sign of the standard reaction Gibbs energy.

SELF-TEST 4.7 The overall reaction for the glycolysis reaction (Section 4.8) is $C_6H_{12}O_6(aq) + 2\ NAD^+(aq) + 2\ ADP^{3-}(aq) + 2\ HPO_4^{2-}(aq) + 2\ H_2O(l) \longrightarrow 2\ CH_3COCO_2^-(aq) + 2\ NADH(aq) + 2\ ATP^{4-}(aq) + 2\ H_3O^+(aq)$. For this reaction, $\Delta_r G^{\oplus} = -80.6$ kJ mol^{-1} at 298 K. What is the value of $\Delta_r G^{\ominus}$?

Answer: -0.7 kJ mol^{-1} ■

For a reaction of the form

$$\text{Reactants} + \nu\ H_3O^+(aq) \longrightarrow \text{products}$$

the biological and thermodynamic standard states are related by

$$\Delta_r G^{\oplus} = \Delta_r G^{\ominus} - \nu RT \times \ln 10^{-7} = \Delta_r G^{\ominus} + 7\nu RT \ln 10 \qquad \textbf{(4.15)}$$

where we have used the relation $\ln x^a = a \ln x$. It follows that at 298.15 K

$$\Delta_r G^{\oplus} = \Delta_r G^{\ominus} + (39.96\ \text{kJ mol}^{-1})\nu$$

and at 37°C (310 K, body temperature)

$$\Delta_r G^{\oplus} = \Delta_r G^{\ominus} + (41.5\ \text{kJ mol}^{-1})\nu$$

There is no difference between thermodynamic and biological standard values if hydrogen ions are not involved in the reaction ($\nu = 0$).

Now we are ready to explore the action of ATP quantitatively. The biological standard values for the hydrolysis of ATP at 37°C are

$$\Delta_r G^{\oplus} = -31 \text{ kJ mol}^{-1} \quad \Delta_r H^{\oplus} = -20 \text{ kJ mol}^{-1} \quad \Delta_r S^{\oplus} = +34 \text{ J K}^{-1} \text{ mol}^{-1}$$

The hydrolysis is therefore exergonic ($\Delta_r G < 0$) under these conditions, and 31 kJ mol^{-1} is available for driving other reactions. On account of its exergonic character, the ADP-phosphate bond has been called a "high-energy phosphate bond." The name is intended to signify a high tendency to undergo reaction and should not be confused with "strong" bond in its normal chemical sense (that of a high bond enthalpy). In fact, even in the biological sense it is not of very "high energy." The action of ATP depends on the bond being intermediate in strength. Thus ATP acts as a phosphate donor to a number of acceptors (such as glucose) but is recharged with a new phosphate group by more powerful phosphate donors in the phosphorylation steps in the respiration cycle.

CASE STUDY 4.2 The biosynthesis of proteins

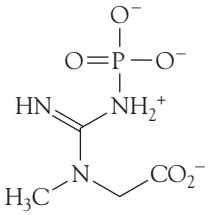
In the cell, each ATP molecule can be used to drive an endergonic reaction for which $\Delta_r G^{\oplus}$ does not exceed +31 kJ mol^{-1}. For example, the biosynthesis of sucrose from glucose and fructose can be driven by plant enzymes because the reaction is endergonic to the extent $\Delta_r G^{\oplus} = +23$ kJ mol^{-1}. The biosynthesis of proteins is strongly endergonic, not only on account of the enthalpy change but also on account of the large decrease in entropy that occurs when many amino acids are assembled into a precisely determined sequence. For instance, the formation of a peptide link is endergonic, with $\Delta_r G^{\oplus} = +17$ kJ mol^{-1}, but the biosynthesis occurs indirectly and is equivalent to the consumption of three ATP molecules for each link. In a moderately small protein such as myoglobin, with about 150 peptide links, the construction alone requires 450 ATP molecules and therefore about 12 mol of glucose molecules for 1 mol of protein molecules. ■

COMMENT 4.2 From now on, we shall represent biochemical reactions with chemical equations written with a shorthand method, in which some substances are given "nicknames" and charges are not always given explicitly. For example, $H_2PO_4^{2-}$ is written as P_i, ATP^{4-} as ATP, and so on. ■

SELF-TEST 4.8 Fats yield almost twice as much energy per gram as carbohydrates. What mass of fat would need to be metabolized to synthesize 1.0 mol of myoglobin molecules?

Answer: 7.6 kg

Adenosine triphosphate is not the only phosphate species capable of driving other less exergonic reactions. For instance, creatine phosphate (**6**) can release its phosphate group in a hydrolysis reaction, and $\Delta_r G^{\oplus} = -43$ kJ mol^{-1}. These different exergonicities give rise to the concept of **transfer potential**, which is the negative of the value of $\Delta_r G^{\oplus}$ for the hydrolysis reaction. Thus, the transfer potential of creatine phosphate is 43 kJ mol^{-1}. Just as one exergonic reaction can drive a less exergonic reaction, so the hydrolysis of a species with a high transfer potential can drive the phosphorylation of a species with a lower transfer potential (Table 4.3).



6 Creatine phosphate

4.8 The oxidation of glucose

The oxidation of glucose to CO_2 and H_2O by O_2 represents the process by which the breakdown of foods leads to the formation of ATP.

The breakdown of glucose in the cell begins with **glycolysis**, a partial oxidation of glucose by nicotinamide adenine dinucleotide (NAD^+, **7**) to pyruvate ion,

Table 4.3 Transfer potentials at 298.15 K

Substance	Transfer potential, $-\Delta_r G^{\oplus}$/(kJ mol^{-1})
AMP	14
ATP, ADP	31
1,3-Bis(phospho)glycerate	49
Creatine phosphate	43
Glucose-6-phosphate	14
Glycerol-1-phosphate	10
Phosphoenolpyruvate	62
Pyrophosphate, $HP_2O_7^{3-}$	33

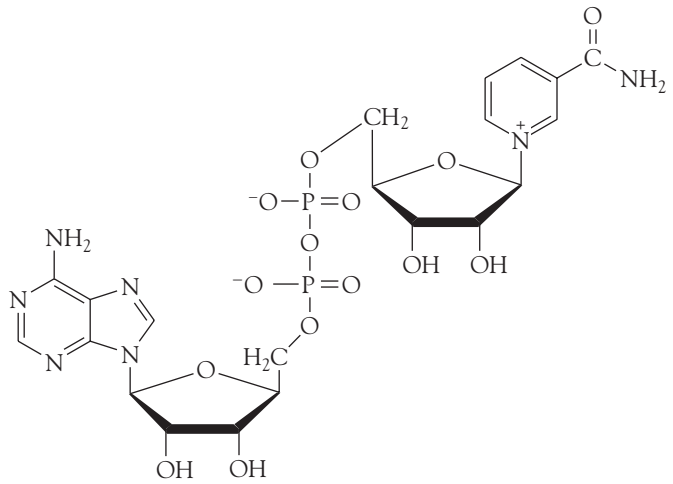
$CH_3COCO_2^-$. Metabolism continues in the form of the **citric acid cycle**, in which pyruvate ions are oxidized to CO_2, and ends with **oxidative phosphorylation**, in which O_2 is reduced to H_2O. Glycolysis is the main source of energy during **anaerobic metabolism**, a form of metabolism in which inhaled O_2 does not play a role. The citric acid cycle and oxidative phosphorylation are the main mechanisms for the extraction of energy from carbohydrates during **aerobic metabolism**, a form of metabolism in which inhaled O_2 does play a role.

Glycolysis occurs in the *cytosol*, the aqueous material encapsulated by the cell membrane, and consists of 10 enzyme-catalyzed reactions (Fig 4.10). The process needs to be initiated by consumption of two molecules of ATP per molecule of glucose. The first ATP molecule is used to drive the phosphorylation of glucose to glucose-6-phosphate (G6P):

$$\text{glucose(aq)} + \text{ATP (aq)} \longrightarrow \text{G6P(aq)} + \text{ADP(aq)} \qquad \Delta_r G^{\oplus} = -17 \text{ kJ mol}^{-1}$$

As we saw in Section 4.1, the next step is the isomerization of G6P to fructose-6-phosphate (F6P). The second ATP molecule consumed during glycolysis drives the phosphorylation of F6P to fructose-1,6-diphosphate (FDP):

$$\text{F6P(aq)} + \text{ATP(aq)} \longrightarrow \text{FDP(aq)} + \text{ADP(aq)} \qquad \Delta_r G^{\oplus} = -14 \text{ kJ mol}^{-1}$$



7 NAD^+

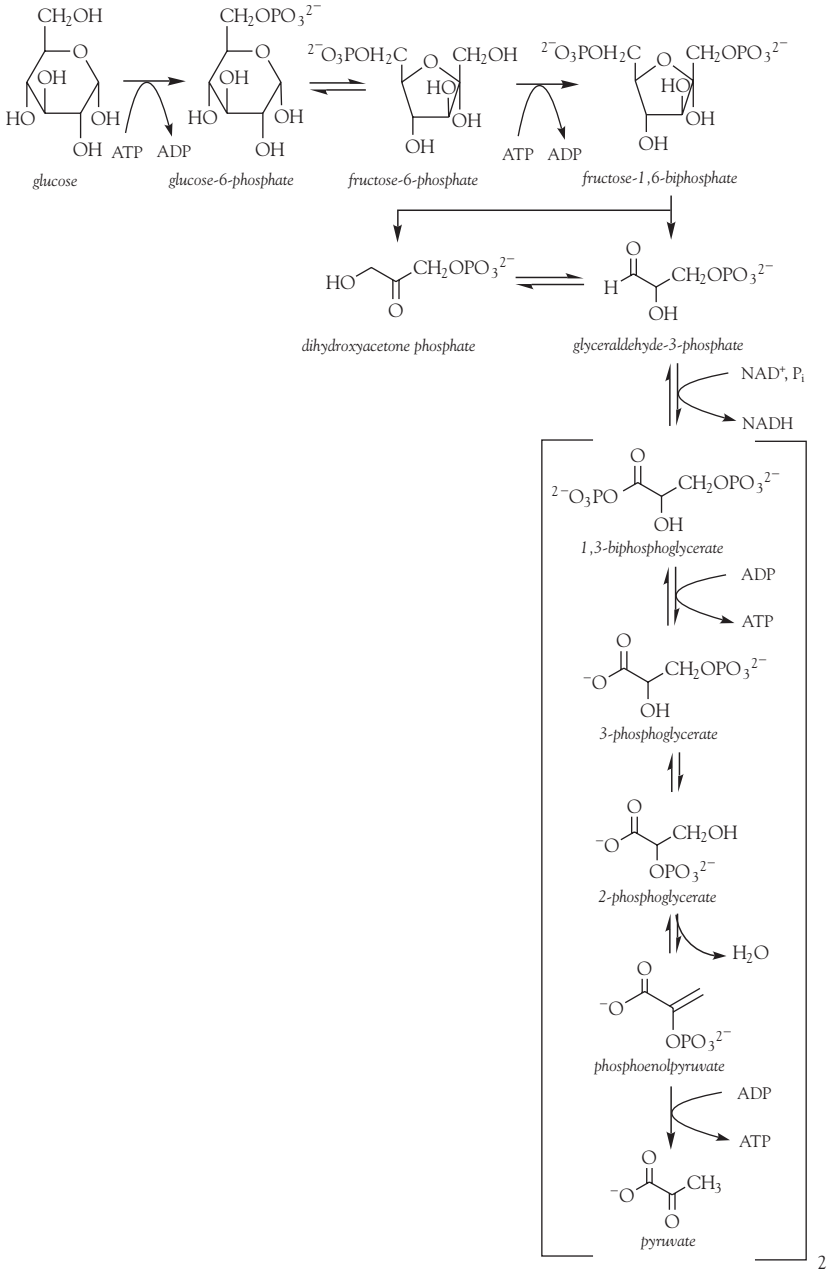
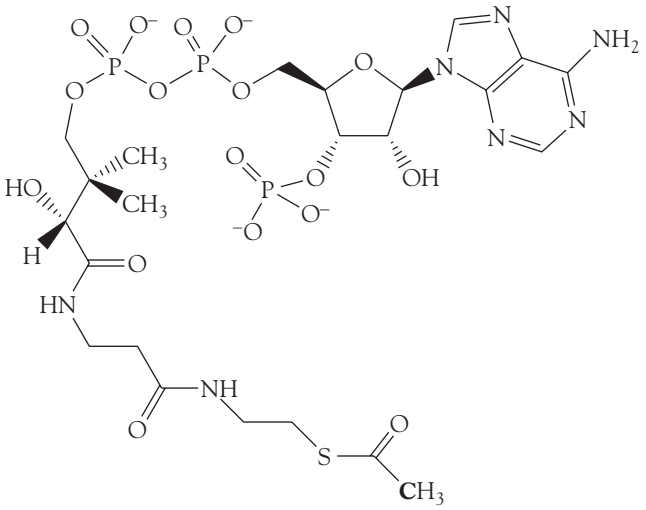


Fig. 4.10 The reactions of glycolysis, in which glucose is partially oxidized by nicotinamide adenine dinucleotide (NAD^+) to pyruvate ion.

In the next step, FDP is broken into two three-carbon units, dihydroxyacetone phosphate (1,3-dihydroxypropanone phosphate, $CH_2OHCOCH_2OPO_3^{2-}$) and glyceraldehyde-3-phosphate, which exist in mutual equilibrium. Only the glyceraldehyde-3-phosphate is oxidized by NAD^+ to pyruvate ion, with formation of two ATP molecules. As glycolysis proceeds, all the dihydroxyacetone phosphate is converted to glyceraldehyde-3-phosphate, so the result is the consumption of two NAD^+ molecules and the formation of four ATP molecules per molecule of glucose.

COMMENT 4.3 The text's web site contains links to databases of structures of many of the enzymes involved in glycolysis, the citric acid cycle, and oxidative phosphorylation. ■

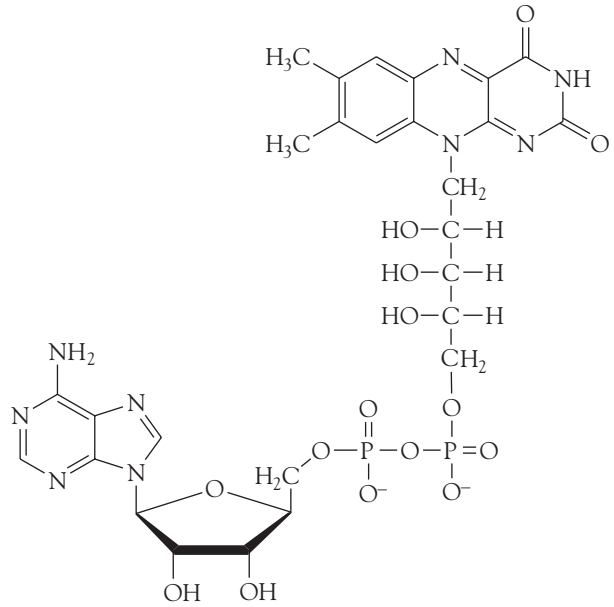


8 Acetyl coenzyme A (acetyl CoA), with the carbon derived from pyruvate in boldface

The oxidation of glucose by NAD^+ to pyruvate ions has $\Delta_r G^{\oplus} = -147$ kJ mol^{-1} at blood temperature. In glycolysis, the oxidation of one glucose molecule is coupled to the *net* conversion of two ADP molecules to two ATP molecules (two ATP molecules are consumed and four are formed), so the net reaction of glycolysis is

$$\text{glucose(aq)} + 2\ \text{NAD}^+\text{(aq)} + 2\ \text{ADP(aq)} + 2\ \text{P}_i\text{(aq)} + 2\ \text{H}_2\text{O(l)} \longrightarrow$$
$$2\ \text{CH}_3\text{COCO}_2^-\text{(aq)} + 2\ \text{NADH(aq)} + 2\ \text{ATP(aq)} + 2\ \text{H}_3\text{O}^+\text{(aq)}$$

The biological standard reaction Gibbs energy is $(-147) - 2(-31)$ kJ mol^{-1} = -85 kJ mol^{-1}. The reaction is exergonic and therefore spontaneous under



9 FAD

biological standard conditions: the oxidation of glucose is used to "recharge" the ATP.

In cells that are deprived of O_2, pyruvate ion is reduced to lactate ion, $CH_3C(OH)CO_2^-$, by NADH.[3] Very strenuous exercise, such as bicycle racing, can decrease sharply the concentration of O_2 in muscle cells, and the condition known as muscle fatigue results from increased concentrations of lactate ion.

The standard Gibbs energy of combustion of glucose is -2880 kJ mol^{-1}, so terminating its oxidation at pyruvate is a poor use of resources, akin to the partial combustion of hydrocarbon fuels in a badly tuned engine. In the presence of O_2, pyruvate is oxidized further during the citric acid cycle and oxidative phosphorylation, which occur in the mitochondria of cells.

The further oxidation of carbon derived from glucose begins with a reaction between pyruvate ion, NAD^+, and coenzyme A (CoA) to give acetyl CoA (**8**), NADH, and CO_2. Acetyl CoA is then oxidized by NAD^+ and flavin adenine dinucleotide (FAD, **9**) in the citric acid cycle (Fig. 4.11), which requires eight enzymes and results in the synthesis of GTP (**10**) from GDP or ATP from ADP:

$$\begin{aligned}\text{Acetyl CoA(aq)} + 3\,\text{NAD}^+\text{(aq)} + \text{FAD(aq)} + \text{GDP(aq)} \\ + \text{P}_\text{i}\text{(aq)} + 2\,\text{H}_2\text{O(l)} \longrightarrow 2\,\text{CO}_2\text{(g)} + 3\,\text{NADH(aq)} + 2\,\text{H}_3\text{O}^+\text{(aq)} \\ + \text{FADH}_2\text{(aq)} + \text{GTP(aq)} + \text{CoA(aq)} \\ \Delta_\text{r}G^\oplus = -57\ \text{kJ mol}^{-1}\end{aligned}$$

In cells that produce GTP, the enzyme nucleoside diphosphate kinase catalyzes the transfer of a phosphate group to ADP to form ATP:

$$\text{GTP(aq)} + \text{ADP(aq)} \longrightarrow \text{GDP(aq)} + \text{ATP(aq)}$$

[3]In yeast, the terminal products are ethanol and CO_2.

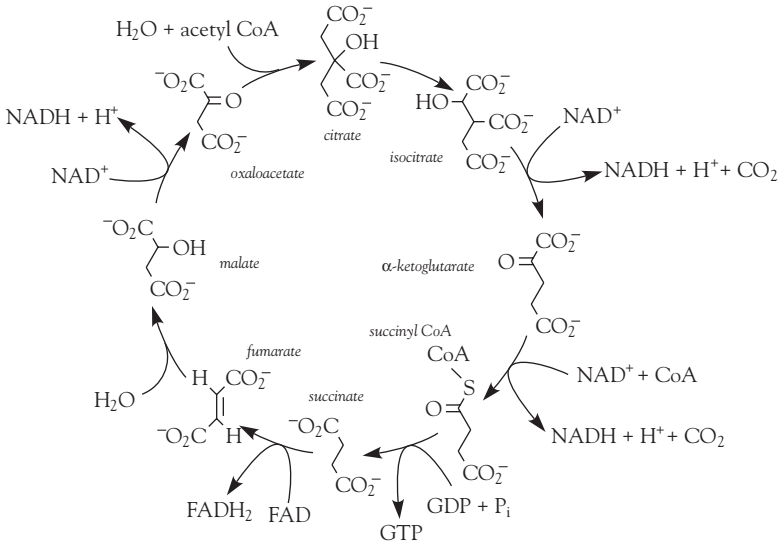
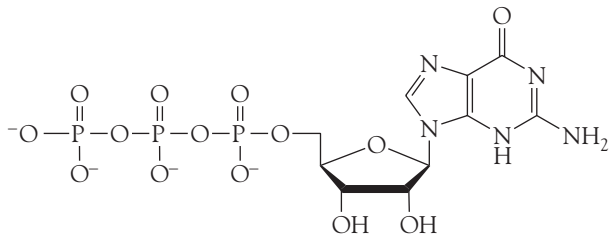


Fig. 4.11 The reactions of the citric acid cycle, in which acetyl CoA is oxidized by NAD^+ and FAD, resulting in the synthesis of GTP (shown) or ATP, depending on the type of cell. The GTP molecules are eventually converted to ATP.



10 GTP

For this reaction, $\Delta_r G^{\oplus} = 0$ because the phosphate group transfer potentials for GTP and ATP are essentially identical. Overall, we write the oxidation of glucose as a result of glycolysis and the citric acid cycle as

$$\begin{aligned}\text{glucose(aq)} + 10\ \text{NAD}^+\text{(aq)} + 2\ \text{FAD(aq)} + 4\ \text{ADP(aq)} \\ + 4\ \text{P}_i\text{(aq)} + 2\ \text{H}_2\text{O(l)} \longrightarrow 6\ \text{CO}_2\text{(g)} + 10\ \text{NADH(aq)} \\ + 6\ \text{H}_3\text{O}^+\text{(aq)} + 2\ \text{FADH}_2\text{(aq)} + 4\ \text{ATP(aq)}\end{aligned}$$

The NADH and $FADH_2$ go on to reduce O_2 during oxidative phosphorylation (Section 5.11), which also produces ATP. The citric acid cycle and oxidative phosphorylation generate as many as 38 ATP molecules for each glucose molecule consumed. Each mole of ATP molecules extracts 31 kJ from the 2880 kJ supplied by 1 mol $C_6H_{12}O_6$ (180 g of glucose), so 1178 kJ is stored for later use. Therefore, aerobic oxidation of glucose is much more efficient than glycolysis.

Proton transfer equilibria

An enormously important biological aspect of chemical equilibrium is that involving the transfer of protons (hydrogen ions, H^+) between species in aqueous environments, such as living cells. Even small drifts in the equilibrium concentration of hydrogen ions can result in disease, cell damage, and death. In this section we see how the general principles outlined earlier in the chapter are applied to proton transfer equilibria. Throughout our discussion, keep in mind that a free hydrogen ion does not exist in water: it is always attached to a water molecule and exists as H_3O^+, a *hydronium ion*.

4.9 Brønsted-Lowry theory

Cells have elaborate procedures for using proton transfer equilibria, and this function cannot be understood without knowing which species provide protons and which accept them and how to express the concentration of hydrogen ions in solution.

According to the **Brønsted-Lowry theory** of acids and bases, an **acid** is a proton donor and a **base** is a proton acceptor. The proton, which in this context means a hydrogen ion, H^+, is highly mobile and acids and bases in water are always in equilibrium with their deprotonated and protonated counterparts and hydronium ions (H_3O^+). Thus, an acid HA, such as HCN, immediately establishes the equilibrium

$$\text{HA(aq)} + \text{H}_2\text{O(l)} \rightleftharpoons \text{H}_3\text{O}^+\text{(aq)} + \text{A}^-\text{(aq)} \qquad K = \frac{a_{\text{H}_3\text{O}^+} a_{\text{A}^-}}{a_{\text{HA}} a_{\text{H}_2\text{O}}}$$

A base B, such as NH_3, immediately establishes the equilibrium

$$B(aq) + H_2O(l) \rightleftharpoons BH^+(aq) + OH^-(aq) \qquad K = \frac{a_{BH^+}a_{OH^-}}{a_B a_{H_2O}}$$

In these equilibria, A^- is the **conjugate base** of the acid HA, and BH^+ is the **conjugate acid** of the base B. Even in the absence of added acids and bases, proton transfer occurs between water molecules, and the **autoprotolysis equilibrium**[4]

$$2\ H_2O(l) \rightleftharpoons H_3O^+(aq) + OH^-(aq) \qquad K = \frac{a_{H_3O^+}a_{OH^-}}{a_{H_2O}^2}$$

is always present.

As will be familiar from introductory chemistry, the hydronium ion concentration is commonly expressed in terms of the pH, which is defined formally as

$$pH = -\log a_{H_3O^+} \tag{4.16}$$

where the logarithm is to base 10. In elementary work, the hydronium ion activity is replaced by the numerical value of its molar concentration, $[H_3O^+]$, which is equivalent to setting the activity coefficient γ equal to 1. For example, if the molar concentration of H_3O^+ is 2.0 mmol L^{-1} (where 1 mmol = 10^{-3} mol), then

$$pH \approx -\log(2.0 \times 10^{-3}) = 2.70$$

If the molar concentration were 10 times less, at 0.20 mmol L^{-1}, then the pH would be 3.70. Notice that *the higher the pH, the lower the concentration of hydronium ions in the solution* and that a change in pH by 1 unit corresponds to a 10-fold change in their molar concentration. However, it should never be forgotten that the replacement of activities by molar concentration is invariably hazardous. Because ions interact over long distances, the replacement is unreliable for all but the most dilute solutions.

SELF-TEST 4.9 Death is likely if the pH of human blood plasma changes by more than ±0.4 from its normal value of 7.4. What is the approximate range of molar concentrations of hydrogen ions for which life can be sustained?

Answer: 16 nmol L^{-1} to 100 nmol L^{-1} (1 nmol = 10^{-9} mol)

4.10 Protonation and deprotonation

The protonation and deprotonation of molecules are key steps in many biochemical reactions, and we need to be able to describe procedures for treating protonation and deprotonation processes quantitatively.

All the solutions we consider are so dilute that we can regard the water present as being a nearly pure liquid and therefore as having unit activity (see Table 3.3).

[4]Autoprotolysis is also called *autoionization*.

When we set $a_{H_2O} = 1$ for all the solutions we consider, the resulting equilibrium constant is called the **acidity constant**, K_a, of the acid HA:[5]

$$K_a = \frac{a_{H_3O^+} a_{A^-}}{a_{HA}} \approx \frac{[H_3O^+][A^-]}{[HA]} \qquad \textbf{(4.17)}$$

Data are widely reported in terms of the negative common (base 10) logarithm of this quantity:

$$pK_a = -\log K_a \qquad \textbf{(4.18)}$$

It follows from eqn 4.8 ($\Delta_r G^\ominus = -RT \ln K$) that pK_a is proportional to $\Delta_r G^\ominus$ for the proton transfer reaction. More explicitly, $pK_a = \Delta_r G^\ominus/(RT \ln 10)$, with $\ln 10 = 2.303\ldots$. Therefore, manipulations of pK_a and related quantities are actually manipulations of standard reaction Gibbs energies in disguise.

SELF-TEST 4.10 Show that $pK_a = \Delta_r G^\ominus/(RT \ln 10)$. *Hint:* $\ln x = \ln 10 \times \log x$.

The value of the acidity constant indicates the extent to which proton transfer occurs at equilibrium in aqueous solution. The smaller the value of K_a, and therefore the larger the value of pK_a, the lower is the concentration of deprotonated molecules. Most acids have $K_a < 1$ (and usually much less than 1), with $pK_a > 0$, indicating only a small extent of deprotonation in water. These acids are classified as **weak acids**. A few acids, most notably, in aqueous solution, HCl, HBr, HI, HNO_3, H_2SO_4 and $HClO_4$, are classified as **strong acids** and are commonly regarded as being completely deprotonated in aqueous solution.[6]

The corresponding expression for a base is called the **basicity constant**, K_b:

$$K_b = \frac{a_{BH^+} a_{OH^-}}{a_B} \approx \frac{[BH^+][OH^-]}{[B]} \qquad pK_b = -\log K_b \qquad \textbf{(4.19)}$$

A **strong base** is fully protonated in solution in the sense that $K_b > 1$. One example is the oxide ion, O^{2-}, which cannot survive in water but is immediately and fully converted into its conjugate acid OH^-. A **weak base** is not fully protonated in water in the sense that $K_b < 1$ (and usually much less than 1). Ammonia, NH_3, and its organic derivatives the amines are all weak bases in water, and only a small proportion of their molecules exist as the conjugate acid (NH_4^+ or RNH_3^+).

The **autoprotolysis constant** for water, K_w, is

$$K_w = a_{H_3O^+} a_{OH^-} \qquad \textbf{(4.20)}$$

At 25°C, the only temperature we consider in this chapter, $K_w = 1.0 \times 10^{-14}$ and $pK_w = -\log K_w = 14.00$. As may be confirmed by multiplying the two constants together, the acidity constant of the conjugate acid, BH^+, and the basicity constant of a base B (the equilibrium constant for the reaction $B + H_2O \rightleftharpoons BH^+ + OH^-$) are related by

$$K_a K_b = \frac{a_{H_3O^+} a_B}{a_{BH^+}} \times \frac{a_{BH^+} a_{OH^-}}{a_B} = a_{H_3O^+} a_{OH^-} = K_w \qquad \textbf{(4.21a)}$$

[5] Acidity constants are also called *acid ionization constants* and, less appropriately, *dissociation constants*.

[6] Sulfuric acid, H_2SO_4, is strong with respect only to its first deprotonation; HSO_4^- is weak.

The implication of this relation is that K_a increases as K_b decreases to maintain a product equal to the constant K_w. That is, *as the strength of a base decreases, the strength of its conjugate acid increases* and vice versa. On taking the negative common logarithm of both sides of eqn 4.21a, we obtain

$$pK_a + pK_b = pK_w \qquad \textbf{(4.21b)}$$

The great advantage of this relation is that the pK_b values of bases may be expressed as the pK_a of their conjugate acids, so the strengths of all weak acids and bases may be listed in a single table (Table 4.4). For example, if the acidity constant of the conjugate acid ($CH_3NH_3^+$) of the base methylamine (CH_3NH_2) is reported as $pK_a = 10.56$, we can infer that the basicity constant of methylamine itself is

$$pK_b = pK_w - pK_a = 14.00 - 10.56 = 3.44$$

Another useful relation is obtained by taking the negative common logarithm of both sides of the definition of K_w in eqn 4.20, which gives

$$pH + pOH = pK_w \qquad \textbf{(4.22)}$$

where $pOH = -\log a_{OH^-}$. This enormously important relation means that the activities (in elementary work, the molar concentrations) of hydronium and hydroxide ions are related by a seesaw relation: as one goes up, the other goes down to preserve the value of pK_w.

SELF-TEST 4.11 The molar concentration of OH^- ions in a certain solution is 0.010 mmol L^{-1}. What is the pH of the solution?

Answer: 9.00

The extent of deprotonation of a weak acid in solution depends on the acidity constant and the initial concentration of the acid, its concentration as prepared. The **fraction deprotonated**, the fraction of acid molecules HA that have donated a proton, is

$$\text{Fraction deprotonated} = \frac{\text{equilibrium molar concentration of conjugate base}}{\text{molar concentration of acid as prepared}}$$

$$f = \frac{[A^-]_{\text{equilibrium}}}{[HA]_{\text{as prepared}}} \qquad \textbf{(4.23)}$$

The extent to which a weak base B is protonated is reported in terms of the **fraction protonated**:

$$\text{Fraction protonated} = \frac{\text{equilibrium molar concentration of conjugate acid}}{\text{molar concentration of base as prepared}}$$

$$f = \frac{[BH^+]_{\text{equilibrium}}}{[B]_{\text{as prepared}}} \qquad \textbf{(4.24)}$$

The most precise way to estimate the pH of a solution of a weak acid is to consider the contributions from deprotonation of the acid and autoprotolysis of water

Table 4.4 Acidity and basicity constants* at 298.15 K

Acid/Base	K_b	pK_b	K_a	pK_a
Strongest weak acids				
Trichloroacetic acid, CCl_3COOH	3.3×10^{-14}	13.48	3.0×10^{-1}	0.52
Benzenesulfonic acid, $C_6H_5SO_3H$	5.0×10^{-14}	13.30	2×10^{-1}	0.70
Iodic acid, HIO_3	5.9×10^{-14}	13.23	1.7×10^{-1}	0.77
Sulfurous acid, H_2SO_3	6.3×10^{-13}	12.19	1.6×10^{-2}	1.81
Chlorous acid, $HClO_2$	1.0×10^{-12}	12.00	1.0×10^{-2}	2.00
Phosphoric acid, H_3PO_4	1.3×10^{-12}	11.88	7.6×10^{-3}	2.12
Chloroacetic acid, $CH_2ClCOOH$	7.1×10^{-12}	11.15	1.4×10^{-3}	2.85
Lactic acid, $CH_3CH(OH)COOH$	1.2×10^{-11}	10.92	8.4×10^{-4}	3.08
Nitrous acid, HNO_2	2.3×10^{-11}	10.63	4.3×10^{-4}	3.37
Hydrofluoric acid, HF	2.9×10^{-11}	10.55	3.5×10^{-4}	3.45
Formic acid, HCOOH	5.6×10^{-11}	10.25	1.8×10^{-4}	3.75
Benzoic acid, C_6H_5COOH	1.5×10^{-10}	9.81	6.5×10^{-5}	4.19
Acetic acid, CH_3COOH	5.6×10^{-10}	9.25	5.6×10^{-5}	4.75
Carbonic acid, H_2CO_3	2.3×10^{-8}	7.63	4.3×10^{-7}	6.37
Hypochlorous acid, HClO	3.3×10^{-7}	6.47	3.0×10^{-8}	7.53
Hypobromous acid, HBrO	5.0×10^{-6}	5.31	2.0×10^{-9}	8.69
Boric acid, $B(OH)_3H^{\dagger}$	1.4×10^{-5}	4.86	7.2×10^{-10}	9.14
Hydrocyanic acid, HCN	2.0×10^{-5}	4.69	4.9×10^{-10}	9.31
Phenol, C_6H_5OH	7.7×10^{-5}	4.11	1.3×10^{-10}	9.89
Hypoiodous acid, HIO	4.3×10^{-4}	3.36	2.3×10^{-11}	10.64
Weakest weak acids				
Weakest weak bases				
Urea, $CO(NH_2)_2$	1.3×10^{-14}	13.90	7.7×10^{-1}	0.10
Aniline, $C_6H_5NH_2$	4.3×10^{-10}	9.37	2.3×10^{-5}	4.63
Pyridine, C_5H_5N	1.8×10^{-9}	8.75	5.6×10^{-6}	5.35
Hydroxylamine, NH_2OH	1.1×10^{-8}	7.97	9.1×10^{-7}	6.03
Nicotine, $C_{10}H_{11}N_2$	1.0×10^{-6}	5.98	1.0×10^{-8}	8.02
Morphine, $C_{17}H_{19}O_3N$	1.6×10^{-6}	5.79	6.3×10^{-9}	8.21
Hydrazine, NH_2NH_2	1.7×10^{-6}	5.77	5.9×10^{-9}	8.23
Ammonia, NH_3	1.8×10^{-5}	4.75	5.6×10^{-10}	9.25
Trimethylamine, $(CH_3)_3N$	6.5×10^{-5}	4.19	1.5×10^{-10}	9.81
Methylamine, CH_3NH_2	3.6×10^{-4}	3.44	2.8×10^{-11}	10.56
Dimethylamine, $(CH_3)_2NH$	5.4×10^{-4}	3.27	1.9×10^{-11}	10.73
Ethylamine, $C_2H_5NH_2$	6.5×10^{-4}	3.19	1.5×10^{-11}	10.81
Triethylamine, $(C_2H_5)_3N$	1.0×10^{-3}	2.99	1.0×10^{-11}	11.01
Strongest weak bases				

*Values for polyprotic acids—those capable of donating more than one proton—refer to the first deprotonation.

†The proton transfer equilibrium is $B(OH)_3(aq) + 2\ H_2O(l) \rightleftharpoons H_3O^+(aq) + B(OH)_4^-(aq)$.

to the total concentration of hydronium ion in solution (see *Further information* 4.1). Autoprotolysis may be ignored if the weak acid is the main contributor of hydronium ions, a condition that is satisfied if the acid is not very weak and is present at not too low a concentration. Then we can estimate the pH of a solution of a weak acid and calculate either of these fractions by using the following strategy.

We organize the necessary work into a table with columns headed by the species and, in successive rows:

1. The initial molar concentrations of the species, ignoring any contributions to the concentration of H_3O^+ or OH^- from autoprotolysis of water
2. The changes in these quantities that must take place for the system to reach equilibrium
3. The resulting equilibrium values

Similar arguments apply to the estimation of the pH of a solution of a weak base. In most cases, we do not know the change that must occur for the system to reach equilibrium, so the change in the concentration is written as x and the reaction stoichiometry is used to write the corresponding changes in the other species. When the values at equilibrium (the last row of the table) are substituted into the expression for the equilibrium constant, we obtain an equation for x in terms of K. This equation can be solved for x, and hence the concentrations of all the species at equilibrium can be found. In general, solution of the equation for x results in several mathematically possible values of x. We select the chemically acceptable solution by considering the signs of the predicted concentrations: they must be positive.

EXAMPLE 4.5 Assessing the extent of deprotonation of a weak acid

Acetic acid lends a sour taste to vinegar and is produced by aerobic oxidation of ethanol by bacteria in fermented beverages, such as wine and cider:

$$CH_3CH_2OH(aq) + O_2(g) \longrightarrow CH_3COOH(aq) + H_2O(l)$$

Estimate the pH and the fraction of CH_3COOH molecules deprotonated in 0.15 M $CH_3COOH(aq)$.

Strategy The aim is to calculate the equilibrium composition of the solution. To do so, set up an equilibrium table with x as the change in molar concentration of H_3O^+ ions required to reach equilibrium. We ignore the tiny concentration of hydronium ions present in pure water. In this example, the equation for x is quadratic:

$$ax^2 + bx + c = 0 \quad \text{with the roots } x = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}$$

However, because we can anticipate that the extent of deprotonation is small (the acid is weak, $K_a << 1$), use the approximation that x is very small to simplify the equations. Once x has been found, calculate pH $= -\log x$. Confirm the accuracy of the calculation by substituting the calculated equilibrium concentrations into the expression for K_a to verify that the value so calculated is equal to the experimental value used in the calculation.

Solution We draw up the following equilibrium table:

Species	CH_3COOH	H_3O^+	$CH_3CO_2^-$
Initial concentration/(mol L^{-1})	0.15	0	0
Change to reach equilibrium/(mol L^{-1})	$-x$	$+x$	$+x$
Equilibrium concentration/(mol L^{-1})	$0.15 - x$	x	x

The value of x is found by inserting the equilibrium concentrations into the expression for the acidity constant:

$$K_a = \frac{[H_3O^+][CH_3CO_2^-]}{[CH_3COOH]} = \frac{x \times x}{0.15 - x}$$

We could arrange the expression into a quadratic equation. However, it is more instructive to make use of the smallness of x to replace $0.15 - x$ by 0.15 (this approximation is valid if $x << 0.15$). Then the simplified equation rearranges first to $0.15 \times K_a = x^2$ and then to

$$x = (0.15 \times K_a)^{1/2} = (0.15 \times 1.8 \times 10^{-5})^{1/2} = 1.6 \times 10^{-3}$$

where we have used $K_a = 1.8 \times 10^{-5}$ (Table 4.4). Therefore, pH = 2.80. Calculations of this kind are rarely accurate to more than one decimal place in the pH (and even that may be too optimistic) because the effects of ion-ion interactions have been ignored, so this answer would be reported as pH = 2.8. The fraction deprotonated, f, is

$$f = \frac{[CH_3CO_2^-]_{equilibrium}}{[CH_3COOH]_{added}} = \frac{x}{0.15} = \frac{1.6 \times 10^{-3}}{0.15} = 0.011$$

That is, only 1.1% of the acetic acid molecules have donated a proton.

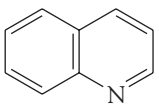
A note on good practice: When an approximation has been made, verify at the end of the calculation that the approximation is consistent with the result obtained. In this case, we assumed that $x << 0.15$ and have found that $x = 1.6 \times 10^{-3}$, which is consistent.

Another note on good practice: Acetic acid (ethanoic acid) is written CH_3COOH because the two O atoms are inequivalent; its conjugate base, the acetate ion (ethanoate ion), is written $CH_3CO_2^-$ because the two O atoms are now equivalent (by resonance).

SELF-TEST 4.12 Estimate the pH of 0.010 M $CH_3CH(OH)COOH(aq)$ (lactic acid) from the data in Table 4.4. Before carrying out the numerical calculation, decide whether you expect the pH to be higher or lower than that calculated for the same concentration of acetic acid.

Answer: 2.5 ■

The calculation of the pH of a solution of a base involves an additional step. The first step is to calculate the concentration of OH^- ions in the solution from the value of K_b by using the equilibrium-table technique and to express it as the pOH of the solution. The additional step is to convert that pOH into a pH by using the water autoprotolysis equilibrium, eqn 4.22, in the form $pH = pK_w - pOH$, with $pK_w = 14.00$ at 25°C.



11 Quinoline

SELF-TEST 4.13 The base quinoline (**11**) has $pK_b = 9.12$. Estimate the pH and the fraction of molecules protonated in an 0.010 M aqueous solution of quinoline.

Answer: 8.4; 1/3571

The ions present when a salt is added to water may themselves be either acids or bases and consequently affect the pH of the solution. For example, when ammonium chloride is added to water, it provides both an acid (NH_4^+) and a base (Cl^-). The solution consists of a weak acid (NH_4^+) and a very weak base (Cl^-). The net effect is that the solution is acidic. Similarly, a solution of sodium acetate consists of a neutral ion (the Na^+ ion) and a base ($CH_3CO_2^-$). The net effect is that the solution is basic, and its pH is greater than 7.

To estimate the pH of the solution, we proceed in exactly the same way as for the addition of a "conventional" acid or base, for in the Brønsted-Lowry theory, there is no distinction between "conventional" acids such as acetic acid and the conjugate acids of bases (such as NH_4^+). For example, to calculate the pH of 0.010 M NH_4Cl(aq) at 25°C, we proceed exactly as in *Example* 4.5, taking the initial concentration of the acid (NH_4^+) to be 0.010 mol L^{-1}. The K_a to use is the acidity constant of the acid NH_4^+, which is listed in Table 4.4. Alternatively, we use K_b for the conjugate base (NH_3) of the acid and convert that quantity to K_a by using eqn 4.21 ($K_aK_b = K_w$). We find pH = 5.63, which is on the acid side of neutral. Exactly the same procedure is used to find the pH of a solution of a salt of a weak acid, such as sodium acetate. The equilibrium table is set up by treating the anion $CH_3CO_2^-$ as a base (which it is) and using for K_b the value obtained from the value of K_a for its conjugate acid (CH_3COOH).

SELF-TEST 4.14 Estimate the pH of 0.0025 M $NH(CH_3)_3Cl$(aq) at 25°C.

Answer: 6.2

4.11 Polyprotic acids

Many biological macromolecules, such as the nucleic acids, contain multiple proton donor sites, and we need to see how to handle this complication quantitatively.

A **polyprotic acid** is a molecular compound that can donate more than one proton. Two examples are sulfuric acid, H_2SO_4, which can donate up to two protons, and phosphoric acid, H_3PO_4, which can donate up to three. A polyprotic acid is best considered to be a molecular species that can give rise to a series of Brønsted acids as it donates its succession of protons. Thus, sulfuric acid is the parent of two Brønsted acids, H_2SO_4 itself and HSO_4^-, and phosphoric acid is the parent of three Brønsted acids, namely H_3PO_4, $H_2PO_4^-$, and HPO_4^{2-}.

For a species H_2A with two acidic protons (such as H_2SO_4), the successive equilibria we need to consider are

$$H_2A(aq) + H_2O(l) \rightleftharpoons H_3O^+(aq) + HA^-(aq) \qquad K_{a1} = \frac{a_{H_3O^+}a_{HA^-}}{a_{H_2A}}$$

$$HA^-(aq) + H_2O(l) \rightleftharpoons H_3O^+(aq) + A^{2-}(aq) \qquad K_{a2} = \frac{a_{H_3O^+}a_{A^{2-}}}{a_{HA^-}}$$

In the first of these equilibria, HA^- is the conjugate base of H_2A. In the second, HA^- acts as the acid and A^{2-} is its conjugate base. Values are given in Table 4.5. In all cases, K_{a2} is smaller than K_{a1}, typically by three orders of magnitude for small molecular species, because the second proton is more difficult to remove, partly on account

Table 4.5 Successive acidity constants of polyprotic acids at 298.15 K

Acid	K_{a1}	pK_{a1}	K_{a2}	pK_{a2}	K_{a3}	pK_{a3}
Carbonic acid, H_2CO_3	4.3×10^{-7}	6.37	5.6×10^{-11}	10.25		
Hydrosulfuric acid, H_2S	1.3×10^{-7}	6.88	7.1×10^{-15}	14.15		
Oxalic acid, $(COOH)_2$	5.9×10^{-2}	1.23	6.5×10^{-5}	4.19		
Phosphoric acid, H_3PO_4	7.6×10^{-3}	2.12	6.2×10^{-8}	7.21	2.1×10^{-13}	12.67
Phosphorous acid, H_2PO_3	1.0×10^{-2}	2.00	2.6×10^{-7}	6.59		
Sulfuric acid, H_2SO_4	Strong		1.2×10^{-2}	1.92		
Sulfurous acid, H_2SO_3	1.5×10^{-2}	1.81	1.2×10^{-7}	6.91		
Tartaric acid, $C_2H_4O_2(COOH)_2$	6.0×10^{-4}	3.22	1.5×10^{-5}	4.82		

of the negative charge on HA^-. Enzymes are polyprotic acids, for they possess many protons that can be donated to a substrate molecule or to the surrounding aqueous medium of the cell. For them, successive acidity constants vary much less because the molecules are so large that the loss of a proton from one part of the molecule has little effect on the ease with which another some distance away may be lost.

EXAMPLE 4.6 Calculating the concentration of carbonate ion in carbonic acid

Groundwater contains dissolved carbon dioxide, carbonic acid, hydrogencarbonate ions, and a very low concentration of carbonate ions. Estimate the molar concentration of CO_3^{2-} ions in a solution in which water and $CO_2(g)$ are in equilibrium. We must be very cautious in the interpretation of calculations involving carbonic acid because equilibrium between dissolved CO_2 and H_2CO_3 is achieved only very slowly. In organisms, attainment of equilibrium is facilitated by the enzyme carbonic anhydrase.

Strategy We start with the equilibrium that produces the ion of interest (such as A^{2-}) and write its activity in terms of the acidity constant for its formation (K_{a2}). That expression will contain the activity of the conjugate acid (HA^-), which we can express in terms of the activity of *its* conjugate acid (H_2A) by using the appropriate acidity constant (K_{a1}). This equilibrium dominates all the rest provided the molecule is small and there are marked differences between its acidity constants, so it may be possible to make an approximation at this stage.

Solution The CO_3^{2-} ion, the conjugate base of the acid HCO_3^- is produced in the equilibrium

$$HCO_3^-(aq) + H_2O(l) \rightleftharpoons H_3O^+(aq) + CO_3^{2-}(aq) \qquad K_{a2} = \frac{a_{H_3O^+}a_{CO_3^{2-}}}{a_{HCO_3^-}}$$

Hence,

$$a_{CO_3^{2-}} = \frac{a_{HCO_3^-}K_{a2}}{a_{H_3O^+}}$$

The HCO_3^- ions are produced in the equilibrium

$$H_2CO_3(aq) + H_2O(l) \rightleftharpoons H_3O^+(aq) + HCO_3^-(aq)$$

One H_3O^+ ion is produced for each HCO_3^- ion produced. These two concentrations are not exactly the same, because a little HCO_3^- is lost in the second deprotonation and the amount of H_3O^+ has been increased by it. Also, HCO_3^- is a weak base and abstracts a proton from water to generate H_2CO_3 (see Section 4.12). However, those secondary changes can safely be ignored in an approximate calculation. Because the molar concentrations of HCO_3^- and H_3O^+ are approximately the same, we can suppose that their activities are also approximately the same and set $a_{HCO_3^-} \approx a_{H_3O^+}$. When this equality is substituted into the expression for $a_{CO_3^{2-}}$, we obtain

$$[CO_3^{2-}] \approx K_{a2}$$

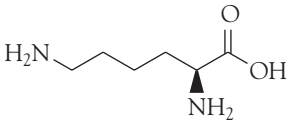
Because we know from Table 4.5 that $pK_{a2} = 10.25$, it follows that $[CO_3^{2-}] = 5.6 \times 10^{-11}$ and therefore that the molar concentration of CO_3^{2-} ions is 5.6×10^{-11} mol L^{-1}.

SELF-TEST 4.15 Calculate the molar concentration of S^{2-} ions in H_2S(aq).

Answer: 7.1×10^{-15} mol L^{-1} ■

CASE STUDY 4.3 The fractional composition of a solution of lysine

The amino acid lysine (Lys, **12**) can accept two protons on its nitrogen atoms and donate one from its carboxyl group. Let's see how the composition of an aqueous solution that contains 0.010 mol L^{-1} of lysine varies with pH. The pK_a values of amino acids are given in Table 4.6.



12 Lysine (Lys)

We expect the fully protonated species (H_3Lys^{2+}) at low pH, the partially protonated species (H_2Lys^+ and HLys) at intermediate pH, and the fully deprotonated species (Lys^-) at high pH. The three acidity constants (using the notation in Table 4.6) are

$$H_3Lys^{2+}(aq) + H_2O(l) \rightleftharpoons H_3O^+(aq) + H_2Lys^+(aq)$$
$$K_{a1} = \frac{[H_3O^+][H_2Lys^+]}{[H_3Lys^{2+}]} = \frac{H[H_2Lys^+]}{[H_3Lys^{2+}]}$$

$$H_2Lys^+(aq) + H_2O(l) \rightleftharpoons H_3O^+(aq) + HLys(aq)$$
$$K_{a2} = \frac{[H_3O^+][HLys]}{[H_2Lys^+]} = \frac{H[HLys]}{[H_2Lys^+]}$$

$$HLys(aq) + H_2O(l) \rightleftharpoons H_3O^+(aq) + Lys^-(aq)$$
$$K_{a3} = \frac{[H_3O^+][Lys^-]}{[HLys]} = \frac{H[Lys^-]}{[HLys]}$$

where, for the sake of simplifying the forms of the expressions, we have set $[H_3O]^+$ equal to H. We also know that the total concentration of lysine in all its forms is

$$[H_3Lys^{2+}] + [H_2Lys^+] + [HLys] + [Lys^-] = L$$

Table 4.6 Acidity constants of amino acids at 298.15 K*

Acid	pK_{a1}	pK_{a2}	pK_{a3}
Ala	2.33	9.71	
Arg	2.03	9.00	12.10
Asn	2.16	8.73	
Asp	1.95	3.71	9.66
Cys	1.91	8.14	10.28
Gln	2.18	9.00	
Glu	2.16	4.15	9.58
Gly	2.34	9.58	
His	1.70	6.04	9.09
Ile	2.26	9.60	
Leu	2.32	9.58	
Lys	2.15	9.16	10.67
Met	2.16	9.08	
Phe	2.18	9.09	
Pro	1.95	10.47	
Ser	2.13	9.05	
Thr	2.20	9.96	
Trp	2.38	9.34	
Tyr	2.24	9.04	10.10
Val	2.27	9.52	

*For the identities of the acids, see the *Data section*. The acidity constants refer, respectively, to the most highly protonated form, the next most, and so on. So the values for Lys, for instance, refer to H_3Lys^{2+}, H_2Lys^{+}, and HLys (the electrically neutral molecule).

We now have four equations for four unknown concentrations. To solve the equations, we proceed systematically, using K_{a3} to express $[Lys^-]$ in terms of [HLys], then K_{a2} to express [HLys] in terms of $[H_2Lys^+]$, and so on:

$$[Lys^-] = \frac{K_{a3}[HLys]}{H} = \frac{K_{a3}K_{a2}[H_2Lys^+]}{H^2} = \frac{K_{a3}K_{a2}K_{a1}[H_3Lys^{2+}]}{H^3}$$

$$[HLys] = \frac{K_{a2}[H_2Lys^+]}{H} = \frac{K_{a2}K_{a1}[H_3Lys^{2+}]}{H^2}$$

$$[H_2Lys^+] = \frac{K_{a1}[H_3Lys^{2+}]}{H}$$

Then the expression for the total concentration can be written in terms of $[H_3Lys^{2+}]$, H, and L. If we write

$$K = H^3 + H^2K_{a1} + HK_{a1}K_{a2} + K_{a1}K_{a2}K_{a3}$$

then it follows that

$$L = \frac{K}{H^3}\,[H_3Lys^{2+}]$$

and the fractions of each species present in the solution are

$$f(H_3Lys^{2+}) = \frac{[H_3Lys^{2+}]}{L} = \frac{H^3}{K}$$

$$f(H_2Lys^{+}) = \frac{[H_2Lys^{+}]}{L} = \frac{H^2K_{a1}}{K}$$

$$f(HLys) = \frac{[HLys]}{L} = \frac{HK_{a2}K_{a1}}{K}$$

$$f(Lys^{-}) = \frac{[HLys^{-}]}{L} = \frac{K_{a3}K_{a2}K_{a1}}{K}$$

These fractions are plotted against pH $= -\log H$ in Fig. 4.12. Note how H_3Lys^{2+} is dominant for pH $<$ pK_{a1}, that H_3Lys^{2+} and H_2Lys^{+} have the same concentration at pH = pK_{a1}, and that H_2Lys^{+} is dominant for pH $>$ pK_{a1}, until HLys becomes dominant, and so on. In a neutral solution at pH = 7, the dominant species is H_2Lys^{+}, for pH = 7 lies between pK_{a1} and pK_{a2}: below pK_{a1}, H_3Lys^{2+} is dominant and above pK_{a2}, HLys is dominant.

A note on good practice: Take note of the symmetry of the expressions derived here. By doing so, it is easy to write down the corresponding expressions for species with different numbers of acidic protons without repeating the lengthy calculation. ■

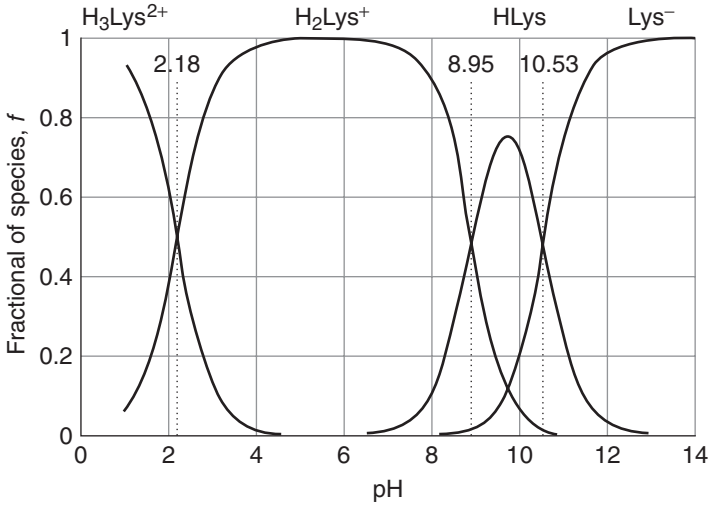
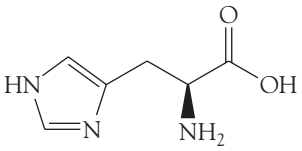


Fig. 4.12 The fractional composition of the protonated and deprotonated forms of lysine (Lys) in aqueous solution as a function of pH. Note that conjugate pairs are present at equal concentrations when the pH is equal to the pK_a of the acid member of the pair.



13 Histidine (His)

SELF-TEST 4.16 Construct the diagram for the fraction of protonated species in an aqueous solution of histidine (**13**).

Answer: Fig. 4.13

We can summarize the behavior discussed in *Case study* 4.3 and illustrated in Figs. 4.12 and 4.13 as follows. Consider each conjugate acid-base pair, with acidity constant K_a; then:

The acid form is dominant for $pH < pK_a$

The conjugate pair have equal concentrations at $pH = pK_a$

The base form is dominant for $pH > pK_a$

In each case, the other possible forms of a polyprotic system can be ignored, provided the pK_a values are not too close together.

4.12 Amphiprotic systems

Many molecules of biochemical significance (including the amino acids) can act as both proton donors and proton acceptors, and we need to be able to treat this dual function quantitatively.

An **amphiprotic** species is a molecule or ion that can both accept and donate protons. For instance, HCO_3^- can act as an acid (to form CO_3^{2-}) and as a base (to form H_2CO_3). Among the most important amphiprotic compounds are the amino acids, which can act as proton donors by virtue of their carboxyl groups and as bases by virtue of their amino groups. Indeed, in solution, amino acids are present largely in their **zwitterionic** ("double ion") form, in which the amino group is protonated and the carboxyl group is deprotonated: the acidic proton of the carboxyl group has been donated to the basic amino group (but not necessarily of the same molecule). The zwitterionic form of glycine, NH_2CH_2COOH, for instance, is $^+H_3NCH_2CO_2^-$.

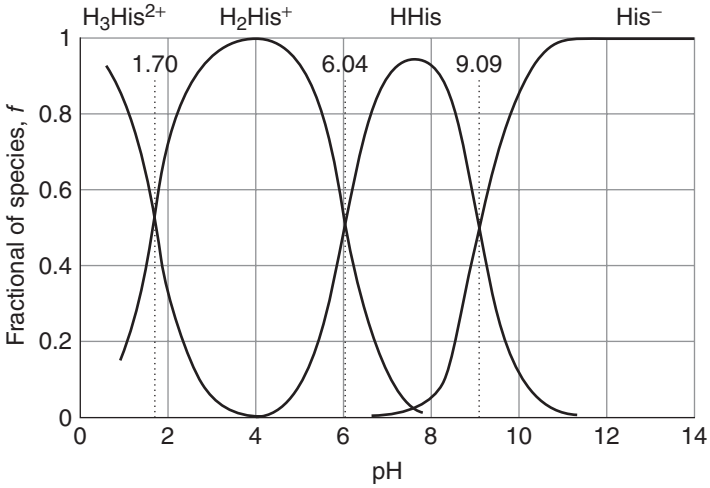


Fig. 4.13 The fractional composition of the protonated and deprotonated forms of histidine (His) in aqueous solution as a function of pH.

We can suppose that in an aqueous solution of glycine, the species present are NH_2CH_2COOH (and in general BAH, where B represents the basic amino group and AH the carboxylic acid group), $NH_2CH_2CO_2^-$ (BA^-), $^+NH_3CH_2COOH$ (^+HBAH), and the zwitterion $^+NH_3CH_2CO_2^-$ ($^+HBA^-$). The proton transfer equilibria in water are

$$\begin{aligned}
BAH(aq) + H_2O(l) &\rightleftharpoons H_3O^+(aq) + BA^-(aq) && K_1\\
{}^+HBAH(aq) + H_2O(l) &\rightleftharpoons H_3O^+(aq) + BAH(aq) && K_2\\
{}^+HBA^-(aq) + H_2O(l) &\rightleftharpoons H_3O^+(aq) + BA^-(aq) && K_3
\end{aligned}$$

By following the same procedure as in *Case study* 4.3, we find the following expressions for the composition of the solution, with $H = [H_3O^+]$:

$$f(BA^-) = \frac{K_1K_2K_3}{K} \qquad f(BAH) = \frac{HK_2K_3}{K}$$
$$f(^+HBA^-) = \frac{HK_1K_2}{K} \qquad f(^+HBAH) = \frac{H^2K_3}{K} \tag{4.25}$$

with $K = H^2K_3 + H(K_1 + K_3)K_2 + K_1K_2K_3$. The variation of composition with pH is shown in Fig. 4.14. Because we can expect the zwitterion to be a much weaker acid than the neutral molecule (because the negative charge on the carboxylate group hinders the escape of the proton from the conjugate acid of the amino group), we can anticipate that $K_3 << K_1$ and therefore that $f(BAH) << f(^+HBA^-)$ at all values of pH.

The further question we need to tackle is the pH of a solution of a salt with an amphiprotic anion, such as a solution of $NaHCO_3$. Is the solution acidic on

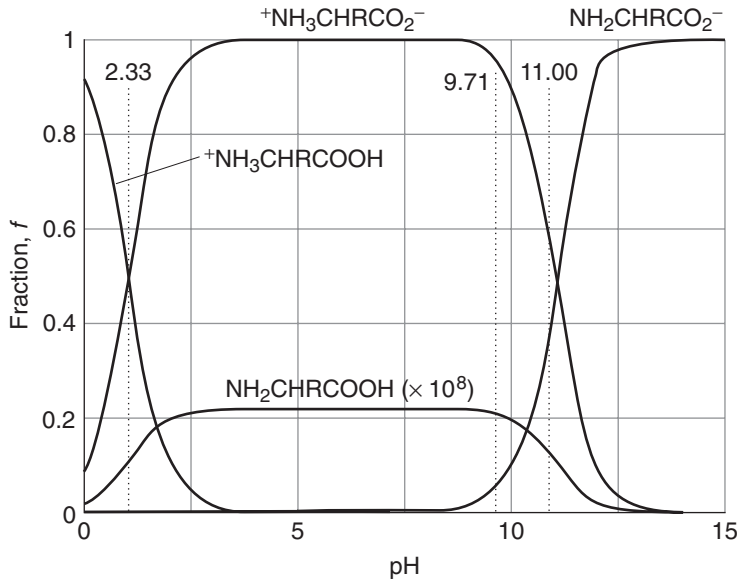


Fig. 4.14 The fractional composition of the protonated and deprotonated forms of an amino acid $NH_2CHRCOOH$, in which the group R does not participate in proton transfer reactions.

account of the acid character of HCO_3^-, or is it basic on account of the anion's basic character? As we show in the following *Derivation*, the pH of such a solution is given by

$$pH = \tfrac{1}{2}(pK_{a1} + pK_{a2}) \qquad \textbf{(4.26)}$$

DERIVATION 4.2 The pH of an amphiprotic salt solution

Let's suppose that we make up a solution of the salt MHA, where HA^- is the amphiprotic anion (such as HCO_3^-) and M^+ is a cation (such as Na^+). To reach equilibrium, in which HA^-, A^{2-}, and H_2A are all present, some HA^- (we write it x) is protonated to form H_2A and some HA^- (this we write y) deprotonates to form A^{2-}. The equilibrium table is as follows:

Species	H_2A	HA^-	A^{2-}	H_3O^+
Initial molar concentration/(mol L^{-1})	0	A	0	0
Change to reach equilibrium/(mol L^{-1})	$+x$	$-(x + y)$	$+y$	$+(y - x)$
Equilibrium concentration/(mol L^{-1})	x	$A - x - y$	y	$y - x$

The two acidity constants are

$$K_{a1} = \frac{[H_3O^+][HA^-]}{[H_2A]} = \frac{(y - x)(A - x - y)}{x}$$

$$K_{a2} = \frac{[H_3O^+][A^{2-}]}{[HA^-]} = \frac{(y - x)y}{A - x - y}$$

Multiplication of these two expressions, noting from the equilibrium table that at equilibrium $y - x$ is just $[H_3O^+]$, gives

$$K_{a1}K_{a2} = \frac{(y - x)^2 y}{x} = [H_3O^+]^2 \times \frac{y}{x}$$

Next, we show that, to a good approximation, $y/x \approx 1$ and therefore that $[H_3O^+] = (K_{a1}K_{a2})^{1/2}$. For this step we expand K_{a1} as follows:

$$xK_{a1} = Ay - y^2 - Ax + x^2$$

Because xK_{a1}, x^2, and y^2 are all very small compared with terms that have A in them, this expression reduces to

$$0 \approx Ay - Ax$$

We conclude that $x \approx y$ and therefore that $y/x \approx 1$, as required. Equation 4.26 now follows by taking the negative common logarithm of both sides of $[H_3O^+] = (K_{a1}K_{a2})^{1/2}$.

As an application of eqn 4.26, consider the pH of an aqueous solution of sodium hydrogencarbonate. Using values from Table 4.5, we can immediately conclude that the pH of the solution *of any concentration* is

$$pH = \tfrac{1}{2}(6.37 + 10.25) = 8.31$$

The solution is basic. We can treat a solution of potassium dihydrogenphosphate in the same way, taking into account only the second and third acidity constants of H_3PO_4 because protonation as far as H_3PO_4 is negligible (see Table 4.5):

$$pH = \tfrac{1}{2}(7.21 + 12.67) = 9.94$$

4.13 Buffer solutions

Cells cease to function and may be damaged irreparably if the pH changes significantly, so we need to understand how the pH is stabilized by a buffer.

Suppose that we make an aqueous solution by dissolving known amounts of a weak acid and its conjugate base. To calculate the pH of this solution, we make use of the expression for K_a of the weak acid and write

$$K_a = \frac{a_{H_3O^+}a_{base}}{a_{acid}} \approx \frac{a_{H_3O^+}[base]}{[acid]}$$

which rearranges first to

$$a_{H_3O^+} \approx \frac{K_a[acid]}{[base]}$$

and then, by taking negative common logarithms, to the **Henderson-Hasselbalch equation**:

$$pH \approx pK_a - \log\frac{[acid]}{[base]} \qquad \textbf{(4.27)}$$

When the concentrations of the conjugate acid and base are equal, the second term on the right of eqn 4.27 is $\log 1 = 0$, so under these conditions $pH = pK_a$.

ILLUSTRATION 4.3 Using the Henderson-Hasselbalch equation

To calculate the pH of a solution formed from equal amounts of $CH_3COOH(aq)$ and $NaCH_3CO_2(aq)$, we note that the latter dissociates (in the sense of separating into ions) fully in water, yielding the ions $Na^+(aq)$ and $CH_3CO_2^-(aq)$, the conjugate base of $CH_3COOH(aq)$. The equilibrium of interest is

$$CH_3COOH(aq) + H_2O(l) \rightleftharpoons H_3O^+(aq) + CH_3CO_2^-(aq)$$

$$K_a = \frac{[H_3O^+][CH_3CO_2^-]}{[CH_3COOH]}$$

Because the pK_a of $CH_3COOH(aq)$ is 4.75 (Table 4.4), it follows from eqn 4.27 that pH = 4.8 (more realistically, pH = 5). ■

SELF-TEST 4.17 Calculate the pH of an aqueous buffer solution that contains equal amounts of NH_3 and NH_4Cl.

Answer: 9.25; more realistically: 9

It is observed that solutions containing known amounts of an acid and that acid's conjugate base show **buffer action**, the ability of a solution to oppose changes in pH when small amounts of strong acids and bases are added. An **acid buffer** solution, one that stabilizes the solution at a pH below 7, is typically prepared by making a solution of a weak acid (such as acetic acid) and a salt that supplies its conjugate base (such as sodium acetate). A **base buffer**, one that stabilizes a solution at a pH above 7, is prepared by making a solution of a weak base (such as ammonia) and a salt that supplies its conjugate acid (such as ammonium chloride). Physiological buffers are responsible for maintaining the pH of blood within a narrow range of 7.37 to 7.43, thereby stabilizing the active conformations of biological macromolecules and optimizing the rates of biochemical reactions.

An acid buffer stabilizes the pH of a solution because the abundant supply of A^- ions (from the salt) can remove any H_3O^+ ions brought by additional acid; furthermore, the abundant supply of HA molecules (from the acid component of the buffer) can provide H_3O^+ ions to react with any base that is added. Similarly, in a base buffer the weak base B can accept protons when an acid is added and its conjugate acid BH^+ can supply protons if a base is added. The following example explores the quantitative basis of buffer action.

EXAMPLE 4.7 Assessing buffer action

Estimate the effect of addition of 0.020 mol of hydronium ions (from a solution of a strong acid, such as hydrochloric acid) on the pH of 1.0 L of (a) 0.15 M $CH_3COOH(aq)$ and (b) a buffer solution containing 0.15 M $CH_3COOH(aq)$ and 0.15 M $NaCH_3CO_2(aq)$.

Strategy Before addition of hydronium ions, the pHs of solutions (a) and (b) are 2.8 (*Example* 4.5) and 4.8 (*Illustration* 4.3). After addition to solution (a) the initial molar concentration of $CH_3COOH(aq)$ is 0.15 M and that of $H_3O^+(aq)$ is (0.020 mol)/(1.0 L) = 0.020 M. After addition to solution (b), the initial molar concentrations of $CH_3COOH(aq)$, $CH_3CO_2^-(aq)$, and $H_3O^+(aq)$ are 0.15 M, 0.15 M, and 0.020 M, respectively. The weak base already present in solution, $CH_3CO_2^-(aq)$, reacts immediately with the added hydronium ion:

$$CH_3CO_2^-(aq) + H_3O^+(aq) \longrightarrow CH_3COOH(aq) + H_2O(l)$$

We use the adjusted concentrations of $CH_3COOH(aq)$ and $CH_3CO_2^-(aq)$ and eqn 4.27 to calculate a new value of the pH of the buffer solution.

Solution For addition of a strong acid to solution (a), we draw up the following equilibrium table to show the effect of the addition of hydronium ions:

Species	CH_3COOH	H_3O^+	$CH_3CO_2^-$
Initial concentration/(mol L^{-1})	0.15	0.02	0
Change to reach equilibrium/(mol L^{-1})	$-x$	$+x$	$+x$
Equilibrium concentration/(mol L^{-1})	$0.15 - x$	$0.020 + x$	x

The value of x is found by inserting the equilibrium concentrations into the expression for the acidity constant:

$$K_a = \frac{[H_3O^+][CH_3CO_2^-]}{[CH_3COOH]} = \frac{(0.020 + x) \times x}{0.15 - x}$$

As in *Example* 4.5, we assume that x is very small; in this case $x << 0.020$, and write

$$K_a \approx \frac{0.020 \times x}{0.15}$$

Then

$$x = (0.15/0.020) \times K_a = 7.5 \times 1.8 \times 10^{-5} = 1.4 \times 10^{-4}$$

We see that our approximation is valid and, therefore, $[H_3O^+] = 0.020 + x \approx 0.020$ and pH = 1.7. It follows that the pH of the unbuffered solution (a) changes dramatically from 4.8 to 1.7 upon addition of 0.020 M $H_3O^+(aq)$.

Now we consider the addition of 0.020 M $H_3O^+(aq)$ to solution (b). Reaction between the strong acid and weak base consumes the added hydronium ions and changes the concentration of $CH_3CO_2^-(aq)$ to 0.13 M and the concentration of $CH_3COOH(aq)$ to 0.17 M. It follows from eqn 4.27 that

$$pH = pK_a - \log \frac{[CH_3COOH]}{[CH_3CO_2^-]} = 4.75 - \log \frac{0.17}{0.13} = 4.6$$

The pH of the buffer solution (b) changes only slightly from 4.8 to 4.6 upon addition of 0.020 M $H_3O^+(aq)$.

SELF-TEST 4.18 Estimate the change in pH of solution (b) from *Example* 4.7 after addition of 0.020 mol of $OH^-(aq)$.

Answer: 4.9 ■

CASE STUDY 4.4 Buffer action in blood

The pH of blood in a healthy human being varies from 7.37 to 7.43. There are two buffer systems that help maintain the pH of blood relatively constant: one arising from a carbonic acid/bicarbonate (hydrogencarbonate) ion equilibrium and another involving protonated and deprotonated forms of hemoglobin, the protein responsible for the transport of O_2 in blood (*Case study* 4.1).

Carbonic acid forms in blood from the reaction between water and CO_2 gas, which comes from inhaled air and is also a by-product of metabolism (Section 4.8):

$$CO_2(g) + H_2O(l) \rightleftharpoons H_2CO_3(aq)$$

In red blood cells, this reaction is catalyzed by the enzyme carbonic anhydrase. Aqueous carbonic acid then deprotonates to form bicarbonate (hydrogencarbonate) ion:

$$H_2CO_3(aq) \rightleftharpoons H^+(aq) + HCO_3^-(aq)$$

The fact that the pH of normal blood is approximately 7.4 implies that $[HCO_3^-]/[H_2CO_3] \approx 20$. The body's control of the pH of blood is an example of **homeostasis**, the ability of an organism to counteract environmental changes with

physiological responses. For instance, the concentration of carbonic acid can be controlled by respiration: exhaling air depletes the system of $CO_2(g)$ and $H_2CO_3(aq)$ so the pH of blood rises when air is exhaled. Conversely, inhalation increases the concentration of carbonic acid in blood and lowers its pH. The kidneys also play a role in the control of the concentration of hydronium ions. There, ammonia formed by the release of nitrogen from some amino acids (such as glutamine) combines with excess hydronium ions and the ammonium ion is excreted through urine.

The condition known as *alkalosis* occurs when the pH of blood rises above about 7.45. *Respiratory alkalosis* is caused by hyperventilation, or excessive respiration. The simplest remedy consists of breathing into a paper bag in order to increase the levels of inhaled CO_2. *Metabolic alkalosis* may result from illness, poisoning, repeated vomiting, and overuse of diuretics. The body may compensate for the increase in the pH of blood by decreasing the rate of respiration.

Acidosis occurs when the pH of blood falls below about 7.35. In *respiratory acidosis*, impaired respiration increases the concentration of dissolved CO_2 and lowers the blood's pH. The condition is common in victims of smoke inhalation and patients with asthma, pneumonia, and emphysema. The most efficient treatment consists of placing the patient in a ventilator. *Metabolic acidosis* is caused by the release of large amounts of lactic acid or other acidic by-products of metabolism (Section 4.8), which react with bicarbonate ion to form carbonic acid, thus lowering the blood's pH. The condition is common in patients with diabetes and severe burns.

The concentration of hydronium ion in blood is also controlled by hemoglobin, which can exist in deprotonated (basic) or protonated (acidic) forms, depending on the state of protonation of several histidines (**13**) on the protein's surface (see Fig. 4.13 for a diagram of the fraction of protonated species in an aqueous solution of histidine). The carbonic acid/bicarbonate ion equilibrium and proton equilibria in hemoglobin also regulate the oxygenation of blood. The key to this regulatory mechanism is the **Bohr effect**, the observation that hemoglobin binds O_2 strongly when it is deprotonated and releases O_2 when it is protonated. It follows that when dissolved CO_2 levels are high and the pH of blood falls slightly, hemoglobin becomes protonated and releases bound O_2 to tissue. Conversely, when CO_2 is exhaled and the pH rises slightly, hemoglobin becomes deprotonated and binds O_2. ■

Checklist of Key Ideas

You should now be familiar with the following concepts:

- ☐ **1.** The reaction Gibbs energy, $\Delta_r G$, is the slope of a plot of Gibbs energy against composition.
- ☐ **2.** The condition of chemical equilibrium at constant temperature and pressure is $\Delta_r G = 0$.
- ☐ **3.** The reaction Gibbs energy is related to the composition by $\Delta_r G = \Delta_r G^\ominus + RT \ln Q$, where Q is the reaction quotient.
- ☐ **4.** The standard reaction Gibbs energy is the difference of the standard Gibbs energies of formation of the products and reactants weighted by the stoichiometric coefficients in the chemical equation $\Delta_r G^\ominus = \sum \nu \Delta_f G^\ominus(\text{products}) - \sum \nu \Delta_f G^\ominus(\text{reactants})$.
- ☐ **5.** The equilibrium constant is the value of the reaction quotient at equilibrium; it is related to the standard Gibbs energy of reaction by $\Delta_r G^\ominus = -RT \ln K$.
- ☐ **6.** A compound is thermodynamically stable with respect to its elements if $\Delta_f G^\ominus < 0$.
- ☐ **7.** The equilibrium constant of a reaction is independent of the presence of a catalyst.

- □ **8.** The variation of an equilibrium constant with temperature is expressed by the van 't Hoff equation, $\ln K' - \ln K = (\Delta_r H^\ominus/R)\{(1/T) - (1/T')\}$.
- □ **9.** The equilibrium constant K increases with temperature if $\Delta_r H^\ominus > 0$ (an endothermic reaction) and decreases if $\Delta_r H^\ominus < 0$ (an exothermic reaction).
- □ **10.** An endergonic reaction has a positive Gibbs energy; an exergonic reaction has a negative Gibbs energy.
- □ **11.** The biological standard state corresponds to pH = 7; the biological and thermodynamic standard reaction Gibbs energies of the reaction Reactants + ν $H_3O^+(aq) \longrightarrow$ products are related by $\Delta_r G^\oplus = \Delta_r G^\ominus + 7\nu RT \ln 10$.
- □ **12.** An endergonic reaction may be driven forward by coupling it to an exergonic reaction.
- □ **13.** The strength of an acid HA is reported in terms of its acidity constant, $K_a = a_{H_3O^+}a_{A^-}/a_{HA}$, and that of a base B in terms of its basicity constant, $K_b = a_{BH^+}a_{OH^-}/a_B$.
- □ **14.** The autoprotolysis constant of water is $K_w = a_{H_3O^+}a_{OH^-}$; this relation implies that pH + pOH = pK_w.
- □ **15.** The basicity constant of a base is related to the acidity constant of its conjugate acid by $K_aK_b = K_w$ (or pK_a + pK_b = pK_w).
- □ **16.** The acid form of a species is dominant if pH < pK_a, and the base form is dominant if pH > pK_a.
- □ **17.** The pH of the solution of an amphiprotic salt is pH = ½(pK_{a1} + pK_{a2}).
- □ **18.** The pH of a mixed solution of a weak acid and its conjugate base is given by the Henderson-Hasselbalch equation, pH = pK_a − log([acid]/[base]).
- □ **19.** The pH of a buffer solution containing equal concentrations of a weak acid and its conjugate base is pH = pK_a.

Further information 4.1 The complete expression for the pH of a solution of a weak acid

Some acids are so weak and undergo so little deprotonation that the autoprotolysis of water can contribute significantly to the pH. We must also take autoprotolysis into account when we find by using the procedures in *Example* 4.5 that the pH of a solution of a weak acid is greater than 6.

We begin the calculation by noting that, apart from water, there are four species in solution, HA, A^-, H_3O^+, and OH^-. Because there are four unknown quantities, we need four equations to solve the problem. Two of the equations are the expressions for K_a and K_w (eqns 4.17 and 4.20), written here in terms of molar concentrations:

$$K_a = \frac{[H_3O^+][A^-]}{[HA]} \quad \textbf{(4.28)}$$

$$K_w = [H_3O^+][OH^-] \quad \textbf{(4.29)}$$

A third equation takes **charge balance**, the requirement that the solution be electrically neutral, into account. That is, the sum of the concentrations of the cations must be equal to the sum of the concentrations of the anions. In our case, the charge balance equation is

$$[H_3O^+] = [OH^-] + [A^-] \quad \textbf{(4.30)}$$

We also know that the total concentration of A groups in all forms in which they occur, which we denote as A, must be equal to the initial concentration of the weak acid. This condition, known as **material balance**, gives our final equation:

$$A = [HA] + [A^-] \quad \textbf{(4.31)}$$

Now we are ready to proceed with a calculation of the hydronium ion concentration in the solution. First, we combine eqns 4.29 and 4.31 and write

$$[A^-] = [H_3O^+] - \frac{K_w}{[H_3O^+]} \quad \textbf{(4.32)}$$

We continue by substituting this expression into eqn 4.31 and solving for [HA]:

$$[HA] = A - [H_3O^+] + \frac{K_w}{[H_3O^+]} \quad \textbf{(4.33)}$$

Upon substituting the expressions for $[A^-]$ (eqn 4.32) and HA (eqn 4.33) into eqn 4.28, we obtain

$$K_a = \frac{[H_3O^+]\left([H_3O^+] - \dfrac{K_w}{[H_3O^+]}\right)}{A - [H_3O^+] + \dfrac{K_w}{[H_3O^+]}} \quad \textbf{(4.34)}$$

Rearrangement of this expression gives

$$[H_3O^+]^3 + K_a[H_3O^+]^2 - (K_w + K_aA)[H_3O^+] - K_aK_w = 0 \quad \textbf{(4.35)}$$

and we see that $[H_3O^+]$ is determined by solving this cubic equation, a task that is best accomplished with a calculator or mathematical software.

There are several experimental conditions that allow us to simplify eqn 4.34. For example, when $[H_3O^+] > 10^{-6}$ M (or pH < 6), $K_w/[H_3O^+] < 10^{-8}$ M and we can ignore this term in eqn 4.34. The resulting expression is

$$K_a = \frac{[H_3O^+]^2}{A - [H_3O^+]} \quad \textbf{(4.36)}$$

Rearrangement of eqn 4.36 gives a quadratic equation:

$$[H_3O^+]^2 + K_a[H_3O^+] - K_aA = 0 \quad \textbf{(4.37)}$$

which can be solved for $[H_3O^+]$. If the extent of deprotonation is very small, we let $[H_3O^+] \ll A$ and write

$$K_a = \frac{[H_3O^+]^2}{A} \quad \textbf{(4.38a)}$$

$$[H_3O^+] = (K_aA)^{1/2} \quad \textbf{(4.38b)}$$

Equations 4.36 and 4.38 are similar to the expressions used in *Example* 4.5, where we set $[H_3O^+]$ equal to x.

Discussion questions

4.1 Explain how the mixing of reactants and products affects the position of chemical equilibrium.

4.2 Explain how a reaction that is not spontaneous may be driven forward by coupling to a spontaneous reaction.

4.3 At blood temperature, $\Delta_rG^\oplus = -218$ kJ mol^{-1} and $\Delta_rH^\oplus = -120$ kJ mol^{-1} for the production of lactate ion during glycolysis. Provide a molecular interpretation for the observation that the reaction is more exergonic than it is exothermic.

4.4 Explain Le Chatelier's principle in terms of thermodynamic quantities.

4.5 Describe the basis of buffer action.

4.6 State the limits to the generality of the following expressions: **(a)** $pH = \frac{1}{2}(pK_{a1} + pK_{a2})$, **(b)** $pH = pK_a - \log([acid]/[base])$, and **(c)** the van 't Hoff equation, written as

$$\ln K' - \ln K = \frac{\Delta_rH^\ominus}{R}\left(\frac{1}{T} - \frac{1}{T'}\right)$$

Exercises

4.7 Write the expressions for the equilibrium constants for the following reactions, making the approximation of replacing activities by molar concentrations or partial pressures:

(a) G6P(aq) + H_2O(l) ⇌ G(aq) + P_i(aq), where G6P is glucose-6-phosphate, G is glucose, and P_i is inorganic phosphate.

(b) Gly(aq) + Ala(aq) ⇌ Gly–Ala(aq) + H_2O(l)

(c) Mg^{2+}(aq) + ATP^{4-}(aq) ⇌ $MgATP^{2-}$(aq)

(d) 2 $CH_3COCOOH$(aq) + 5 O_2(g) ⇌ 6 CO_2(g) + 4 H_2O(l)

4.8 The equilibrium constant for the reaction A + B ⇌ 2 C is reported as 3.4×10^4. What would it be for the reaction written as **(a)** 2 C ⇌ A + B, **(b)** 2 A + 2 B ⇌ 4 C, **(c)** ½ A + ½ B ⇌ C?

4.9 The equilibrium constant for the hydrolysis of the dipeptide alanylglycine by a peptidase enzyme is $K = 8.1 \times 10^2$ at 310 K. Calculate the standard reaction Gibbs energy for the hydrolysis.

4.10 One enzyme-catalyzed reaction in a biochemical cycle has an equilibrium constant that is 10 times the equilibrium constant of a second reaction. If the standard Gibbs energy of the former reaction is −300 kJ mol^{-1}, what is the standard reaction Gibbs energy of the second reaction?

4.11 What is the value of the equilibrium constant of a reaction for which $\Delta_rG^\ominus = 0$?

4.12 The standard reaction Gibbs energies (at pH = 7) for the hydrolysis of glucose-1-phosphate, glucose-6-phosphate, and glucose-3-phosphate are −21, −14, and −9.2 kJ mol^{-1}, respectively. Calculate the equilibrium constants for the hydrolyses at 37°C.

4.13 The standard Gibbs energy for the hydrolysis of ATP to ADP is −31 kJ mol^{-1}; what is the Gibbs energy of reaction in an environment at 37°C in which the ATP, ADP, and P_i concentrations are all **(a)** 1.0 mmol L^{-1}, **(b)** 1.0 μmol L^{-1}?

4.14 The distribution of Na^+ ions across a typical biological membrane is 10 mmol L^{-1} inside the cell and 140 mmol L^{-1} outside the cell. At equilibrium the concentrations are equal. What is the Gibbs energy difference across the membrane at 37°C? The difference in concentration must be sustained by coupling to reactions that have at least that difference of Gibbs energy.

4.15 For the hydrolysis of ATP at 37°C, $\Delta_r H^\oplus$ = −20 kJ mol^{-1} and $\Delta_r S^\oplus$ = +34 J K^{-1} mol^{-1}. Assuming that these quantities remain constant, estimate the temperature at which the equilibrium constant for the hydrolysis of ATP becomes greater than 1.

4.16 Two polynucleotides with sequences A_nU_n (where A and U denote adenine and uracil, respectively) interact through A–U base pairs, forming a double helix. When $n = 5$ and $n = 6$, the equilibrium constants for formation of the double helix are 5.0×10^3 and 2.0×10^5, respectively. **(a)** Suggest an explanation for the increase in the value of the equilibrium constant with n. **(b)** Calculate the contribution of a single A–U base pair to the Gibbs energy of formation of a double helix between A_nU_n polypeptides.

4.17 Under biochemical standard conditions, aerobic respiration produces approximately 38 molecules of ATP per molecule of glucose that is completely oxidized. **(a)** What is the percentage efficiency of aerobic respiration under biochemical standard conditions? **(b)** The following conditions are more likely to be observed in a living cell: $p_{CO_2} = 5.3 \times 10^{-2}$ atm, $p_{O_2} = 0.132$ atm, [glucose] = 5.6×10^{-2} mol L^{-1}, [ATP] = [ADP] = [P_i] = 1.0×10^{-4} mol L^{-1}, pH = 7.4, T = 310 K. Assuming that activities can be replaced by the numerical values of molar concentrations, calculate the efficiency of aerobic respiration under these physiological conditions.

4.18 The second step in glycolysis is the isomerization of glucose-6-phosphate (G6P) to fructose-6-phosphate (F6P). *Example* 4.2 considered the equilibrium between F6P and G6P. Draw a graph to show how the reaction Gibbs energy varies with the fraction f of F6P in solution. Label the regions of the graph that correspond to the formation of F6P and G6P being spontaneous, respectively.

4.19 The saturation curves shown Fig. 4.7 may also be modeled mathematically by the equation

$$\log \frac{s}{1-s} = \nu \log p - \nu \log K$$

where s is the saturation, p is the partial pressure of O_2, K is a constant (not the equilibrium constant for binding of one ligand), and ν is the *Hill coefficient*, which varies from 1, for no cooperativity, to N for all-or-none binding of N ligands ($N = 4$ in Hb). The Hill coefficient for Mb is 1, and for Hb it is 2.8. **(a)** Determine the constant K for both Mb and Hb from the graph of fractional saturation (at $s = 0.5$) and then calculate the fractional saturation of Mb and Hb for the following values of p/kPa: 1.0, 1.5, 2.5, 4.0, 8.0. **(b)** Calculate the value of s at the same p values assuming ν has the theoretical maximum value of 4.

4.20 Classify the following compounds as endergonic or exergonic: **(a)** glucose, **(b)** urea, **(c)** octane, **(d)** ethanol.

4.21 Consider the combustion of sucrose:

$$C_{12}H_{22}O_{11}(s) + 12\ O_2(g) \rightleftharpoons 12\ CO_2(g) + 11\ H_2O(l)$$

(a) Combine the standard reaction entropy with the standard reaction enthalpy and calculate the standard reaction Gibbs energy at 298 K. **(b)** In assessing metabolic processes, we are usually more interested in the work that may be performed for the consumption of a given mass of compound than the heat it can produce (which merely keeps the body warm). Recall

from Chapter 2 that the change in Gibbs energy can be identified with the maximum non-expansion work that can be extracted from a process. What is the maximum energy that can be extracted as **(i)** heat, **(ii)** non-expansion work when 1.0 kg of sucrose is burned under standard conditions at 298 K?

4.22 Is it more energy effective to ingest sucrose or glucose? Calculate the non-expansion work, the expansion work, and the total work that can be obtained from the combustion of 1.0 kg of glucose under standard conditions at 298 K when the product includes liquid water. Compare your answer with your results from *Exercise* 4.21b.

4.23 The oxidation of glucose in the mitochondria of energy-hungry brain cells leads to the formation of pyruvate ions, which are then decarboxylated to ethanal (acetaldehyde, CH_3CHO) in the course of the ultimate formation of carbon dioxide. **(a)** The standard Gibbs energies of formation of pyruvate ions in aqueous solution and gaseous ethanal are −474 and −133 kJ mol^{-1}, respectively. Calculate the Gibbs energy of the reaction in which pyruvate ions are converted to ethanal by the action of pyruvate decarboxylase with the release of carbon dioxide. **(b)** Ethanal is soluble in water. Would you expect the standard Gibbs energy of the enzyme-catalyzed decarboxylation of pyruvate ions to ethanal in solution to be larger or smaller than the value for the production of gaseous ethanal?

4.24 Calculate the standard biological Gibbs energy for the reaction

$$\text{Pyruvate}^- + \text{NADH} + \text{H}^+ \longrightarrow \text{lactate}^- + \text{NAD}^+$$

at 310 K given that $\Delta_r G^\ominus = -66.6$ kJ mol^{-1}. (NAD^+ is the oxidized form of nicotinamide dinucleotide.) This reaction occurs in muscle cells deprived of oxygen during strenuous exercise and can lead to cramping.

4.25 The standard biological reaction Gibbs energy for the removal of the phosphate group from adenosine monophosphate is −14 kJ mol^{-1} at 298 K. What is the value of the thermodynamic standard reaction Gibbs energy?

4.26 Estimate the values of the biological standard Gibbs energies of the following phosphate transfer reactions:

(a) GTP(aq) + ADP(aq) ⟶ GDP(aq) + ATP(aq)

(b) Glycerol(aq) + ATP(aq) ⟶ glycerol-1-phosphate + ADP(aq)

(c) 3-Phosphoglycerate(aq) + ATP(aq) ⟶ 1,3-bis(phospho)glycerate(aq) + ADP(aq)

4.27 Show that if the logarithm of an equilibrium constant is plotted against the reciprocal of the temperature, then the standard reaction enthalpy may be determined.

4.28 The conversion of fumarate ion to malate ion is catalyzed by the enzyme fumarase:

$$\text{Fumarate}^{2-}(\text{aq}) + \text{H}_2\text{O}(\text{l}) \longrightarrow \text{malate}^-(\text{aq})$$

Use the following data to determine the standard reaction enthalpy:

θ/°C	15	20	25	30	35	40	45	50
K	4.786	4.467	4.074	3.631	3.311	3.090	2.754	2.399

4.29 What is the standard enthalpy of a reaction for which the equilibrium constant is **(a)** doubled, **(b)** halved when the temperature is increased by 10 K at 298 K?

4.30 Numerous acidic species are found in living systems. Write the proton transfer equilibria for the following biochemically important acids in aqueous solution: **(a)** $H_2PO_4^-$ (dihydrogenphosphate ion), **(b)** lactic acid ($CH_3CHOHCOOH$), **(c)** glutamic acid ($HOOCCH_2CH_2CH(NH_2)COOH$), **(d)** glycine (NH_2CH_2COOH), **(e)** oxalic acid ($HOOCCOOH$).

4.31 For biological and medical applications we often need to consider proton transfer equilibria at body temperature (37°C). The value of K_w for water at body temperature is 2.5×10^{-14}. **(a)** What is the value of $[H_3O^+]$ and the pH of neutral water at 37°C? **(b)** What is the molar concentration of OH^- ions and the pOH of neutral water at 37°C?

4.32 Suppose that something had gone wrong in the Big Bang, and instead of ordinary hydrogen there was an abundance of deuterium in the universe. There would be many subtle changes in equilibria, particularly the deuteron transfer equilibria of heavy atoms and bases. The K_w for D_2O, heavy water, at 25°C is 1.35×10^{-15}.

(a) Write the chemical equation for the autoprotolysis (more precisely, autodeuterolysis) of D_2O. **(b)** Evaluate pK_w for D_2O at 25°C. **(c)** Calculate the molar concentrations of D_3O^+ and OD^- in neutral heavy water at 25°C. **(d)** Evaluate the pD and pOD of neutral heavy water at 25°C. **(e)** Formulate the relation between pD, pOD, and $pK_w(D_2O)$.

4.33 The molar concentration of H_3O^+ ions in the following solutions was measured at 25°C. Calculate the pH and pOH of the solution: **(a)** 1.5×10^{-5} mol L^{-1} (a sample of rainwater), **(b)** 1.5 mmol L^{-1}, **(c)** 5.1×10^{-14} mol L^{-1}, **(d)** 5.01×10^{-5} mol L^{-1}.

4.34 Calculate the molar concentration of H_3O^+ ions and the pH of the following solutions: **(a)** 25.0 cm^3 of 0.144 M HCl(aq) was added to 25.0 cm^3 of 0.125 M NaOH(aq), **(b)** 25.0 cm^3 of 0.15 M HCl(aq) was added to 35.0 cm^3 of 0.15 M KOH(aq), **(c)** 21.2 cm^3 of 0.22 M HNO_3(aq) was added to 10.0 cm^3 of 0.30 M NaOH(aq).

4.35 Determine whether aqueous solutions of the following salts have a pH equal to, greater than, or less than 7; if pH > 7 or pH < 7, write a chemical equation to justify your answer. **(a)** NH_4Br, **(b)** Na_2CO_3, **(c)** KF, **(d)** KBr.

4.36 **(a)** A sample of potassium acetate, KCH_3CO_2, of mass 8.4 g is used to prepare 250 cm^3 of solution. What is the pH of the solution? **(b)** What is the pH of a solution when 3.75 g of ammonium bromide, NH_4Br, is used to make 100 cm^3 of solution? **(c)** An aqueous solution of volume 1.0 L contains 10.0 g of potassium bromide. What is the percentage of Br^- ions that are protonated?

4.37 There are many organic acids and bases in our cells, and their presence modifies the pH of the fluids inside them. It is useful to be able to assess the pH of solutions of acids and bases and to make inferences from measured values of the pH. A solution of equal concentrations of lactic acid and sodium lactate was found to have pH = 3.08. **(a)** What are the values of pK_a and K_a of lactic acid? **(b)** What would the pH be if the acid had twice the concentration of the salt?

4.38 Calculate the pH, pOH, and fraction of solute protonated or deprotonated in the following aqueous solutions: **(a)** 0.120 M $CH_3CH(OH)COOH$(aq) (lactic acid), **(b)** 1.4×10^{-4} M $CH_3CH(OH)COOH$(aq), **(c)** 0.15 M NH_4Cl(aq), **(d)** 0.15 M $NaCH_3CO_2$(aq), **(e)** 0.112 M $(CH_3)_3N$(aq) (trimethylamine).

4.39 Show how the composition of an aqueous solution that contains 0.010 mol L^{-1} glycine varies with pH.

4.40 Show how the composition of an aqueous solution that contains 0.010 mol L^{-1} tyrosine varies with pH.

4.41 Calculate the pH of the following acid solutions at 25°C; ignore second deprotonations only when that approximation is justified. **(a)** 1.0×10^{-4} M H_3BO_3(aq) (boric acid acts as a monoprotic acid), **(b)** 0.015 M H_3PO_4(aq), **(c)** 0.10 M H_2SO_3(aq).

4.42 The amino acid tyrosine has $pK_a = 2.20$ for deprotonation of its carboxylic acid group. What are the relative concentrations of tyrosine and its conjugate base at a pH of **(a)** 7, **(b)** 2.2, **(c)** 1.5?

4.43 Appreciable concentrations of the potassium and calcium salts of oxalic acid, $(COOH)_2$, are found in many leafy green plants, such as rhubarb and spinach. **(a)** Calculate the molar concentrations of $HOOCCO_2^-$, $(CO_2)_2^{2-}$, H_3O^+, and OH^- in 0.15 M $(COOH)_2$(aq). **(b)** Calculate the pH of a solution of potassium hydrogenoxalate.

4.44 In green sulfur bacteria, hydrogen sulfide, H_2S, is the agent that brings about the reduction of CO_2 to carbohydrates during photosynthesis. Calculate the molar concentrations of H_2S, HS^-, S^{2-}, H_3O^+, and OH^- in 0.065 M H_2S(aq).

4.45 The *isoelectric point*, pI, of an amino acid is the pH at which the predominant species in solution is the zwitterionic form of the amino acid and only small but equal concentrations of positively and negatively charged forms of the amino acid are present. It follows that at the isoelectric point, the average charge on the amino acid is zero. Show that **(a)** $pI = \frac{1}{2}(pK_{a1} + pK_{a2})$ for amino acids with side chains that are neither acidic nor basic (such as glycine and alanine), **(b)** $pI = \frac{1}{2}(pK_{a1} + pK_{a2})$ for amino acids with acidic side chains (such as aspartic acid and glutamic acid), and **(c)** $pI = \frac{1}{2}(pK_{a2} + pK_{a3})$ for amino acids with basic side chains (such as lysine and histidine), where pK_{a1}, pK_{a2}, and pK_{a3} are given in Table 4.6. *Hint:* See *Case study* 4.3 and *Derivation* 4.2.

4.46 Predict the pH region in which each of the following buffers will be effective, assuming equal

molar concentrations of the acid and its conjugate base: **(a)** sodium lactate and lactic acid, **(b)** sodium benzoate and benzoic acid, **(c)** potassium hydrogenphosphate and potassium phosphate, **(d)** potassium hydrogenphosphate and potassium dihydrogenphosphate, **(e)** hydroxylamine and hydroxylammonium chloride.

4.47 From the information in Tables 4.4 and 4.5, select suitable buffers for **(a)** pH = 2.2 and **(b)** pH = 7.0.

4.48 The weak base colloquially known as Tris, and more precisely as tris(hydroxymethyl)aminomethane, has $pK_a = 8.3$ at 20°C and is commonly used to produce a buffer for biochemical applications. **(a)** At what pH would you expect Tris to act as a buffer in a solution that has equal molar concentrations of Tris and its conjugate acid? **(b)** What is the pH after the addition of 3.3 mmol NaOH to 100 cm^3 of a buffer solution with equal molar concentrations of Tris and its conjugate acid form? **(c)** What is the pH after the addition of 6.0 mmol HNO_3 to 100 cm^3 of a buffer solution with equal molar concentrations of Tris and its conjugate acid?

Projects

4.49 Here we continue our exploration of the thermodynamics of unfolding of biological macromolecules. Our focus is the thermal and chemical denaturation of chymotrypsin, one of many enzymes that catalyze the cleavage of polypeptides (see *Case study* 8.1).

(a) The denaturation of a biological macromolecule can be described by the equilibrium

$$\text{macromolecule in native form} \rightleftharpoons \text{macromolecule in denatured form}$$

Show that the fraction θ of denatured macromolecules is related to the equilibrium constant K_d for the denaturation process by

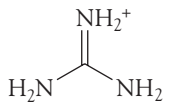
$$\theta = \frac{1}{1 + K_d}$$

(b) Now explore the thermal denaturation of a biological macromolecule. **(i)** Write an expression for the temperature dependence of K_d in terms of the standard enthalpy and standard entropy of denaturation. **(ii)** At pH = 2, the standard enthalpy and entropy of denaturation of chymotrypsin are +418 kJ mol^{-1} and +1.32 kJ K^{-1} mol^{-1}, respectively. Using these data and your results from parts (a) and (b.i), plot θ against T. Compare the shape of your plot with that of the plot shown in Fig. 3.16. **(iii)** The "melting temperature" of a biological macromolecule is the temperature at which $\theta = \frac{1}{2}$. Use your results from part (ii) to calculate the melting temperature of chymotrypsin at pH = 2. **(iv)** Calculate the standard Gibbs energy and the equilibrium constant for the denaturation of chymotrypsin at pH = 2.0 and T = 310 K (body temperature). Is the protein stable under these conditions?

(c) We saw in *Exercise* 3.35 that the unfolding of a protein may also be brought about by treatment with *denaturants*, substances such as guanidinium hydrochloride (GuHCl; the guanidinium ion is shown in **14**) that disrupt the intermolecular interactions responsible for the native three-dimensional conformation of a biological macromolecule. Data for a number of proteins denatured by urea or guanidinium hydrochloride suggest a linear relationship between the Gibbs energy of denaturation of a protein, ΔG_d, and the molar concentration of a denaturant [D]:

$$\Delta G_d^{\ominus} = \Delta G^{\ominus}_{d,water} - m[D]$$

where m is an empirical parameter that measures the sensitivity of unfolding to denaturant concentration and $\Delta G^{\ominus}_{d,water}$ is the Gibbs energy of denaturation of the protein in the absence of denaturant and is a measure of the thermal stability of the macromolecule. **(i)** At 27°C and



14 The guanidinium ion

pH 6.5, the fraction θ of denatured chymotrypsin molecules varies with the concentration of GuHCl as follows:

θ	1.00	0.99	0.78	0.44	0.23	0.08	0.06	0.01
[GuHCl]/(mol L^{-1})	0.00	0.75	1.35	1.70	2.00	2.35	2.70	3.00

Calculate m and $\Delta G^{\ominus}_{d,water}$ for chymotrypsin under these experimental conditions. **(ii)** Using the same data, plot θ against [GnHCl]. Comment on the shape of the curve. **(iii)** To gain insight into your results from part (c.ii), you will now derive an equation that relates θ to [D]. Begin by showing that $\Delta G^{\ominus}_{d,water} = m[D]_{1/2}$, where $[D]_{1/2}$ is the concentration of denaturant corresponding to $\theta = \frac{1}{2}$. Then write an expression for θ as a function of [D], $[D]_{1/2}$, m, and T. Finally, plot the expression using the values of $[D]_{1/2}$, m, and T from part (c.i). Is the shape of your plot consistent with your results from part (c.ii)?

4.50 In *Case study* 4.4, we discussed the role of hemoglobin in regulating the pH of blood. Now we explore the mechanism of regulation in detail.

(a) If we denote the protonated and deprotonated forms of hemoglobin as HbH and Hb^-, respectively, then the proton transfer equilibria for deoxygenated and fully oxygenated hemoglobin can be written as:

$$HbH \rightleftharpoons Hb^- + H^+ \qquad pK_a = 6.62$$
$$HbHO_2 \rightleftharpoons HbO_2^- + H^+ \qquad pK_a = 8.18$$

where we take the view (for the sake of simplicity) that the protein contains only one acidic proton. **(i)** What fraction of deoxygenated hemoglobin is deprotonated at pH = 7.4, the value for normal blood? **(ii)** What fraction of oxygenated hemoglobin is deprotonated at pH = 7.4? **(iii)** Use your results from parts (a.i) and (a.ii) to show that deoxygenation of hemoglobin is accompanied by the uptake of protons by the protein.

(b) It follows from the discussion in *Case study* 4.4 and part (a) that the exchange of CO_2 for O_2 in tissue is accompanied by complex proton transfer equilibria: the release of CO_2 into blood produces hydronium ions that can be bound tightly to hemoglobin once it releases O_2. These processes prevent changes in the pH of blood. To treat the problem more quantitatively, let us calculate the amount of CO_2 that can be transported by blood without a change in pH from its normal value of 7.4. **(i)** Begin by calculating the amount of hydronium ion bound per mole of oxygenated hemoglobin molecules at pH = 7.4. **(ii)** Now calculate the amount of hydronium ion bound per mole of deoxygenated hemoglobin molecules at pH = 7.4. **(iii)** From your results for parts (b.i) and (b.ii), calculate the amount of hydronium ion that can be bound per mole of hemoglobin molecules as a result of the release of O_2 by the fully oxygenated protein at pH = 7.4. **(iv)** Finally, use the result from part (b.iii) to calculate the amount of CO_2 that can be released into the blood per mole of hemoglobin molecules at pH = 7.4.