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Introduction to Apparent Equilibrium Constants

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Two types of equilibrium constant expressions are needed in biochemistry. The thermodynamics of biochemical reactions can be discussed in terms of species like ATP^{4-} , HATP^{3-} , and MgATP^{2-} or in terms of reactants (sums of species) like ATP. The use of species corresponds with writing chemical reactions that balance atoms of elements and electric charges; the corresponding equilibrium constants are represented by K . This approach is required when chemical details are being discussed, as in considering the mechanism of enzymatic catalysis. But discussion in terms of metabolism must involve, in great deal detail, acid dissociation constants and dissociation constants of complexes with metal ions. Therefore metabolism is discussed by writing biochemical reactions in terms of reactants — that is, sums of species, like ATP — at a specified pH and perhaps specified concentrations of free metal ions that are bound reversibly by reactants. Biochemical reactions do not balance hydrogen ions because the pH is held constant, and they do not balance metal ions for which free concentrations are held constant. When the pH is held constant, there is the implication that acid or alkali will be added to the system to hold the pH constant if the reaction produces or consumes hydrogen ions. In actual practice a buffer is used to hold the pH nearly constant, and the pH is measured at equilibrium. The corresponding equilibrium constants are represented by K' , which are referred to as apparent equilibrium constants because they are functions of pH and perhaps the free concentrations

of one or more free metal ions. Biochemical thermodynamics is more complicated than the chemical thermodynamics of reactions in aqueous solutions because there are more independent variables that have to be specified. This introductory chapter is primarily concerned with the hydrolysis of ATP at specified T , P , pH, pMg, and ionic strength. The thermodynamics of the hydrolysis of ATP and closely related reactions have received a good deal of attention because of the importance of these reactions in energy metabolism.

■ 1.1 BRIEF HISTORY OF THE THERMODYNAMICS OF BIOCHEMICAL REACTIONS

The first major publication on the thermodynamics of biochemical reactions was by Burton in Krebs and Kornberg, *Energy Transformations in Living Matter*, 1957. Before that time, apparent equilibrium constants had been measured for a number of enzyme-catalyzed reactions, but Burton recognized that these apparent equilibrium constants together with standard Gibbs energies of formation $\Delta_f G^0$ of species determined by chemical methods can yield $\Delta_r G^0$ for biochemical species to make a table that can be used to calculate equilibrium constants of biochemical reactions that have not been studied (Burton and Krebs, 1953). In retrospect it is easy to see that in 1953 to 1957 there were some problems that were apparently not clearly recognized or solved. Since Burton was the first, it is worth saying a little more about his 1957 thermodynamic tables. The first table gives $\Delta_r G^0$ values for about 100 species in biochemical reactions. A large number of these values were taken from chemical thermodynamic tables available in the 1950s, but a number were new values calculated from measured apparent equilibrium constants for enzyme-catalyzed reactions. $\Delta_f G^0$ values of species can be readily calculated when the reactants in the enzyme-catalyzed reaction are all single species and $\Delta_r G^0$ values are known for all of the reactants except one. It is noteworthy that Burton omitted the species of orthophosphate from his table and that he was not able to include species of ATP, ADP, NAD_{ox} , and NAD_{red} . His second table gives standard Gibbs energy changes at pH 7 for oxidation-reduction reactions that were calculated using the convention that $[\text{H}^+] = 1 \text{ mol L}^{-1}$ at pH 7; the symbol $\Delta G'$ was used for this quantity. This table also gives the corresponding standard cell potentials for these reactions. The third table gives $\Delta G'$ values at pH 7 for a number of reactions in glycolysis and alcoholic fermentation. The fourth table is on the citric acid cycle, and the fifth table is on Gibbs energies of hydrolysis. When a biochemical reaction is studied at a pH where there is a predominant chemical reaction, it is possible to discuss thermodynamics in terms of species. But when some reactants are represented by an equilibrium distribution of several species with different numbers of hydrogen atoms, this approach is not satisfactory. The quantitative treatment of reactions involving reactants with pKs in the neighborhood of pH 7 was not possible until acid dissociation constants of these reactants had been determined. Some measurements of acid dissociation constants of ATP and related substances (Alberty, Smith, and Bock, 1951) and dissociation constants of ionic complexes of these substances with divalent cations (Smith and Alberty, 1956) were made in this period.

In the 1960s there was a good deal of interest in the thermodynamics of the hydrolysis of ATP and of other organic phosphates (Alberty, 1968, 1969; Phillips, George, and Rutman, 1969), but standard Gibbs energies of species were not calculated. The measurement of apparent equilibrium constants for biochemical reactions was extended in the 1970s (Guynn and Veech, 1973; Veech et al., 1979) and 1980s (Tewari and Goldberg, 1988).

In 1969 Wilhoit picked up where Burton had left off and compiled the standard thermodynamic properties $\Delta_f G^0$ and $\Delta_f H^0$ of species involved in biochemical reactions. He recognized the problems involved in including species

of ATP in such a table and made a suggestion as to how to handle it. In 1977 Thauer, Jungermann, and Decker published a table of standard Gibbs energies of formation of many species of biochemical interest, and showed how to adjust standard Gibbs energies of reaction to pH 7 by adding $m\Delta_r G^0(\text{H}^+)$, where m is the net number of protons in the reaction.

During the 1960s and 1970s, new nomenclature for treating the thermodynamics of biochemical reactions was developed, including the use of K' for the apparent equilibrium constant written in terms of sums of species, but omitting $[\text{H}^+]$. These changes led to the publication of *Recommendations for Measurement and Presentation of Biochemical Equilibrium Data* by an IUPAC-IUB Committee (Wadsö, Gutfreund, Privalov, Edsall, Jencks, Armstrong, and Biltonen, 1976).

Goldberg and Tewari published an evaluation of thermodynamic and transport properties of carbohydrates and their monophosphates in 1989 and of the ATP series in 1991. Miller and Smith-Magowan published on the thermodynamics of the Krebs cycle and related compounds in 1990.

Alberty (1992a, b) pointed out that when the pH or the free concentration of a metal ion is specified, the Gibbs energy G does not provide the criterion for spontaneous change and equilibrium. When intensive variables in addition to the temperature and pressure are held constant, it is necessary to define a transformed Gibbs energy G' by use of a Legendre transform, as discussed in Chapters 2 and 4. This leads to a complete set of transformed thermodynamic properties at specified pH, that is, a transformed entropy S' , transformed enthalpy H' , and a transformed heat capacity at constant pressure $C'_{p,m}$. These changes led to the publication of *Recommendations for Nomenclature and Tables in Biochemical Thermodynamics* by an IUPAC-IUBMB Committee (Alberty, Cornish-Bowden, Gibson, Goldberg, Hammes, Jencks, Tipton, Vecch, Westerhoff, and Webb, 1994).

This introductory chapter describes the thermodynamics of biochemical reactions in terms of equilibrium constants and apparent equilibrium constants and avoids references to other thermodynamic properties, which are introduced later.

■ 1.2 ACID DISSOCIATION CONSTANTS AND DISSOCIATION CONSTANTS OF COMPLEX IONS

Strictly speaking, equilibrium constant expressions for chemical reactions involving ions in aqueous solutions should be written in terms of activities a_i of species, rather than concentrations. The **activity** of species i is given by $a_i = \gamma_i c_i$, where γ_i is the **activity coefficient**, which is a function of ionic strength. Activity coefficients of neutral molecules are close to unity in dilute aqueous solutions, but the activity coefficients of ions may deviate significantly from unity, depending on their electric charges and the ionic strength. The **ionic strength** of a solution is defined by $I = (\frac{1}{2})\sum z_i^2 c_i$, where z_i is the charge on ion i and c_i is its concentration on the molar scale. When dilute aqueous solutions are studied, the ionic strength is under the control of the investigator and is essentially constant when the composition changes during a reaction. Thus it is convenient to take equilibrium constants and other thermodynamic properties to be functions of the ionic strength so that equilibrium constant expressions can be written in terms of concentrations. The exception to this statement is H_2O . In dilute aqueous solutions the convention in thermodynamics is to omit $[\text{H}_2\text{O}]$ in the expression for the equilibrium constant because its activity remains essentially at unity.

In 1923 Debye and Hckel showed that the activity coefficient γ_i of an ion decreases with increasing ionic strength, according to

$$\log \gamma_i = -Az_i^2 I^{1/2} \quad (1.2-1)$$

where $A = 0.510651 \text{ L}^{-1/2} \text{ mol}^{1/2}$ at 298.15 K in water at a pressure of 1 bar. This

is referred to as a limiting law because it becomes more accurate as the ionic strength approaches zero. At ionic strengths in the physiological range, 0.05 to 0.25 M, there are significant deviations from equation 1.2-1. Of the several ways to extend this equation empirically to provide approximate activity coefficients in the physiological range, the most widely used equation is

$$\log \gamma_i = -\frac{Az_i^2 I^{1/2}}{1 + BI^{1/2}} \quad (1.2-2)$$

This is referred to as the extended Debye-Hückel equation. It is an approximation that gives a good fit of data at low ionic strengths (Goldberg and Tewari, 1991) when $B = 1.6 \text{ L}^{1/2} \text{ mol}^{-1/2}$. Better fits can be obtained with more complicated equations with more parameters, but these parameters are not known for solutions involved in studying biochemical reactions. The way that thermodynamic properties vary with the ionic strength is discussed in more detail in Section 3.6.

Since hydrogen ions and metal ions, like Mg^{2+} , are often reactants, it is convenient to define the pH_c as $-\log[\text{H}^+]$, where c refers to concentrations, and pMg as $-\log[\text{Mg}^{2+}]$. However, a glass electrode measures $\text{pH}_a = -\log\{\gamma(\text{H}^+)[\text{H}^+]\}$ where a refers to activity. Thus

$$\text{pH}_a = -\log\{\gamma(\text{H}^+)\} + \text{pH}_c \quad (1.2-3)$$

Substituting the extended Debye-Hückel equation in this equation yields (Alberty, 2001d)

$$\text{pH}_a - \text{pH}_c = \frac{AI^{1/2}}{1 + 1.6I^{1/2}} \quad (1.2-4)$$

The differences between the measured pH_a and the pH_c used in biochemical thermodynamics are given as a function of ionic strength and temperature in Table 1.1.

These are the adjustments to be subtracted from pH_a obtained with a pH meter to obtain pH_c , which is used in the equations in this book. pH_c is lower than pH_a because the ion atmosphere of H^+ reduces its activity. In the rest of the book, the subscript “ c ” on pH will be omitted so that $\text{pH} = -\log[\text{H}^+]$.

In considering reactions in biochemical systems it is convenient to move the activity coefficients into the equilibrium constants. For example, the equilibrium constant expression for the dissociation of a weak acid can be written as follows:

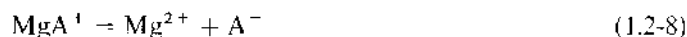


$$K_a(\text{HA}) = \frac{a(\text{H}^+)a(\text{A}^-)}{a(\text{HA})} = \frac{\gamma(\text{H}^+)[\text{H}^+]\gamma(\text{A}^-)[\text{A}^-]}{\gamma(\text{HA})[\text{HA}]} \quad (1.2-6)$$

$$K_c(\text{HA}) = \frac{K_a(\text{HA})\gamma(\text{HA})}{\gamma(\text{H}^+)\gamma(\text{A}^-)} = \frac{[\text{H}^+][\text{A}^-]}{[\text{HA}]} = \frac{10^{-\text{pH}}[\text{A}^-]}{[\text{HA}]} \quad (1.2-7)$$

The acid dissociation constant K_a is independent of ionic strength, but the acid dissociation constant K_c depends on the ionic strength, as indicated by equation 1.2-7. The equilibrium constant expression in equation 1.2-7 will be used in the rest of the book, but the subscript “ c ” will be omitted. This will make it possible for us to deal with concentrations of species, rather than activities.

The same considerations apply to the dissociations of complex ions. For example, the equilibrium expression for the dissociation of a complex ion with a magnesium ion can be written as follows:



$$K_{\text{MgA}} = \frac{[\text{Mg}^{2+}][\text{A}^-]}{[\text{MgA}^+]} = \frac{10^{-\text{pMg}}[\text{A}^-]}{[\text{MgA}^+]} \quad (1.2-9)$$

Table 1.1 $\text{pH}_a - \text{pH}_c$ as a Function of Ionic Strength and Temperature

I/M	10°C	25°C	40°C
0	0	0	0
0.05	0.082	0.084	0.086
0.1	0.105	0.107	0.110
0.15	0.119	0.122	0.125
0.2	0.130	0.133	0.137
0.25	0.138	0.142	0.146

Source: R. A. Alberty, *J. Phys. Chem. B* 105, 7865 (2001).
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where $\text{pMg} = -\log[\text{Mg}^{2+}]$ and K_{MgA} is a function of the ionic strength, as well as temperature.

Strictly speaking, equations 1.2-7 and 1.2-9 should have c° in the denominator, where $c^\circ = 1 \text{ M}$ is the standard concentration, to make the equilibrium constant dimensionless (Mills et al., 1993). However, the c° is omitted in this book in order to simplify expressions for equilibrium constants. Nevertheless, equilibrium constants are still considered to be dimensionless, so their logarithm can be taken.

In using acid dissociation constants and the dissociation constants of complex ions, it is convenient to take the base 10 logarithms of equations 1.2-7 and 1.2-9 to obtain

$$\text{pH} = \text{p}K_{\text{HA}} + \log\left(\frac{[\text{A}^-]}{[\text{HA}]}\right) \quad (1.2-10)$$

$$\text{pMg} = \text{p}M_{\text{MgA}} + \log\left(\frac{[\text{A}^-]}{[\text{MgA}^-]}\right) \quad (1.2-11)$$

where $\text{p}K_{\text{HA}} = -\log K_{\text{HA}}$ and $\text{p}M_{\text{MgA}} = -\log K_{\text{MgA}}$ are functions of ionic strength at constant temperature. Table 1.3 in the last section of this chapter gives the $\text{p}K$ s of some weak acids of interest in biochemistry as a function of ionic strength. Note that the effect of ionic strength is larger for acids with larger charges. For polyprotic acids $\text{p}K_1$ applies to the weakest acid group, $\text{p}K_2$ to the second weakest, and so on, in the pH range considered (usually 5 to 9). The calculation of Table 1.3 is based on the extended Debye-Hückel equation.

■ 1.3 BINDING OF HYDROGEN IONS AND MAGNESIUM IONS BY ADENOSINE TRIPHOSPHATE

Acid dissociation constants and dissociation constants of complex ions determine the concentrations of species that are present in a solution at equilibrium under specified conditions. Ionic dissociation reactions occur rapidly and tend to remain at equilibrium during an enzyme-catalyzed reaction. Since ATP (see Fig. 1.1) is the primary carrier of energy in biochemical systems and since a good deal is known about its binding properties, these properties are considered here in some detail.

An ATP ion with four negative charges can bind five hydrogen ions in strongly acidic solutions, but biochemistry is primarily concerned with the neutral region. We will consider only the hydrogen ion bindings that affect equilibrium in this region, namely the terminal phosphate group with a $\text{p}K$ about 7 and the

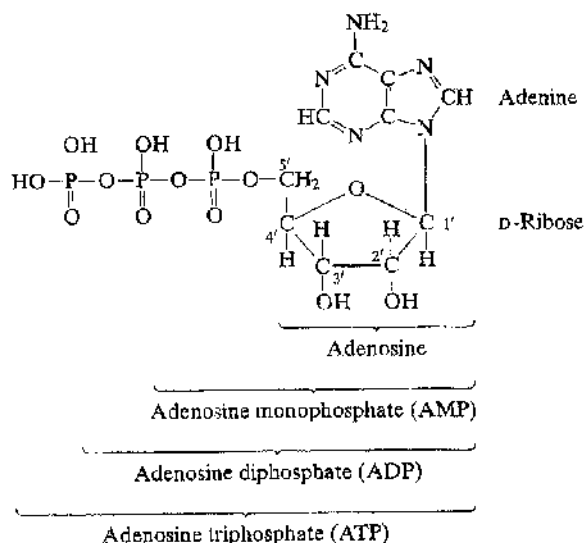


Figure 1.1 Structure of adenosine triphosphate.

adenine group with pK about 4. The other three pK s are in the neighborhood of 2 or below and can be ignored in treating biochemical reactions. The anions of ATP bind metal ions as well as hydrogen ions. The dissociation constants for the complex ions that are formed can be determined by use of acid titrations because the binding of a metal ion reduces the apparent pK for the phosphate group (Alberty, Smith, and Bock, 1951; Smith and Alberty, 1956; Silbey and Alberty, 2001). The apparent pK of the phosphate group is the midpoint of the titration of $H_2PO_4^-$ in the presence of magnesium ions at the desired concentration of free metal ions. Because of the importance of ATP in energy metabolism, a great deal of data on the acid dissociation constants and the dissociation constants of complex ions of ATP, ADP, AMP, and P_i are available. Goldberg and Tewari (1991) and Larson, Tewari, and Goldberg (1993) critically evaluated these data including that on glucose 6-phosphate (G6P). The values for acid dissociation constants and magnesium complex ion dissociation constants involved in the ATP series are given in Table 1.2.

Since ATP is made up of three species in the physiological pH range in the absence of metal ions that are bound, its concentration is given by

$$[ATP] = [ATP^{4-}] + [HATP^{3-}] + [H_2ATP^{2-}] \quad (1.3-1)$$

Substituting the expressions for the two acid dissociation constants yields

$$[ATP] = [ATP^{4-}] \left(1 + \frac{[H^+]}{K_{1ATP}} + \frac{[H^+]^2}{K_{1ATP}K_{2ATP}} \right) \quad (1.3-2)$$

The mole fraction r of the ATP in the ATP^{4-} form at a specified concentration of hydrogen ions is given by

$$r(ATP^{4-}) = \frac{1}{1 + \frac{[H^+]}{K_{1ATP}} + \frac{[H^+]^2}{K_{1ATP}K_{2ATP}}} \quad (1.3-3)$$

The mole fractions of ATP in the other two forms are readily derived:

$$r(HATP^{3-}) = \frac{\frac{[H^+]}{K_{1ATP}}}{1 + \frac{[H^+]}{K_{1ATP}} + \frac{[H^+]^2}{K_{1ATP}K_{2ATP}}} \quad (1.3-4)$$

Table 1.2 Equilibrium Constants in the ATP Series at 298.15 K

Reaction	$pK(I=0)$	$K(I=0)$	$K(I=0.25\text{ M})$
$\text{HAMP}^- = \text{H}^+ + \text{AMP}^{2-}$	6.73	1.862×10^{-7}	6.877×10^{-7}
$\text{H}_2\text{AMP}^- = \text{H}^+ + \text{HAMP}^-$	3.99	1.023×10^{-4}	1.966×10^{-4}
$\text{MgAMP}^- = \text{Mg}^{2+} + \text{AMP}^{2-}$	2.79	1.622×10^{-3}	2.212×10^{-2}
$\text{HADP}^{2-} = \text{H}^+ + \text{ADP}^{3-}$	$K_{1\text{ADP}}$ 7.18	6.607×10^{-8}	4.689×10^{-7}
$\text{H}_2\text{ADP}^- = \text{H}^+ + \text{HADP}^{2-}$	$K_{2\text{ADP}}$ 4.36	4.365×10^{-5}	1.612×10^{-4}
$\text{MgADP}^- = \text{Mg}^{2+} + \text{ADP}^{3-}$	$K_{3\text{ADP}}$ 4.65	2.239×10^{-5}	1.128×10^{-3}
$\text{MgHADP}^- = \text{Mg}^{2+} + \text{HADP}^{2-}$	$K_{4\text{ADP}}$ 2.50	3.162×10^{-3}	4.313×10^{-2}
$\text{HAATP}^{3-} = \text{H}^+ + \text{ATP}^{4-}$	$K_{1\text{ATP}}$ 7.60	2.512×10^{-8}	3.426×10^{-7}
$\text{H}_2\text{ATP}^{2-} = \text{H}^+ + \text{HAATP}^{3-}$	$K_{2\text{ATP}}$ 4.68	2.089×10^{-5}	1.483×10^{-4}
$\text{MgATP}^{2-} = \text{Mg}^{2+} + \text{ATP}^{4-}$	$K_{3\text{ATP}}$ 6.18	6.607×10^{-7}	1.229×10^{-4}
$\text{MgHAATP}^- = \text{Mg}^{2+} + \text{HAATP}^{3-}$	$K_{4\text{ATP}}$ 3.63	2.344×10^{-4}	1.181×10^{-2}
$\text{Mg}_2\text{ATP}^- = \text{Mg}^{2+} + \text{MgATP}^{2-}$	$K_{5\text{ATP}}$ 2.69	2.042×10^{-3}	2.785×10^{-2}
$\text{H}_2\text{PO}_4^- = \text{H}^+ + \text{HPO}_4^{2-}$	7.22	6.026×10^{-8}	2.225×10^{-7}
$\text{MgHPO}_4^- = \text{Mg}^{2+} + \text{HPO}_4^{2-}$	2.71	1.950×10^{-3}	2.66×10^{-2}
$\text{HG6P}^- = \text{H}^+ + \text{G6P}^{2-}$	6.42	3.802×10^{-7}	1.404×10^{-6}
$\text{MgG6P}^- = \text{Mg}^{2+} + \text{G6P}^{2-}$	2.60	2.512×10^{-3}	3.462×10^{-2}
$\text{Adenosine}^1 = \text{H}^+ + \text{adenosine}$	3.50	3.162×10^{-4}	3.162×10^{-4}
$\text{ATP}^{4-} + \text{H}_2\text{O} = \text{ADP}^{3-} + \text{HPO}_4^{2-} + \text{H}^+$		2.946×10^{-1}	
$\text{ADP}^{3-} + \text{H}_2\text{O} = \text{AMP}^{2-} + \text{HPO}_4^{2-} + \text{H}^+$		6.622×10^{-2}	
$\text{AMP}^{2-} + \text{H}_2\text{O} = \text{adenosine} + \text{HPO}_4^{2-}$		1.894×10^2	
$\text{G6P}^{2-} + \text{H}_2\text{O} = \text{glucose} + \text{HPO}_4^{2-}$		8.023×10^4	
$\text{ATP}^{4-} + \text{glucose} = \text{ADP}^{3-} + \text{G6P}^{2-} + \text{H}^+$		3.671×10^{-3}	
$2\text{ADP}^{3-} = \text{ATP}^{4-} + \text{AMP}^{2-}$		2.248×10^{-1}	

Source: R. A. Alberty and R. N. Goldberg, *Biochem.*, 31, 10612 (1992). Copyright 1992 American Chemical Society.

$$f(\text{H}_2\text{ATP}^{2-}) = \frac{[\text{H}^+]^2}{1 + \frac{[\text{H}^+]}{K_{1\text{ATP}}} + \frac{[\text{H}^+]^2}{K_{1\text{ATP}}K_{2\text{ATP}}}} \quad (1.3-5)$$

These mole fractions are plotted versus pH at 298.15 K and $I = 0.25\text{ M}$ in Fig. 1.2.

Since it is possible to calculate the mole fractions of the various species of ATP at a specified pH, the **average binding of hydrogen ions** \bar{N}_H can be calculated by use of

$$\bar{N}_H = \frac{0[\text{ATP}^{4-}] + 1[\text{HAATP}^{3-}] + 2[\text{H}_2\text{ATP}^{2-}]}{[\text{ATP}]} \quad (1.3-6)$$

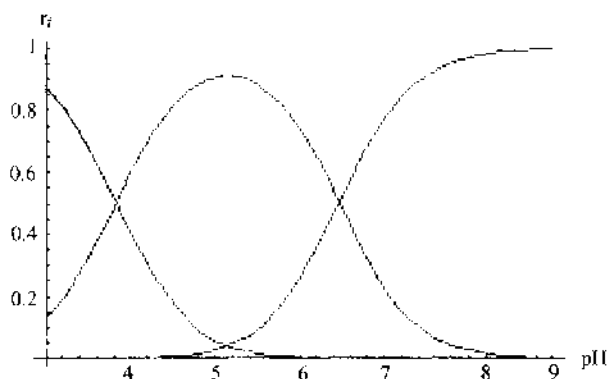


Figure 1.2 Mole fractions of three species of ATP plotted versus pH at 298.15 K and $I = 0.25\text{ M}$ (see Problem 1.1).

The numbers of hydrogen ions bound that are calculated using this equation are based on the arbitrary convention of not counting the additional 12 hydrogen atoms in ATP. Thus, the average number \bar{N}_H of hydrogen ions bound by ATP is given by

$$\bar{N}_H = \frac{\frac{[H^+]}{K_{1ATP}} + \frac{2[H^+]^2}{K_{1ATP}K_{2ATP}}}{1 + \frac{[H^+]}{K_{1ATP}} + \frac{[H^+]^2}{K_{1ATP}K_{2ATP}}} \quad (1.3-7)$$

At very high pH, the binding of H^+ approaches zero, and below pH 4 it approaches 2.

In dealing with binding, it is convenient to use the concept of a binding polynomial (Wyman 1948, 1964, 1965, 1975; Edsall and Wyman, 1958; Hermans and Scheraga, 1961; Schellman, 1975, 1976; Wyman and Gill, 1990). The polynomial in the denominator of equation 1.3-7 is referred to as the **binding polynomial** P . It is actually a kind of partition function because it gives the partition of a reactant between the various species that make it up. The binding polynomial for the binding of hydrogen ions by ATP is given by

$$P = 1 + \frac{[H^+]}{K_{1ATP}} + \frac{[H^+]^2}{K_{1ATP}K_{2ATP}} \quad (1.3-8)$$

The average binding of hydrogen ions is given by

$$\bar{N}_H = \frac{[H^+]}{P} \frac{dP}{d[H^+]} = \frac{d \ln P}{d \ln [H^+]} = \frac{-1}{\ln(10)} \frac{d \ln P}{d \text{pH}} \quad (1.3-9)$$

Equation 1.3-7 is readily obtained from equation 1.3-8 by use of equation 1.3-9.

Substituting the values of the two acid dissociation constants of ATP at 298.15 K, 1 bar, and $I = 0.25$ M from Table 1.2 into equation 1.3-7 or 1.3-9 yields the plot of \bar{N}_H versus pH that is shown in Fig. 1.3.

Figure 1.3 shows that the acid titration curve for a weak acid can be calculated from its pKs, and this raises the question as to how the pKs can be calculated from the titration curve. This can be done by first integrating equation 1.3-9 to obtain the natural logarithm of the binding potential P :

$$\int \frac{\bar{N}_H}{[H^+]} d[H^+] - \int d \ln P = \ln P + \text{const.} \quad (1.3-10)$$

or

$$-\ln(10) \int \bar{N}_H \text{pH} = \int d \ln P = \ln P + \text{const.} \quad (1.3-11)$$

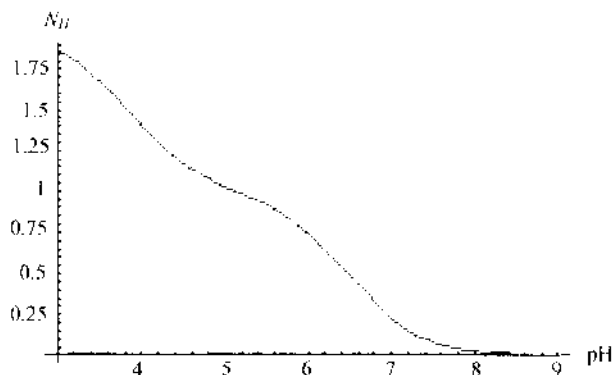


Figure 1.3 Binding of hydrogen ions by ATP at 298.15 K and $I = 0.25$ M (see Problem 1.2).

The acid dissociation constants can be calculated from $\ln P$ by fitting the plot of P versus $[H^+]$ with a power series in $[H^+]$.

ATP also binds magnesium ions as shown by the three complex ion dissociation constants in Table 1.2. Incorporating these species into equations 1.3-1 and 1.3-2 yields the following binding polynomial for ATP:

$$P = 1 + \frac{[H^+]}{K_{1ATP}} + \frac{[H^+]^2}{K_{1ATP}K_{2ATP}} + \frac{[Mg^{2+}]}{K_{3ATP}} + \frac{[Mg^{2+}][H^+]}{K_{1ATP}K_{4ATP}} + \frac{[Mg^{2+}]^2}{K_{3ATP}K_{5ATP}} \quad (1.3-12)$$

Now the binding of hydrogen ions is given by the following partial derivatives of the binding polynomial:

$$\bar{N}_H = \frac{[H^+]}{P} \left(\frac{\partial P}{\partial [H^+]} \right)_{pMg} = \frac{-1}{\ln(10)} \left(\frac{\partial \ln P}{\partial pH} \right)_{pMg} = [H^+] \left(\frac{\partial \ln P}{\partial [H^+]} \right)_{pMg} \quad (1.3-13)$$

The average binding of magnesium ions \bar{N}_{Mg} is given by the following partial derivatives of the binding polynomial:

$$\bar{N}_{Mg} = \frac{[Mg^{2+}]}{P} \left(\frac{\partial P}{\partial [Mg^{2+}]} \right)_{pH} = \frac{-1}{\ln(10)} \left(\frac{\partial \ln P}{\partial pMg} \right)_{pH} = [Mg^{2+}] \left(\frac{\partial \ln P}{\partial [Mg^{2+}]} \right)_{pH} \quad (1.3-14)$$

These differentiations yield

$$\bar{N}_H = \frac{\frac{[H^+]}{K_{1ATP}} + \frac{2[H^+]^2}{K_{1ATP}K_{2ATP}} + \frac{[Mg^{2+}][H^+]}{K_{1ATP}K_{4ATP}}}{1 + \frac{[H^+]}{K_{1ATP}} + \frac{[H^+]^2}{K_{1ATP}K_{2ATP}} + \frac{[Mg^{2+}]}{K_{3ATP}} + \frac{[Mg^{2+}][H^+]}{K_{1ATP}K_{4ATP}} + \frac{[Mg^{2+}]^2}{K_{3ATP}K_{5ATP}}} \quad (1.3-15)$$

$$\bar{N}_{Mg} = \frac{\frac{[Mg^{2+}]}{K_{3ATP}} + \frac{[Mg^{2+}][H^+]}{K_{1ATP}K_{4ATP}} + \frac{2[Mg^{2+}]^2}{K_{3ATP}K_{5ATP}}}{1 + \frac{[H^+]}{K_{1ATP}} + \frac{[H^+]^2}{K_{1ATP}K_{2ATP}} + \frac{[Mg^{2+}]}{K_{3ATP}} + \frac{[Mg^{2+}][H^+]}{K_{1ATP}K_{4ATP}} + \frac{[Mg^{2+}]^2}{K_{3ATP}K_{5ATP}}} \quad (1.3-16)$$

Figure 1.4 shows a plot of \bar{N}_H versus pH at several values of pMg. It is evident that the apparent pK of ATP in the neighborhood of 7 is reduced to about 5

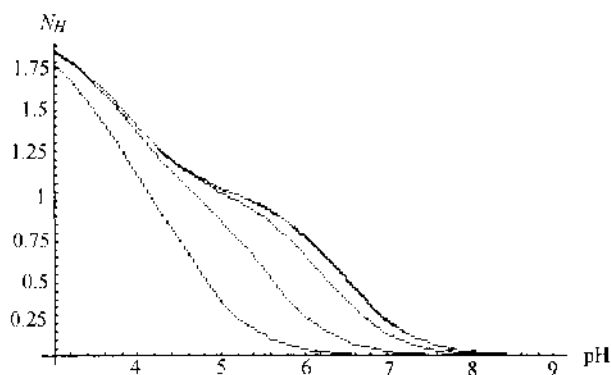


Figure 1.4 Binding of hydrogen ions by ATP at 298.15 K, $I = 0.25$ M, and pMg 2, 3, 4, 5, and 6 (see Problem 1.3).

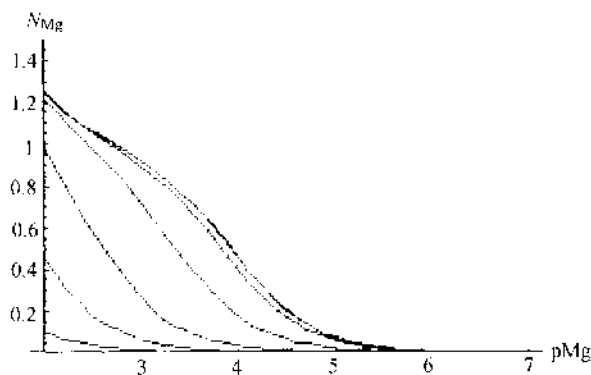


Figure 1.5 Binding of magnesium ions by ATP at 298.15 K, $I = 0.25$ M, and pH 3, 4, 5, 6, 7, 8, and 9 (see Problem 1.4).

a MgCl_2 solution with $[\text{Mg}^{2+}] = 10^{-2}$ M at ionic strength 0.25 M. Experimental plots of this type make it possible to calculate $K_{4\text{ATP}}$ and $K_{5\text{ATP}}$ (Smith and Alberty, 1956).

Figure 1.5 shows a plot of \bar{N}_{Mg} versus pMg at several values of pH. As the hydrogen ion concentration is increased, the binding of magnesium ions is decreased because of the competition for the same sites.

Equations 1.3-15 and 1.3-16 can be used to make three-dimensional plots of \bar{N}_{Mg} and as functions of pH and pMg. These plots are given in Figs. 1.6 and 1.7. The back plane of Fig. 1.6 gives the hydrogen ion binding of ATP in the essential absence of Mg (more accurately, pMg > 6). At pMg 2 the apparent second pK of ATP is less than 5. Figure 1.7 shows that below pMg 5 there is essentially no binding of magnesium ion and that binding increases to a number a little greater than 1 at pMg 2 and pH > 6 but is eliminated by further reduction of the pH. Figures 1.4 to 1.7 can also be obtained by plotting derivatives of the binding potential (see equations 1.3-13 and 1.3-14), rather than by use of equations 1.3-15 and 1.3-16 (see Problems 1.5 and 1.6).

A remarkable fact about Figs. 1.6 and 1.7 is that at any given pH and pMg, in Fig. 1.6, the slope in the pMg direction is the same as the slope in pH direction in Fig. 1.7 at that pH and pMg. This is because the mixed partial derivatives of

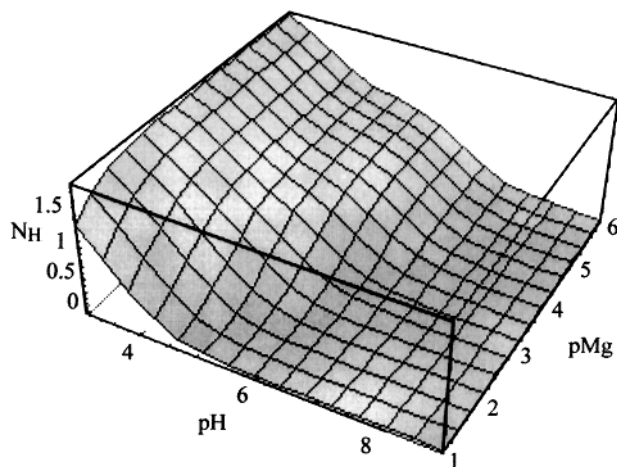


Figure 1.6 Plot of \bar{N}_{H} versus pH and pMg for ATP at 298.15 K and $I = 0.25$ M (see Problem 1.5).

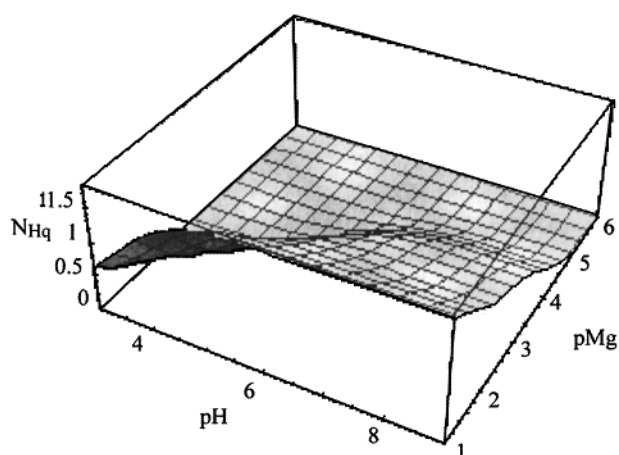


Figure 1.7 Plot of N_{Mg} versus pH and pMg at 298.15 K and $I = 0.25$ M (see Problem 1.6).

a function like P are equal.

$$\left(\frac{\partial \bar{N}_H}{\partial pMg} \right)_{T,P,pH} = \left(\frac{\partial \bar{N}_{Mg}}{\partial pH} \right)_{T,P,pMg} \quad (1.3-17)$$

In thermodynamics, this is referred to as a **Maxwell equation**. This equation is derived later in Section 4.8. Thus the effect of pMg on the binding of hydrogen ions is the same as the effect of pH on the binding of magnesium ions; in short, these are **reciprocal effects**. The bindings of these two ions are referred to as **linked functions**. Equation 1.3-17 can be confirmed by plotting these two derivatives, and the same plot is obtained in both cases. This would be a lot of work to do by hand, but since *Mathematica*[®] can take partial derivatives, this can be done readily with a computer. The two plots are identical and are given in Fig. 1.8.

■ 1.4 APPARENT EQUILIBRIUM CONSTANTS OF BIOCHEMICAL REACTIONS

In this section we consider the hydrolysis of adenosine triphosphate to adenosine diphosphate and inorganic phosphate, first at a specified pH in the absence of metal ions that are bound and then in the presence of magnesium ions. At

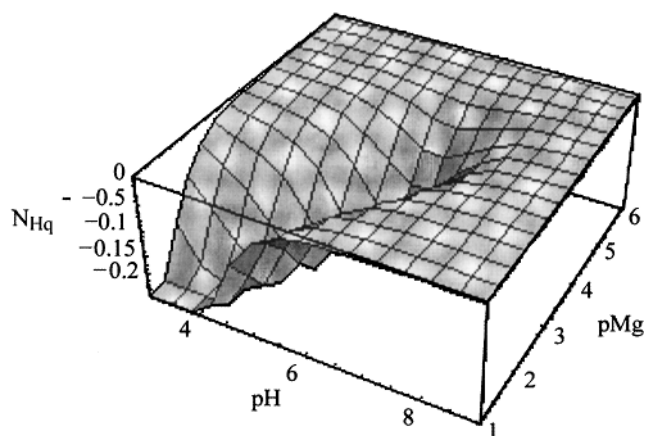
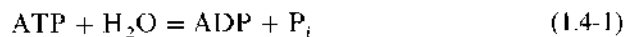


Figure 1.8 Plot of $(\partial \bar{N}_{Mg} / \partial pH)$ or $(\partial N_H / \partial pMg)$ versus pH and pMg at 298.15 K and $I = 0.25$ M (see Problem 1.6).

specified pH (and pMg) the **biochemical reaction** is written in terms of sums of species:



Biochemical textbooks often add a H^- on the right-hand side, but this is stoichiometrically incorrect when the pH is held constant, as we will see in the next section. It is also wrong, in principle, as we will see in Chapter 4, since hydrogen atoms are not balanced by biochemical reactions because the pH is held constant. The statement that the pH is constant means that in principle acid or alkali is added to the reaction system as the reaction occurs to hold the pH constant. In practice, a buffer is used to hold the pH nearly constant, and the pH is measured at equilibrium.

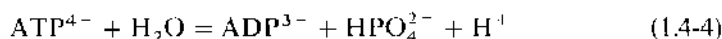
The expression for the apparent equilibrium constant K' for reaction 1.4-1 is

$$K' = \frac{[\text{ADP}][\text{P}_i]}{[\text{ATP}]} \quad (1.4-2)$$

because the activity of water is taken as unity in dilute aqueous solutions at each temperature. The apparent equilibrium constant K' is a function of T , P , pH, pMg, and ionic strength. In the neutral region in the absence of magnesium ions, ATP, ADP, and P_i each consist of two species, and so

$$\begin{aligned} K' &= \frac{([\text{ADP}^{3-}] + [\text{HADP}^{2-}])([\text{HPO}_4^{2-}] + [\text{H}_2\text{PO}_4^-])}{[\text{ATP}^{4-}] - [\text{HATP}^{3-}]} \\ &= \frac{[\text{ADP}^{3-}][\text{HPO}_4^{2-}]}{[\text{ATP}^{4-}]} \frac{(1 + [\text{H}^+]/K_{1\text{ADP}})(1 + [\text{H}^+]/K_{1\text{Pi}})}{(1 + [\text{H}^+]/K_{1\text{ATP}})} \\ &= \frac{K_{\text{ref}}}{[\text{H}^+]} \frac{(1 + [\text{H}^+]/K_{1\text{ADP}})(1 + [\text{H}^+]/K_{1\text{Pi}})}{(1 + [\text{H}^+]/K_{1\text{ATP}})} \end{aligned} \quad (1.4-3)$$

where K_{ref} is the chemical equilibrium constant for the chemical **reference reaction**



$$K_{\text{ref}} = \frac{[\text{ADP}^{3-}][\text{HPO}_4^{2-}][\text{H}^+]}{[\text{ATP}^{4-}]} \quad (1.4-5)$$

Since the acid dissociation constants are known, the value of K_{ref} can be calculated from the value of K' at a pH in the neutral region in the absence of metal ions by using equation 1.4-3. Values of K_{ref} at zero ionic strength are given in Table 1.2 for six reference reactions.

When magnesium ions or other metal ions are bound reversibly and a wider range of pH is considered, equation 1.4-3 becomes more complicated. Therefore it is convenient to use the nomenclature of binding polynomials introduced in equation 1.3-8. The binding polynomial of ATP is given in equation 1.3-12, and the binding potentials for ADP and P_i are as follows:

$$P_{\text{ADP}} = 1 + \frac{[\text{H}^+]}{K_{1\text{ADP}}} + \frac{[\text{H}^+]^2}{K_{1\text{ADP}}K_{2\text{ADP}}} + \frac{[\text{Mg}^{2+}]}{K_{3\text{ADP}}} + \frac{[\text{Mg}^{2+}][\text{H}^+]}{K_{1\text{ADP}}K_{4\text{ADP}}} \quad (1.4-6)$$

$$P_{\text{P}_i} = 1 + \frac{[\text{H}^+]}{K_{1\text{P}_i}} + \frac{[\text{Mg}^{2+}]}{K_{2\text{P}_i}} \quad (1.4-7)$$

Thus the apparent equilibrium constant for the hydrolysis of ATP as a function of $[\text{H}^+]$ and $[\text{Mg}^{2+}]$ is given by

$$K' = \frac{K_{\text{ref}}P_{\text{ADP}}P_{\text{P}_i}}{[\text{H}^+]P_{\text{ATP}}} \quad (1.4-8)$$

Since the chemical equilibrium constants in this equation are known at zero ionic strength at 298.15 K and are given in Table 1.2, K' can be calculated at any pH

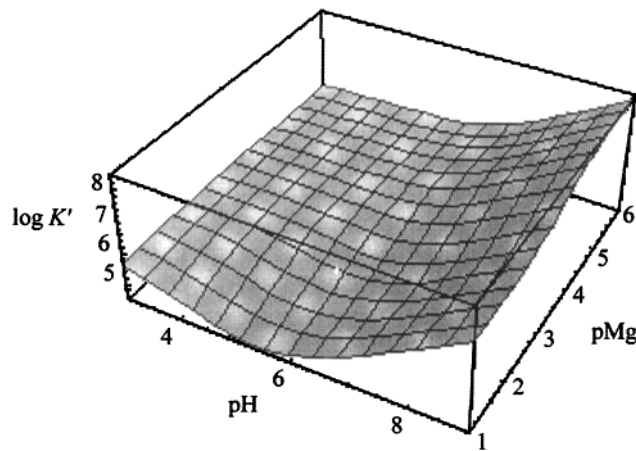


Figure 1.9 Plot of the base 10 logarithm of the apparent equilibrium constant for the hydrolysis of ATP to ADP and P_i at 298.15 K and 0.25 M ionic strength (see Problem 1.7).

in the range 3 to 9 and any pMg in the range 2 to 7 at a specified ionic strength. The dependence of K' on pH and pMg is shown in Fig. 1.9; $\log K'$ is plotted versus pH and pMg since K' varies over many powers of ten in these ranges of pH and pMg.

In Chapter 4 we will be interested in $-RT \ln K'$, where the gas constant R is $8.31451 \text{ J K}^{-1} \text{ mol}^{-1}$, and so this quantity in kJ mol^{-1} is plotted versus pH and pMg in Fig. 1.10. The pH dependencies of the apparent equilibrium constants of biochemical reactions were discussed by Alberty and Cornish-Bowden in 1993.

■ 1.5 PRODUCTION OF HYDROGEN IONS AND MAGNESIUM IONS IN THE HYDROLYSIS OF ADENOSINE TRIPHOSPHATE

The calculation of the binding of hydrogen ions \bar{N}_H for ATP discussed in Section 1.3 can be applied to ADP and P_i so that the change in binding of H^+ in the hydrolysis of ATP can be calculated using

$$\Delta_r N_H = \bar{N}_H(\text{ADP}) + \bar{N}_H(P_i) - \bar{N}_H(\text{ATP}) - 1 \quad (1.5-1)$$

where the -1 is for the two protons in water minus the proton in HPO_4^- , which is treated as the base species of inorganic phosphate in the reference reaction. The

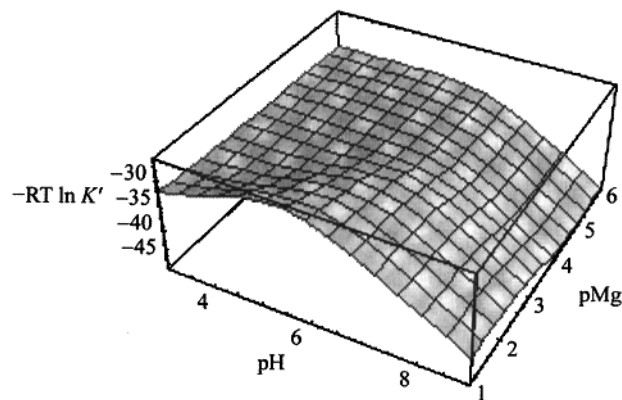


Figure 1.10 Plot of $-RT \ln K'$ in kJ mol^{-1} versus pH at 298.15 K and 0.25 M ionic strength (see Problem 1.7).

change in the binding of magnesium ions in the hydrolysis of ATP at specified pH is given by

$$\Delta_r N_{\text{Mg}} = \bar{N}_{\text{Mg}}(\text{ADP}) + \bar{N}_{\text{Mg}}(\text{P}_i) - \bar{N}_{\text{Mg}}(\text{ATP}) \quad (1.5-2)$$

Since \bar{N}_{H} and N_{Mg} can be calculated for these reactants, $\Delta_r N_{\text{H}}$ and $\Delta_r N_{\text{Mg}}$ can be calculated as a function of pH and pMg. However, when a computer is available there is an easier way to do this by using equation 1.3-13 for the binding of hydrogen ions and 1.3-14 for the binding of magnesium ions. For example, equation 1.5-1 can be written

$$\begin{aligned} \Delta_r N_{\text{H}} = & -\frac{1}{\ln(10)} \left(\frac{\partial \ln P_{\text{ADP}}}{\partial \text{pH}} \right)_{\text{pMg}} - \frac{1}{\ln(10)} \left(\frac{\partial \ln P_{\text{P}_i}}{\partial \text{pH}} \right)_{\text{pMg}} \\ & + \frac{1}{\ln(10)} \left(\frac{\partial \ln P_{\text{ATP}}}{\partial \text{pH}} \right)_{\text{pMg}} - 1 \end{aligned} \quad (1.5-3)$$

where of course T and P are also held constant. Note that this same result is obtained by simply differentiating the expression for $\ln K'$ (equation 1.4-8) with respect to pH. Thus

$$\Delta_r N_{\text{H}} = -\frac{1}{\ln(10)} \left(\frac{\partial \ln K'}{\partial \text{pH}} \right)_{\text{pMg}} \quad (1.5-4)$$

The change in binding of Mg^{2+} ions can be calculated using

$$\Delta_r N_{\text{Mg}} = -\frac{1}{\ln(10)} \left(\frac{\partial \ln K'}{\partial \text{pMg}} \right)_{\text{pH}} \quad (1.5-5)$$

Since K' is a pretty complicated function of pH and pMg, it would be very difficult to carry these calculations out by hand. However, with *Mathematica* the calculations can be done quickly. Figure 1.11 shows the change in the binding of hydrogen ions in the hydrolysis of ATP as a function of pH and pMg. At high pH the change in binding is -1 mole of H^+ per mole of ATP hydrolyzed, as expected from the reference reaction, which predominates at high pH. The products bind fewer hydrogen ions, and so there is a net production of hydrogen ions in the biochemical reaction. In the presence of magnesium ions there are conditions where the change in binding is positive, which indicates that hydrogen ions are consumed in the hydrolysis of ATP under these conditions.

Figure 1.12 shows the change in binding of magnesium ions as a function of pH and pMg. Magnesium ions are always produced in the hydrolysis because

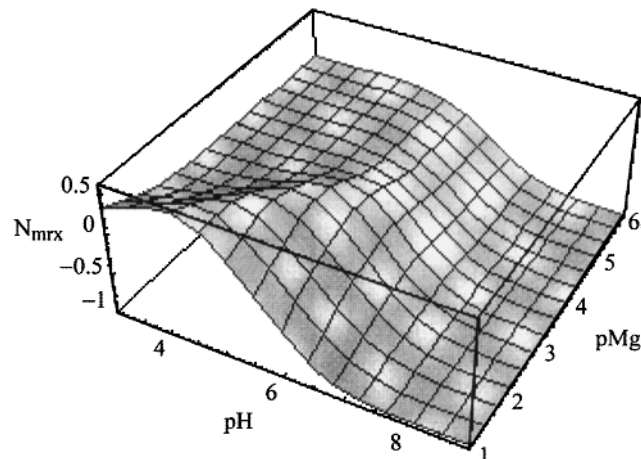


Figure 1.11 Change in the binding of hydrogen ions in the hydrolysis of ATP as a function of pH and pMg at 298.15 K and 0.25 M ionic strength (see Problem 1.8).

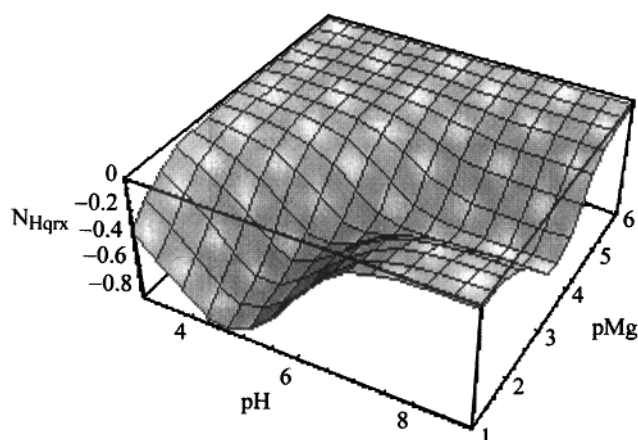


Figure 1.12 Change in the binding of magnesium ions in the hydrolysis of ATP at 298.15 K and 0.25 M ionic strength (see Problem 1.9).

they are more strongly bound by ATP than by ADP and P_i . The change in binding approaches zero as the concentration of free magnesium ions approaches zero, and it also approaches zero at high concentrations of magnesium ion and high pH, where the principal reaction is $Mg_2ATP + H_2O = MgADP + MgHPO_4 + H^+$.

Figures 1.11 and 1.12 are related in the same way as the binding curves for a single reactant (see equation 1.3-15); that is, the slope of the plot of $\Delta_r N_H$ in the pMg direction is the same as the slope of the plot of $\Delta_r N_{Mg}$ in the pH direction. This is a consequence of the reciprocity relation:

$$\left(\frac{\partial \Delta_r N_H}{\partial pMg}\right)_{T,P,pH} = \left(\frac{\partial \Delta_r N_{Mg}}{\partial pH}\right)_{T,P,pMg} \quad (1.5-6)$$

This equation is derived later in Section 4.8.

The change in the value of the apparent equilibrium constant with pH and pMg and the production or consumption of hydrogen ions and magnesium ions by the biochemical reaction are really two sides of the same coin. The effects of pH and pMg on K' are due to the fact that the biochemical reaction produces or consumes these ions. This is an example of **Le Chatelier's principle**, which states that when an independent variable of a system at equilibrium is changed, the equilibrium shifts in the direction that tends to reduce the effect of the change. If the reaction produces hydrogen ions, lowering the pH will cause K' to decrease because the system is doing what it can to reduce the effect of the pH change.

■ 1.6 pKs OF WEAK ACIDS

In this chapter we have seen that acid dissociation constants are needed to calculate the dependence of apparent equilibrium constants on pH. In Chapter 3 we will discuss the calculation of the effects of ionic strength and temperature on acid dissociation constants. The database described later can be used to calculate pKs of reactants at 298.15 K at desired ionic strengths. Because of the importance of pKs of weak acids, Table 1.3 is provided here. More experimental measurements of acid dissociation constants and dissociation constants of complex ions with metal ions are needed because they are essential for the interpretation of experimental equilibrium constants and heats of reactions. A major database of acid dissociation constants and dissociation constants of metal ion complexes is provided by Martell, Smith, and Motekaitis (2001).

Table 1.3 pKs of Weak Acids at 298.15 K in Dilute Aqueous Solutions as a Function of Ionic Strength (See Problem 1.10)

	$I = 0 \text{ M}$	$I = 0.10 \text{ M}$	$I = 0.25 \text{ M}$
acetate	4.75	4.54	4.47
acetylphosphate K_1	8.69	8.26	8.12
acetylphosphate K_2	5.11	4.90	4.83
adenine	4.20	4.20	4.20
ammonia	9.25	9.25	9.25
ATP K_1	7.60	6.74	6.47
ATP K_2	4.68	4.04	3.83
ADP K_1	7.18	6.53	6.33
ADP K_2	4.36	3.93	3.79
AMP K_1	6.73	6.30	6.16
AMP K_2	3.99	3.77	3.71
adenosine	3.47	3.47	3.47
bisphosphoglycerate	7.96	7.10	6.83
citrate K_1	6.39	5.75	5.54
citrate K_2	4.76	4.33	4.19
isocitrate K_1	6.40	5.76	5.55
socitrate K_2	4.76	4.33	4.19
coenzyme A	8.38	8.16	8.10
HCO_3^- K_1	10.30	9.90	9.76
H_2CO_3 K_2	6.37	6.15	6.08
cysteine	8.38	8.16	8.09
dihydroxyacetone phosphate	5.70	5.27	5.13
fructose 6-phosphate K_1	6.27	5.84	5.70
fructose-1,6-biphosphate K_1	6.65	5.79	5.52
fructose-1,6-biphosphate K_2	6.05	5.41	5.20
fumarate K_1	4.60	4.17	4.03
fumarate K_2	3.09	2.88	2.81
galactose 1-phosphate K_1	6.15	5.72	5.58
glucose 6-phosphate K_1	6.42	5.99	5.85
glutathione _{red}	8.34	7.91	7.77
glucose 1-phosphate K_1	6.50	6.07	5.93
glyceraldehyde phosphate	5.70	5.27	5.13
glycerol 3-phosphate	6.67	6.24	6.10
malate K_1	5.26	4.83	4.69
oxalate K_1	4.28	3.85	3.71
phosphoenolpyruvate	7.00	6.36	6.15
2-phosphoglycerate	7.64	7.00	6.79
3-phosphoglycerate	7.53	6.89	6.68
phosphate K_1	7.22	6.79	6.65
pyrophosphate K_1	9.46	8.60	8.33
pyrophosphate K_2	6.72	6.08	5.87
pyrophosphate K_3	2.26	1.83	1.69
ribose 1-phosphate K_1	6.69	6.26	6.12
ribose 5-phosphate K_1	6.69	6.26	6.12
succinate K_1	5.64	5.21	5.07
succinate K_2	4.21	3.99	3.92
succinylcoA	4.21	4.00	3.93
thioredoxin _{red} K_1	8.64	8.21	8.09
thioredoxin _{red} K_2	8.05	7.83	7.76

The equations and calculations described in this chapter are very useful, but so far we have not discussed thermodynamic properties other than equilibrium constants. The other properties introduced in the next three chapters provide a better understanding of the energetics and equilibria of reactions. We will consider the basic structure of thermodynamics in Chapter 2 and then to apply these ideas to chemical reactions in Chapter 3 and biochemical reactions in Chapter 4.

