Chapter 1 Biochemical Thermodynamics

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1.1 Introduction

It is necessary to start a book on rapid-equilibrium enzyme kinetics with a chapter on biochemical thermodynamics for two reasons: (1) The complete rate equation for an enzyme-catalyzed reaction has to contain the biochemical thermodynamics of the reaction that is catalyzed. Setting the velocity in the complete rate equation equal to zero yields the Haldane relation for the apparent equilibrium constant K' in terms of the kinetic parameters at the specified temperature, pH and ionic strength. (2) The equilibrim concentration of the enzyme-substrate complex in the rate-determining reaction is calculated with a set of independent biochemical reactions. When an independent set of reactions is at equilibrium, other reactions that are the sums or differences of the reactions in the independent set are also at equilibrium.

In chemical thermodynamics, reactions are written in terms of species, and chemical reactions must balance the various atoms and electric charges. But in biochemical thermodynamics, reactions are written in terms of reactants (sums of species like ATP), and all atoms are balanced except for hydrogen atoms because the pH is held constant. Electric charges are also not balanced in a biochemical reaction. Conceptually, hydrogen ions are added or removed during the reaction so that the pH is held constant. In practice a buffer is used to hold the pH approximately constant.

This book is about rapid-equilibrium rate equations because they are simpler than steady-state rate equations. This is not to say that all enzyme-catalyzed reactions involve a rate-determining reaction, but simply to say that rapid-equilibrium rate equations are the place to start in the investigation of rate equations. When the kinetic data for an enzyme-catalyzed reaction cannot be represented by a rapid-equilibrium rate equation, steady-state rate equations or empirical rate equations have to be used.

The thermodynamics of enzyme-catalyzed reactions is based on chemical thermodynamics because it is the species of substrates, enzymatic sites, and enzyme-substrate complexes that react. Important new concepts are involved in biochemical thermodynamics because the pH is an independent variable like T and P; that is, the pH is chosen by the investigator. In chemical thermodynamics, on the other hand, the pH is calculated using the chemical equilibrium constants for an independent set of chemical reactions and the conservation of all atoms and electric charges.

To see how the specification of pH is handled in biochemical thermodynamics, it is necessary to consider the foundations of chemical thermodynamics. The most basic criterion for spontaneous change and equilibrium in chemical thermodynamics is provided by the entropy S because the second law specifies that the entropy of an isolated system can only increase and has its maximum value at equilibrium: thus $(\mathrm{d}S)_{U,V} \geq 0$, where U is the internal energy and V is the volume. This criterion is only applicable to isolated systems because this is the only way to hold the internal energy and volume constant. The internal energy U provides the criterion for spontaneous change and equilibrium at constant volume and entropy: $(\mathrm{d}U)_{V,S} \leq 0$. The internal energy decreases to a minimum when the system goes to equilibrium at constant V and S, but there is no way to hold the entropy constant. The enthalpy H is defined by the Legendre transform $H = U + PV$, and it provides the criterion for spontaneous change and equilibrium at specified P and S: $(\mathrm{d}H)_{P,S} \leq 0$. Again there is no way to hold the entropy constant. A Legendre transform is the defintion of a new thermodynamic property by subtracting the product of two conjugate variables from an existing thermodynamic property [12,17,19]. Gibbs defined what we now call the Gibbs energy G by use of the Legendre transform $G = H - TS$. The Gibbs energy is so useful in chemistry because it provides the criterion for spontaneous change at specified temperature and pressure: $(\mathrm{d}G)_{T,P} \leq 0$. Thus a chemical reaction at specified T and P can spontaneously go in the direction that decreases the Gibbs energy, and the Gibbs energy of a chemical reaction system is at its lowest value at equilibrium.

1.2 Chemical Thermodynamics

The fundamental equation for the Gibbs energy G of a chemical reaction system is given by [22]

$$\mathrm{d}G = -S\mathrm{d}T + V\mathrm{d}P + \sum_{j=1}^{N}\mu_j\,\mathrm{d}n_j \tag{1.2-1}$$

where μ_j is the chemical potential of species j, n_j is the amount of species j, and N is the number of different species. Equation 1.2-1 shows that T, P, and $\{n_j\}$ are the independent variables for the Gibbs energy of a chemical reaction system. For ideal solutions, the chemical potential of a species is given by

$$\mu_j = \mu_j{}^\circ + RT\ln[j] \tag{1.2-2}$$

where $\mu_j{}^\circ$ is the standard chemical potential of species j and $[j]$ is the molar concentration of species j. When there is a single chemical reaction involving N species, the differential of the amount of species j is given by $\mathrm{d}n_j = \nu_j\,\mathrm{d}\xi$, where ν_j is the stoichiometric number for species j and ξ is the extent of the single reaction. In working with the fundamental equation, the chemical potential of a species is used, but, in working with experimental data, the symbol for the Gibbs energy of formation $\Delta_\mathrm{f}\,G_j$ is used rather than μ_j. When there is a single chemical reaction in a system, equation 1.2-1 becomes

$$\mathrm{d}G = -S\mathrm{d}T + V\mathrm{d}P + \sum_{j=1}^{N}\nu_j\,\Delta_\mathrm{f}\,G_j\,\mathrm{d}\xi \tag{1.2-3}$$

At constant temperature and pressure, the Gibbs energy of reaction is given by

$$\Delta_\mathrm{r}G = \mathrm{d}G/\mathrm{d}\xi = \sum_{j=1}^{N}\nu_j\Delta_\mathrm{f}\,G_j \tag{1.2-4}$$

Equation 1.2-2 for a chemical species can be written as

$$\Delta_\mathrm{f}\,G_j = \Delta_\mathrm{f}\,G_j{}^\circ + RT\ln[j] \tag{1.2-5}$$

where $\Delta_\mathrm{f}\,G_j{}^\circ$ is the standard Gibbs energy of formation of species j. In chemical thermodynamics, this equation is used for ideal solutions and activity coefficients are introduced, but in biochemical thermodynamics this equation is used at the specified ionic strength and the Debye-Huckel equation is used to account for the effects of ionic strength. Substituting equation 1.2-5 into equation 1.2-4 yields

$$\Delta_r G = \Delta_r G^\circ + RT\ln Q \tag{1.2-6}$$

where $\Delta_r G^\circ$ is the standard Gibbs energy of reaction $\sum \nu_j \Delta_f G_j{}^\circ$, and Q is the reaction quotient. At equilibrium, $\Delta_r G = 0$ and Q becomes the chemical equilibrium constant K.

$$\Delta_r G^\circ = -RT\ln K = \sum_{j=1}^{N} \nu_j \Delta_f G_j{}^\circ \tag{1.2-7}$$

Chemists have developed tables of values of $\Delta_f G_j{}^\circ$ for species with respect to the elements. In other words, the $\Delta_f G_j{}^\circ$ for elements are taken to be zero in a defined reference state. These tables can be used to calculate a number of chemical equilibrium constants K that are of biochemical interest, but the Natonal Bureau of Standards Tables [10] are limited to C_2.

More information about chemical thermodynamics is given in text books on chemical thermodynamics like Beattie and Oppenheim [8], and in my two books on biochemical thermodynamics [20,23].

The first publication that applied chemical thermodynamics to biochemical reactions was by Burton and Krebs [2], and the first table of thermodynamic properties was published by Burton in Krebs and Kornberg, Energy Transformations in Living Matter [4]. Burton recognized that the equilibrium constants for enzyme-catalyzed reactions together with the standard Gibbs energies $\Delta_f G_j{}^\circ$ of species determined with chemical methods can yield $\Delta_f G_j{}^\circ$ biochemical species. He made a table that could be used to calculate equilibrium constants of biochemical reactions that have not been studied. But he ran into problems with reactants like ATP that are sums of species at pH 7. Wilhoit [5] extended these tables, but ATP remained a problem. I became involved with ATP through electrophoresis, and my group determined acid dissociation constants and magnesium complex dissociation constants of ATP [1,3]. I worked on the thermodynamics of petroleum processing in the 1980-1990 period and learned that when the concentration of a species (like H^+) is held constant, the criterion for equilibrium is provided by a transformed Gibbs energy [13,15].

There is one more aspect of chemical thermodynamics that needs to be mentioned. If the equilibrium constants of a set of chemical reactions are known, the equilibrium composition can be calculated, but an independent set of chemical reactions must be used in the calculation. A set of chemical reactions is independent if no reaction in the set can be obtained by adding and subtracting reactions in the set. Linear algebra is needed for a complete discussion, but it leads to a simple equation: $N = C + R$, where N is the number of different species, C is the number of components, and R is the number of independent reactions [20,23]. The number of components in chemical thermodynamics is the number of elements involved.

1.3 Transformed Thermodynamic Properties of Biochemical Reactants at a Specified pH

To obtain the criterion for spontaneous change when the pH is held constant in addition to the temperature and presssure, it is necessary to use another Legendre transform. Two examples of Legendre transforms are $H = U + PV$ and $G = H - TS$ that have been discussed in Section 1.1. The conjugate variables required to introduce the pH as an independent variable are the amount of hydrogen atoms in the system $n_c(\mathrm{H})$, which is an extensive property, and the chemical potential of hydrogen ions $\mu(\mathrm{H}^+)$, which is the intensive property corrresponding to the specified pH. The amount of the hydrogen component $n_c(\mathrm{H})$ is expressed in moles, and $\mu(\mathrm{H}^+)$ is expressed kJ mol^{-1}. The product of conjugate variables is always an energy, and so $n_c(\mathrm{H})\mu(\mathrm{H}^+)$ has the units of kJ, as does G. The relation between the chemical potential of hydrogen ions and the pH is

$$\mu(\mathrm{H}^+) = \Delta_f G°(\mathrm{H}^+) - RT\ln(10)\,\mathrm{pH} \qquad (1.3\text{-}1)$$

where $\Delta_f G°(\mathrm{H}^+)$ is the standard Gibbs energy of formation of hydrogen ions and $R = 8.31451$ J mol^{-1}. Thus the transformed Gibbs energy G' of a thermodynamic system at a specified pH is defined by the Legendre transform

$$G' = G - n_c(\mathrm{H})\,\mu(\mathrm{H}^+) = G - n_c(\mathrm{H})\{\Delta_f G°(\mathrm{H}^+) - RT\ln(10)\,\mathrm{pH}\} \qquad (1.3\text{-}2)$$

The transformed Gibbs energy is needed in biochemical thermodynamics because it provides the criterion for spontaneous change and equilibrium at specified temperature, pressure, and pH: $(\mathrm{d}G')_{T,P,\mathrm{pH}} \leq 0$. Thus a biochemical reaction at specified T, P, and pH can react spontaneously in the direction that decreases G', and G' has its minimum value at equilibrium.

The fundamental equation for the transformed Gibbs energy G' for a biochemical reaction system when N' reactants are present is given by [13,20,23]

$$\mathrm{d}G' = -S'\mathrm{d}T + V\mathrm{d}P + \sum_{i=1}^{N'} \Delta_f G_i'\,\mathrm{d}n_i' + RT\ln(10)\,n_c(\mathrm{H})\,\mathrm{dpH} \qquad (1.3\text{-}3)$$

where $\Delta_f G_i'$ is the transformed Gibbs energy of formation of reactant i (sum of species), and n_i' is the amount of reactant i. A number of steps are involved in deriving this equation. Equation 1.3-3 shows that there is a new type of term in the fundamental equation for the transformed Gibbs energy that is proportional to dpH.

When the reactants in a biochemical reaction system are involved in a single biochemical reaction, $\mathrm{d}n_i' = \nu_i'\mathrm{d}\xi'$, where ν_i' is the stoichiometric number of reactant i in the biochemical reaction and $\mathrm{d}\xi'$ is the differential of the extent of the biochemical reaction. The extent ξ' of a biochemical reaction is defined by $n_i' = (n_i')_0 + \nu_i'\xi'$, where $(n_i')_0$ the amount of reactant i when $\xi' = 0$. Replacing n_i' in equation 1.3-3 with this equation at constant T, P, and pH leads to the expression for the change in the transformed Gibbs energy in the biochemical reaction.

$$\Delta_r G' = \sum_{i=1}^{N'} \nu_i' \Delta_f G_i' \qquad (1.3\text{-}4)$$

Thus equation 1.3-3 for a biochemical reaction system with a single biochemical reaction can be written as

$$dG' = -S'dT + VdP + \Delta_r G' d\xi' + RT\ln(10) n_c(\mathrm{H})\mathrm{dpH} \qquad (1.3\text{-}5)$$

Equation 1.3-5 can be used to derive the expression for the apparent equilibrium constant K' for a biochemical reaction at a specified pH. Substituting equation 1.3-4 in equation 1.3-5 yields

$$dG' = -S'dT + VdP + \sum_{i=1}^{N'} \nu_i' \Delta_f G_i' d\xi' + RT\ln(10) n_c(\mathrm{H})\mathrm{dpH} \qquad (1.3\text{-}6)$$

At specified T, P, and pH,

$$dG'/d\xi' = \sum_{i=1}^{N'} \nu_i' \Delta_f G_i' = \Delta_r G' \qquad (1.3\text{-}7)$$

This is very much like equation 1.2-4 in chemical thermodynamics except that the i reactants are sums of species and ν_i' is the stoichiometric number of reactant i in the biochemical reaction at a specified pH. Substituting

$$\Delta_f G_i' = \Delta_f G_i'^{\circ} + RT\ln[i] \qquad (1.3\text{-}8)$$

in equation 1.3-7 yields

$$\Delta_r G' = \sum_{i=1}^{N'} \nu_i' \Delta_f G'^{\circ} + RT\ln Q' = \Delta_r G'^{\circ} + RT\ln Q' \qquad (1.3\text{-}9)$$

where $\Delta_r G'^{\circ}$ is the standard transformed Gibbs energy of reaction that is given by equation 1.3-8. Q' is the apparent reaction quotient. At equilibrium, $\Delta_r G' = 0$, and so

$$\Delta_r G'^{\circ} = -RT\ln K' \qquad (1.3\text{-}10)$$

where K' is the apparent equilibrium constant for the biochemical reaction. The expression for K' is written in terms of reactant concentrations, except for H_2O.

Now we need to discuss the calculation of the standard transformed Gibbs energy of formation of a reactant $\Delta_f G_i{}'^\circ$ that was introduced in equation 1.3-8. The standard transformed Gibbs energies of formation $\Delta_f G_j{}'^\circ$ of the species that make up a reactant are given by

$$\Delta_f G_j{}'^\circ = \Delta_f G_j{}^\circ - N_H(j)\,\Delta_f G(H^+) \tag{1.3-11}$$

where $N_H(j)$ is the number of hydrogen atoms in species j. Since dilute solutions are assumed to be ideal, the Gibbs energy of species j is given by

$$\Delta_f G_j = \Delta_f G_j{}^\circ + RT\ln[j] \tag{1.3-12}$$

This equation can be applied to hydrogen ions at a specified pH.

$$\Delta_f G(H^+) = \Delta_f G^\circ(H^+) + RT\ln\left[10^{-pH}\right] \;= \Delta_f G^\circ(H^+) - RT\ln(10)\,pH \tag{1.3-13}$$

Substituting this equation into equation 1.3-11 yields

$$\Delta_f G_j{}'^\circ = \Delta_f G_j{}^\circ - N_H(j)\,(\Delta_f G^\circ(H^+) - RT\ln(10)pH) \tag{1.3-14}$$

When species have electric charges their standard thermodynamic properties need to be adjusted for the ionic strength I according to the extended Debye-Huckel theory.

$$\Delta_f G_j{}'^\circ(I) \;=\; \Delta_f G_j{}^\circ(I{=}0)\; - N_H(j)\,RT\ln(10)\,pH \;-\; RT\alpha\left(z_j{}^2 \;-\; N_H(j)\right)I^{1/2}/\left(1 \;+\; 1.6\,I^{1/2}\right) \tag{1.3-15}$$

In the ionic strength term, z_j is the electric charge of species j, and $\alpha = 1.17582\ \mathrm{kg}^{1/2}\ \mathrm{mol}^{-1/2}$ at 298.15 K. This equation makes it possible to produce tables of standard transformed Gibbs energies of formation $\Delta_f G_j{}'^\circ$ of species at specified temperature, pH and ionic strength.

Now we can consider the relation between the standard transformed Gibbs energies of formation of the species and the standard transformed Gibbs energy of formation of the reactant at a specified temperature, pH, and ionic strength. Because of the entropy of mixing the species, the standard transformed Gibbs energy of formation is more negative than any of the species. The standard transformed Gibbs energy of formation of a reactant $\Delta_f G_i{}'^\circ$ is given in terms of the standard transformed Gibbs energies of formation $\Delta_f G_j{}'^\circ$ of the species (pseudoisomers) by [11]

$$\Delta_f G_i{}'^\circ = -\,RT\ln\sum_{j=1}^{N}\exp(-\Delta_f G_j{}'^\circ/RT) \tag{1.3-16}$$

where N is the number of different species in the pseudoisomer group. This summation is a partition function. The same result can be obtained by taking the mole-fraction-weighted average of the standard transformed Gibbs energies of formation of the species and adding a term for the transformed Gibbs energy of mixing. This equation made it possible to produce tables of standard transformed Gibbs energies of biochemical reactants at specified temperature, pH, and ionic strength. The equilibrium mole fractions r_j of the species in the reactant at a specified pH are given by

$$r_j = \exp[(\Delta_f G_i'^\circ - \Delta_f G_j'^\circ)/RT] \tag{1.3-17}$$

When the pH is specified, equation 1.2-7 is replaced with

$$\Delta_r G'^\circ = -RT\ln K' = \sum_{i=1}^{N'} \nu_i' \Delta_f G_i'^\circ \tag{1.3-18}$$

This makes it possible to produce tables of standard transformed Gibbs energies of reaction and tables of apparent equilibrium constants K' for biochemical reactions for which $\Delta_f G_i'^\circ$ (see equation 1.3-16) are known for all the reactants. It is important to notice that this does not require that K' has been measured for this reaction. More detailed derivations of these equations are given in references [20] and [23].

When the pH has been specified, the relation $N = C + R$ of chemical thermodynamics no longer applies; see the end of Section 1.2. It is replaced with $N' = C' + R'$, where N' is the number of reactants (sums of species), C' is the number of components, excluding hydrogen, and R' is the number of independent biochemical reactions [20,23]. The equilibrium composition of a system of biochemical reactions can be calculated using an independent set of biochemical reactions. A set of reactions is independent if no reaction in the set can be obtained by adding or subtracting reactions in the set. This relation for determining the number R' of independent reactions will be used often in this book.

1.4 Calculation of the change in binding of hydrogen ions in a biochemical reaction at a specified pH by taking the derivative of logK' or $\Delta_r G'^\circ$ with respect to pH

The fourth term in equation 1.3-5 shows how the transformed Gibbs energy of a biochemical reaction system with one reaction changes with the pH, but there is no direct way to determine G' for a reaction system. However, the terms on the right hand side of equation 1.3-5 are related by Maxwell equations. The derivative of $\Delta_r G'$ with respect to pH is equal to the derivative of $RT\ln(10)n_c(\mathrm{H})$ with respect to the extent of reaction ξ'.

$$\partial \Delta_r G'/\partial \mathrm{pH} = \partial RT\ln(10) n_c(\mathrm{H})/\partial \xi' = RT\ln(10)\partial n_c(\mathrm{H})/\partial \xi' \tag{1.4-1}$$

Because of equation 1.3-9, the derivative $\partial \Delta_r G'/\partial \mathrm{pH}$ is the same as $\partial \Delta_r G'^\circ/\partial \mathrm{pH}$. Since $\Delta_r G'^\circ = -RT\ln K'$, equation 1.4-1 can be written as

$$\partial \ln K'/\partial \mathrm{pH} = -\ln(10)\partial n_c(\mathrm{H})/\partial \xi' \tag{1.4-2}$$

This shows that when the apparent equilibrium constant depends on the pH, the amount of hydrogen atoms in the system changes when the reaction occurs. $\partial n_c(\mathrm{H})/\partial \xi'$ is the increase in the binding of hydrogen ions, and so it is represented by $\Delta_r N_\mathrm{H}$.

$$\Delta_r N_\mathrm{H} = -\partial \log K'/\partial \mathrm{pH} \tag{1.4-3}$$

When the apparent equilibrium constant increases with the pH, $\Delta_r N_H$ is negative, and the amount of hydrogen atoms in the system decreases as the reaction occurs. This means that the reaction produces hydrogen ions. When the apparent equilibrium constant decreases with the pH, $\Delta_r N_H$ is positive, and the amount of hydrogen atoms in the system increases as the reaction occurs. This means that the reaction consumes hydrogen ions. This is an example of Le Chatelier's principle that if the conditions are changed on a system at equilibrium it will shift in the direction to oppose the change.

The change in binding of hydrogen ions in a biochemical reaction can also be calculated from the acid dissociation constants of the reactants using

$$\Delta_r N_H = \sum \nu_i{}' \overline{N}_H(i) \qquad (1.4\text{-}4)$$

where $\overline{N}_H(i)$ is the average number of hydrogen atoms bound by the species of reactant i. $\Delta_r N_H$ can be calculated for almost all enzyme-catalyzed reactions in the EC list [Web2] because the p*K*s of reactants can generally be estimated, and the number of hydrogen atoms in species are known from their structures [26].

When we get to kinetics, we will see that $\Delta_r N_H$ of thermodynamics is related to the consumption n of hydrogen ions in the rate-determining reaction [27]. When it is necessary to use the steady-state rate equation the relation between the consumption of hydrogen ions and the velocity is more complicated.

1.5 Calculation of the standard transformed Gibbs energies of formation of biochemical reactants

To calculate the standard transformed Gibbs energy of a biochemical reactant $\Delta_f G_i{}'^{\circ}$, it is necessary to use equation 1.3-15 to calculate the standard transformed Gibbs energy of formation of each species at the desired temperature, pH, and ionic strength. The standard transformed Gibbs energy of formation of a reactant is not a mole fraction average of the $\Delta_f G_j{}'^{\circ}$ of the species that make up the reactant because of the entropy of mixing. Equation 1.3-16 has to be used to calculate the standard transformed Gibbs energy of the sum of species (the reactant) at the desired temperature, pH and ionic strength. The database BasicBiochemData3 [*MathSource3*] contains $\{\Delta_f G^\circ, \Delta_f H^\circ, z_j, N_H\}$ for each species of 199 biochemical reactants. The energies are in kJ mol^{-1}at 298.15 K. In order to demonstrate the calculation of standard transformed Gibbs energies of reactants and the calculation of the apparent equilibrium constants of biochemical reactions, the species properties of the species of inorganic phosphate, ATP, ADP, and H_2O from BasicBiochemData3 are given here

The species properties for the hydrolysis of ATP are given by [14]:

```
pisp = {{-1096.1, -1299., -2, 1}, {-1137.3, -1302.6, -1, 2}};

atpsp =
  {{-2768.1, -3619.21, -4, 12}, {-2811.48, -3612.91, -3, 13}, {-2838.18, -3627.91, -2, 14}};

adpsp =
  {{-1906.13, -2626.54, -3, 12}, {-1947.1, -2620.94, -2, 13}, {-1971.98, -2638.54, -1, 14}};

h2osp = {{-237.19, -285.83, 0, 2}};
```

The following program derives the expression for the standard transformed Gibbs energy of formation of a reactant at 298.15 K.

```
calcdGmat[speciesmat_] :=
Module[{dGzero,dHzero, zi, nH, pHterm, isterm,gpfnsp},(*This program derives the
function of pH and ionic strength (is) that gives the standard transformed Gibbs
energy of formation of a reactant (sum of species) at 298.15 K.  The input speciesmat
is a matrix that gives the standard Gibbs energy of formation, the standard enthalpy
of formation, the electric charge, and the number of hydrogen atoms in each species.
There is a row in the matrix for each species of the reactant. gpfnsp is a list of
the functions for the species.  Energies are expressed in kJ mol^-1.*)
{dGzero,dHzero,zi,nH}=Transpose[speciesmat];
pHterm = nH*8.31451*.29815*Log[10^-pH];
isterm = 2.91482*((zi^2) - nH)*(is^.5)/(1 + 1.6*is^.5);
gpfnsp=dGzero - pHterm - isterm;
-8.31451*.29815*Log[Apply[Plus,Exp[-1*gpfnsp/(8.31451*.29815)]]]]
```

The function that expresses the standard transformed Gibbs energy of formation of inorganic phosphate at 298.15 K in the range pH 5 to 9 and ionic strength is calculated as follows:

```
piG = calcdGmat[pisp]
```

$$-2.47897\,\mathrm{Log}\left[e^{-0.403393\left(-1137.3+\frac{2.91482\,is^{0.5}}{1+1.6\,is^{0.5}}-4.95794\,\mathrm{Log}\left[10^{-pH}\right]\right)}+e^{-0.403393\left(-1096.1+\frac{8.74446\,is^{0.5}}{1+1.6\,is^{0.5}}-2.47897\,\mathrm{Log}\left[10^{-pH}\right]\right)}\right]$$

Table 1.1 Standard transformed Gibbs energies of formation $\Delta_f G'^{\circ}$ of inorganic phosphate in kJ mol^{-1} at 298.15 K.

```
TableForm[piG /. is → {0, .1, .25} /. pH → {5, 6, 7, 8, 9}, TableHeadings →
  {{"I=0", "I=0.10", "I=0.25"}, {"  pH 5", "  pH 6", "  pH 7", "  pH 8", "  pH 9"}}]
```

	pH 5	pH 6	pH 7	pH 8	pH 9
I=0	-1080.23	-1068.95	-1058.56	-1050.81	-1044.77
I=0.10	-1079.65	-1068.56	-1059.17	-1052.42	-1046.58
I=0.25	-1079.46	-1068.49	-1059.49	-1052.97	-1047.17

The function that expresses the standard transformed Gibbs energy of formation of ATP in the range pH 5 to 9 is calculated as follows:

```
atpG = calcdGmat[atpsp]
```

$$-2.47897\,\mathrm{Log}\left[e^{-0.403393\left(-2838.18+\frac{29.1482\,is^{0.5}}{1+1.6\,is^{0.5}}-34.7056\,\mathrm{Log}\left[10^{-pH}\right]\right)}+\right.$$
$$\left.e^{-0.403393\left(-2811.48+\frac{11.6593\,is^{0.5}}{1+1.6\,is^{0.5}}-32.2266\,\mathrm{Log}\left[10^{-pH}\right]\right)}+e^{-0.403393\left(-2768.1+\frac{11.6593\,is^{0.5}}{1+1.6\,is^{0.5}}-29.7477\,\mathrm{Log}\left[10^{-pH}\right]\right)}\right]$$

Table 1.2 Standard transformed Gibbs energies of formation $\Delta_f G'^{\circ}$ of ATP in kJ mol^{-1} at 298.15 K.

```
TableForm[atpG /. is → {0, .1, .25} /. pH → {5, 6, 7, 8, 9}, TableHeadings →
  {{"I=0", "I=0.10", "I=0.25"}, {"  pH 5", "  pH 6", "  pH 7", "  pH 8", "  pH 9"}}]
```

	pH 5	pH 6	pH 7	pH 8	pH 9
I=0	-2441.43	-2366.43	-2292.61	-2220.96	-2151.73
I=0.10	-2438.3	-2364.24	-2292.16	-2222.71	-2154.09
I=0.25	-2437.46	-2363.76	-2292.5	-2223.44	-2154.88

The function that expresses the standard transformed Gibbs energy of formation of ADP in the range pH 5 to 9 is calculated as follows:

```
adpG = calcdGmat[adpsp]
```

$$-2.47897\,\mathrm{Log}\left[e^{-0.403393\left(-1971.98+\frac{37.8927\,is^{0.5}}{1+1.6\,is^{0.5}}-34.7056\,\mathrm{Log}\left[10^{-pH}\right]\right)}+\right.$$
$$\left.e^{-0.403393\left(-1947.1+\frac{26.2334\,is^{0.5}}{1+1.6\,is^{0.5}}-32.2266\,\mathrm{Log}\left[10^{-pH}\right]\right)}+e^{-0.403393\left(-1906.13+\frac{8.74446\,is^{0.5}}{1+1.6\,is^{0.5}}-29.7477\,\mathrm{Log}\left[10^{-pH}\right]\right)}\right]$$

Table 1.3 Standard transformed Gibbs energies of formation $\Delta_f G'^{\circ}$ of ADP in kJ mol^{-1} at 298.15 K.

```
TableForm[adpG /. is → {0, .1, .25} /. pH → {5, 6, 7, 8, 9}, TableHeadings →
  {{"I=0", "I=0.10", "I=0.25"}, {"  pH 5", "  pH 6", "  pH 7", "  pH 8", "  pH 9"}}]
```

```
        |  pH 5      pH 6      pH 7      pH 8      pH 9
I=0     | -1576.6   -1502.08  -1428.93  -1358.51  -1289.7
I=0.10  | -1570.84  -1497.02  -1425.55  -1356.41  -1287.83
I=0.25  | -1569.05  -1495.55  -1424.7   -1355.78  -1287.24
```

The function that expresses the standard transformed Gibbs energy of formation of H_2O in the range pH 5 to 9 is calculated as follows:

```
h2oG = calcdGmat[h2osp]
```

$$-2.47897\ \mathrm{Log}\left[e^{-0.403393\left(-237.19+\frac{5.82964\,\mathrm{is}^{0.5}}{1+1.6\,\mathrm{is}^{0.5}}-4.95794\,\mathrm{Log}\left[10^{-\mathrm{pH}}\right]\right)}\right]$$

Table 1.4 Standard transformed Gibbs energies of formation $\Delta_f G'^\circ$ of H_2O in kJ mol^{-1} at 298.15 K.

```
TableForm[h2oG /. is → {0, .1, .25} /. pH → {5, 6, 7, 8, 9}, TableHeadings →
  {{"I=0", "I=0.10", "I=0.25"}, {"  pH 5", "  pH 6", "  pH 7", "  pH 8", "  pH 9"}}]
```

```
        |  pH 5      pH 6      pH 7      pH 8      pH 9
I=0     | -180.11   -168.693  -157.277  -145.861  -134.445
I=0.10  | -178.885  -167.469  -156.053  -144.637  -133.221
I=0.25  | -178.49   -167.074  -155.658  -144.242  -132.826
```

1.6 Calculation of the standard transformed Gibbs energy of hydrolysis of ATP to ADP and the apparent equilibrium constant *K* '

The biochemical reaction is

$$\mathrm{ATP} + H_2O = \mathrm{ADP} + P_i \tag{1.6-1}$$

For the hydrolysis of ATP, the change in standard transformed Gibbs energy of reaction is given by

$$\Delta_r G'^\circ = \Delta_f G'^\circ(\mathrm{ADP}) + \Delta_f G'^\circ(P_i) - \Delta_f G'^\circ(\mathrm{ATP}) - \Delta_f G'^\circ(H_2O) \tag{1.6-2}$$

The apparent equilibrium constant K' is given by

$$K' = \exp(-\Delta_r G'^\circ/RT) = \frac{[\mathrm{ADP}][\mathrm{Pi}]}{[\mathrm{ATP}]} \tag{1.6-3}$$

The concentration of H_2O is omitted in the expression of the apparent equilibrium constant, but $\Delta_f G'^\circ(H_2O)$ is needed to calculate K' at the desired temperature, pH, and ionic strength. The biochemical reactant ATP is made up of three species (ATP^{4-}, HATP^{3-}, $H_2\mathrm{ATP}^{2-}$) in the pH range 5 to 9.

The following program calculates the function of pH and ionic strength that yields the change in a standard thermodynamic property for a biochemical reaction at 298.15 K [23].

```
deriverxfn[eq_] :=
 Module[{function}, (*Derives the function of pH and ionic strength that gives the
     thermodynamic properties of a biochemical reaction typed in the form atpG+h2oG+de==
   adpG+piG.  Other suffixes can be used for H, S, and NH.*)
  function = Solve[eq, de]; function[[1, 1, 2]]]
```

```
atphydfnG = deriverxfn[atpG + h2oG + de == adpG + piG]
```

$$
\begin{aligned}
&2.47897\,\mathrm{Log}\left[e^{-0.403393\left(-237.19-\frac{5.82964\,\mathrm{is}^{0.5}}{1.+1.6\,\mathrm{is}^{0.5}}-4.95794\,\mathrm{Log}\left[10.^{-1.\,\mathrm{pH}}\right]\right)}\right]+\\
&\quad 2.47897\,\mathrm{Log}\left[e^{-0.403393\left(-2838.18+\frac{29.1482\,\mathrm{is}^{0.5}}{1.+1.6\,\mathrm{is}^{0.5}}-34.7056\,\mathrm{Log}\left[10.^{-1.\,\mathrm{pH}}\right]\right)}+\right.\\
&\qquad \left.e^{-0.403393\left(-2811.48+\frac{11.6593\,\mathrm{is}^{0.5}}{1.+1.6\,\mathrm{is}^{0.5}}-32.2266\,\mathrm{Log}\left[10.^{-1.\,\mathrm{pH}}\right]\right)}+e^{-0.403393\left(-2768.1-\frac{11.6593\,\mathrm{is}^{0.5}}{1.+1.6\,\mathrm{is}^{0.5}}-29.7477\,\mathrm{Log}\left[10.^{-1.\,\mathrm{pH}}\right]\right)}\right]-\\
&\quad 2.47897\,\mathrm{Log}\left[e^{-0.403393\left(-1971.98+\frac{37.8927\,\mathrm{is}^{0.5}}{1.+1.6\,\mathrm{is}^{0.5}}-34.7056\,\mathrm{Log}\left[10.^{-1.\,\mathrm{pH}}\right]\right)}+e^{-0.403393\left(-1947.1+\frac{26.2334\,\mathrm{is}^{0.5}}{1.+1.6\,\mathrm{is}^{0.5}}-32.2266\,\mathrm{Log}\left[10.^{-1.\,\mathrm{pH}}\right]\right)}+\right.\\
&\qquad \left.e^{-0.403393\left(-1906.13+\frac{8.74446\,\mathrm{is}^{0.5}}{1.+1.6\,\mathrm{is}^{0.5}}-29.7477\,\mathrm{Log}\left[10.^{-1.\,\mathrm{pH}}\right]\right)}\right]-\\
&\quad 2.47897\,\mathrm{Log}\left[e^{-0.403393\left(-1137.3+\frac{2.91482\,\mathrm{is}^{0.5}}{1.+1.6\,\mathrm{is}^{0.5}}-4.95794\,\mathrm{Log}\left[10.^{-1.\,\mathrm{pH}}\right]\right)}+e^{-0.403393\left(-1096.1-\frac{8.74446\,\mathrm{is}^{0.5}}{1.+1.6\,\mathrm{is}^{0.5}}-2.47897\,\mathrm{Log}\left[10.^{-1.\,\mathrm{pH}}\right]\right)}\right]
\end{aligned}
$$

This function of the pH and ionic strength can be used to make tables or plots of $\Delta_r G'^\circ$ for the hydrolysis of ATP at 298.15 K.

Table 1.5 Standard transformed Gibbs energies of reaction in kJ mol^{-1} for ATP + H_2O = ADP + P_i at 298.15 K

```
PaddedForm[TableForm[atphydfnG /. is → {0, .1, .25} /. pH → {5, 6, 7, 8, 9}, TableHeadings →
    {{"I=0", "I=0.10", "I=0.25"}, {"   pH 5", "   pH 6", "   pH 7", "   pH 8", "   pH 9"}}], {4, 2}]
```

```
       |  pH 5    pH 6    pH 7    pH 8    pH 9
-------+---------------------------------------
I=0    | -35.30  -35.91  -37.60  -42.50  -48.29
I=0.10 | -33.30  -33.87  -36.50  -41.48  -47.10
I=0.25 | -32.56  -33.22  -36.04  -41.07  -46.70
```

This calculation can be checked by using the values $\Delta_r G'^\circ$ at pH 7 and 0.25 M ionic strength for the four reactants.

```
-1059.49 - 1424.7 + 155.66 + 2292.5
```

```
-36.03
```

The corresponding apparent equilibrium constants are calculated as follows:

```
kprimeATPhyd = Exp[-atphydfnG / (8.31451 * .29815)]
```

$$
e^{0.403393\left(-2.47897\,\mathrm{Log}\left[e^{-0.403393\left(-237.19-\frac{5.82964\,\mathrm{is}^{0.5}}{1.+1.6\,\mathrm{is}^{0.5}}-4.95794\,\mathrm{Log}\left[10.^{-1.\,\mathrm{pH}}\right]\right)}\right]-2.47897\,\mathrm{Log}\left[e^{-0.403393\left(-2838.18+\frac{29.1482\,\mathrm{is}^{0.5}}{1.+1.6\,\mathrm{is}^{0.5}}-34.7056\,\mathrm{Log}\left[10.^{-1.\,\mathrm{pH}}\right]\right)}+e^{-0.403393\left(-2811.48\right.}\right.\right.}
$$

Table 1.6 Apparent equilibrium constants K' at 298.15 K

```
PaddedForm[TableForm[kprimeATPhyd /. is → {0, .1, .25} /. pH → {5, 6, 7, 8, 9},
  TableHeadings → {{"I=0", "I=0.10", "I=0.25"},
    {"     pH 5", "     pH 6", "     pH 7", "     pH 8", "     pH 9"}}]]
```

```
       |    pH 5            pH 6            pH 7            pH 8            pH 9
-------+------------------------------------------------------------------------------
I=0    | 1.52785 × 10^6  1.95781 × 10^6  3.87287 × 10^6  2.79036 × 10^7  2.88781 × 10^8
I=0.10 | 680808.         859076.         2.48023 × 10^6  1.8478 × 10^7   1.78325 × 10^8
I=0.25 | 506774.         659585.         2.05626 × 10^6  1.5698 × 10^7   1.51989 × 10^8
```

The apparent equilibrium constants for about 200 enzyme-catalyzed reactions are given in Chapter 12 of reference [23] and in BasicBiochemData3 [*MathSource*3]. $\Delta_r H'^\circ$ and $\Delta_r S'^\circ$ can also be calculated as functions of pH and ionic strength.

1.7 Calculation of the change in the binding of hydrogen ions $\Delta_r N_H$ in ATP + H_2O = ADP + P_i at 298.15 K

Equation 1.4-3 shows that the change in binding of hydrogen ions in the hydrolysis of ATP to ADP can be calculated using $\Delta_r N_H = -\partial\log[\text{kprimeATPhyd}]/\partial\text{pH}$.

Table 1.7 Change in the binding of hydrogen ions in ATP + H_2O = ADP + P_i at 298.15 K

```
PaddedForm[TableForm[
  -D[Log[10, kprimeATPhyd], pH] /. is → {0, .1, .25} /. pH → {5, 6, 7, 8, 9}, TableHeadings →
   {{"I=0", "I=0.10", "I=0.25"}, {"  pH 5", "  pH 6", "  pH 7", "  pH 8", "  pH 9"}}], {4, 2}]
```

```
        |  pH 5    pH 6    pH 7    pH 8    pH 9
--------+----------------------------------------
I=0     | -0.15   -0.12   -0.58   -1.01   -1.01
I=0.10  | -0.05   -0.22   -0.72   -0.96   -1.00
I=0.25  | -0.04   -0.25   -0.74   -0.96   -1.00
```

In Biochemical Thermodynamics: Applications of *Mathematica* [23] $\Delta_r N_H$ is calculated at 298.15 K and 0.25 M ionic strength for 229 enzyme-catalyzed reactions.

1.8 Calculation of the change in the binding of hydrogen ions in a biochemical reaction at a specified pH without information on standard Gibbs energies of formation of species

The calculation of $\Delta_r N_H$ without knowing the standard Gibbs energies of formation of the species in a reaction is a two-step process. First, the average number of hydrogen ions $\overline{N}_H$ in various reactants are calculated. Second, these average values are added and subtracted to obtain $\Delta_r N_H$ for various biochemical reactions. The calculation of the average binding of hydrogen ions by ATP as a function of pH and ionic strength is discussed on p. 29-32 in Biochemical Thermodynamics: Applications of *Mathematica* [23]. Since ATP is made up of three species in the range pH 5 to 9, its concentration is given by

$$[\text{ATP}] = [\text{ATP}^{4-}] + [\text{HATP}^{3-}] + [\text{H}_2\,\text{ATP}^{2-}] \tag{1.8-1}$$

When the acid dissociations are at equilibrium, substituting the expressions for the two acid dissociation constants yields

$$[\text{ATP}] = [\text{ATP}^{4-}](1 + 10^{\text{pK1ATP-pH}} + 10^{\text{pK1ATP+pK2ATP-2 pH}}) = [\text{ATP}^{4-}]*\text{p} \tag{1.8-2}$$

where $\text{pK}_{1\,\text{ATP}} = -\log\text{K}_{1\,\text{ATP}}$ is the highest pK that has to be considered in the range pH 5 to 9. The factor multiplying $\left[\text{ATP}^{4-}\right]$ is referred to as a binding polynomial and is represented here by p. Equation 1.8-2 shows that the equilibrium mole fraction r_1 for $\left[\text{ATP}^{4-}\right]$ is given by

$$r_1 = 1/\text{p} \tag{1.8-3}$$

The other two equilibrium mole fractions are given by

$$r_2 = 10^{\text{pK1ATP-pH}}/\text{p} \tag{1.8-4}$$

$$r_3 = 10^{\text{pK1ATP+pK2ATP-2 pH}}/\text{p} \tag{1.8-5}$$

The pKs are at the desired temperature and ionic strength. The following calculations of the equilibrium concentrations of the species of ATP are for 298.15 K and 0.25 M ionic strength.

```
patp = 1 + 10^(pK1ATP - pH) + 10^(pK1ATP + pK2ATP - 2 * pH);
```

```
pK1ATP = 6.47;

pK2ATP = 3.83;
```

The equilibrium mole fractions of the three species of ATP are given by

```
r1 = 1 / patp;

r2 = 10^ (pK1ATP - pH) / patp;

r3 = 10^ (pK1ATP + pK2ATP - 2 * pH) / patp;
```

ATP^{4-}has 12 hydrogen atoms. $HATP^{3-}$ has 13 hydrogen atoms. H_2ATP^{2-} has 14 hydrogen atoms. The average number of hydrogen atoms in a biochemical reactant is given by

$$\overline{N}_H(i) = \sum r_j N_H(j) \qquad (1.8\text{-}6)$$

The average number of hydrogen atoms in ATP is given by

```
nHATP = 12 * r1 + 13 * r2 + 14 * r3;

Plot[{nHATP}, {pH, 3, 9}, AxesLabel → {"pH", "N̄H"}]
```

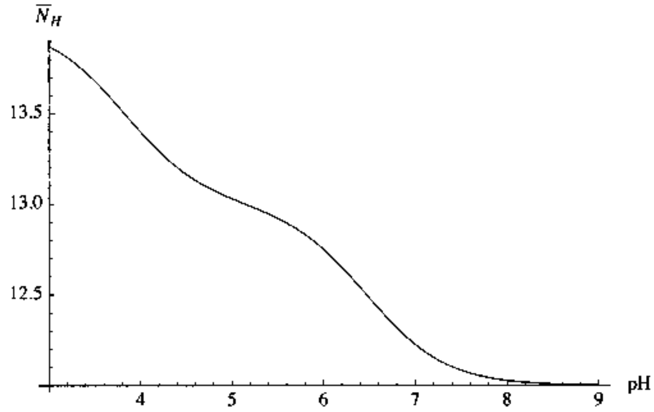

Fig. 1.1 Titration curve for ATP at 298.15 K and ionic strength 0.25 M.

In contrast with the way titration curves are usually plotted, this plot gives the total number of hydrogen atoms.

The following program is used to derive the function of pH and ionic strength that will yield the average number of hydrogen atoms in a reactant with two dissociable hydrogen atoms.

```
calcavnoH[pK1_, pK2_, nHbasicform_] :=
  Module[{p, r1, r2, r3}, (*This program derives the function of pH that gives the average
    number of hydrogen atoms in a reactant with three species.  The pKs are for the
    desired temperature and ionic strength.  nHbasicform is the number of hydrogen
    atoms in the species that dominates at the highest pH.  If there is a single pK,
   pK2 can be set equal to zero.  When there is a single species in the range pH 5 to 9,
   both pK1 and pK2 are set equal to zero.*)
   p = 1 + 10^ (pK1 - pH) + 10^ (pK1 + pK2 - 2 * pH);
   r1 = 1 / p;
r2 = 10^ (pK1 - pH) / p;
r3 = 10^ (pK1 + pK2 - 2 * pH) / p;
   nHbasicform * r1 + (nHbasicform + 1) * r2 + (nHbasicform + 2) * r3];
```

The function of pH that yields $\overline{N}_H$(ATP) is obtained as follows:

```
atpnhfun = calcavnoH[6.47, 3.83, 12]
```

$$\frac{12}{1+10^{10.3-2\,\mathrm{pH}}+10^{6.47-\mathrm{pH}}}+\frac{7\;2^{11.3-2\,\mathrm{pH}}\;5^{10.3-2\,\mathrm{pH}}}{1+10^{10.3-2\,\mathrm{pH}}+10^{6.47-\mathrm{pH}}}+\frac{13\;10^{6.47-\mathrm{pH}}}{1+10^{10.3-2\,\mathrm{pH}}+10^{6.47-\mathrm{pH}}}$$

Note that the function for ATP is named atpnhfun because the name atpNH is already used in BasicBiochemData3.nb [*MathSource*3] for the average number of hydrogen atoms in ATP calculated from its standard transformed Gibbs energy of formation.

```
Plot[{atpnhfun}, {pH, 3, 9}, AxesLabel → {"pH", "N̄H"}]
```

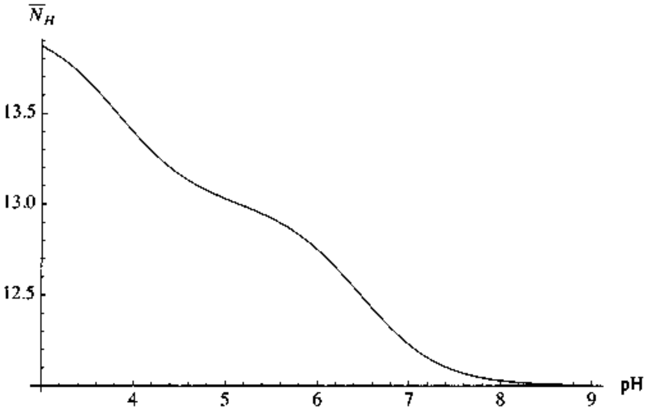

Fig. 1.2 This is the same plot as in Fig. 1.1 that was obtained without using the program calcavnoH.

The function of pH that yields $\bar{N}_H$(ADP) is obtained as follows:

```
adpnhfun = calcavnoH[6.33, 3.79, 12];
```

The function of pH that yields $\bar{N}_H$(AMP) is obtained as follows:

```
ampnhfun = calcavnoH[6.16, 3.71, 12];
```

These pKs in the ATP series were published by Alberty and Goldberg [14] in 1992.

The function of pH that yields $\bar{N}_H$ (P_i) is obtained as follows:

```
pinhfun = calcavnoH[6.65, 0, 1];
```

The second pK is set to zero to take it out of the calculation.

The function of pH that yields $\bar{N}_H$ `(adenosine)` is obtained as follows:

```
adenosinenhfun = calcavnoH[3.47, 0, 13];
```

The function of pH that yields $\bar{N}_H$ (H_2O) is obtained as follows:

```
h2onhfun = calcavnoH[0, 0, 2];
```

Since water does not have a p*K* in the range pH 5 to 9, both pK1 and pK2 are set equal to zero.

The average numbers of hydrogen atoms in these six reactants are summarized in Table 1.8.1.

```
listnames = {"ATP", "ADP", "AMP", "Adenosine", "Pi", "H₂O"};
```

Table 1.8 Average numbers of hydrogen atoms $\overline{N}_H$ in six reactants at 298.15 K and 0.25 M ionic strength

```
PaddedForm[TableForm[{atpnhfun, adpnhfun, ampnhfun, adenosinenhfun, pinhfun, h2onhfun} /.
    pH → {5, 6, 7, 8, 9}, TableHeadings →
    {listnames, {"  pH 5", "  pH 6", "  pH 7", "  pH 8", "  pH 9"}}] // N, {4, 2}]
```

```
          |  pH 5   pH 6   pH 7   pH 8   pH 9
----------+-----------------------------------
ATP       | 13.03  12.75  12.23  12.03  12.00
ADP       | 13.01  12.69  12.18  12.02  12.00
AMP       | 12.98  12.60  12.13  12.01  12.00
Adenosine | 13.03  13.00  13.00  13.00  13.00
Pi        |  1.98   1.82   1.31   1.04   1.00
H2O       |  2.00   2.00   2.00   2.00   2.00
```

These numbers can be added and subtracted to obtain $\Delta_r N_H$ for a number of enzyme-catalyzed reactions, as shown in the next section.

The change in binding of hydrogen ions is given by

$$\Delta_r N_H = \sum \nu_i{}' \overline{N}_H(i) \qquad (1.8\text{-}7)$$

where $\nu_i{}'$ is the stoichiometric number for reactant i.

This type of equation applies to $\Delta_r G'^\circ$, $\Delta_r H'^\circ$, and $\Delta_r S'^\circ$. Therefore, correct stoichiometry for biochemical equations is very important.

It is convenient to rewrite the program trGibbsRxSummary (in BasicBiochemData3.nb [*MathSource*3]) to calculate $\Delta_r N_H$ for reactions that are typed in. The program chgNHbindSummary does not require information on the standard Gibbs energies of formation of species. The short program "round" rounds changes to two digits. The names of reactants are those used in *Mathematica* where names have to start with lower case letters and connot contain dashes, spaces or periods. The scientific names of reactants are given on p. 425 of Biochemical Thermodynamics: Applications of *Mathematica* [23].

```
round[vec_, params_:{6, 2}] :=(*When a list of numbers has more digits to the
right of the decimal point than you want, say 6, you can request 2 by using
round[vec,{6,2}],*)
  Flatten[Map[NumberForm[#1, params] & , {vec}, {2}]]
```

```
chgNHbindSummary[eq_, title_, reaction_, pHlist_] :=
 Module[{functiom, vectorNH}, (*When this program is given the equation for a biochemical
     reaction in the form acetaldehydenhfun+nadrednhfun+de==ethanolnhfun+nadoxnhfun,
  it calculates the change in binding of hydrogen ions in the reaction.  The
   temperature and ionic strength are the temperature and ionic strength for the
   pKs.  title_ is in the form "EC 1.1.1.1 Alcohol dehydrogenase".  reaction_
   is in the form "acetaldehyde+nadred=ethanol+nadox".*)
  function = Solve[eq, de];
  vectorNH = round[function[[1, 1, 2]] /. pH -> pHlist, {4, 2}];
  Print[title]; Print[reaction]; Print[vectorNH]]
```

The following calculations give $\Delta_r N_H$ at pHs 5, 6, 7, 8, and 9 at 298.15 K and 0.25 M ionic strength:

```
chgNHbindSummary[atpnhfun + h2onhfun + de == adpnhfun + pinhfun,
 "EC 3.6.1.3 Adenosinetriphosphatase", "atp+h2o=adp+pi", {5, 6, 7, 8, 9}]
```

```
EC 3.6.1.3 Adenosinetriphosphatase

atp+h2o=adp+pi

{-0.04, -0.25, -0.74, -0.96, -1.00}
```

This agrees with the preceding section, but it was obtained without information on Gibbs energies of formation of species.

1.9 Another way to consider the pH dependence of the apparent equilibrium constant for ATP + H_2O = ADP + Pi

It has been known for some time [16] that the pH dependence of the apparent equilibrium can be represented by

$$K' = \frac{[\mathrm{ADP}][\mathrm{Pi}]}{[\mathrm{ATP}]} = K_{\mathrm{ref}} 10^{n\mathrm{pH}} f(\mathrm{pH}) \qquad (1.9\text{-}1)$$

where K_{ref} is the chemical equilibrium constant for a reference chemical reaction and n (positive integer if H^+ is produced and negative integer if H^+ is consumed) is the number of hydrogen ions in the reference reaction. The function $f(\mathrm{pH})$ introduces the effects of the p*K*s of the reactants. For the hydrolysis of ATP to ADP, the reference reaction can be taken to be

$$\mathrm{ATP}^{4-} + \mathrm{H_2O} = \mathrm{ADP}^{3-} + \mathrm{HPO_4^{2-}} + \mathrm{H^+} \qquad K_{\mathrm{ref1}} = \frac{[\mathrm{ADP}^{3-}][\mathrm{HPO_4^{2-}}][\mathrm{H^+}]}{[\mathrm{ATP}^{4-}]} \qquad (1.9\text{-}2)$$

When this is the reference reaction, the apparent equilibrium constant is given by $K' = K_{\mathrm{ref1}} 10^{\mathrm{pH}} f(\mathrm{pH})$.

If the reference reaction is taken to be

$$\mathrm{ATP}^{4-} + \mathrm{H_2O} = \mathrm{ADP}^{3-} + \mathrm{H_2PO_4^{-}} \qquad K_{\mathrm{ref2}} = \frac{[\mathrm{ADP}^{3-}][\mathrm{H_2PO_4^{2-}}]}{[\mathrm{ATP}^{4-}]} \qquad (1.9\text{-}3)$$

The pH dependence of K' is given by $K' = K_{\mathrm{ref2}} f(\mathrm{pH})$. In biochemical thermodynamics the choice of reference reaction is arbitrary, but in rapid-equilibrium enzyme kinetics the rate is proportional to $10^{n\mathrm{pH}}$ where n is -1, -2, ... when one, two, ... hydrogen ions are consumed in the rate-determining reaction [27]. The following sections show how to calculate the distributions of species in Pi, ATP, and ADP, and how the apparent equilibrium constant for ATP + H_2O = ADP + Pi depends on pH.

1.9.1 Distributions of species of inorganic phosphate as functions of pH

Inorganic phosphate consists of 2 species in the range pH 5-9. The p*K* is pKpi = 6.65 at 298.15 K and ionic strength 0.25 M. The fractions of the two species in total phosphate as a function of pH can be calculated by hand, but it is convenient to use *Mathematica,* which is needed when there are more dissociable hydrogen ions. The fraction of a species can be calculated by using Solve[eqs,vars,elims] to derive the function of hydrogen ion concentration h that yields the equilibrium concentration for each species. In using Solve it is necesssary to use the hydrogen ion concentration, rather than the pH, because Solve was primarily developed to solve sets of simultaneous polynomial equations. In *Mathematica*, the initial letter for a property has to be a lower case letter, and so the following abbreviations are used to represent concentrations:

$[HPO_4^{2-}]$ = pi
$[H_2PO_4^-]$ = hpi
$[HPO_4^{2-}] + [H_2PO_4^-]$ = pi + hpi = pit
$[ATP\ ^{4-}]$ = atp
$[HATP\ ^{3-}]$ = hatp
$[H_2\ ATP\ ^{3-}]$ = h2atp
$[ATP\ ^{4-}] + [HATP\ ^{3-}] + [H_2\ ATP\ ^{2-}]$ = atp + hatp + h2atp = atpt
$[ADP\ ^{3-}]$ = adp
$[HADP\ ^{2-}]$ = hadp
$[H_2\ ADP\ ^{-}]$ = h2adp
$[ADP\ ^{3-}] + [HADP\ ^{2-}] + [H_2\ ADP\ ^{-}]$ = adp + hadp + h2adp = adpt

Calculation of the fraction of [Pi] that is $[HPO_4^{2-}]$

The dissociation constant K_1 for $H_2PO_4^- = H^+ + HPO_4^{2-}$ is represented in *Mathematica* with k1pi = h×pi/hpi. The following calculation yields the function of h for the equilibrium concentration of HPO_4^{2-}. The total concentration of inorganic phosphate $[Pi]_t$ is represented by pit.

```
Solve[{k1pi == h * pi / hpi, pit == pi + hpi}, {pi}, {hpi}]
```

$$\left\{\left\{\mathtt{pi} \to \frac{\mathtt{k1pi\ pit}}{\mathtt{h + k1pi}}\right\}\right\}$$

The fraction pi/pit is

$$\frac{\mathtt{k1pi}}{\mathtt{h + k1pi}};$$

In calculating equilibrium concentrations of species, it is more convienent to use pHs and p*K*s. The ReplaceAll operation (/.x->) is used to make this change.

$$\frac{\mathtt{k1pi}}{\mathtt{h + k1pi}}\ \mathtt{/.\ h \to 10\hat{\ }{-pH}\ /.\ k1pi \to 10\hat{\ }{-pKpi}}$$

$$\frac{10^{-\mathtt{pKpi}}}{10^{-\mathtt{pH}} + 10^{-\mathtt{pKpi}}}$$

This is the expression for $[HPO_4^{2-}]/[Pi]$. To calculate this fraction as a function of pH at 298.15 K and 0.25 M ionic strength, pKpi is taken to be 6.65 [23].

$$\frac{\mathbf{k1pi}}{\mathbf{h+k1pi}}\ \mathbf{/.\ h \to 10\hat{}\text{-}pH\ /.\ k1pi \to 10\hat{}\text{-}pKpi\ /.\ pKpi \to 6.65}$$

$$\frac{2.23872\times 10^{-7}}{2.23872\times 10^{-7}+10^{-\mathrm{pH}}}$$

Plot the fraction pi/pit as a function of pH.

$$\mathbf{plot1 = Plot}\left[\frac{\mathbf{2.2387211385683377\grave{}*\hat{}\text{-}7}}{\mathbf{2.2387211385683377\grave{}*\hat{}\text{-}7 + 10^{-pH}}},\ \mathbf{\{pH,\ 5,\ 9\},\ AxesLabel \to \left\{"pH",\ "[HPO_4^{2-}]/[Pi]"\right\}}\right];$$

This plot is in composite Fig. 1.3.

The reciprocal of the fraction $[\mathrm{HPO_4^{2-}}]/[\mathrm{Pi}]$ is given by

$$\frac{10^{-\mathrm{pH}}+10^{-\mathrm{pKpi}}}{10^{-\mathrm{pKpi}}} = 1 + 10^{-\mathrm{pH}+\mathrm{pKpi}} = [\mathrm{Pi}]/[\mathrm{HPO_4^{2-}}]$$

Thus $[\mathrm{Pi}] = (1 + 10^{-\mathrm{pH}+\mathrm{pKpi}})[\mathrm{HPO_4^{2-}}]$.

[Pi] is of course always greater than $[\mathrm{HPO_4^{2-}}]$. This equation will be used in Section 1.9.5.

Calculation of the fraction of [Pi] that is $[\mathrm{H_2PO_4^-}]$

The fraction of inorganic phosphate in the form $[\mathrm{H_2PO_4^-}]$ is calculated as a function of pH as follows:

```
Solve[{k1pi == h * pi / hpi, pit == pi + hpi}, {hpi}, {pi}]
```

$$\left\{\left\{\mathrm{hpi} \to \frac{\mathrm{h\ pit}}{\mathrm{h + k1pi}}\right\}\right\}$$

The fraction hpi/pit is

$$\frac{\mathbf{h}}{\mathbf{h+k1pi}};$$

It is more convienent to use pHs and pKs.

$$\frac{\mathbf{h}}{\mathbf{h+k1pi}}\ \mathbf{/.\ h \to 10\hat{}\text{-}pH\ /.\ k1pi \to 10\hat{}\text{-}pKpi}$$

$$\frac{10^{-\mathrm{pH}}}{10^{-\mathrm{pH}}+10^{-\mathrm{pKpi}}}$$

This is the expression for $[\mathrm{H_2PO_4^-}]/[\mathrm{Pi}]$.

To calculate the fraction of $\mathrm{H_2PO_4^-}$ as a function of pH at 298.15 K and 0.25 M ionic strength, pKpi is taken to be 6.65.

$$\frac{\mathbf{h}}{\mathbf{h+k1pi}}\ \mathbf{/.\ h \to 10\hat{}\text{-}pH\ /.\ k1pi \to 10\hat{}\text{-}pKpi\ /.\ pKpi \to 6.65}$$

$$\frac{10^{-\mathrm{pH}}}{2.23872\times 10^{-7}+10^{-\mathrm{pH}}}$$

The reciprocal $[\mathrm{Pi}]/[\mathrm{H_2PO_4^-}]$ is

$$1\Big/\frac{10^{-pH}}{10^{-pH}+10^{-pKpi}}$$

$$10^{pH}\left(10^{-pH}+10^{-pKpi}\right)$$

$$\mathbf{Simplify}\left[1\Big/\frac{10^{-pH}}{10^{-pH}+10^{-pKpi}}\right]$$

$$1+10^{pH-pKpi}$$

This is the expression for [Pi]/[$H_2PO_4^-$], which is needed in Section 1.9.5. Notice that
[Pi] = $10^{pH}(10^{-pH}+10^{-pKi})[H_2PO_4^-] = \left(1+10^{pH-pKpi}\right)[H_2PO_4^-]$
This shows that [Pi] > [$H_2PO_4^-$].

Plot the fraction hpi/pit as a function of pH.

$$\mathbf{plot2 = Plot}\left[\frac{10^{-pH}}{\texttt{2.2387211385683377`*\textasciicircum-7} + 10^{-pH}}, \{pH, 5, 9\}, \mathbf{AxesLabel} \to \{\text{"pH"}, \text{"}[H_2PO_4^-]/[Pi]\text{"}\}\right];$$

This plot is given in composite Fig. 1.3 (a) on the next page.

These two plots can be superimposed.

$$\mathbf{plot3 = Plot}\left[\left\{\frac{\texttt{2.2387211385683377`*\textasciicircum-7}}{\texttt{2.2387211385683377`*\textasciicircum-7} + 10^{-pH}}, \frac{10^{-pH}}{\texttt{2.2387211385683377`*\textasciicircum-7} + 10^{-pH}}\right\}, \{pH, 5, 9\}, \mathbf{AxesLabel} \to \{\text{"pH"}, \text{"fractions"}\}, \mathbf{PlotStyle} \to \{\mathbf{Black}\}\right];$$

This plot is given in composite Fig. 1.3.

These three plots can be shown together as follows:

```
GraphicsArray[{{plot1, plot2, plot3}}]
```

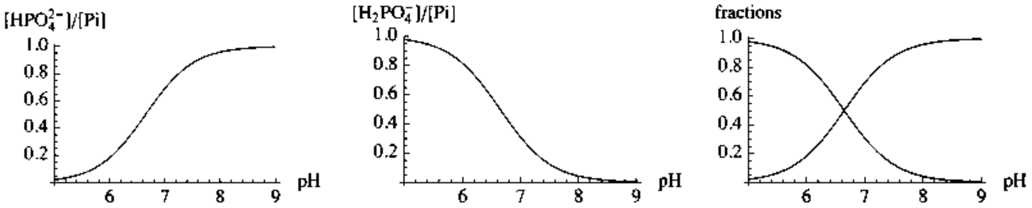

Fig. 1.3 (a) $[HPO_4^{2-}]/[Pi]$ versus pH at 298.15 K and ionic strength 0.25 M. (b) $[H_2PO_4^-]/[Pi]$ versus pH at 298.15 K and ionic strength 0.25 M. (c) Superposition of (a) and (b).

▪ 1.9.2 Distributions of species of ATP as functions of pH

ATP is composed of 3 species in the range pH 5-9. At 298.15 K 0.25 M ionic strength, pK1atp = 6.47 and pK2atp = 3.83

Calculation of the fraction of [ATP] that is [ATP $^{4-}$]

ATP has 2 acid dissociations and one conservation equation. First, the equlibrium concentration of ATP^{4-} is calculated:

```
Solve[{k1atp == h * atp / hatp, k2atp == h * hatp / h2atp, atpt == atp + hatp + h2atp},
 {atp}, {hatp, h2atp}]
```

$$\left\{\left\{\text{atp}\to\frac{\text{atpt k1atp k2atp}}{\text{h}^2+\text{h k2atp}+\text{k1atp k2atp}}\right\}\right\}$$

The fraction (atp/atpt) = [ATP^{4-}]/[ATP] is given by

$$\frac{\textbf{k1atp k2atp}}{\textbf{h}^2+\textbf{h k2atp}+\textbf{k1atp k2atp}};$$

It is more convienent to use pHs and p*K*s.

$$\frac{\textbf{k1atp k2atp}}{\textbf{h}^2+\textbf{h k2atp}+\textbf{k1atp k2atp}}\ \texttt{/. h -> 10^-pH /. k1atp -> 10^-pK1atp /. k2atp -> 10^-pK2atp}$$

$$\frac{10^{-\text{pK1atp}-\text{pK2atp}}}{10^{-2\,\text{pH}}+10^{-\text{pH}-\text{pK2atp}}+10^{-\text{pK1atp}-\text{pK2atp}}}$$

The fraction (atp/atpt) can be plotted versus pH.

$$\frac{10^{-\text{pK1atp}-\text{pK2atp}}}{10^{-2\,\text{pH}}+10^{-\text{pH}-\text{pK2atp}}+10^{-\text{pK1atp}-\text{pK2atp}}}\ \texttt{/. pK1atp -> 6.47 /. pK2atp -> 3.83}$$

$$\frac{5.01187\times10^{-11}}{5.01187\times10^{-11}+10^{-3.83-\text{pH}}+10^{-2\,\text{pH}}}$$

$$\texttt{plot1atp = Plot}\left[\frac{10^{-\text{pK1atp}-\text{pK2atp}}}{10^{-2\,\text{pH}}+10^{-\text{pH}-\text{pK2atp}}+10^{-\text{pK1atp}-\text{pK2atp}}}\ \texttt{/. pK1atp -> 6.47 /. pK2atp -> 3.83,}\right.$$
$$\left.\texttt{\{pH, 5, 9\}, AxesLabel -> }\{\texttt{"pH", "[ATP}^{4-}\texttt{]/[ATP]"}\}\texttt{, PlotLabel -> "(a)"}\right];$$

This plot is given later in Fig. 1.4.

The reciprocal of [ATP^{4-}]/[ATP] is given by

$$1\Big/\frac{10^{-\text{pK1atp}-\text{pK2atp}}}{10^{-2\,\text{pH}}+10^{-\text{pH}-\text{pK2atp}}+10^{-\text{pK1atp}-\text{pK2atp}}}$$

$$10^{\text{pK1atp}+\text{pK2atp}}\left(10^{-2\,\text{pH}}+10^{-\text{pH}-\text{pK2atp}}+10^{-\text{pK1atp}-\text{pK2atp}}\right)$$

$$\texttt{Simplify}\left[1\Big/\frac{10^{-\text{pK1atp}-\text{pK2atp}}}{10^{-2\,\text{pH}}+10^{-\text{pH}-\text{pK2atp}}+10^{-\text{pK1atp}-\text{pK2atp}}}\right]$$

$$1+10^{-\text{pH}+\text{pK1atp}}+10^{-2\,\text{pH}+\text{pK1atp}+\text{pK2atp}}$$

This is [ATP]/[ATP^{4-}], and so the concentration of ATP is always greater than the concentration of ATP^{4-}.

Calculation of the fraction of [ATP] that is [HATP $^{3-}$]

```
Solve[{k1atp == h * atp / hatp, k2atp == h * hatp / h2atp, atpt == atp + hatp + h2atp},
 {hatp}, {atp, h2atp}]
```

$$\left\{\left\{\text{hatp}\to\frac{\text{atpt h k2atp}}{\text{h}^2+\text{h k2atp}+\text{k1atp k2atp}}\right\}\right\}$$

The fraction (hatp/atpt) is given by

$$\frac{\mathbf{h\ k2atp}}{\mathbf{h^2 + h\ k2atp + k1atp\ k2atp}};$$

It is more convienent to use pHs and p*K*s.

$$\frac{\mathbf{h\ k2atp}}{\mathbf{h^2 + h\ k2atp + k1atp\ k2atp}}\ \mathbf{/.\ h \to 10\hat{}\text{-}pH\ /.\ k1atp \to 10\hat{}\text{-}pK1atp\ /.\ k2atp \to 10\hat{}\text{-}pK2atp}$$

$$\frac{10^{-pH-pK2atp}}{10^{-2\,pH} + 10^{-pH-pK2atp} + 10^{-pK1atp-pK2atp}}$$

Plot the fraction hatp/atpt = $\left[\text{HATP}^{3-}\right] \big/ [\text{ATP}]$ as a function of pH

$$\mathbf{plot2atp = Plot}\left[\frac{10^{-pH-pK2atp}}{10^{-2\,pH} + 10^{-pH-pK2atp} + 10^{-pK1atp-pK2atp}}\ \mathbf{/.\ pK1atp \to 6.47\ /.\ pK2atp \to 3.83,}\right.$$
$$\left.\mathbf{\{pH,\ 5,\ 9\},\ AxesLabel \to \left\{\text{"pH"},\ \text{"[HATP}^{3-}\text{]/[ATP]"}\right\},\ PlotLabel \to \text{"(b)"}}\right];$$

Calculation of the fraction of [ATP] that is $[H_2\ ATP^{\ 2-}]$

```
Solve[{k1atp == h * atp / hatp, k2atp == h * hatp / h2atp, atpt == atp + hatp + h2atp},
  {h2atp}, {atp, hatp}]
```

$$\left\{\left\{\text{h2atp} \to \frac{\text{atpt h}^2}{\text{h}^2 + \text{h k2atp} + \text{k1atp k2atp}}\right\}\right\}$$

The fraction (h2atp/atpt) is given by

$$\frac{\mathbf{h^2}}{\mathbf{h^2 + h\ k2atp + k1atp\ k2atp}};$$

It is more convienent to use pHs and p*K*s.

$$\frac{\mathbf{h^2}}{\mathbf{h^2 + h\ k2atp + k1atp\ k2atp}}\ \mathbf{/.\ h \to 10\hat{}\text{-}pH\ /.\ k1atp \to 10\hat{}\text{-}pK1atp\ /.\ k2atp \to 10\hat{}\text{-}pK2atp}$$

$$\frac{10^{-2\,pH}}{10^{-2\,pH} + 10^{-pH-pK2atp} + 10^{-pK1atp-pK2atp}}$$

Plot the fraction h2atp/atpt = $[H_2\ ATP^{\ 2-}]/[ATP]$ as a function of pH

$$\mathbf{plot3atp = Plot}\left[\frac{10^{-2\,pH}}{10^{-2\,pH} + 10^{-pH-pK2atp} + 10^{-pK1atp-pK2atp}}\ \mathbf{/.\ pK1atp \to 6.47\ /.\ pK2atp \to 3.83,}\right.$$
$$\left.\mathbf{\{pH,\ 5,\ 9\},\ AxesLabel \to \left\{\text{"pH"},\ \text{"}\left[\text{H}_2\ \text{ATP}^{2-}\right] \big/ \text{[ATP]"}\right\},\ PlotLabel \to \text{"(c)"}}\right];$$

These three plots can be superimposed.

$$\mathbf{plot4atp = Plot}\left[\left\{\frac{10^{-pK1atp-pK2atp}}{10^{-2\,pH} + 10^{-pH-pK2atp} + 10^{-pK1atp-pK2atp}},\ \frac{10^{-pH-pK2atp}}{10^{-2\,pH} + 10^{-pH-pK2atp} + 10^{-pK1atp-pK2atp}},\right.\right.$$
$$\left.\frac{10^{-2\,pH}}{10^{-2\,pH} + 10^{-pH-pK2atp} + 10^{-pK1atp-pK2atp}}\right\}\ \mathbf{/.\ pK1atp \to 6.47\ /.\ pK2atp \to 3.83,}$$
$$\left.\mathbf{\{pH,\ 5,\ 9\},\ AxesLabel \to \{\text{"pH"},\ \text{"fractions"}\},\ PlotLabel \to \text{"(d)"}}\right];$$

```
GraphicsArray[{{plot1atp, plot2atp}, {plot3atp, plot4atp}}]
```

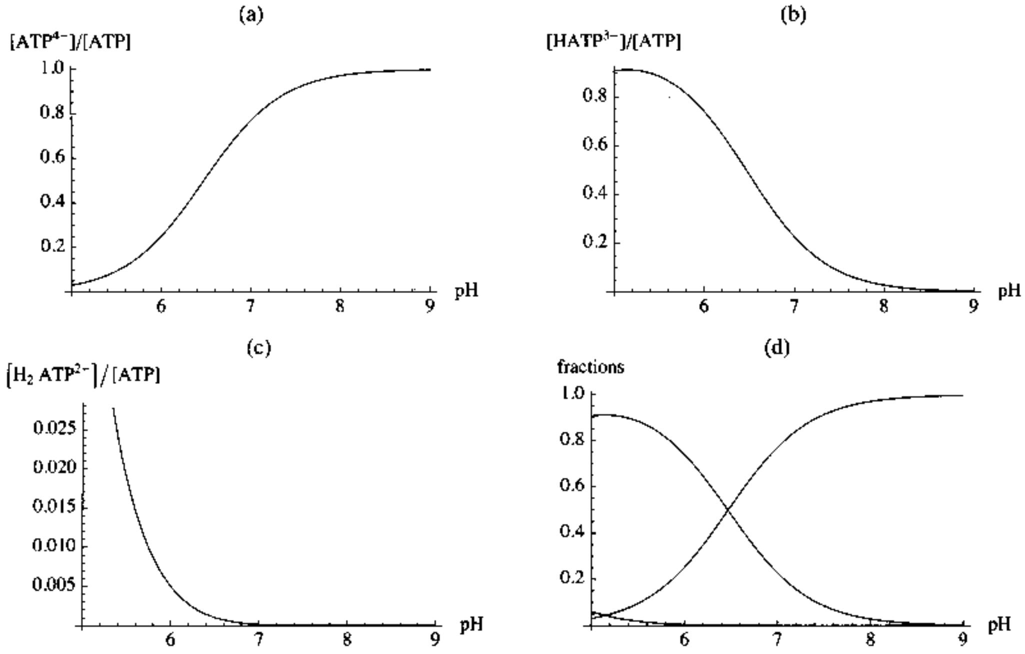

Fig. 1.4 Plots of fractions of [ATP] in (a) $[ATP^{4-}]$, (b) $[HATP^{3-}]$, (c) $[H_2 ATP^{2-}]$, and (d) fractions at 298.15 K and ionic strength 0.25 M.

■ 1.9.3 Distributions of species of ADP as functions of pH

ADP is composed of 3 species in the range pH 5-9. At 298.15 K and 0.25 M ionic strength, pK1adp = 6.33 and pK2adp = 3.79.

Calculation of the fraction of [ADP] that is $[ADP^{3-}]$

ADP has 2 acid dissociations and one conservation equation. First, the equlibrium concentration of ADP^{3-} is calculated:

```
Solve[{k1adp == h * adp / hadp, k2adp == h * hadp / h2adp, adpt == adp + hadp + h2adp},
  {adp}, {hadp, h2adp}]
```

$$\left\{\left\{\text{adp} \to \frac{\text{adpt k1adp k2adp}}{\text{h}^2 + \text{h k2adp} + \text{k1adp k2adp}}\right\}\right\}$$

The fraction (adp/adpt) = $[ADP^{4-}]$/[ADP] is given by

$$\frac{\textbf{k1adp k2adp}}{\textbf{h}^2 + \textbf{h k2adp} + \textbf{k1adp k2adp}};$$

It is more convienent to use pHs and p*K*s.

$$\frac{\textbf{k1adp k2adp}}{\textbf{h}^2 + \textbf{h k2adp} + \textbf{k1adp k2adp}} \;\textbf{/. h} \to \textbf{10\^{}-pH /. k1adp} \to \textbf{10\^{}-pK1adp /. k2adp} \to \textbf{10\^{}-pK2adp}$$

$$\frac{10^{-\text{pK1adp}-\text{pK2adp}}}{10^{-2\,\text{pH}} + 10^{-\text{pH}-\text{pK2adp}} + 10^{-\text{pK1adp}-\text{pK2adp}}}$$

The fraction (adp/adpt) can be plotted versus pH.

$$\frac{10^{-\mathrm{pK1adp}-\mathrm{pK2adp}}}{10^{-2\,\mathrm{pH}}+10^{-\mathrm{pH}-\mathrm{pK2adp}}+10^{-\mathrm{pK1adp}-\mathrm{pK2adp}}}\ \text{/. pK1adp} \to 6.33\ \text{/. pK2adp} \to 3.79$$

$$\frac{7.58578\times 10^{-11}}{7.58578\times 10^{-11}+10^{-3.79-\mathrm{pH}}+10^{-2\,\mathrm{pH}}}$$

Plot the fraction adp/adpt as a function of pH

$$\text{plot1adp = Plot}\left[\frac{10^{-\mathrm{pK1adp}-\mathrm{pK2adp}}}{10^{-2\,\mathrm{pH}}+10^{-\mathrm{pH}-\mathrm{pK2adp}}+10^{-\mathrm{pK1adp}-\mathrm{pK2adp}}}\ \text{/. pK1adp} \to 6.33\ \text{/. pK2adp} \to 3.79,\ \{\mathrm{pH}, 5, 9\},\ \text{AxesLabel} \to \{\text{"pH"}, \text{"[ADP}^{3-}\text{]/[ADP]"}\},\ \text{PlotLabel} \to \text{"(a)"}\right];$$

Similar plots can be made for the equilibrium concentrations of hadp and h2adp.

The reciprocal of $[\mathrm{ADP}^{3-}]/[\mathrm{ADP}]$ is given by

$$1\Big/\frac{10^{-\mathrm{pK1adp}-\mathrm{pK2adp}}}{10^{-2\,\mathrm{pH}}+10^{-\mathrm{pH}-\mathrm{pK2adp}}+10^{-\mathrm{pK1adp}-\mathrm{pK2adp}}}$$

$$10^{\mathrm{pK1adp}+\mathrm{pK2adp}}\left(10^{-2\,\mathrm{pH}}+10^{-\mathrm{pH}-\mathrm{pK2adp}}+10^{-\mathrm{pK1adp}-\mathrm{pK2adp}}\right)$$

$$\text{Simplify}\left[1\Big/\frac{10^{-\mathrm{pK1adp}-\mathrm{pK2adp}}}{10^{-2\,\mathrm{pH}}+10^{-\mathrm{pH}-\mathrm{pK2adp}}+10^{-\mathrm{pK1adp}-\mathrm{pK2adp}}}\right]$$

$$1+10^{-\mathrm{pH}+\mathrm{pK1adp}}+10^{-2\,\mathrm{pH}+\mathrm{pK1adp}+\mathrm{pK2adp}}$$

This is $[\mathrm{ADP}]/[\mathrm{ADP}^{3-}]$, and so the concentration of ADP is always greater than the concentration of ADP^{3-}.

Calculation of the fraction of [ADP] that is $[\mathrm{HADP}^{\ 2-}]$

```
Solve[{k1adp == h * adp / hadp, k2adp == h * hadp / h2adp, adpt == adp + hadp + h2adp},
 {hadp}, {adp, h2adp}]
```

$$\left\{\left\{\mathrm{hadp}\to\frac{\mathrm{adpt\ h\ k2adp}}{\mathrm{h}^2+\mathrm{h\ k2adp}+\mathrm{k1adp\ k2adp}}\right\}\right\}$$

The fraction (hadp/adpt) is given by

$$\frac{\mathrm{h\ k2adp}}{\mathrm{h}^2+\mathrm{h\ k2adp}+\mathrm{k1adp\ k2adp}};$$

It is more convienent to use pHs and pKs.

$$\frac{\mathrm{h\ k2adp}}{\mathrm{h}^2+\mathrm{h\ k2adp}+\mathrm{k1adp\ k2adp}}\ \text{/. h} \to 10\text{^}-\mathrm{pH}\ \text{/. k1adp} \to 10\text{^}-\mathrm{pK1adp}\ \text{/. k2adp} \to 10\text{^}-\mathrm{pK2adp}$$

$$\frac{10^{-\mathrm{pH}-\mathrm{pK2adp}}}{10^{-2\,\mathrm{pH}}+10^{-\mathrm{pH}-\mathrm{pK2adp}}+10^{-\mathrm{pK1adp}-\mathrm{pK2adp}}}$$

Plot the fraction hadp/adpt = $\left[\mathrm{HADP}^{2-}\right]\Big/[\mathrm{ADP}]$ as a function of pH

$$\text{plot2adp = Plot}\left[\frac{10^{-\mathrm{pH}-\mathrm{pK2adp}}}{10^{-2\,\mathrm{pH}}+10^{-\mathrm{pH}-\mathrm{pK2adp}}+10^{-\mathrm{pK1adp}-\mathrm{pK2adp}}}\ \text{/. pK1adp} \to 6.33\ \text{/. pK2adp} \to 3.79,\ \{\mathrm{pH}, 5, 9\},\ \text{AxesLabel} \to \{\text{"pH"}, \text{"[HADP}^{2-}\text{]/[ADP]"}\},\ \text{PlotLabel} \to \text{"(b)"}\right];$$

Calculation of the fraction of [ADP] that is [H_2 ADP $^-$]

```
Solve[{k1adp == h * adp / hadp, k2adp == h * hadp / h2adp, adpt == adp + hadp + h2adp},
 {h2adp}, {adp, hadp}]
```

$$\left\{\left\{\text{h2adp} \to \frac{\text{adpt}\ h^2}{h^2 + h\ \text{k2adp} + \text{k1adp}\ \text{k2adp}}\right\}\right\}$$

The fraction (h2adp/adpt) is given by

$$\frac{\mathbf{h^2}}{\mathbf{h^2 + h\ k2adp + k1adp\ k2adp}};$$

It is more convienent to use pHs and pKs.

$$\frac{\mathbf{h^2}}{\mathbf{h^2 + h\ k2adp + k1adp\ k2adp}}\ \texttt{/. h} \to \texttt{10\^{}-pH /. k1adp} \to \texttt{10\^{}-pK1adp /. k2adp} \to \texttt{10\^{}-pK2adp}$$

$$\frac{10^{-2\,\text{pH}}}{10^{-2\,\text{pH}} + 10^{-\text{pH}-\text{pK2adp}} + 10^{-\text{pK1adp}-\text{pK2adp}}}$$

Plot the fraction h2adp/adpt = [H_2 ADP $^-$]/[ADP] as a function of pH

$$\texttt{plot3adp = Plot}\left[\frac{10^{-2\,\text{pH}}}{10^{-2\,\text{pH}} + 10^{-\text{pH}-\text{pK2adp}} + 10^{-\text{pK1adp}-\text{pK2adp}}}\ \texttt{/. pK1adp} \to \texttt{6.33 /. pK2adp} \to \texttt{3.79,}\ \{\texttt{pH, 5, 9}\}\texttt{, AxesLabel} \to \{\texttt{"pH", "[}\mathrm{H_2}\texttt{ ADP}^-\texttt{] / [ADP]"}\}\texttt{, PlotLabel} \to \texttt{"(c)"}\right];$$

These three plots can be superimposed.

$$\texttt{plot4adp = Plot}\left[\left\{\frac{10^{-\text{pK1adp}-\text{pK2adp}}}{10^{-2\,\text{pH}} + 10^{-\text{pH}-\text{pK2adp}} + 10^{-\text{pK1adp}-\text{pK2adp}}}, \frac{10^{-\text{pH}-\text{pK2adp}}}{10^{-2\,\text{pH}} + 10^{-\text{pH}-\text{pK2adp}} + 10^{-\text{pK1adp}-\text{pK2adp}}}, \frac{10^{-2\,\text{pH}}}{10^{-2\,\text{pH}} + 10^{-\text{pH}-\text{pK2adp}} + 10^{-\text{pK1adp}-\text{pK2adp}}}\right\}\ \texttt{/. pK1adp} \to \texttt{6.47 /. pK2adp} \to \texttt{3.83,}\ \{\texttt{pH, 5, 9}\}\texttt{, AxesLabel} \to \{\texttt{"pH", "fractions"}\}\texttt{, PlotLabel} \to \texttt{"(d)"}\right];$$

```
GraphicsArray[{{plot1adp, plot2adp}, {plot3adp, plot4adp}}]
```

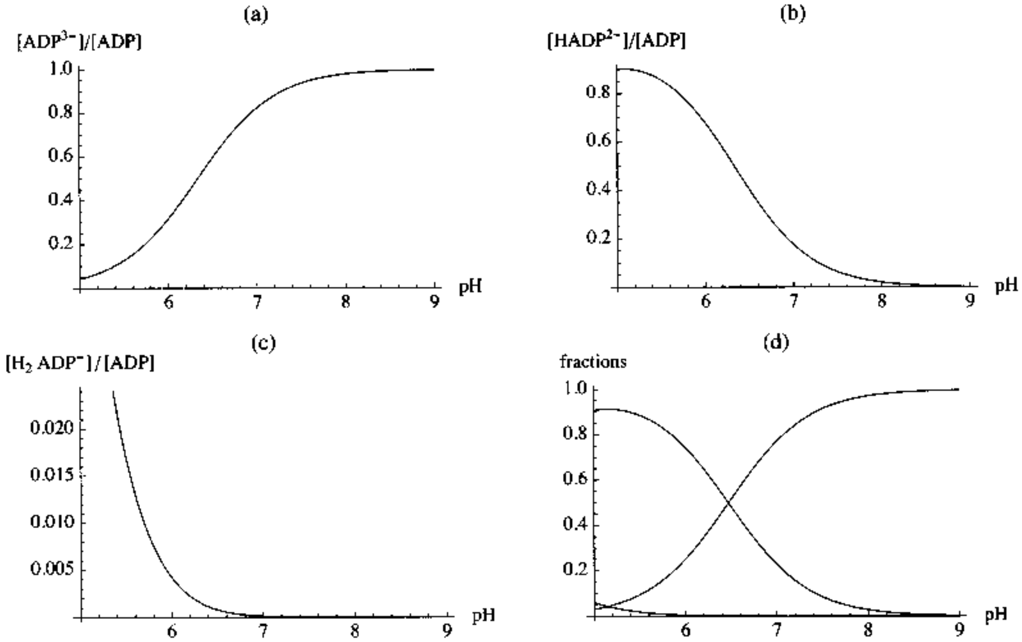

Fig. 1.5 Plots of fractions of [ADP] in (a) $[ADP^{3-}]$, (b) $[HADP^{2-}]$, (c) $[H_2ADP^-]$, and (d) fractions at 298.15 K and ionic strength 0.25 M.

■ 1.9.4 Calculations of the apparent equlibrium constant for ATP + H_2O = ADP + Pi as a function of pH

The apparent equilibrium constant K' is given by [ADP][Pi]/[ATP].

```
adpt * pit / atpt;
```

These 3 equilibrium expressions are used to replace adpt, pit, and atpt.

$$\left(1+10^{-\text{pH}+\text{pK1adp}}+10^{-2\,\text{pH}+\text{pK1adp}+\text{pK2adp}}\right)*\text{adp};$$

$$\left(1+10^{-\text{pH}+\text{pKpi}}\right)*\text{pi};$$

$$\left(1+10^{-\text{pH}+\text{pK1atp}}+10^{-2\,\text{pH}+\text{pK1atp}+\text{pK2atp}}\right)*\text{atp};$$

These three expressions are used to replace atpt, adpt, and pit in the expression for the apparent equilibrium constant K'.

$$\text{(adpt * pit / atpt) /. adpt -> }\left(1+10^{-\text{pH}+\text{pK1adp}}+10^{-2\,\text{pH}+\text{pK1adp}+\text{pK2adp}}\right)*\text{adp /. pit -> }\left(1+10^{-\text{pH}+\text{pKpi}}\right)*\text{pi /.}$$
$$\text{atpt} \to \left(1+10^{-\text{pH}+\text{pK1atp}}+10^{-2\,\text{pH}+\text{pK1atp}+\text{pK2atp}}\right)*\text{atp}$$

$$\frac{\left(1+10^{-\text{pH}+\text{pK1adp}}+10^{-2\,\text{pH}+\text{pK1adp}+\text{pK2adp}}\right)\left(1+10^{-\text{pH}+\text{pKpi}}\right)\text{adp pi}}{\left(1+10^{-\text{pH}+\text{pK1atp}}+10^{-2\,\text{pH}+\text{pK1atp}+\text{pK2atp}}\right)\text{atp}}$$

The chemical reference reaction is $ATP^{4-} + H_2O = ADP^{3-} + HPO_4{}^{2-} + H^+$ k_{ref1} = adp*pi*h/atp
Replace adp*pi/atp with kref1/h = kref1*10^pH. The apparent equilibrium constant is given by

$$\frac{\left(1+10^{-\text{pH}+\text{pK1adp}}+10^{-2\,\text{pH}+\text{pK1adp}+\text{pK2adp}}\right)\left(1+10^{-\text{pH}+\text{pKpi}}\right)*\text{kref1}*10\,\hat{}\,\text{pH}}{\left(1+10^{-\text{pH}+\text{pK1atp}}+10^{-2\,\text{pH}+\text{pK1atp}+\text{pK2atp}}\right)}$$

$$\frac{10^{\text{pH}}\left(1+10^{-\text{pH}+\text{pK1adp}}+10^{-2\,\text{pH}+\text{pK1adp}+\text{pK2adp}}\right)\left(1+10^{-\text{pH}+\text{pKpi}}\right)\text{kref1}}{1+10^{-\text{pH}+\text{pK1atp}}+10^{-2\,\text{pH}+\text{pK1atp}+\text{pK2atp}}}$$

$$\frac{\left(1+10^{-\text{pH}+\text{pK1adp}}+10^{-2\,\text{pH}+\text{pK1adp}+\text{pK2adp}}\right)\left(1+10^{-\text{pH}+\text{pKpi}}\right)*\text{kref1}*10\,\hat{}\,\text{pH}}{\left(1+10^{-\text{pH}+\text{pK1atp}}+10^{-2\,\text{pH}+\text{pK1atp}+\text{pK2atp}}\right)}\;/.\;\text{pK1adp}\to 6.33\;/.\;\text{pK2adp}\to 3.79\;/.\;\text{pKpi}\to 6.65\;/.\;\text{pK1atp}\to 6.47\;/.\;\text{pK2atp}\to 3.83\;/.\;\text{pH}\text{ -> }7$$

$$1.35578\times 10^{7}\,\text{kref1}$$

The apparent equibrium constant K' for ATP + H_2O = ADP + Pi at 298.15 K, pH 7 and 0.25 M ionic strength (5,19) is

```
Exp[36.04 / (8.31451 * .29815)]
```

$$2.06015\times 10^{6}$$

Therefore, kref1 is given by

```
(2.06015 * 10^6) / (1.35578 * 10^7)
```

```
0.151953
```

The apparent equilibrium constant is given as a function of pH by

$$\frac{\left(1+10^{-\text{pH}+\text{pK1adp}}+10^{-2\,\text{pH}+\text{pK1adp}+\text{pK2adp}}\right)\left(1+10^{-\text{pH}+\text{pKpi}}\right)*\text{kref1}*10\,\hat{}\,\text{pH}}{\left(1+10^{-\text{pH}+\text{pK1atp}}+10^{-2\,\text{pH}+\text{pK1atp}+\text{pK2atp}}\right)}\;/.\;\text{pK1adp}\to 6.33\;/.\;\text{pK2adp}\to 3.79\;/.\;\text{pKpi}\to 6.65\;/.\;\text{pK1atp}\to 6.47\;/.\;\text{pK2atp}\to 3.83\;/.\;\text{kref1}\to .151953$$

$$\frac{0.151953\;10^{\text{pH}}\left(1+10^{10.12-2\,\text{pH}}+10^{6.33-\text{pH}}\right)\left(1+10^{6.65-\text{pH}}\right)}{1+10^{10.3-2\,\text{pH}}+10^{6.47-\text{pH}}}$$

The apparent equilibrium constants at pHs 5, 6, 7, 8, and 9 are given by

$$\frac{\left(1+10^{-\text{pH}+\text{pK1adp}}+10^{-2\,\text{pH}+\text{pK1adp}+\text{pK2adp}}\right)\left(1+10^{-\text{pH}+\text{pKpi}}\right)*\text{kref1}*10\,\hat{}\,\text{pH}}{\left(1+10^{-\text{pH}+\text{pK1atp}}+10^{-2\,\text{pH}+\text{pK1atp}+\text{pK2atp}}\right)}\;/.\;\text{pK1adp}\to 6.33\;/.\;\text{pK2adp}\to 3.79\;/.\;\text{pKpi}\to 6.65\;/.\;\text{pK1atp}\to 6.47\;/.\;\text{pK2atp}\to 3.83\;/.\;\text{kref1}\to .151953\;/.\;\text{pH}\to\{5,\,6,\,7,\,8,\,9\}$$

$$\{505886.,\;659168.,\;2.06015\times 10^{6},\;1.57486\times 10^{7},\;1.52508\times 10^{8}\}$$

The changes in the standard transformed Gibbs energies of reaction $\Delta_r G'^\circ$ are given by

$$-8.31451*.29815*\text{Log}\left[\frac{\left(1+10^{-\text{pH}+\text{pK1adp}}+10^{-2\,\text{pH}+\text{pK1adp}+\text{pK2adp}}\right)\left(1+10^{-\text{pH}+\text{pKpi}}\right)*\text{kref1}*10\,\hat{}\,\text{pH}}{\left(1+10^{-\text{pH}+\text{pK1atp}}+10^{-2\,\text{pH}+\text{pK1atp}+\text{pK2atp}}\right)}\;/.\;\text{pK1adp}\to 6.33\;/.\;\text{pK2adp}\to 3.79\;/.\;\text{pKpi}\to 6.65\;/.\;\text{pK1atp}\to 6.47\;/.\;\text{pK2atp}\to 3.83\;/.\;\text{kref1}\to .151953\;/.\;\text{pH}\to\{5,\,6,\,7,\,8,\,9\}\right]$$

```
{-32.559, -33.2151, -36.04, -41.0822, -46.7106}
```

These values are in agreement with BasicBiochemData3 [*MathSource*3, p. 227].

The expression for the apparent equilibrium constant for ATP + H_2O can be summarized by $K' = 10^{\mathrm{pH}} K_{\mathrm{ref1}} f(\mathrm{pH})$ where $f(\mathrm{pH})$ brings in the effects of the p*K*s of the reactants. However, the choice of a reference reaction is arbitrary. For example, the reference reaction can be taken to be $\mathrm{ATP}^{4-} + H_2O = \mathrm{ADP}^{3-} + H_2PO_4^{-}$ kref2 = adp*hpi/atp. In this case, pit is given as a function of pH by

```
(1 + 10^(pH - pKpi)) * hpi
```

$$\left(1+10^{\mathrm{pH}-\mathrm{pKpi}}\right)\mathrm{hpi}$$

Functions of pH are introduced.

$$(\mathrm{adpt} * \mathrm{pit} / \mathrm{atpt}) \;/.\; \mathrm{adpt} \text{ -> } \left(1+10^{-\mathrm{pH}+\mathrm{pK1adp}}+10^{-2\,\mathrm{pH}+\mathrm{pK1adp}+\mathrm{pK2adp}}\right) * \mathrm{adp} \;/.\; \mathrm{pit} \text{ -> } \left(1+10^{\mathrm{pH}-\mathrm{pKpi}}\right) * \mathrm{hpi} \;/.\; \mathrm{atpt} \to \left(1+10^{-\mathrm{pH}+\mathrm{pK1atp}}+10^{-2\,\mathrm{pH}+\mathrm{pK1atp}+\mathrm{pK2atp}}\right) * \mathrm{atp}$$

$$\frac{\left(1+10^{-\mathrm{pH}+\mathrm{pK1adp}}+10^{-2\,\mathrm{pH}+\mathrm{pK1adp}+\mathrm{pK2adp}}\right)\left(1+10^{\mathrm{pH}-\mathrm{pKpi}}\right)\mathrm{adp\;hpi}}{\left(1+10^{-\mathrm{pH}+\mathrm{pK1atp}}+10^{-2\,\mathrm{pH}+\mathrm{pK1atp}+\mathrm{pK2atp}}\right)\mathrm{atp}}$$

The expression for the chemical reference reaction is replaced with kref2.

$$\frac{\left(1+10^{-\mathrm{pH}+\mathrm{pK1adp}}+10^{-2\,\mathrm{pH}+\mathrm{pK1adp}+\mathrm{pK2adp}}\right)\left(1+10^{\mathrm{pH}-\mathrm{pKpi}}\right)\mathrm{kref2}}{\left(1+10^{-\mathrm{pH}+\mathrm{pK1atp}}+10^{-2\,\mathrm{pH}+\mathrm{pK1atp}+\mathrm{pK2atp}}\right)}$$

$$\frac{\left(1+10^{-\mathrm{pH}+\mathrm{pK1adp}}+10^{-2\,\mathrm{pH}+\mathrm{pK1adp}+\mathrm{pK2adp}}\right)\left(1+10^{\mathrm{pH}-\mathrm{pKpi}}\right)\mathrm{kref2}}{1+10^{-\mathrm{pH}+\mathrm{pK1atp}}+10^{-2\,\mathrm{pH}+\mathrm{pK1atp}+\mathrm{pK2atp}}}$$

The apparent equilibrium constant is expressed as a function of pH by

$$\frac{\left(1+10^{-\mathrm{pH}+\mathrm{pK1adp}}+10^{-2\,\mathrm{pH}+\mathrm{pK1adp}+\mathrm{pK2adp}}\right)\left(1+10^{\mathrm{pH}-\mathrm{pKpi}}\right)\mathrm{kref2}}{\left(1+10^{-\mathrm{pH}+\mathrm{pK1atp}}+10^{-2\,\mathrm{pH}+\mathrm{pK1atp}+\mathrm{pK2atp}}\right)} \;/.\; \mathrm{pK1adp}\to 6.33 \;/.\; \mathrm{pK2adp}\to 3.79 \;/.\; \mathrm{pKpi}\to 6.65 \;/.\; \mathrm{pK1atp}\to 6.47 \;/.\; \mathrm{pK2atp}\to 3.83$$

$$\frac{\left(1+10^{10.12-2\,\mathrm{pH}}+10^{6.33-\mathrm{pH}}\right)\left(1+10^{-6.65+\mathrm{pH}}\right)\mathrm{kref2}}{1+10^{10.3-2\,\mathrm{pH}}+10^{6.47-\mathrm{pH}}}$$

$$\frac{\left(1+10^{10.120000000000001`-2\,\mathrm{pH}}+10^{6.33`-\mathrm{pH}}\right)\left(1+10^{-6.65`+\mathrm{pH}}\right)\mathrm{kref2}}{1+10^{10.3`-2\,\mathrm{pH}}+10^{6.47`-\mathrm{pH}}} \;/.\; \mathrm{pH}\to 7$$

```
3.03521 kref2
```

The apparent equibrium constant K' at 298.15 K, pH 7 and 0.25 M ionic strength is

```
Exp[36.04 / (8.31451 * .29815)]
```

$$2.06015\times 10^{6}$$

Therefore, kref2 is given by

```
(2.06015 * 10^6) / (3.03521)
```

```
678750.
```

The apparent equilibrium constant is given as a function of pH by

$$\frac{\left(1+10^{10.120000000000001\text{`}-2\,\mathrm{pH}}+10^{6.33\text{`}-\mathrm{pH}}\right)\left(1+10^{-6.65\text{`}+\mathrm{pH}}\right)\mathbf{kref2}}{1+10^{10.3\text{`}-2\,\mathrm{pH}}+10^{6.47\text{`}-\mathrm{pH}}}\ \mathtt{/.\ kref2} \rightarrow 678\,750$$

$$\frac{678\,750\left(1+10^{10.12-2\,\mathrm{pH}}+10^{6.33-\mathrm{pH}}\right)\left(1+10^{-6.65+\mathrm{pH}}\right)}{1+10^{10.3-2\,\mathrm{pH}}+10^{6.47-\mathrm{pH}}}$$

$$\frac{\left(1+10^{10.120000000000001\text{`}-2\,\mathrm{pH}}+10^{6.33\text{`}-\mathrm{pH}}\right)\left(1+10^{-6.65\text{`}+\mathrm{pH}}\right)\mathbf{kref2}}{1+10^{10.3\text{`}-2\,\mathrm{pH}}+10^{6.47\text{`}-\mathrm{pH}}}\ \mathtt{/.\ kref2} \rightarrow 678\,750\ \mathtt{/.\ pH} \rightarrow \{5, 6, 7, 8, 9\}$$

$$\{505\,886., 659\,169., 2.06015\times 10^{6}, 1.57487\times 10^{7}, 1.52508\times 10^{8}\}$$

The standard transformed Gibbs energy of reaction $\Delta_r G'^\circ$ in kJ mol^{-1} are given by

```
-8.31451 * .29815 * Log[{505886.48270386615`, 659168.7875151065`,
   2.0601513062382448`*^6, 1.5748664776306028`*^7, 1.5250818400661832`*^8}]
```

```
{-32.559, -33.2151, -36.04, -41.0822, -46.7106}
```

The important point here is that although $K' = 10^{npH} K_{ref} f(\mathrm{pH})$, where n is zero or a positive or negative integer, the value of n is arbitrary in thermodynamics. In rapid-equilibrium enzyme kinetics the velocity is proportional to 10^{npH}, where n is the number of hydrogen ions consumed in the rate-determining reaction. When one hydrogen ion is consumed, $n = -1$.

1.10 Data on the thermodynamics of enzyme-catalyzed reactions in the literature and on the web

There are two types of thermodynamic data on enzyme-catalyzed reactions that are of interest in biochemical kinetics: (1) Experimental measurements of apparent equilibrium constants and heats of reaction. (2) Data on thermodynamic properties of species obtained chemically or from experimental measurements of apparent equilibrium constants and heats of reaction. This second type of data has the advantage that it can be used to calculate apparent equilibrium constants and heats of enzyme-catalyzed reactions under conditions that have not been studied experimentally. This second type of thermodynamic data can be used to calculate properties of biochemical reactions that are difficult to measure directly, but may be of interest in connection with enzyme kinetics. An example of this is checking the Haldane relation to calculate the apparent equilibrium constant with the kinetic parameters for the forward and reverse reactions. Another example is comparing the change in binding of hydrogen ions $\Delta_r N_H$ calculated using thermodynamics using equation 1.4-3 or 1.4-4 with the integer n for the number of hydrogen ions consumed in the rate-determining reaction in a rapid-equilibrium mechanism [27].

1.10.1 Experimental data on apparent equilibrium constants and heats of reaction of enzyme-catalyzed reactions

The place to look for information on experimental measurements of apparent equilibrium costants and heats of reaction of enzyme-catalyzed reactions are the 10 compilations by Goldberg and Tewari of experimental data on apparent equilibrium constants and heats of reaction. These data are summarized on an interactive site at the National Institute Science and Technology [Web3]. The EC numbers [Web2] of the reactions are given, and the quality of the data is evaluated.

▪ 1.10.2 Data on thermodynamic properties of species obtained chemically or from experimental measurements of apparent equilibrium constants and heats of enzyme-catalyzed reactions

At the time of Krebs [2,4], it was recognized that values of equilibrium constants on enzyme-catalyzed reactions and heats of reaction could be used to calculate standard Gibbs energies of formation and standard enthalpies of formation of species to augment the tables produced by chemists from the study of chemical reactions (2). This means that the most basic way to store information on apparent equlibrium constants and heats of biochemical reactions is to make tables of $\Delta_f G°$ and $\Delta_f H°$ of the species involved at 298.15 K and zero ionic strength. This makes it possible to calculate standard transformed Gibbs energies of formation $\Delta_f G_i{}'°$ and standard transformed enthalpies of formation $\Delta_f H_i{}'°$ of biochemical reactants at desired temperatures, pHs, and ionic strengths. These values can be used to calculate standard transformed Gibbs energies $\Delta_r G'°$ of reaction and standard transformed enthalpies $\Delta_r H'°$ of reaction. The crtical evaluations of apparent equilibrium constants and heats of reaction of enzyme catalyzed reactions by Goldberg and Tewari [Web3] have been used in developing BasicBiochemData3 [*MathSource*3] that gives $\Delta_f G°$ and $\Delta_f H°$ for species of199 biochemical reactants at 298.15 K in *Mathematica*. This list of species data can be extended by analyzing more data from the Goldberg-Tewari tables. As explained in Section 1.4, the fact that the pH is specified in biochemical thermodynamics makes it possible to calculate another reaction property that is not analogous to any property in chemical thermodynamics, and that is the change in binding of hydrogen ions $\Delta_r N_H$ in the enzyme-catalyzed reaction.

▪ 1.10.3 Calculated values of standard transformed Gibbs energies of reaction, standard transformed enthalpies of reaction, apparent equilibrium constants, and changes in the binding of hydrogen ions

Species data on 199 reactants have been used to calculate these properties for about 300 biochemical reactions at 298.15 K and 0.25 M ionic strength [23]. For 94 of these reactants, the standard enthalpies of formation of the species are known at 298.15 K, and this makes it possible to calculate $\Delta_r G'°$, $\Delta_r H'°$, and $\Delta_r N_H$ at temperatures in the range 273.15 K to about 313.15 K. BasicBiochemData3 [*MathSource*3] gives the following functions in *Mathematica*:

(1) Functions of pH and ionic strength at 298.15 K for $\Delta_f G_i{}'°$ for 199 reactants, for example, atp.
(2) Functions of pH and ionic strength at 298.15 K for $\overline{N}_H$ for 199 reactants, for example, atpNH.
(3) Functions of temperature, pH and ionic strength for $\Delta_f G_i{}'°$ for 94 reactants, for example, atpGT.
(4) Functions of temperature, pH and ionic strength for $\Delta_f H_i{}'°$ for 94 reactants, for example, atpHT
(5) Functions of temperature, pH and ionic strength for $\Delta_f S_i{}'°$ for 94 reactants, for example, atpST
(6) Functions of temperature, pH and ionic strength for $\overline{N}_H$ for 94 reactants, for example, atpNHT

These 774 functions are not given here, but enough information is given to calculate the $\Delta_r G'°$ for ATP + H_2O = ADP + Pi at 273.15 K, 298.15 K, and 313.15 K, pHs 5, 6, 7, 8, and 9, and ionic strengths 0, 0.10, and 0.25 M. The following program derives the function of *T* (in Kelvin), pH, and ionic strength (is) that gives the standard transformed Gibbs energy of formation of a reactant (sum of species).

```
derivetrGibbsT[speciesmat_] := Module[{dGzero, dGzeroT, dHzero, zi, nH, gibbscoeff
, pHterm, isterm, gpfnsp}, (*This program derives the function of T (in Kelvin),
pH, and ionic strength (is) that gives the standard transformed Gibbs energy
of formation of a reactant (sum of species). The input speciesmat is a matrix
that gives the standard Gibbs energy of formation in kJ mol^-1 at 298.15 K and
zero ionic strength, the standard enthalpy of formation in kJ mol^-1 at 298.15 K
and zero ionic strength, the electric charge, and the number of hydrogen atoms in
each species. There is a row in the matrix for each species of the reactant.
gpfnsp is a list of the functions for the standard transformed Gibbs energies
of the species. The corresponding functions for other transformed properties
can be obtained by taking partial derivatives. The standard transformed Gibbs
energy of formation of a reactant in kJ mol^-1 can be calculated at any temperature
in the range 273.15 K to 313.15 K, any pH in the range 5 to 9, and any ionic
strength in the range 0 to 0.35 M by use of ReplaceAll (/.).*)
{dGzero, dHzero, zi, nH} = Transpose[speciesmat];
gibbscoeff = (9.20483 * t) / 10^3 - (1.284668 * t^2) / 10^5 + (4.95199 * t^3) / 10^8;
dGzeroT = (dGzero * t) / 298.15 + dHzero * (1 - t / 298.15);
pHterm = (nH * 8.31451 * t * Log[10^(-pH)]) / 1000;
istermG = (gibbscoeff * (zi^2 - nH) * is^0.5) / (1 + 1.6 * is^0.5);
gpfnsp = dGzeroT - pHterm - istermG;
-((8.31451 * t * Log[Plus @@ (E^(-(gpfnsp / ((8.31451 * t) / 1000))))]) / 1000)]
```

Since these properties are calculated here in Chapter 1, the functions are named atpGTch1, ...

```
atpGTch1 = derivetrGibbsT[atpsp]
```

$$-0.00831451\,t\,\mathrm{Log}\Big[e^{-\frac{120.272\left(-3627.91\,(1-0.00335402\,t)-9.5193\,t+\frac{10\,is^{0.5}\,(0.00920483\,t-0.0000128467\,t^{2}+4.95199\times10^{-8}\,t^{3})}{1+1.6\,is^{0.5}}-0.116403\,t\,\mathrm{Log}[10^{-pH}]\right)}{t}}+e^{-\frac{120.272\left(-3612.91\,(1-0.00335402\,t)-9.42975\,t+\frac{4\,is^{0.5}\,(0.00920483\,t-0.0000128467\,t^{2}+4.95199\times10^{-8}\,t^{3})}{1+1.6\,is^{0.5}}-0.108089\,t\,\mathrm{Log}[10^{-pH}]\right)}{t}}+e^{-\frac{120.272\left(-3619.21\,(1-0.00335402\,t)-9.28425\,t-\frac{4\,is^{0.5}\,(0.00920483\,t-0.0000128467\,t^{2}+4.95199\times10^{-8}\,t^{3})}{1+1.6\,is^{0.5}}-0.0997741\,t\,\mathrm{Log}[10^{-pH}]\right)}{t}}\Big]$$

```
adpGTch1 = derivetrGibbsT[adpsp]
```

$$-0.00831451\,t\,\mathrm{Log}\Big[e^{-\frac{120.272\left(-2638.54\,(1-0.00335402\,t)-6.61405\,t+\frac{13\,is^{0.5}\,(0.00920483\,t-0.0000128467\,t^{2}+4.95199\times10^{-8}\,t^{3})}{1+1.6\,is^{0.5}}-0.116403\,t\,\mathrm{Log}[10^{-pH}]\right)}{t}}+e^{-\frac{120.272\left(-2620.94\,(1-0.00335402\,t)-6.53061\,t+\frac{9\,is^{0.5}\,(0.00920483\,t-0.0000128467\,t^{2}+4.95199\times10^{-8}\,t^{3})}{1+1.6\,is^{0.5}}-0.108089\,t\,\mathrm{Log}[10^{-pH}]\right)}{t}}+e^{-\frac{120.272\left(-2626.54\,(1-0.00335402\,t)-6.39319\,t+\frac{3\,is^{0.5}\,(0.00920483\,t-0.0000128467\,t^{2}+4.95199\times10^{-8}\,t^{3})}{1+1.6\,is^{0.5}}-0.0997741\,t\,\mathrm{Log}[10^{-pH}]\right)}{t}}\Big]$$

```
piGTch1 = derivetrGibbsT[pisp]
```

$$-0.00831451\,t\,\mathrm{Log}\Big[e^{-\frac{120.272\left(-1302.6\,(1-0.00335402\,t)-3.81452\,t+\frac{is^{0.5}\,(0.00920483\,t-0.0000128467\,t^{2}+4.95199\times10^{-8}\,t^{3})}{1+1.6\,is^{0.5}}-0.016629\,t\,\mathrm{Log}[10^{-pH}]\right)}{t}}+e^{-\frac{120.272\left(-1299.\,(1-0.00335402\,t)-3.67634\,t-\frac{3\,is^{0.5}\,(0.00920483\,t-0.0000128467\,t^{2}+4.95199\times10^{-8}\,t^{3})}{1+1.6\,is^{0.5}}-0.00831451\,t\,\mathrm{Log}[10^{-pH}]\right)}{t}}\Big]$$

```
h2oGTch1 = derivetrGibbsT[h2osp]
```

$$-0.00831451\,t\,\mathrm{Log}\left[e^{-\frac{120.272\left(-285.83\,(1-0.00335402\,t)-0.795539\,t+\frac{2\,is^{0.5}\,(0.00920483\,t-0.0000128467\,t^2+4.95199\times10^{-8}\,t^3)}{1+1.6\,is^{0.5}}\;0.016629\,t\,\mathrm{Log}\left[10^{\,pH}\right]\right)}{t}}\right]$$

Table 1.9 Standard transformed Gibbs energies of reaction for ATP + H_2O = ADP + Pi as a function of temperature, pH, and ionic strength

```
PaddedForm[
 TableForm[(piGTch1 + adpGTch1 - (atpGTch1 + h2oGTch1)) /. t → {273.15, 298.15, 313.15} /.
   pH → {5, 6, 7, 8, 9} /. is → {0, .1, .25},
  TableHeadings → {{"273.15 K", "298.15 K", "313.15 K"}, {"     pH 5", "     pH 6",
     "     pH 7", "     pH 8", "     pH 9"}, {"I=0", "I=0.10", "I=0.25"}}], 4]
```

```
          |    pH 5            pH 6            pH 7            pH 8            pH 9
----------+-----------------------------------------------------------------------------------
          |I=0   |-34.29 I=0   |-34.95 I=0   | -36.5 I=0   |-40.78 I=0   |-45.98
273.15 K  |I=0.10|-32.66 I=0.10|-33.17 I=0.10| -35.4 I=0.10|-39.79 I=0.10|-44.92
          |I=0.25|-32.03 I=0.25|-32.58 I=0.25|-34.94 I=0.25|-39.43 I=0.25|-44.57
          |I=0   | -35.3 I=0   |-35.91 I=0   | -37.6 I=0   | -42.5 I=0   |-48.29
298.15 K  |I=0.10| -33.3 I=0.10|-33.87 I=0.10| -36.5 I=0.10|-41.48 I=0.10| -47.1
          |I=0.25|-32.56 I=0.25|-33.22 I=0.25|-36.04 I=0.25|-41.07 I=0.25| -46.7
          |I=0   | -35.9 I=0   |-36.49 I=0   |-38.27 I=0   |-43.53 I=0   |-49.68
313.15 K  |I=0.10|-33.67 I=0.10|-34.28 I=0.10|-37.16 I=0.10|-42.48 I=0.10| -48.4
          |I=0.25|-32.87 I=0.25|-33.59 I=0.25|-36.69 I=0.25|-42.05 I=0.25|-47.97
```

Tables like this can also be made for standard transformed enthalpies of reaction, standard transformed entropies of reaction, and changes in the binding of hydrogen ions in a reaction.

1.11 Discussion

This chapter on biochemical thermodynamics has been included because when there is a rate-determining reaction, all the reactions in the mechanism of catalysis prior to the rate-determining reaction are at equilibrium. When these reactions are at equilibrium, the composition can be calculated by use of a set of independent reactions and the conservation equation for enzymatic sites. Fortunately, this can be done using Solve in a personal computer, even for complicated mechanisms. Examples of complicated mechanisms are reactions like random A + B + C → products and mechanisms that include p*K*s of the enzymatic site, enzyme-substrate complexes, and the substrates.

Biochemical thermodynamics is different from chemical thermodynamics because the pH (and perhaps pMg) has to treated like temperature and pressure in chemical thermodynamics. In biochemical thermodynamics, T, P, and pH are independent variables set by the investigator. Therefore, the thermodynamic properties like equilibrium constants are functions of these variables. The way this is done is to use a Legendre transform to define a transformed Gibbs energy $G\,'$. There are corresponding transformed enthaplies $H\,'$ and transformed entropies $S\,'$. When the pH is specified, it is necessary to deal with reactants like ATP, which are sums of species that are in equilibrium with each other. This is exactly what biochemists do when they write ATP + H_2O = ADP + *P*i.