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RECOMMENDATIONS FOR NOMENCLATURE
AND TABLES IN BIOCHEMICAL
THERMODYNAMICS

(IUPAC Recommendations 1994)

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Recommendations for nomenclature and tables in biochemical thermodynamics

Chemical equations are written in terms of specific ionic and elemental species and balance elements and charge, whereas biochemical equations are written in terms of reactants that often consist of species in equilibrium with each other and do not balance elements that are assumed fixed, such as hydrogen at constant pH. Both kinds of reaction equations are needed in biochemistry. When the pH and the free concentrations of certain metal ions are specified, the apparent equilibrium constant K' for a biochemical reaction is written in terms of sums of species and can be used to calculate a standard transformed Gibbs energy of reaction $\Delta_r G'^{\circ}$. Transformed thermodynamic properties can be calculated directly from conventional thermodynamic properties of species. Calorimetry or the dependence of K' on temperature can be used to obtain the standard transformed enthalpy of reaction $\Delta_r H'^{\circ}$. Standard transformed Gibbs energies of formation $\Delta_f G'^{\circ}(i)$ and standard transformed enthalpies of formation $\Delta_f H'^{\circ}(i)$ for reactants (sums of species) can be calculated at various T , pH, pMg, and ionic strength (I) if sufficient information about the chemical reactions involved is available. These quantities can also be calculated from measurement of K' for a number of reactions under the desired conditions. Tables can be used to calculate $\Delta_r G'^{\circ}$ and $\Delta_r H'^{\circ}$ for many more reactions.

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1. PREAMBLE

In 1976 an Interunion Commission on Biothermodynamics (IUPAC, IUB, IUPAB) published *Recommendations for Measurement and Presentation of Biochemical Equilibrium Data* (ref. 1). This report recommended symbols, units, and terminology for biochemical equilibrium data and standard conditions for equilibrium measurements. These recommendations have served biochemistry well, but subsequent developments indicate that new recommendations and an expanded nomenclature are needed. In 1985 the Interunion Commission on Biothermodynamics published *Recommendations for the Presentation of Thermodynamic and Related Data in Biology* (1985) (ref. 2).

Before discussing the new recommendations, some of the basic recommendations of 1976 are reviewed and the recommended changes in these basic matters are given.

2. BASIC 1976 RECOMMENDATIONS ON SYMBOLS AND NOMENCLATURE

In the 1976 *Recommendations* (ref. 1), the overall reaction for the hydrolysis of ATP to ADP was written as*



and the expressions for the apparent equilibrium constant K' and the apparent standard Gibbs energy change $\Delta G^{\circ'}$ were written as

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

$$\Delta G^{\circ'} = -RT \ln \frac{[\text{total ADP}][\text{total P}_i]}{[\text{total ATP}]} \quad (3)$$

where these are equilibrium concentrations, recommended to be molar concentrations. The 1976 *Recommendations* further recommended that information about the experimental conditions could be indicated by writing K_c' (pH = x , etc.) and $\Delta G_c^{\circ'}$ (pH = x , etc.), where the subscript c indicates that molar concentrations are used. The 1976 *Recommendations* pointed out that the hydrolysis of ATP can also be formulated in terms of particular species of reactants and products. For example, at high pH and in the absence of magnesium ion



leads to the equilibrium constant expression

$$K_{\text{ATP}^{4-}} = \frac{[\text{ADP}^{3-}][\text{P}_i^{2-}][\text{H}^+]}{[\text{ATP}^{4-}]} \quad (5)$$

where the equilibrium constant $K_{\text{ATP}^{4-}}$ is independent of pH. The 1976 *Recommendations* went on to show how K' is related to $K_{\text{ATP}^{4-}}$.

* Abbreviations used in this document are: AMP, adenosine 5'-monophosphate; ADP, adenosine 5'-diphosphate; ATP, adenosine 5'-triphosphate; Glc, glucose; Glc-6-P, glucose 6-phosphate; P_i , orthophosphate. The designator (aq) is understood as being appended to all species that exist in aqueous solution.

3. CORRESPONDING NEW RECOMMENDATIONS

The new recommendation is that reaction 1 be should be written as



where ATP refers to an equilibrium mixture of ATP^{4-} , HATP^{3-} , $\text{H}_2\text{ATP}^{2-}$, MgATP^{2-} , MgHATP^- , and Mg_2ATP at the specified pH and pMg. This is referred to as a biochemical equation to emphasize that it describes the reaction that occurs at specified pH and pMg. The apparent equilibrium constant K' is made up of the equilibrium concentrations of the reactants relative to the standard state concentration c° , which is 1 M; note that M is an abbreviation for mol L⁻¹.

$$K' = \frac{([\text{ADP}]/c^\circ)([\text{P}_i]/c^\circ)}{([\text{ATP}]/c^\circ)} = \frac{[\text{ADP}][\text{P}_i]}{[\text{ATP}]c^\circ} \quad (7)$$

The term c° arises in the derivation of this equilibrium constant expression from the fundamental equation of thermodynamics and makes the equilibrium constant dimensionless. The logarithm of K' can only be taken if it is dimensionless (ref. 3). The standard state concentration used is an absolutely essential piece of information for the interpretation of the numerical value of an equilibrium constant. The apparent equilibrium constant K' is a function of T , P , pH, pMg, and I (ionic strength). Various metal ions may be involved, but Mg^{2+} is used as an example. As described below,

$$\Delta_r G'^\circ = -RT \ln K' \quad (8)$$

where $\Delta_r G'^\circ$ is the standard transformed Gibbs energy of reaction. The important point is that when the pH, and sometimes the free concentrations of certain metal ions, are specified, the criterion of equilibrium is the transformed Gibbs energy G' (ref. 4, 5). The reason for this name is discussed later in the section on transformed thermodynamic properties. Since the apparent equilibrium constant K' yields the standard value (the change from the initial state with the separated reactants at c° to the final state with separated products at c°) of the change in the transformed Gibbs energy G' , the superscript \circ comes after the prime in $\Delta_r G'^\circ$. The subscript r (recommended in ref. 3) refers to a reaction and is not necessary, but it is useful in distinguishing the standard transformed Gibbs energy of reaction from the standard transformed Gibbs energy of formation $\Delta_f G'^\circ(i)$ of reactant i , which is discussed below.

The hydrolysis of ATP can also be described by means of a chemical equation such as



A chemical equation balances atoms and charge, but a biochemical equation does not balance H if the pH is specified or Mg if pMg is specified, and therefore does not balance charge. Equation 9 differs from equation 4 in one way that is significant but not of major importance. Writing HPO_4^{2-} , rather than P_i^{2-} is a move in the direction of showing that atoms and charge balance in equation 9. Strictly speaking ATP^{4-} ought to be written $\text{C}_{10}\text{H}_{12}\text{O}_{13}\text{N}_5\text{P}_3^{4-}$. That is not necessary or advocated here, but we will see later that the atomic composition of a biochemical species is used in calculating standard transformed thermodynamic properties. Chemical equation 9 leads to the following equilibrium constant expression

$$K = \frac{[\text{ADP}^{3-}][\text{HPO}_4^{2-}][\text{H}^+]}{[\text{ATP}^{4-}](c^\circ)^2} \quad (10)$$

where $c^\circ = 1$ M. The equilibrium constant K is a function of T , P , and I . This equilibrium constant expression does not completely describe the equilibrium that is reached except at high pH and in the absence of Mg^{2+} . Chemical equations like equation 4 are useful in analyzing biochemical reactions and are often referred to as reference equations; thus, the corresponding equilibrium constants may be represented by K_{ref} . The effect of Mg^{2+} is discussed here, but this should be taken as only an example because the effects of other metal ions can be handled in the same manner.

4. ADDITIONAL NEW RECOMMENDATIONS

4.1 RECOMMENDATIONS CONCERNING CHEMICAL REACTIONS

The thermodynamics of reactions of species in aqueous solution is discussed in every textbook on physical chemistry, but this section is included to contrast the nomenclature with that of the next section and to respond to the special needs of biochemistry. As mentioned in Section 3, equilibrium constants of chemical reactions that are used in biochemistry are taken to be functions of T , P , and I . Therefore, the standard thermodynamic properties are also functions of T , P , and I . The standard Gibbs energy of reaction $\Delta_r G^\circ$ for reaction 9 is calculated using

$$\Delta_r G^\circ = -RT \ln K \quad (11)$$

and there are corresponding values of $\Delta_r H^\circ$ and $\Delta_r S^\circ$ that are related by

$$\Delta_r G^\circ = \Delta_r H^\circ - T\Delta_r S^\circ \quad (12)$$

The standard enthalpy of reaction is given by

$$\Delta_r H^\circ = RT^2 \left[\frac{\partial \ln K}{\partial T} \right]_{P,I} \quad (13)$$

If $\Delta_r H^\circ$ is independent of temperature in the range considered, it can be calculated using

$$\Delta_r H^\circ = [RT_1 T_2 / (T_2 - T_1)] \ln(K_2 / K_1) \quad (14)$$

If the standard molar heat capacity change $\Delta_r C_p^\circ$ is not equal to zero and is independent of temperature, the standard molar enthalpy of reaction varies with temperature according to

$$\Delta_r H^\circ(T) = \Delta_r H^\circ(T^*) + \Delta_r C_p^\circ(T - T^*) \quad (15)$$

The reference temperature T^* is usually taken as 298.15 K. In this case, $\Delta_r G^\circ$ and K vary with temperature according to (ref. 6)

$$\begin{aligned} \Delta_r G^\circ(T) &= -RT \ln K(T) \\ &= \Delta_r H^\circ(T^*) + \Delta_r C_p^\circ(T - T^*) + T\{\Delta_r G^\circ(T^*) - \Delta_r H^\circ(T^*)\}/T^* - T\Delta_r C_p^\circ \ln(T/T^*) \end{aligned} \quad (16)$$

Additional terms containing $(\partial \Delta_r C_p^\circ / \partial T)_P$ and higher order derivatives may be needed for extremely accurate data or for a very wide temperature range.

Equations 13 and 14 are exact only when the equilibrium constants are based on a molality standard state. If the equilibrium constants were determined with a standard state based on molarity, these equilibrium constants should be converted to a molality basis prior to using equation 13. For dilute aqueous solutions, $m_i = c_i/\rho$ where m_i and c_i are, respectively, the molality and the molarity of substance i and ρ is the mass density of water in kg L^{-1} . If this conversion is not made, there is an error of $RT^2(\partial \ln \rho / \partial T)_{P,I}$ for each unsymmetrical term in the equilibrium constant. This quantity is equal to $0.187 \text{ kJ mol}^{-1}$ for dilute aqueous solutions at 298.15 K. Similar statements pertain to equations 27 and 28, which are given later in this document.

Since the standard thermodynamic properties $\Delta_r G^\circ$ and $\Delta_r H^\circ$ apply to the change from the initial state with the separated reactants at c° to the final state with separated products at c° , it is of interest to calculate the changes in the thermodynamic properties under conditions where the reactants and products have specified concentrations other than c° . The change in Gibbs energy $\Delta_r G$ in an isothermal reaction in which the reactants and products are not all in their standard states, that is, not all at 1 M, is given by

$$\Delta_r G = \Delta_r G^\circ + RT \ln Q \quad (17)$$

where Q is the reaction quotient of specified concentrations of species. The reaction quotient has the same form as the equilibrium constant expression, but the concentrations are arbitrary, rather than being equilibrium concentrations. Ideal solutions are assumed. The change in Gibbs energy $\Delta_r G$ in an isothermal reaction is related to the change in enthalpy $\Delta_r H$ and change in entropy $\Delta_r S$ by

$$\Delta_r G = \Delta_r H - T\Delta_r S \quad (18)$$

The corresponding changes in entropy and enthalpy are given by

$$\Delta_r S = \Delta_r S^\circ - R \ln Q \quad (19)$$

$$\Delta_r H = \Delta_r H^\circ \quad (20)$$

The standard reaction entropy can be calculated from the standard molar entropies of the reacting species:

$\Delta_r S^\circ = \sum v_i \bar{S}^\circ(i)$, where v_i is the stoichiometric number (positive for products and negative for reactants) of species i .

The electromotive force E of an electrochemical cell is proportional to the $\Delta_r G$ for the cell reaction.

$$\Delta_r G = - |v_e| F E \quad (21)$$

where $|v_e|$ is the number of electrons transferred in the cell reaction and F is the Faraday constant (96 485.31 C mol⁻¹). Substituting equation 17 yields

$$E = E^\circ - \frac{RT}{|v_e|F} \ln Q \quad (22)$$

where $E^\circ = - \Delta_r G^\circ / |v_e| F$ is the standard electromotive force, that is the electromotive force when all of the species are in their standard states, but at the ionic strength specified for $\Delta_r G^\circ$. The electromotive force for a cell is equal to the difference in the electromotive forces of the half cells.

The standard Gibbs energy and enthalpy of reaction can be calculated from the formation properties of the species.

$$\Delta_r G^\circ = \sum v_i \Delta_f G^\circ(i) \quad (23)$$

$$\Delta_r H^\circ = \sum v_i \Delta_f H^\circ(i) \quad (24)$$

where the v_i is the stoichiometric numbers of species i . The standard entropy of formation of species i can be calculated using

$$\Delta_f S^\circ(i) = [\Delta_f H^\circ(i) - \Delta_f G^\circ(i)] / T \quad (25)$$

Two special needs of biochemistry are illustrated by considering the seven species in Table I. The first part of Table I gives the standard thermodynamic properties as they are found in the standard thermodynamic tables (see Appendix). The standard thermodynamic tables give the standard formation properties for the standard state, which is the state in a hypothetical ideal solution with a concentration of 1 M but the properties of an infinitely dilute solution and the activity of the solvent equal to unity. This means that the tabulated thermodynamic properties apply at $I = 0$. Since many biochemical reactions are studied at about $I = 0.25$ M, the tabulated values of $\Delta_f G^\circ(i)$ and $\Delta_f H^\circ(i)$ in The NBS Tables of Chemical Thermodynamic Properties and the CODATA Key Values for Thermodynamics have to be corrected to ionic strength 0.25 M, as described in Section 5.3. The values at $I = 0.25$ M are given in the second part of Table I. No adjustments are made for H₂O, MgHPO₄, and glucose because the ionic strength adjustment is negligible for neutral species. We will see in Section 5.1 that the transformed Gibbs energy G' is the criterion of equilibrium at specified pH and pMg. In Section 5.4, we will see that the calculation of transformed thermodynamic properties involves the adjustment of the standard formation properties of species to the desired pH and pMg by use of formation reactions involving H⁺ and Mg²⁺. The standard transformed formation properties of species can be calculated at any given pH and pMg in the range for which the acid dissociation constants and magnesium complex dissociation constants are known. However, for the purpose of making tables, it is necessary to choose a pH and pMg that is of general interest. For the tables given here, pH = 7 and pMg = 3 are used because they are close to the values in many living cells. Table II shows the result of these calculations for the species in Table I. The ions H⁺ and Mg²⁺ do not appear in this table because it applies at pH = 7 and pMg = 3. These methods have been applied to calculate the

transformed formation properties of P_i (ref. 4); glucose 6-phosphate (ref. 5); adenosine, AMP, ADP, and ATP (ref. 7).

Table I. Standard Formation Properties of Aqueous Species at 298.15 K.

	$\frac{\Delta_f H^\circ}{\text{kJ mol}^{-1}}$	$\frac{\Delta_f G^\circ}{\text{kJ mol}^{-1}}$
$I = 0 \text{ M}$		
H ₂ O	-285.83	-237.19
H ⁺	0.00	0.00
Mg ²⁺	-467.00	-455.30
HPO ₄ ²⁻	-1299.00	-1096.10
H ₂ PO ₄ ⁻	-1302.60	-1137.30
MgHPO ₄	-1753.80	-1566.87
Glucose	-1262.19	-915.90
$I = 0.25 \text{ M}$		
H ₂ O	-285.83	-237.19
H ⁺	0.41	-0.81
Mg ²⁺	-465.36	-458.54
HPO ₄ ²⁻	-1297.36	-1099.34
H ₂ PO ₄ ⁻	-1302.19	-1138.11
MgHPO ₄	-1753.80	-1566.87
Glucose	-1262.19	-915.90

Table II. Standard Transformed Formation Properties of Species at 298.15 K, pH = 7, pMg = 3, and $I = 0.25 \text{ M}$.

	$\frac{\Delta_f H'^\circ}{\text{kJ mol}^{-1}}$	$\frac{\Delta_f G'^\circ}{\text{kJ mol}^{-1}}$
H ₂ O	-286.65	-155.66
HPO ₄ ²⁻	-1297.77	-1058.57
H ₂ PO ₄ ⁻	-1303.01	-1056.58
MgHPO ₄	-1288.85	-1050.44
Glucose	-1267.11	-426.70

Tables I and II can be extended by use of measured equilibrium constants and enthalpies of reaction for enzyme-catalyzed reactions. For example, the species of glucose 6-phosphate can be added to these two tables because the equilibrium constant for the hydrolysis of glucose 6-phosphate has been measured at several temperatures, and because the acid dissociation constant and magnesium complex dissociation constant for glucose 6-phosphate are known at more than one temperature. However, as the standard

thermodynamic properties are not known for any species of adenosine, AMP, ADP, or ATP, it is necessary to adopt the convention that $\Delta_f G^\circ = \Delta_f H^\circ = 0$ for adenosine in dilute aqueous solution at each temperature. This convention was introduced for H^+ a long time ago. This method has been used to calculate the standard enthalpies and standard Gibbs energies of formation of adenosine phosphate species relative to H_2ADP^- at 298.15 K (ref. 8). When this convention is used, it is not possible to calculate the enthalpy of combustion of adenosine, but it is possible to calculate $\Delta_r G^\circ$ and $\Delta_r H^\circ$ for reactions of adenosine that do not reduce it to CO_2 , H_2O , and N_2 . If the standard thermodynamic properties of all of the species of a reactant are known, $\Delta_r G^\circ$ and $\Delta_r H^\circ$ can be calculated at any specified pH and pMg, as described in the next section. When $\Delta_r G^\circ$ and $\Delta_r H^\circ$ are eventually determined for adenosine in dilute aqueous solution, the values of $\Delta_r G^\circ$ and $\Delta_r H^\circ$ of the other species in the ATP series can be calculated, but this will not alter the equilibrium constants and enthalpies of reaction that can be calculated using the tables calculated using the assumption that $\Delta_f G^\circ = \Delta_f H^\circ = 0$ for adenosine.

In making these calculations, the pH has been defined by $pH = -\log_{10}([H^+]/c^\circ)$, rather than in terms of the activity, as it is in more precise measurements. The reason for doing this is that approximations are involved in the interpretation of equilibrium experiments on biochemical reactions at the electrolyte concentrations of living cells. Even Na^+ and K^+ ions are bound weakly by highly charged species of biochemical reactants, like ATP. As an approximation the acid dissociation constants and magnesium complex dissociation constants are taken to be functions of the ionic strength and the different effects of Na^+ and K^+ are ignored. These approximations can be avoided in more precise work, but only at the cost of a large increase in the number of parameters and the amount of experimental work required.

4.2 RECOMMENDATIONS CONCERNING BIOCHEMICAL REACTIONS

When pH and pMg are specified, a whole new set of transformed thermodynamic properties come into play (ref. 4, 5). These properties are different from the usual Gibbs energy G , enthalpy H , entropy S , and heat capacity at constant pressure C_p , and they are referred to as the transformed Gibbs energy G' , transformed enthalpy H' , transformed entropy S' , and transformed heat capacity at constant pressure C_p' . The standard transformed Gibbs energy of reaction $\Delta_r G'^\circ$ is made up of contributions from the standard transformed enthalpy of reaction $\Delta_r H'^\circ$ and the standard transformed entropy of reaction $\Delta_r S'^\circ$.

$$\Delta_r G'^\circ = \Delta_r H'^\circ - T\Delta_r S'^\circ \quad (26)$$

The standard transformed enthalpy of reaction is given by

$$\Delta_r H'^\circ = RT^2 \left(\frac{\partial \ln K'}{\partial T} \right)_{P, pH, pMg, I} \quad (27)$$

If $\Delta_r H'^\circ$ is independent of temperature in the range considered, it can be calculated using

$$\Delta_r H'^\circ = [RT_1 T_2 / (T_2 - T_1)] \ln(K_2' / K_1') \quad (28)$$

where K_2' and K_1' are measured at the same P , pH, pMg, and I . If $\Delta_r H'^\circ$ is dependent on temperature, a more complicated equation involving an additional parameter $\Delta_r C_p'^\circ$, the standard transformed heat capacity of reaction at constant pressure, can be used with the assumption that

$$\Delta_r H'^\circ(T) = \Delta_r H'^\circ(298.15 \text{ K}) + (T - 298.15 \text{ K})\Delta_r C_p'^\circ \quad (29)$$

This more complicated equation is analogous to equation 16. The standard transformed reaction entropy can be calculated from the standard transformed molar entropies of the reacting species:

$\Delta_r S'^0 = \sum v_i' \bar{S}'^0(i)$, where the v_i' are the apparent stoichiometric numbers (positive for products and negative for reactants) of the reactants i in a biochemical reaction written in terms of reactants (sums of species) (for example, reaction 6).

The standard transformed enthalpy of reaction $\Delta_r H'^0$ can also be calculated from calorimetric measurements. When that is done it is necessary to make corrections for the enthalpies of reaction caused by the change $\Delta_r N(\text{H}^+)$ in the binding of H^+ and in the change $\Delta_r N(\text{Mg}^{2+})$ in the binding of Mg^{2+} in the reaction (see equation 38 below). The change in binding of an ion in a biochemical reaction is equal to the number of ions bound by the products at the specified pH and pMg minus the number of the ions bound by the reactants. Note that $\Delta_r N(\text{H}^+)$ and $\Delta_r N(\text{Mg}^{2+})$ are dimensionless.

The change in binding of H^+ and Mg^{2+} in a biochemical reaction can be calculated if the acid dissociation constants and magnesium complex dissociation constants for the reactants are known; the equilibrium constant for the biochemical reaction itself does not have to be known for this calculation. Earlier calculations of the production of H^+ and Mg^{2+} used a different sign convention (ref. 9). The changes in binding $\Delta_r N(\text{H}^+)$ and $\Delta_r N(\text{Mg}^{2+})$ are given by

$$\Delta_r N(\text{H}^+) = \sum v_i' \bar{N}_{\text{H}}(i) \quad (30)$$

$$\Delta_r N(\text{Mg}^{2+}) = \sum v_i' \bar{N}_{\text{Mg}}(i) \quad (31)$$

where the v_i' are the apparent stoichiometric numbers of the reactants i in a biochemical reaction. $\bar{N}_{\text{H}}(i)$ is the number of H bound by an average molecule of reactant i at $T, P, \text{pH}, \text{pMg}$, and I . $\bar{N}_{\text{H}}(i)$ is calculated from $\sum r_i N_{\text{H}}(i)$ where r_i is the mole fraction of species i in the equilibrium mixture of the species of the reactant at the specified pH and pMg. $N_{\text{H}}(i)$ is the number of hydrogen atoms in species i . The average numbers $\bar{N}_{\text{H}}(i)$ and $\bar{N}_{\text{Mg}}(i)$ can be included in tables of transformed thermodynamic properties at specified pH and pMg so that $\Delta_r N(\text{H}^+)$ and $\Delta_r N(\text{Mg}^{2+})$ can be readily calculated for biochemical reactions. The sign and magnitude of $\Delta_r N(\text{H}^+)$ and $\Delta_r N(\text{Mg}^{2+})$ are important because they determine the effect of pH and pMg on the apparent equilibrium constant K' . It can be shown (ref. 9, 10) that

$$\Delta_r N(\text{H}^+) = - \left(\frac{\partial \log_{10} K'}{\partial \text{pH}} \right)_{T, P, \text{pMg}, I} \quad (32)$$

$$\Delta_r N(\text{Mg}^{2+}) = - \left(\frac{\partial \log_{10} K'}{\partial \text{pMg}} \right)_{T, P, \text{pH}, I} \quad (33)$$

where the logarithms are \log_{10} . A pHstat can be used to measure $\Delta_r N(\text{H}^+)$ directly.

Since the standard transformed thermodynamic properties $\Delta_r G'^0$ and $\Delta_r H'^0$ apply to the change from the initial state with the separated reactants at c° to the final state with separated products at c° , it is of interest to calculate the changes in the transformed thermodynamic properties under conditions where the reactants and products have the concentrations they do in a living cell. The change in transformed Gibbs energy $\Delta_r G'$ in an isothermal reaction in which the reactants and products are not all in their standard states, that is, not all at 1 M, is given by

$$\Delta_r G' = \Delta_r G'^0 + RT \ln Q' \quad (34)$$

where Q' is the apparent reaction quotient of specified concentrations of reactants (sums of species). The change in transformed Gibbs energy $\Delta_r G'$ in an isothermal reaction at specified pH and pMg is related to the change in transformed enthalpy $\Delta_r H'$ and change in transformed entropy $\Delta_r S'$ by

$$\Delta_r G' = \Delta_r H' - T \Delta_r S' \quad (35)$$

The corresponding changes in transformed entropy and transformed enthalpy are given by

$$\Delta_r S' = \Delta_r S'^0 - R \ln Q' \quad (36)$$

$$\Delta_r H' = \Delta_r H'^{\circ} \quad (37)$$

The calorimetrically determined enthalpy of reaction $\Delta_r H(\text{cal})$ includes the enthalpies of reaction of H^+ and Mg^{2+} (consumed or produced) with the buffer at the specified T , P , pH, pMg, and I . The standard transformed enthalpy of reaction $\Delta_r H'^{\circ}$ can be calculated using (ref. 11)

$$\Delta_r H'^{\circ} = \Delta_r H(\text{cal}) - \Delta_r N(\text{H}^+) \Delta_r H^{\circ}(\text{HBuff}) - \Delta_r N(\text{Mg}^{2+}) \Delta_r H^{\circ}(\text{MgBuff}) \quad (38)$$

$\Delta_r H^{\circ}(\text{HBuff})$ is the standard enthalpy for the acid dissociation of the buffer, and $\Delta_r H^{\circ}(\text{MgBuff})$ is the standard enthalpy for the dissociation of the magnesium complex formed with the buffer. The values of $\Delta_r N(\text{H}^+)$ and $\Delta_r N(\text{Mg}^{2+})$ can be determined experimentally using equations 32 and 33, or they can be calculated if sufficient data on acid and magnesium complex dissociation constants are available.

When the pH is specified, the electromotive force of an electrochemical cell can be discussed in terms of the concentrations of reactants (sums of species) rather than species. When this is done the electromotive force of the cell or of a half cell is referred to as the apparent electromotive force E' . When this is done equation 34 becomes

$$E' = E'^{\circ} - \frac{RT}{|v_e|F} \ln Q' \quad (39)$$

where $E'^{\circ} = -\Delta_r G'^{\circ}/|v_e|F$ is the standard apparent electromotive force at that pH. The symbol E'° is also used for the standard apparent reduction potential for an electrode reaction.

Biochemists have not had the advantage of having tables of standard formation properties of reactants at some standard set of conditions involving pH and pMg. Currently, information on biochemical reactions is tabulated as standard transformed Gibbs energies of reaction $\Delta_r G'^{\circ}$ and, in some cases, standard transformed enthalpies of reaction $\Delta_r H'^{\circ}$ at specified, T , P , pH, pMg, and I . Standard transformed formation properties have not been calculated because of lack of thermodynamic information to connect reactants in aqueous solution with the elements in their standard states and because of lack of knowledge as to how to calculate standard thermodynamic properties for a reactant like ATP that is made up of an equilibrium mixture of species at a given pH and pMg. The solution to the first problem is to assign zeros to a minimum number of species. This is what is done with $\text{H}^+(\text{aq})$ a long time ago. The solution to the second problem is that when pH and pMg are specified, the various species ATP^{4-} , HATP^{3-} , MgATP^{2-} , etc. of ATP become pseudoisomers. That is, the relative concentrations of the various species are then a function of temperature only. At a given T , P , pH, pMg, and I , the relative concentrations can be calculated, and the standard transformed thermodynamic properties of ATP (sum of species) can be calculated. The equations for doing this are given in Section 5.2. Thus, ATP at a given pH and pMg can be treated like a single species with the properties $\Delta_f G'^{\circ}$, $\Delta_f H'^{\circ}$, and $\Delta_f S'^{\circ}$.

When pH and pMg are specified, the transformed formation properties (indicated by a subscript f) of reactants are defined by (ref. 4)

$$\Delta_r G'^{\circ} = \sum v_i' \Delta_f G'^{\circ}(i) \quad (40)$$

$$\Delta_r H'^{\circ} = \sum v_i' \Delta_f H'^{\circ}(i) \quad (41)$$

where the v_i' are the apparent stoichiometric numbers of the reactants i in a biochemical reaction written in terms of reactants. These formation properties apply to reactants like ATP (that is, sums of species) at a specified T , P , pH, pMg, and I . The corresponding standard transformed entropy of formation of a reactant like ATP can be calculated using

$$\Delta_f G'^{\circ}(i) = \Delta_f H'^{\circ}(i) - T \Delta_f S'^{\circ}(i) \quad (42)$$

Table III gives standard transformed enthalpies of formation and standard transformed Gibbs energies of formation that have been calculated at 298.15 K, pH = 7, pMg = 3, and $I = 0.25$ M (ref. 7). The values for creatine phosphate are based on the recent work of Teague and Dobson (ref. 12). The adjustment of

standard formation properties of species to standard transformed formation properties at the desired pH and pMg has been mentioned in connection with Table II. When a reactant exists as a single species at pH = 7 and pMg = 3, the transformed formation properties of the species in Table II go directly into Table III. Water has to be included in this table because its formation properties must be used in equations 40 and 41, even though it does not appear in the expression for the apparent equilibrium constant. When a reactant exists at pH = 7 and pMg = 3 as an equilibrium mixture of species, isomer group thermodynamics (see Section 5.2) has to be used to calculate standard transformed formation properties (Table III) for that reactant.

Table III. Standard Transformed Formation Properties of Reactants (sums of species) at 298.15 K, pH = 7, pMg = 3, and $I = 0.25$ M. This table uses the convention that $\Delta_f G^\circ = \Delta_f H^\circ = 0$ for adenosine in dilute aqueous solution.

	$\frac{\Delta_f H^\circ}{\text{kJ mol}^{-1}}$	$\frac{\Delta_f G^\circ}{\text{kJ mol}^{-1}}$
ATP	-2981.79	-2102.88
ADP	-2000.19	-1231.48
AMP	-1016.59	-360.38
A (adenosine)	-5.34	529.96
Glc-6-P	-2279.09	-1318.99
Glc (glucose)	-1267.11	-426.70
CrP	-1509.75	-750.37
Cr (creatine)	-540.08	107.69
P _i	-1299.13	-1059.55
H ₂ O(l)	-286.65	-155.66

The large number of significant figures in Table I might appear to indicate that these thermodynamic properties are known very accurately, but this is misleading. The values in such a table are used only by subtracting them from other values in the table, and so the only things that are important are differences between values. The values have to be given in the table with enough significant figures so that thermodynamic information in the differences is not lost. The following examples illustrate uses of this table.

This kind of table will make it easier to make thermodynamic calculations on systems of enzymatic reactions, like glycolysis. Currently, to calculate $\Delta_r G^\circ$ for the net reaction of glycolysis, 10 reactions must be added and $\Delta_r G^\circ$ must be multiplied by 2 for some of them.

Example 1. Calculate $\Delta_r G^\circ$, $\Delta_r H^\circ$, $\Delta_r S^\circ$, and K' for the glucokinase reaction (EC 2.7.1.2) (ref. 13) at 298.15 K, pH = 7, pMg = 3, and $I = 0.25$ M.



$$\begin{aligned}\Delta_r G'^{\circ} &= (-1231.48 - 1318.99 + 2102.88 + 426.70) \text{ kJ mol}^{-1} \\ &= -20.89 \text{ kJ mol}^{-1}\end{aligned}$$

$$\begin{aligned}\Delta_r H'^{\circ} &= (-2000.19 - 2279.09 + 2981.79 + 1267.11) \text{ kJ mol}^{-1} \\ &= -30.38 \text{ kJ mol}^{-1}\end{aligned}$$

$$\Delta_r S'^{\circ} = (-30.38 + 20.89) \times 10^3 \text{ J mol}^{-1} / 298.15 \text{ K} = -31.83 \text{ J K}^{-1} \text{ mol}^{-1}$$

$$K' = \exp\left(\frac{20\,890 \text{ J mol}^{-1}}{8.3145 \text{ J K}^{-1} \text{ mol}^{-1} \times 298.15 \text{ K}}\right) = 4.57 \times 10^3$$

Example 2. Calculate $\Delta_r G'$, $\Delta_r H'$, and $\Delta_r S'$ for the glucokinase reaction at 298.15 K, pH = 7, pMg = 3, and $I = 0.25 \text{ M}$ when the reactant concentrations are $[\text{ATP}] = 10^{-5} \text{ M}$, $[\text{ADP}] = 10^{-3} \text{ M}$, $[\text{Glc}] = 10^{-4} \text{ M}$, and $[\text{Glc-6-P}] = 10^{-2} \text{ M}$.

$$Q' = (10^{-3})(10^{-2}) / (10^{-5})(10^{-4}) = 10^4$$

$$\Delta_r G' = \Delta_r G'^{\circ} + RT \ln Q' = -20.89 + (8.3145 \times 10^{-3})(298.15) \ln 10^4 = 1.94 \text{ kJ mol}^{-1}$$

Since $\Delta_r G'$ is positive, the glucokinase reaction cannot occur in the forward direction under these conditions.

$$\Delta_r H' = \Delta_r H'^{\circ} = -30.38 \text{ kJ mol}^{-1}$$

$$\Delta_r S' = \Delta_r S'^{\circ} - R \ln Q' = -108.41 \text{ J K}^{-1} \text{ mol}^{-1}$$

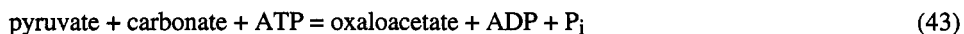
Note that

$$\Delta_r G' = \Delta_r H' - T \Delta_r S' = -30.38 - (298.15)(-0.10841) = 1.94 \text{ kJ mol}^{-1}$$

4.3 THE IMPORTANCE OF DISTINGUISHING BETWEEN CHEMICAL EQUATIONS AND BIOCHEMICAL EQUATIONS

Both types of equations are needed in biochemistry. Chemical equations are needed when it is important to keep track of all of the atoms and charges in a reaction, as in discussing the mechanism of chemical change. Biochemical reactions are needed to answer the question as to whether a reaction goes in the forward or backward direction at specified T , P , pH, pMg, and I , or for calculating the equilibrium extent of such a reaction. Therefore, it is essential to be able to distinguish between these two types of equations on sight. The reaction equations in [Enzyme Nomenclature](#) [ref. 13] are almost exclusively biochemical equations. In the case of the hydrolysis of adenosine triphosphate to adenosine diphosphate and inorganic phosphate, it is clear that equation 9 is a chemical equation and equation 6 is a biochemical equation. Equation 6 does not indicate that hydrogen ions or magnesium ions are conserved, but it is meant to indicate that C, O, N, and P are conserved. Equation 6 indicates the form of the expression for the apparent equilibrium constant K' at specified T , P , pH, pMg, and I . Equation 9 indicates that electric charge is conserved, and the abbreviations ATP^{4-} and ADP^{3-} can be replaced by the atomic compositions of these ions to show that C, H, O, N, and P are conserved. Equation 9 indicates the form of the expression for the equilibrium constant K at specified T , P , and I . Currently, the hydrolysis of ATP is often represented by $\text{ATP} + \text{H}_2\text{O} = \text{ADP} + \text{P}_i + \text{H}^+$ in textbooks and research papers, but this is a hybrid of a chemical equation and a biochemical equation and does not have an equilibrium constant. Furthermore, this "equation" does not give the correct stoichiometry. The correct stoichiometry with respect to H^+ is obtained by use of equation 32 and is $\Delta_r N(\text{H}^+) = 0.62$ at 298.15 K, 1 bar, pH = 7, pMg = 3, and $I = 0.25 \text{ M}$. The convention is that H_2O is omitted in the equilibrium expression for K or K' when reactions in dilute aqueous solutions are considered.

In writing biochemical equations, words are often used to avoid the implication that hydrogen atoms and charge are being balanced, but it is important to understand that all other atoms are balanced. For example,



is a biochemical equation and



is a chemical reaction. There is no unique way to write a chemical reaction; for example, this equation could be written with H_2PO_4^- and no H^+ on the right hand side. It can also be written with CO_2 on the left-hand side, but then it is necessary to be clear about whether this CO_2 is in the solution or gas phase. In equation 43 the word carbonate refers to the sum of the species CO_2 , H_2CO_3 , HCO_3^- , and CO_3^{2-} in aqueous solutions.

It is important to realize that $K' = K$ for reactions where the reactants are nonelectrolytes (ref. 14). An example is the hydrolysis of sucrose to glucose and fructose. Of course sugars do have ionizable groups, but we are usually not interested in the dissociations that occur above $\text{pH} = 12$. For racemases, $K' = K$. There are other reactions for which K' is approximately equal to K because a product has very nearly the same acid dissociation constant as a reactant.

The need to clearly distinguish between biochemical equations and chemical equations raises problems with some abbreviations that are widely used. For example, the use of NAD^+ in a biochemical equation makes it look like this charge should be balanced. NAD^+ is also not a suitable abbreviation for use in a chemical equation because it is actually a negative ion.

Chemical equations and biochemical equations should not be added or subtracted from each other because their sum or difference does not lead to an equation that has an equilibrium constant. On the other hand, chemical equations can be added to chemical equations, and biochemical equations can be added to biochemical equations.

The net equation for a system of biochemical reactions can also be written as a chemical equation or a biochemical equation, but the equilibrium constants are, of course, in general different. Net equations in the form of biochemical equations are especially useful for determining whether the system of reactions goes in the forward or backward direction at specified T , P , pH , pMg , and I .

These recommendations apply also to reactions catalyzed by RNA enzymes (ref. 15), catalytic antibodies (ref. 16), and synthetic enzymes (ref. 17) (sometimes called ribozymes, abzymes, and synzymes, respectively). The reactions catalyzed have apparent equilibrium constants K' that are functions of pH and free concentrations of certain metal ions. Both biochemical equations and chemical equations can be written for these reactions if the reactants are weak acids or bind metal ions.

4.4 EXPERIMENTAL MATTERS

In reporting results on equilibrium measurements on biochemical reactions it is extremely important to give enough information to specify T , P , pH , pMg (or free concentration of any other cation that is bound by reactants), and I at equilibrium. The most difficult of these variables are pMg and I . The calculation of pMg in principle requires information on the composition of the solution in terms of species, and this requires information on the dissociation constants of all of the weak acids and magnesium complex ions. However, if the metal ion binding constants of the buffer are known and the reactants are at low concentrations compared with the buffer, the concentrations of free metal ions can be calculated approximately. It is important to specify the composition of the solution and calculate the ionic strength, even if it can only be done approximately. Other important issues are the purities of materials, the methods of analysis, the

question as to whether the same value of apparent equilibrium constant was obtained from both directions, and assignment of uncertainties. For calorimetric measurements, it is important to measure the extent of reaction. An important part of any thermodynamic investigation is the clear specification of the substances used and the reaction(s) studied. It is very helpful to readers to give Enzyme Nomenclature (ref. 13) identification numbers of enzymes and Chemical Abstracts Services registry numbers for reactants. IUPAC has published a "Guide to the Procedures for the Publication of Thermodynamic Data" (ref. 18), and CODATA has published a "Guide for the Presentation in the Primary Literature of Numerical Data Derived from Experiments" (ref. 19).

It is recommended that equilibrium and calorimetric measurements on biochemical reactions be carried out over as wide a range of temperature, pH, pMg, and I as is practical. For the study of biochemical reactions under "near physiological conditions," the following set of conditions is recommended: $T = 310.15$ K, pH = 7.0, pMg = 3.0, and $I = 0.25$ M. It is also recognized that there is no unique set of physiological conditions and that for many purposes it will be necessary and desirable to study biochemical reactions under different sets of conditions.

For the purpose of relating results obtained on biochemical reactions to the main body of thermodynamic data (NBS Tables and other tables listed in the Appendix) the results of experiments should be treated so as to yield results for a chemical (reference) reaction at $T = 298.15$ K and $I = 0$. If this calculation is done, the method of data reduction and all auxiliary data used should be reported. It is also recognized that while a standard state based upon the concentration scale has been widely used in biochemistry, the molality scale has significant advantages for many purposes and can also be used for the study of biochemical reactions and for the calculation of thermodynamic properties.

5. THERMODYNAMIC BACKGROUND

5.1 TRANSFORMED THERMODYNAMIC PROPERTIES

The definition of a transformed Gibbs energy is a continuation of a process that starts with the first and second laws of thermodynamics, but is not always discussed in terms of Legendre transforms. The combined first and second law for a closed system involving only pressure-volume work is

$$dU = TdS - PdV \quad (45)$$

where U is the internal energy and S is the entropy. The criterion for spontaneous change at specified S and V is $(dU)_{S,V} \leq 0$. That is, if S and V are held constant, U can only decrease and is at a minimum at equilibrium. To obtain a criterion at specified S and P , the enthalpy was defined by the Legendre transform $H = U + PV$ so that $(dH)_{S,P} \leq 0$. To obtain a criterion at specified T and V , the Helmholtz energy was defined by the Legendre transform $A = U - TS$ so that $(dA)_{T,V} \leq 0$. To obtain a criterion at specified T and P , the Gibbs energy was defined by the Legendre transform $G = H - TS$ so that $(dG)_{T,P} \leq 0$. The Gibbs energy is especially useful because it provides the criterion for equilibrium at specified T and P . Two Legendre transforms can be combined. For example, the internal energy can be transformed directly to G by use of $G = U + PV - TS$. Alberty and Oppenheim (ref. 20, 21) used a Legendre transform to develop a criterion for equilibrium for the alkylation of benzene by ethylene at a specified partial pressure of ethylene. Wyman and Gill (ref. 22) have described the use of transformed Gibbs energies in describing macromolecular components in solution.

In 1992, Alberty (refs. 4, 5) used the Legendre transform

$$G' = G - n'(H^+)\mu(H^+) - n'(Mg^{2+})\mu(Mg^{2+}) \quad (46)$$

to define a transformed Gibbs energy G' in terms of the Gibbs energy G . Here $n'(\text{H}^+)$ is the total amount of H^+ in the system (bound and unbound) and $\mu(\text{H}^+)$ is the specified chemical potential for H^+ , which is given for an ideal solution by

$$\mu(\text{H}^+) = \mu(\text{H}^+)^{\circ} + RT \ln([\text{H}^+]/c^{\circ}) \quad (47)$$

where $\mu(\text{H}^+)^{\circ}$ is the chemical potential of H^+ at 1 M in an ideal solution at specified T , P , and I . The transformed Gibbs energy G' is defined in order to obtain a criterion of spontaneous change at T , P , pH, and pMg. It can be shown that $(dG')_{T,P,\text{pH},\text{pMg}} \leq 0$, so that G' is at a minimum when T , P , pH, and pMg are held constant. This is the fundamental justification for the use of G' in biochemistry. Under the appropriate circumstances, the magnesium term can be left out or be replaced by a term in another metal ion. The reaction is generally an enzyme-catalyzed reaction, but these concepts apply to any reaction involving a weak acid or metal ion complex when the pH and concentration of free metal ion at equilibrium are specified.

A consequence of equation 46 is that the chemical potential μ_i of each species in the system is replaced by the transformed chemical potential μ_i' given by

$$\mu_i' = \mu_i - N_{\text{H}}(i)\mu(\text{H}^+) - N_{\text{Mg}}(i)\mu(\text{Mg}^{2+}) \quad (48)$$

where $N_{\text{H}}(i)$ is the number of hydrogen atoms in species i and $N_{\text{Mg}}(i)$ is the number of magnesium atoms in species i .

Although thermodynamic derivations are carried out using the chemical potential, in actual calculations, the chemical potential μ_i of species i is replaced by the Gibbs energy of formation $\Delta_f G_i$ and the transformed chemical potential μ_i' of species i is replaced by the transformed Gibbs energy of formation $\Delta_f G_i'$, where

$$\Delta_f G_i = \Delta_f G_i^{\circ} + RT \ln([i]/c^{\circ}) \quad (49)$$

$$\Delta_f G_i' = \Delta_f G_i^{\circ} + RT \ln([i]/c^{\circ}) \quad (50)$$

for ideal solutions. The calculation of $\Delta_f G_i^{\circ}$ for a species is discussed in Section 5.4 and the calculation of $\Delta_f G_i^{\circ}$ for a reactant is discussed in Section 5.5.

Once the $\Delta_f G_i^{\circ}$ for the species (H_2PO_4^- , HPO_4^{2-} , MgHPO_4) of P_i , for example, have been calculated, the next question is how can these values be combined to obtain the value of $\Delta_f G_i^{\circ}$ for P_i ? The equations for this calculation are given in the next section.

5.2 ISOMER GROUP THERMODYNAMICS

A problem that has to be faced in biochemical thermodynamics at specified pH and pMg is that a reactant may consist of various species in equilibrium at the specified pH and pMg. Fortunately, a group of isomers (or pseudoisomers) in equilibrium with each other have thermodynamic properties just like a species does, but we refer to the properties of a pseudoisomer group as transformed properties. The problem of calculating a standard transformed Gibbs energy of formation of a reactant like ATP also arises when a reactant exists in isomeric forms (or hydrated and unhydrated forms), even if it is not a weak acid and does not complex with metal ions, so first we discuss a simple isomerization. The thing that characterizes an isomer group in ideal solutions is that the distribution within the isomer group is a function of temperature only. For such solutions, the standard Gibbs energy of formation of an isomer group $\Delta_f G^{\circ}(\text{iso})$ can be calculated from the standard Gibbs energies of formation $\Delta_f G_i^{\circ}$ of the various isomers using (ref. 23)

$$\Delta_f G^{\circ}(\text{iso}) = -RT \ln \sum_{i=1}^{N_I} \exp(-\Delta_f G_i^{\circ}/RT) \quad (51)$$

where N_I is the number of isomers in the isomer group. The standard enthalpy of formation $\Delta_f H^{\circ}(\text{iso})$ of the isomer group can be calculated using (ref. 24)

$$\Delta_f H^\circ(\text{iso}) = \sum_{i=1}^{N_I} r_i \Delta_f H_i^\circ \quad (52)$$

where r_i is the equilibrium mole fraction of the i th species within the isomer group that is given by

$$r_i = \exp\{[\Delta_f G^\circ(\text{iso}) - \Delta_f G_i^\circ]/RT\} \quad (53)$$

The standard entropy of formation of the isomer group $\Delta_f S^\circ(\text{iso})$ is given by

$$\Delta_f S^\circ(\text{iso}) = \sum_{i=1}^{N_I} r_i \Delta_f S_i^\circ - R \sum_{i=1}^{N_I} r_i \ln r_i \quad (54)$$

These equations can be used for pseudoisomer groups (for example, the species of ATP at specified pH and pMg) by using the transformed thermodynamic properties of the species.

For pseudoisomer groups, equations 51, 52, and 53 become

$$\Delta_f G^{\circ}(\text{reactant}) = -RT \ln \sum_{i=1}^{N_I} \exp(-\Delta_f G_i^{\circ}/RT) \quad (55)$$

$$\Delta_f H^{\circ}(\text{reactant}) = \sum_{i=1}^{N_I} r_i \Delta_f H_i^{\circ} \quad (56)$$

$$r_i = \exp\{[\Delta_f G^{\circ}(\text{reactant}) - \Delta_f G_i^{\circ}]/RT\} \quad (57)$$

where i refers to a species at specified pH and specified free concentrations of metal ions that are bound.

5.3 ADJUSTMENT FOR IONIC STRENGTH

The ionic strength has a significant effect on the thermodynamic properties of ions, and the extended Debye-Huckel theory can be used to adjust the standard Gibbs energy of formation and the standard enthalpy of formation of ion i to the desired ionic strength (ref. 25-28). At 298.15 K these adjustments can be approximated by

$$\Delta_f G_i^{\circ}(I) = \Delta_f G_i^{\circ}(I=0) - 2.91482 z_i^2 I^{1/2}/(1 + BI^{1/2}) \quad (58)$$

$$\Delta_f H_i^{\circ}(I) = \Delta_f H_i^{\circ}(I=0) + 1.4775 z_i^2 I^{1/2}/(1 + BI^{1/2}) \quad (59)$$

where kJ mol^{-1} are used, z_i is the charge on ion i , and $B = 1.6 \text{ L}^{1/2} \text{ mol}^{-1/2}$. Since for H^+ , $\Delta_f G^\circ = 0$ and $\Delta_f H^\circ = 0$ at each temperature at $I = 0$, $\Delta_f G^\circ(\text{H}^+, 298.15 \text{ K}, I = 0.25 \text{ M}) = -0.81 \text{ kJ mol}^{-1}$ and $\Delta_f H^\circ(\text{H}^+, 298.15 \text{ K}, I = 0.25 \text{ M}) = 0.41 \text{ kJ mol}^{-1}$. For the purpose of these recommendations, $\text{pH} = -\log_{10}([\text{H}^+]/c^\circ)$ and $\text{pMg} = -\log_{10}([\text{Mg}^{2+}]/c^\circ)$, as discussed above in Section 4.1.

The adjustment of thermodynamic quantities from one solution composition to another using ionic strength effects alone is an approximation that works well at low ionic strengths ($< 0.1 \text{ M}$) but it can fail at higher ionic strengths. Rigorous treatments require the use of interaction parameters (ref. 29) and a knowledge of the composition of the solution. While a substantial body of information on these parameters exists for aqueous inorganic solutions, there is very little of this type of data available for biochemical substances. Therefore, it is important that complete information on the compositions of the solutions used in equilibrium and calorimetric measurements be reported so that when values of these interaction parameters eventually become available, the results can be treated in a more rigorous manner. Specific ion effects are especially important when nucleic acids, proteins, and other polyelectrolytes are involved (refs. 30, 31).

5.4 ADJUSTMENT OF STANDARD THERMODYNAMIC PROPERTIES OF SPECIES TO THE DESIRED pH AND pMg

When pH and pMg are specified, the various species of ATP, for example, become pseudoisomers; that is their relative concentrations are a function of temperature only. The procedure for calculating the

transformed chemical potential μ_i' of a species has been indicated in equation 48. For actual calculations the chemical potentials μ_i of species are replaced with $\Delta_f G_i$ (see equation 49), and the transformed chemical potentials μ_i' of species are replaced with $\Delta_f G_i'$ (see equation 50). Thus equation 48 for a species can be written

$$\Delta_f G_i' = \Delta_f G_i^{\circ} - N_H(i)[\Delta_f G^{\circ}(\text{H}^+) + RT \ln([\text{H}^+]/c^{\circ})] - N_{\text{Mg}}(i)[\Delta_f G^{\circ}(\text{Mg}^{2+}) + RT \ln([\text{Mg}^{2+}]/c^{\circ})] \quad (60)$$

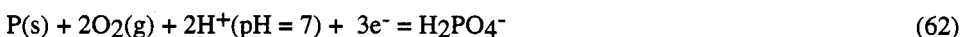
where $N_H(i)$ is the number of hydrogen atoms in species i . The corresponding equation for the standard transformed enthalpy of formation of species i is

$$\Delta_f H_i' = \Delta_f H_i^{\circ} - N_H(i)\Delta_f H^{\circ}(\text{H}^+) - N_{\text{Mg}}(i)\Delta_f H^{\circ}(\text{Mg}^{2+}) \quad (61)$$

since the enthalpy of an ion in an ideal solution is independent of its concentration.

In adjusting standard Gibbs energies of formation to a specified pH, there is the question as to whether to count all of the hydrogens or only those involved in the reaction under consideration. However, the recommendation here is to adjust for all of the hydrogens in a species because all of them may be ultimately removed in biochemical reactions. This has been done in Tables 2 and 3.

There is a simple way to look at the standard transformed Gibbs energy of formation $\Delta_f G_i'^{\circ}$ and the standard transformed enthalpy of formation $\Delta_f H_i'^{\circ}$ of species i , and that is that they are the changes in formation reactions of the species with H^+ at the specified pH and Mg^{2+} at the specified pMg on the left-hand side of the formation reaction (ref. 32). For H_2PO_4^- ,



$$\Delta_f G'^{\circ}(\text{H}_2\text{PO}_4^-) = \Delta_f G^{\circ}(\text{H}_2\text{PO}_4^-) - 2\{\Delta_f G^{\circ}(\text{H}^+) + RT \ln 10^{-\text{pH}}\} \quad (63)$$

$$\Delta_f H'^{\circ}(\text{H}_2\text{PO}_4^-) = \Delta_f H^{\circ}(\text{H}_2\text{PO}_4^-) - 2\Delta_f H^{\circ}(\text{H}^+) \quad (64)$$

The quantities $\Delta_f G^{\circ}(\text{H}^+)$ and $\Delta_f H^{\circ}(\text{H}^+)$ are included because they are equal to zero only at zero ionic strength. The electrons required to balance the formation reaction are assigned $\Delta_f G^{\circ}(\text{e}^-) = \Delta_f H^{\circ}(\text{e}^-) = 0$. This calculation can be made with either the standard thermodynamic properties at $I = 0$ or at some specified ionic strength.

The calculation of $\Delta_f G'^{\circ}$ and $\Delta_f H'^{\circ}$ for HPO_4^{2-} and MgHPO_4 follow this same pattern, with Mg^{2+} (pMg = 3) also on the left-hand side of the formation reaction of MgHPO_4 .

For a pseudoisomer group in which $\Delta_f G^{\circ}$ and $\Delta_f H^{\circ}$ are not known for any species, zero values have to be assigned to one of the species, as described in Section 4.1.

5.5 CALCULATION OF THE STANDARD FORMATION PROPERTIES OF A PSEUDOISOMER GROUP AT SPECIFIED pH AND pMg

H_2PO_4^- , HPO_4^{2-} and MgHPO_4 form a pseudoisomer group when pH and pMg are specified. Therefore, equations 55-57 can be used to calculate $\Delta_f G'^{\circ}(\text{P}_i)$ and $\Delta_f H'^{\circ}(\text{P}_i)$ for inorganic phosphate at the desired pH and pMg. These calculations have been made for inorganic phosphate and glucose 6-phosphate at pH = 7 and pMg = 3 by Alberty (ref. 4), and for adenosine, AMP, ADP, and ATP by Alberty and Goldberg (ref. 7) using the convention that $\Delta_f G^{\circ} = \Delta_f H^{\circ} = 0$ for neutral adenosine.

For less common and more complicated reactants, the acid dissociation constants and magnesium complex dissociation constants may not be known. The $\Delta_f G_i'^{\circ}$ values of the reactants at pH = 7 and pMg = 3 can, however, be calculated if K' has been measured at pH = 7 and pMg = 3 for a reaction in which $\Delta_f G_i'^{\circ}$ is known for the other reactants. For example, this approach can be used to calculate $\Delta_f G_i'^{\circ}$ for the reactants in glycolysis.

5.6 THE ACTUAL EXPERIMENT AND THOUGHT EXPERIMENTS

In the laboratory, a biochemical equilibrium experiment is actually carried out at specified T and P , and the pH is measured at equilibrium. Buffers are used to hold the pH constant, but there may be a change in the pH if the catalyzed reaction produces or consumes acid. pMg at equilibrium has to be calculated, and this can be done accurately only if the acid dissociation constants and magnesium complex dissociation constants are known for all of the reactants and buffer components (ref. 12, 33). In the absence of this information pMg can be calculated approximately if the buffer binds H^+ and Mg^{2+} , these dissociation constants are known, and the concentrations of the reactants are much smaller than the concentration of the buffer components that are primarily responsible for the binding of Mg^{2+} . We can hope that some day there will be a pMg electrode as convenient as the pH electrode.

When we interpret the thermodynamics of a biochemical equilibrium experiment, we use an idealized thought experiment that is equivalent to the laboratory experiment. In the laboratory experiment, the buffer determines the approximate pH, but the pH will drift if H^+ is produced or consumed. The pH should be measured at equilibrium because the composition and $\Delta_r G'^{\circ}$ and $\Delta_r H'^{\circ}$ depend on this pH. Since the experimental results depend on the final pH, we can imagine that the experiment was carried out in a reaction vessel with a semipermeable membrane (permeable to H^+ and an anion, and impermeable to other reactants) with a pH reservoir on the other side. If the binding of H^+ by the products is greater than that of the reactants, H^+ will diffuse in from the pH reservoir as the reaction proceeds. If the binding of H^+ by the reactants is greater, H^+ will diffuse out of the reaction vessel as the reaction proceeds. Thus hydrogen ion is not conserved in the reaction vessel in this idealized thought experiment. Similar statements can be made about Mg^{2+} . In calorimetric experiments, corrections have to be made for the enthalpies of reaction due to the production of H^+ and Mg^{2+} to obtain $\Delta_r H'^{\circ}$, as mentioned earlier.

The thermodynamic interpretation of the apparent equilibrium constant K' uses $\Delta_r G'^{\circ}$ and $\Delta_r H'^{\circ}$. These quantities correspond with another thought experiment in which the separated reactants, each at 1 M at the specified T , P , final pH, final pMg , and I react to form the separated products, each at 1 M at the specified T , P , final pH, final pMg , and I .

5.7 LINEAR ALGEBRA

It is generally understood that chemical equations conserve atoms and charge, but it is not generally known how the conservation equations for a chemical reaction system can be calculated from a set of chemical equations or how an independent set of chemical equations can be calculated from the conservation equations for the system. Nor is it well known that conservation equations in addition to atom and charge balances may arise from the mechanism of reaction. The quantitative treatment of conservation equations and chemical reactions requires the use of matrices and matrix operations (ref. 10, 23). When the equilibrium concentrations of species such as H^+ and Mg^{2+} are specified, these species and electric charge are not conserved, and so a biochemical equation should not indicate that they are conserved. The current practice of using words like acetate and symbols like ATP and P_i is satisfactory provided that people understand the reason for using these words and symbols. It should be possible to distinguish between chemical equations and biochemical equations on sight, and this means that different symbols should be used for the reactants in these two types of equations.

A set of simple chemical equations has been discussed from the viewpoint of linear algebra (ref. 34). The hydrolysis of ATP to ADP and P_i at specified pH has also been discussed from the viewpoint of linear algebra which shows why the 4 chemical equations reduce down to a single biochemical equation (ref. 35). The conservation matrix for a biochemical reaction is especially useful for the identification of the constraints in addition to element balances (ref. 36).

6. RECOMMENDATIONS ON THERMODYNAMIC TABLES

The papers by Alberty (ref. 5) and Alberty and Goldberg (ref. 7) show four types of tables of thermodynamic properties of biochemical reactants: (1) $\Delta_f G^\circ$ and $\Delta_f H^\circ$ for species at 298.15 K, 1 bar (0.1 MPa), $I = 0$. (2) $\Delta_f G^\circ$ and $\Delta_f H^\circ$ for species at 298.15 K, 1 bar, $I = 0.25$ M. (3) $\Delta_f G'^\circ$ and $\Delta_f H'^\circ$ for species at 298.15 K, 1 bar, pH = 7, pMg = 3, and $I = 0.25$ M. (4) $\Delta_f G'^\circ$ and $\Delta_f H'^\circ$ for reactants (sum of species) at 298.15 K, 1 bar, pH = 7, pMg = 3, and $I = 0.25$ M. Table 1 contains the most basic information for calculating $\Delta_f G^\circ$ and $\Delta_f H^\circ$ for reference reactions at $I = 0$ and corresponds with the NBS and CODATA Tables. Table 4 is the most convenient for calculating $\Delta_r G'^\circ$ and $\Delta_r H'^\circ$ under normal experimental conditions of 298.15 K, 1 bar, pH = 7, pMg = 3, and $I = 0.25$ M. Currently, thermodynamic information in biochemistry is stored as K' and $\Delta_r H'^\circ$ when pH and pMg are specified and as K and $\Delta_r H^\circ$ for reactions in terms of species. In order to calculate K' and $\Delta_r H'^\circ$ or K and $\Delta_r H^\circ$ for a reaction that has not been studied, it is currently necessary to add and subtract known reactions. It would be more convenient to be able to look up reactants in a table and add and subtract their formation properties to calculate K' and $\Delta_r H'^\circ$ or K and $\Delta_r H^\circ$, as is usually done for chemical reactions. One reactant can be involved in hundreds of reactions, and so it is more economical to focus on the reactants. The usefulness of such a table increases rapidly with its length. As mentioned in Section 4.3, columns for $\bar{N}_H(i)$ and $\bar{N}_{Mg}(i)$ can be included so that the change in binding of H and Mg at specified T, P, pH, pMg, and I can be readily calculated by use of equations 30 and 31.

The choice of 298.15 K, 1 bar, pH = 7, pMg = 3, and $I = 0.25$ M is arbitrary, but these conditions are often used. Tables can be constructed for other conditions (T , pH, pMg, and I) if sufficient information is available. H_2O has to be included in this table because its $\Delta_f G'^\circ(H_2O)$ and $\Delta_f H'^\circ(H_2O)$ have to be included in the summations in equations 40 and 41 when it is a reactant, even though H_2O is omitted in the expression for the apparent equilibrium constant.

The most basic principle is that thermodynamic tables on biochemical reactants at pH = 7 and pMg = 3 should be consistent with the usual thermodynamic tables to as great an extent as possible. A great deal is already known about the thermodynamics of reactions in aqueous solution, and this is all of potential value in biochemistry. The standard transformed formation properties of inorganic phosphate and glucose 6-phosphate at pH = 7 and pMg = 3 can be calculated since the standard formation properties of inorganic phosphate and glucose are well known and the properties of glucose 6-phosphate can be calculated from $\Delta_r G'^\circ$ and $\Delta_r H'^\circ$ for the glucose 6-phosphatase reaction. This is true for many other biochemical reactants. Sometimes it is necessary to use the convention that $\Delta_f G^\circ = \Delta_f H^\circ = 0$ for a reference species, as described in the discussion of the ATP series. It is difficult and expensive to obtain these missing data because biochemical reactants are often rather large molecules and contain a large number of elements. The methods described here make it possible to calculate $\Delta_f G'^\circ$ and $\Delta_f H'^\circ$ for P_i , glucose 6-phosphate, adenosine, AMP, ADP, and ATP at temperatures in the approximate range 273-320 K, pH in the approximate range 3-10, pMg in the range above about 2, and ionic strengths in the approximate range 0-0.35 M. Since the choice of reference species is arbitrary to a certain extent, it is desirable to have

international agreement on these choices. This agreement is required so that thermodynamic properties in different tables of this type can be used together.

For many biochemical reactants, the acid and magnesium complex dissociation constants have not been measured, but this does not mean that these reactants cannot be included in a table of $\Delta_f G^{\circ}$ and $\Delta_f H^{\circ}$ at 298.15 K, pH = 7, pMg = 3, and $I = 0.25$ M. What is required is that the apparent equilibrium constant K' for a reaction involving this reactant (or pair of reactants) with reactants with known properties has been determined at pH = 7, pMg = 3, and $I = 0.25$ M at more than one temperature. If the pKs of a reactant are unknown, there is a problem in calculating the equilibrium pMg, but this uncertainty may not be large if the concentration of Mg^{2+} is controlled by a buffer with known binding properties and the equilibrium concentration of the reactants is low.

The fact that biochemical reactions are often organized in series will facilitate the construction of thermodynamic tables. When a series starts or ends with a reactant with known formation properties, knowledge of the apparent equilibrium constants in the series makes it possible to calculate $\Delta_f G^{\circ}$ for reactants in the series at pH = 7. For example, consider glycolysis for which the apparent equilibrium constants for the 10 reactions have been known for some time. Since the standard transformed thermodynamic properties of glucose in aqueous solution are known, the $\Delta_f G^{\circ}$ values for the 17 reactants at pH = 7, including pyruvate, can be calculated with just one problem. Since $\Delta_f G^{\circ}$ is not known for NAD or NADH, one of the species has to be assigned $\Delta_f G^{\circ} = \Delta_f H^{\circ} = 0$. Since the thermodynamic properties of pyruvate are known, this provides a check on the calculation of $\Delta_f G_i^{\circ}$ of the reactants in glycolysis.

The following conventions are recommended:

1. When a reactant exists only in an electrically neutral form at pH = 7 and pMg = 3 and $\Delta_f G^{\circ}$ and $\Delta_f H^{\circ}$ for that form in dilute aqueous solution are known, the values of $\Delta_f G^{\circ}$ and $\Delta_f H^{\circ}$ are calculated by adjusting for the content of H. An example is glucose.
2. When a reactant exists in a single ionized form in the neighborhood of pH = 7 and pMg = 3, the values of $\Delta_f G^{\circ}$ and $\Delta_f H^{\circ}$ for that form in the usual tables (which apply at $I = 0$) have to be adjusted to $I = 0.25$ M with the extended Debye-Hückel theory and adjusted for H to obtain the entry to the table of $\Delta_f G^{\circ}$ and $\Delta_f H^{\circ}$ values. Obviously, thermodynamic properties of H^+ and Mg^{2+} will not be found in the table of $\Delta_f G^{\circ}$ values. Also, ions like Ca^{2+} which bind significantly with multiply charged negative species of biochemical reactants cannot be put in the table because they require treatment like Mg^{2+} . Values of $\Delta_f G^{\circ}$ and $\Delta_f H^{\circ}$ for Krebs cycle intermediates that exist at pH = 7 and pMg = 3 in a single ionic form calculated by Miller and Smith-Magowan (Appendix, ref. 8) can be adjusted for H and Mg and used in the proposed table after the values have been corrected to $I = 0.25$ M. An example is succinate.
3. When a reactant exists in several ionized or complexed forms that are at equilibrium at pH = 7 and pMg = 3 and the standard thermodynamic properties of all of the ionized and complexed forms are known, the values of $\Delta_f G^{\circ}$ and $\Delta_f H^{\circ}$ of reactants at pH = 7, pMg = 3, $I = 0.25$ M can be calculated using isomer group thermodynamics. Examples are inorganic phosphate (P_i), pyrophosphate, carbonate, citrate, and glucose 6-phosphate.
4. When a reactant exists in several ionized or complexed forms with known dissociation constants and $\Delta_f G^{\circ}$ and $\Delta_f H^{\circ}$ are not known for any species of the reactant, $\Delta_f G^{\circ}$ and $\Delta_f H^{\circ}$ for the species can only be calculated by assigning one of them $\Delta_f G^{\circ} = \Delta_f H^{\circ} = 0$ in dilute aqueous solution. The $\Delta_f G^{\circ}$ and $\Delta_f H^{\circ}$ values of the various species have to be adjusted to an ionic strength of 0.25 M, and adjusted for H and Mg, so that $\Delta_f G^{\circ}$ and $\Delta_f H^{\circ}$ can be calculated for the reactant (sum of species) by use of isomer group thermodynamics, as illustrated here for the ATP series. NAD and NADH also provide an

example, which is a little different because the acid and magnesium complex dissociation constants are believed to be identical.

5. If acid dissociation and magnesium dissociation constants are not known for a reactant, it can still be put into a table at $\text{pH} = 7$ and $\text{pMg} = 3$ if apparent equilibrium constants have been measured under these conditions for a reaction involving this reactant with other reactants whose transformed thermodynamic properties are known. Examples are the many reactants in glycolysis other than glucose, ATP, ADP, P_i , NAD, NADH, and H_2O .

6. It is not necessary to have columns in tables for $\Delta_f S'^0$ and $\Delta_f S^0$ because these can be treated as dependent properties and can be calculated from equations 25 and 42.

7. NOMENCLATURE

Symbol	Name	Unit
A	extensive Helmholtz energy of a system	kJ
B	parameter in the extended Debye-Hückel theory	$\text{L}^{1/2} \text{mol}^{-1/2}$
c_i	concentration of species i	mol L^{-1}
c^0	standard state concentration (1 M)	mol L^{-1}
$\Delta_r C_P^0$	standard heat capacity at constant pressure of reaction at T , P , and I	$\text{J K}^{-1} \text{mol}^{-1}$
$\Delta_r C_P'^0$	standard transformed heat capacity of reaction at constant T , P , pH , pMg , and I	$\text{J K}^{-1} \text{mol}^{-1}$
E	electromotive force	V
E^0	standard electromotive force of a cell or half cell	V
E'	apparent electromotive force at specified pH	V
E'^0	standard apparent electromotive force of a cell or half cell at specified pH	V
F	Faraday constant ($96\,485.31 \text{ C mol}^{-1}$)	C mol^{-1}
G	extensive Gibbs energy of a system	kJ
G'	extensive transformed Gibbs energy of a system	kJ
$\Delta_r G$	reaction Gibbs energy for specified concentrations of species at specified T , P , and I	kJ mol^{-1}
$\Delta_r G^0$	standard reaction Gibbs energy of a specified reaction in terms of species at specified T , P , and I	kJ mol^{-1}
$\Delta_r G'$	transformed reaction Gibbs energy in terms of reactants (sums of species) for specified concentrations of reactants and products at specified T , P , pH , pMg , and I	kJ mol^{-1}
$\Delta_r G'^0$	standard transformed reaction Gibbs energy of a specified reaction in terms of reactants (sums of species) at specified T , P , pH , pMg and I	kJ mol^{-1}
$\Delta_f G(i)$	Gibbs energy of formation of species i at a specified concentration of i and specified T , P , and I	kJ mol^{-1}
$\Delta_f G^0(i)$	standard Gibbs energy of formation of species i at specified T , P , and I	kJ mol^{-1}
$\Delta_f G'(i)$	transformed Gibbs energy of formation of species i or reactant i (sum of species) at specified concentration and specified T , P , pH , pMg , and I	kJ mol^{-1}
$\Delta_f G'^0(i)$	standard transformed Gibbs energy of formation of species i or reactant i (sum of species) at specified T , P , pH , pMg , and I	kJ mol^{-1}

H	extensive enthalpy of a system	kJ
H'	extensive transformed enthalpy of a system	kJ
$\Delta_r H(\text{cal})$	calorimetrically determined enthalpy of reaction that includes the enthalpies of reaction of H^+ and Mg^{2+} (consumed or produced) with any buffer in solution	kJ mol ⁻¹
$\Delta_r H$	enthalpy of reaction of a specified reaction in terms of species at specified T , P , and I	kJ mol ⁻¹
$\Delta_r H^\circ$	standard enthalpy of reaction of a specified reaction in terms of species at specified T , P , and I	kJ mol ⁻¹
$\Delta_r H'$	transformed enthalpy of reaction of a specified reaction in terms of reactants (sums of species) for specified concentrations of reactants and products at specified T , P , pH, pMg, and I	kJ mol ⁻¹
$\Delta_r H'^\circ$	standard transformed enthalpy of a specified reaction in terms of reactants (sums of species) at specified T , P , pH, pMg and I	kJ mol ⁻¹
$\Delta_f H(i)$	enthalpy of formation of species i at specified T , P , and I	kJ mol ⁻¹
$\Delta_f H^\circ(i)$	standard enthalpy of formation of species i at specified T , P , and I	kJ mol ⁻¹
$\Delta_f H'(i)$	transformed enthalpy of formation of species i or reactant i (sum of species) at specified T , P , pH, pMg, and I	kJ mol ⁻¹
$\Delta_f H'^\circ(i)$	standard transformed enthalpy of formation of species i or reactant i (sum of species) at specified T , P , pH, pMg, and I	kJ mol ⁻¹
I	ionic strength	mol L ⁻¹
K	equilibrium constant for a specified reaction written in terms of concentrations of species at specified T , P , and I (omitting H_2O when it is a reactant)	dimensionless
K'	apparent equilibrium constant for a specified reaction written in terms of concentrations of reactants (sums of species) at specified T , P , pH, pMg, and I (omitting H_2O when it is a reactant)	dimensionless
m_i	molality of i	mol kg ⁻¹
n_i or $n(i)$	amount of species i	mol
$n'(i)$	amount of species (bound and unbound) or amount of reactant i (that is, sum of species)	mol
$N_{\text{H}}(i)$	number of H atoms in species i	dimensionless
$N_{\text{Mg}}(i)$	number of Mg atoms in species i	dimensionless
$\bar{N}_{\text{H}}(i)$	average number of H atoms in reactant i at specified T , P , pH, pMg, and I	dimensionless
$\Delta_r N(\text{H}^+)$	change in binding of H^+ in a biochemical reaction at specified T , P , pH, pMg, and I	dimensionless
$\Delta_r N(\text{Mg}^{2+})$	change in binding of Mg^{2+} in a biochemical reaction at specified T , P , pH, pMg, and I	dimensionless
N_{I}	number of isomers in an isomer group	dimensionless
pH	$-\log_{10}([\text{H}^+]/c^\circ)$	dimensionless
pMg	$-\log_{10}([\text{Mg}^{2+}]/c^\circ)$	dimensionless
pX	$-\log_{10}([\text{X}]/c^\circ)$	dimensionless
P	pressure	bar
Q	reaction quotient of specified concentrations of species in the same form as the equilibrium constant expression	dimensionless

Q'	apparent reaction quotient of specified concentrations of reactants and products (sum of species) in the same form as the apparent equilibrium constant expression	dimensionless
R	gas constant (8.31451 J K ⁻¹ mol ⁻¹)	J K ⁻¹ mol ⁻¹
r_i or $r(i)$	equilibrium mole fraction of i within a specified class of molecules	dimensionless
S	extensive entropy of a system	J K ⁻¹
S'	extensive transformed entropy of a system	J K ⁻¹
$\bar{S}^{\circ}(i)$	standard molar entropy of species i at specified T , P , and I	J K ⁻¹ mol ⁻¹
$\bar{S}'^{\circ}(i)$	standard molar transformed entropy of species i or reactant i at specified T , P , pH, pMg, and I	J K ⁻¹ mol ⁻¹
$\Delta_r S$	entropy of reaction of a specified reaction in terms of species at specified T , P , and I	J K ⁻¹ mol ⁻¹
$\Delta_r S^{\circ}$	standard entropy of reaction of a specified reaction in terms of ionic species at specified T , P , and I	J K ⁻¹ mol ⁻¹
$\Delta_r S'$	transformed entropy of reaction of a specified reaction in terms of reactants (sums of species) for specified concentrations of reactants and products at specified T , P , pH, pMg, and I	J K ⁻¹ mol ⁻¹
$\Delta_r S'^{\circ}$	standard transformed entropy of a specified reaction in terms of sums of species at specified T , P , pH, pMg and I	J K ⁻¹ mol ⁻¹
$\Delta_f S^{\circ}(i)$	standard entropy of formation of species i at specified T , P , and I	J K ⁻¹ mol ⁻¹
$\Delta_f S'^{\circ}(i)$	standard transformed entropy of formation of species i or reactant i (sum of species) at specified T , P , pH, pMg, and I	J K ⁻¹ mol ⁻¹
T	temperature	K
U	extensive internal energy of a system	kJ
V	volume	L
z_i	charge of ion i with sign	dimensionless
ρ	density	kg m ⁻³
$\mu(i)$	chemical potential of species i at specified T , P , and I	kJ mol ⁻¹
$\mu'(i)$	transformed chemical potential of species i or reactant (sum of species) i at specified T , P , pH, pMg, and I [can be replaced by $\Delta_f G'(i)$]	kJ mol ⁻¹
$\mu^{\circ}(i)$	standard chemical potential of species i at specified T , P , and I [can be replaced by $\Delta_f G^{\circ}(i)$]	kJ mol ⁻¹
ν_e	number of electrons in a cell reaction	dimensionless
ν_i or $\nu(i)$	stoichiometric number of species i in a specified chemical reaction	dimensionless
$\nu'(i)$	apparent stoichiometric number of reactant i in a specified biochemical reaction	dimensionless

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9. APPENDIX: SURVEY OF CURRENT BIOCHEMICAL THERMODYNAMIC TABLES (The reader is cautioned on distinguishing chemical reactions from biochemical reactions.)

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