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Systems of biochemical reactions from the point of view of a semigrand partition function

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Abstract

Semigrand partition functions contain all the thermodynamic information on reaction systems. When they are written for systems at specified pH, they yield the transformed Gibbs energy G' of the system and the thermodynamic properties that can be calculated from G'. When they are written for systems at specified pH and specified concentrations of coenzymes, they yield the further transformed Gibbs energy G'' and properties that can be calculated from G''. This is illustrated by considering: (1) a reactant that is a weak monoprotic acid at a specified pH; (2) a reaction between two pseudoisomer groups at a specified pH; and (3) the first five reactions of glycolysis. Equilibrium compositions in glycolysis are calculated at pH 7 and different steady-state concentrations of ATP and ADP.

Keywords: Semigrand partition function; Transformed Gibbs energy; Legendre transform; Glycolysis; Equilibrium composition

1. Introduction

In making thermodynamic calculations on biochemical reactions, it is convenient to hold the pH constant, and when this is done the criterion for spontaneous change and equilibrium is provided by the transformed Gibbs energy G', which is defined by a Legendre transform [1,2]. In making equilibrium calculations on systems of biochemical reactions, it may be of interest to assume in addition that the concentrations of coenzymes like ATP and ADP are constant. When this is done the criterion for spontaneous change and equilibrium is provided by a further transformed Gibbs energy G'', which is defined by another Legendre transform [3,4]. Actually, both types of calculations owe a lot to statistical mechanics because these calculations follow the pat-

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tern of equilibrium calculations on gaseous hydrocarbons at specified partial pressures of molecular hydrogen, ethylene, and acetylene. In 1989 Alberty and Oppenheim [5] used a semigrand partition function to calculate the equilibrium distribution of alkyl benzenes at elevated temperature as a function of the partial pressure of ethylene. The semigrand partition function contains all the thermodynamic information on a system at specified temperature, pressure, and chemical potentials of one or more species or reactants (sums of species) that are considered to be in a large reservoir that is separated from the reaction system by a semipermeable membrane. The thermodynamic properties of biochemical reactants have recently been discussed from the point of view of the semigrand partition function [6].

The transformed Gibbs energy G' of a system of biochemical reactions at specified pH can be calculated from the semigrand partition function Γ', where $G' = -kT\ln\Gamma'$ and k is the Boltzmann constant. This use of semigrand partition functions is extended here to the treatment of a biochemical reaction at specified pH and to biochemical reaction systems at specified pH and steady state concentrations of ATP and ADP. When the concentrations of coenzymes are held constant, the further transformed Gibbs energy G'' of the system can be obtained from the semigrand partition function Γ'', where $G'' = -kT\ln\Gamma''$. This method is used to calculate the equilibrium composition for the first five reactions in glycolysis at specified concentrations of ATP and ADP.

2. Weak acid at a specified *T*, *P* and pH

2.1. Use of the transformed Gibbs energy G′

When the *T*, *P*, and pH are specified, the criterion for spontaneous change and equilibrium is provided by the transformed Gibbs energy G' that is defined by the following Legendre transform [1,2,7]:

$$G' = G - n_c(\mathrm{H})\mu(\mathrm{H}^+) \tag{1}$$

where $n_c(\mathrm{H})$ is the amount of the hydrogen component in the system and $\mu(\mathrm{H}^+)$ is the specified chemical potential of hydrogen ions. The Gibbs energy of the system is given by

$$G = \Sigma\mu_i n_i \tag{2}$$

where n_i is the amount of species i. The amount of the hydrogen component is given by

$$n_c(\mathrm{H}) = \Sigma N_{\mathrm{H}i} n_i \tag{3}$$

where $N_{\mathrm{H}i}$ is the number of hydrogen atoms in species i. Substituting these two relations in Eq. (1) yields

$$G' = \Sigma n'_i \mu'_i \tag{4}$$

where the transformed chemical potential of species i is given by

$$\mu'_i = \mu_i - N_{\mathrm{H}i}\mu(\mathrm{H}^+) \tag{5}$$

At zero ionic strength the chemical potential of hydrogen ions is given by

$$\mu(\mathrm{H}^+) = RT\ln[\mathrm{H}^+] = RT\ln 10^{-\mathrm{pH}} = -RT\ln(10)\mathrm{pH} \tag{6}$$

so that

$$\mu'_i = \mu_i + N_{\mathrm{H}i}RT\ln(10)\mathrm{pH} \tag{7}$$

which shows the effect of pH on the chemical potential of species i. The limitation to zero ionic strength is used here to make some of the following statistical mechanical equations simpler, but the effect of ionic strength can be taken into account and is taken into account later in making numerical calculations. The thermodynamic properties G, G', μ_i, and μ'_i, etc., are taken to be functions of the ionic strength so that it is not necessary to show activity coefficients explicitly.

The fundamental equation of thermodynamics for G' for a dilute aqueous solution containing a weak acid HA and its basic form A^- is [2]

$$dG' = -S'dT + VdP + \mu'_1 dn_1 + \mu'_2 dn_2 + n_c(H)RT\ln(10)dpH \quad (8)$$

where n_1 and n_2 are the amounts of HA and A^-. If HA and A^- are in equilibrium at the specified pH, they have the same transformed chemical potential μ'_i. These two species form a pseudoisomer group at a specified pH, and so Eq. (8) can be written

$$dG' = -S'dT + VdP + \mu'_{iso} dn'_{iso} + n_c(H)RT\ln(10)dpH \quad (9)$$

where $\mu'_{iso} = \mu'_1 = \mu'_2$ is the transformed chemical potential of the pseudoisomer group and the amount of the pseudoisomer group is given by $n'_{iso} = n_1 + n_2$. The criterion for spontaneous change and equilibrium is given by $dG' \leq 0$ at specified T, P, pH, and n'_{iso}. Eq. (9) can be integrated at constant values of the intensive variables T, P, and pH to obtain

$$G' = \mu'_{iso} n'_{iso} \quad (10)$$

Eq. (9) shows that the natural variables of G' for this system are T, P, n'_{iso}, and pH. They are referred to as natural variables because if G' can be determined as a function of these variables, all of the other thermodynamic properties of the system can be calculated by using the four partial derivatives of G' that are indicated by Eq. (9). In addition Eq. (9) provides six Maxwell relations and leads to a Gibbs–Duhem equation that shows that the properties T, P, μ'_{iso}, and pH are not independent. Thus μ'_{iso} is a function of T, P, and pH. This treatment of an aqueous solution containing a weak monoprotic acid can be extended to polyprotic acids, including proteins. However, when a protein such as cytochrome c is involved in an enzyme-catalyzed reaction, its effect on the pH dependence of the apparent equilibrium constant of the reaction is expected to arise primarily from acid groups in the protein that are linked to the reactive site [8].

In dealing with the fundamental equation of thermodynamics, it is customary to use the chemical potential μ_i and the transformed chemical potential μ'_i, but in making numerical calculations it is customary to use the Gibbs energy of formation $\Delta_f G$ and the transformed Gibbs energy of formation $\Delta_f G'$. It can be shown [9] that the standard transformed Gibbs energy of formation $\Delta_f G'^\circ_i$ of a species at 298.15 K can be calculated from the standard Gibbs energy $\Delta_f G^\circ_i$ of the species using

$$\Delta_f G'^\circ_i = \Delta_f G^\circ_i - N_{Hi}RT\ln 10^{-pH} - 2.91482(z_i^2 - N_{Hi})I^{1/2}/(1 + 1.6I^{1/2}) \quad (11)$$

where z_i is the charge number of the species, I is the ionic strength, and the 1.6 $l^{1/2}$ $mol^{-1/2}$ is the empirical constant in the extended Debye–Hückel equation [10].

When a biochemical reactant involves two or more species, as in the case HA and A^-, the standard transformed Gibbs energy of the pseudoisomer group is calculated using [11]

$$\Delta_f G'^\circ(\text{iso}) = -RT\ln\Sigma\exp[-\Delta_f G'^\circ_f/RT] \quad (12)$$

where the summation is a partition function. The equilibrium mole fraction r_i of the ith pseudoisomer in the pseudoisomer group is given by

$$r_i = \exp\left\{\left[\Delta_f G'^\circ(\text{iso}) - \Delta_f G'^\circ_i\right]/RT\right\} \quad (13)$$

which is a kind of Boltzmann distribution.

Eq. (12) for $\Delta_f G'^\circ$(iso) can also be written as [12]

$$\Delta_f G'^\circ(\text{iso}) = \Delta_f G'^\circ_i - RT\ln\left(1 + [H^+]/K_1 + [H^+]^2/K_1K_2 + \ldots\right) \quad (14)$$

where $\Delta_f G'^\circ_1$ is the standard transformed Gibbs energy of formation of the species with the smallest number of dissociable hydrogen atoms and K_1, K_2, … are the successive acid dissociation constants in the order of increasing size. This equation is needed for calculating $\Delta_f G'^\circ_1$, $\Delta_f G'^\circ_2$, … for the species in the pseudoisomer group from the experimental value of $\Delta_f G'^\circ$(iso).

When Mg^{2+} or other cations that are bound reversibly by reactants are present, their free

concentrations exert the same kind of effects on the apparent equilibrium constant and other thermodynamic properties as H^+. These effects can be taken into account in the same way as those of H^+.

2.2. Use of the semigrand partition function Γ'

The semigrand partition function Γ' for a system containing a weak acid and its basic form at a specified pH is given by [5,6]

$$\begin{aligned}\Gamma'(T,P,\mathrm{pH},N'_{\mathrm{iso}}) &= \{\exp(-\beta\mu_1)\exp \\ &\quad (-N_{\mathrm{H1}}\ln(10)\mathrm{pH}) \\ &\quad +\exp(-\beta\mu_2)\exp \\ &\quad (-N_{\mathrm{H2}}\ln(10)\mathrm{pH})\}^{N'_{\mathrm{iso}}} \\ &= \{\exp(-\beta\mu'_1) \\ &\quad +\exp(-\beta\mu'_2)\}^{N'_{\mathrm{iso}}} \end{aligned} \tag{15}$$

where μ_1 and μ_2 are the chemical potentials of species 1 and 2. N_{H1} and N_{H2} are the numbers of hydrogen atoms in these two species. N'_{iso} is the number of molecules in the pseudoisomer group. In statistical mechanics, it is customary to use numbers of molecules rather than amounts and to use $\beta = 1/kT$, where k is the Boltzmann constant. The same symbol is customarily used for the chemical potential as in thermodynamics. This partition function contains a weighted sum of $\exp(-\beta_i\mu_i)$, where $\exp(-N_{\mathrm{H}i}\ln(10)\mathrm{pH})$ is the weighting factor. The number of terms in this sum can be increased indefinitely. This summation is really a Laplace transform [13]. This form of the partition function applies at zero ionic strength, but the ionic strength effect can be taken into account by making the equation more complicated.

When Eq. (15) is substituted in $G' = -kT\ln\Gamma'$, we obtain

$$\begin{aligned}G' = -N'_{\mathrm{iso}}kT\ln\{&\exp(-\beta\mu_1)\exp \\ &(-N_{\mathrm{H1}}\ln(10)\mathrm{pH}) \\ &+\exp(-\beta\mu_2)\exp(N_{\mathrm{H2}}\ln(10)\mathrm{pH})\}\end{aligned} \tag{16}$$

This equation can be simplified by use of Eq. (7), which yields

$$\begin{aligned}G' &= -N'_{\mathrm{iso}}kT\ln\{\exp[-\beta\mu'_1] + \exp[-\beta\mu'_2]\} \\ &= N'_{\mathrm{iso}}\mu'_{\mathrm{iso}}\end{aligned} \tag{17}$$

which is equivalent to Eq. (10).

Since the semigrand partition function provides an expression for the transformed Gibbs energy of the system that is equivalent to the expression obtained in thermodynamics, the values of the dependent variables can be obtained by taking the partial derivatives of G' that are indicated by Eq. (9). Applying $(\partial G'/\partial N'_{\mathrm{iso}})$ at T, P, pH to Eq. (17) indicates that

$$\mu'_{\mathrm{iso}} = -kT\ln\{\exp[-\beta\mu'_1] + \exp[-\beta\mu'_2]\} \tag{18}$$

which can also be written

$$\mu'_{\mathrm{iso}} = \mu'^{\circ}_{\mathrm{iso}} + kT\ln[\mathrm{A}] \tag{19}$$

where $[\mathrm{A}] = [\mathrm{HA}] + [\mathrm{A}^-]$. Eq. (18) leads to

$$\mu'^{\circ}_{\mathrm{iso}} = -kT\ln\{\exp[-\beta\mu'^{\circ}_1] + \exp[-\beta\mu'^{\circ}_2]\} \tag{20}$$

This can be demonstrated by substituting $\mu'_1 = \mu'^{\circ}_1 + kT\ln[\mathrm{HA}]$ and $\mu'_2 = \mu'^{\circ}_2 + kT\ln[\mathrm{A}^-]$ in Eq. (18) and using Eq. (13).

Eq. (9) indicates that the other thermodynamic properties of the system can be obtained from [14]

$$S' = -\left(\frac{\partial G'}{\partial T}\right)_{P,\mathrm{pH},n'_{\mathrm{iso}}} = kT\left(\frac{\partial\ln\Gamma'}{\partial T}\right)_{P,\mathrm{pH},n'_{\mathrm{iso}}} \tag{21}$$

$$V = -\left(\frac{\partial G'}{\partial P}\right)_{T,\mathrm{pH},n'_{\mathrm{iso}}} = -kT\left(\frac{\partial\ln\Gamma'}{\partial P}\right)_{T,\mathrm{pH},n'_{\mathrm{iso}}} \tag{22}$$

$$\begin{aligned}n_{\mathrm{c}}(H)\ln(10) &= \frac{1}{kT}\left(\frac{\partial G'}{\partial\mathrm{pH}}\right)_{T,P,n'_{\mathrm{iso}}} \\ &= -\left(\frac{\partial\ln\Gamma'}{\partial\mathrm{pH}}\right)_{T,P,n'_{\mathrm{iso}}}\end{aligned} \tag{23}$$

This shows that the semigrand partition function Γ' contains all the thermodynamic information about the system. In the remainder of this paper the analogs of these equations will not be given, but the expressions for the various types of Gibbs energies will be derived from the two points of view.

3. Biochemical reaction at specified T, P, and pH

3.1. Use of the transformed Gibbs energy G'

The fundamental equation for G' for a dilute aqueous solution containing two pseudoisomer groups A and B is

$$\mathrm{d}G' = -S'\mathrm{d}T + V\mathrm{d}P + \mu'_{\mathrm{iso}\,A}\mathrm{d}n'_{\mathrm{iso}\,A} + \mu'_{\mathrm{iso}\,B}\mathrm{d}n'_{\mathrm{iso}\,B} + n_{\mathrm{c}}(H)RT\ln(10)\mathrm{dpH} \tag{24}$$

Integrating at constant values of the intensive properties yields

$$G' = \mu'_{\mathrm{iso}\,A}n'_{\mathrm{iso}\,A} + \mu'_{\mathrm{iso}\,B}n'_{\mathrm{iso}\,B} \tag{25}$$

But suppose these two pseudoisomer groups are involved in the following biochemical reaction

$$A = 2B \tag{26}$$

In this case there is a single independent variable: the extent of reaction ξ', which is defined by $n'_i = n_{i0} + \nu'_i\xi'$ where n'_{i0} is the initial amount of a pseudoismer group and ν'_i is its stoichiometric number. Now the fundamental equation for G' can be written

$$\begin{aligned}\mathrm{d}G' &= -S'\mathrm{d}T + V\mathrm{d}P + (2\mu'_{\mathrm{isoB}} - \mu'_{\mathrm{isoA}})\mathrm{d}\xi' + n_{\mathrm{c}}(\mathrm{H})RT\ln(10)\mathrm{dpH}\\ &= -S'\mathrm{d}T + V\mathrm{d}P + \Delta_{\mathrm{r}}G'\mathrm{d}\xi' + n_{\mathrm{c}}(\mathrm{H})RT\ln(10)\mathrm{dpH}\end{aligned} \tag{27}$$

where $\Delta_{\mathrm{r}}G'$ is the change in the transformed Gibbs energy in the reaction. This fundamental equation has some new partial derivatives and Maxwell equation that are not explored here.

The criterion for equilibrium ($\partial G'/\partial\xi' = 0$ at constant T, P, and pH) indicates that $2\mu'_{\mathrm{isoB}} = \mu_{\mathrm{isoA}}$ at equilibrium or

$$2(\Delta_{\mathrm{f}}G'^{\circ}_{\mathrm{B}} + RT\ln[\mathrm{B}]_{\mathrm{eq}}) = \Delta_{\mathrm{f}}G'^{\circ}_{\mathrm{A}} + RT\ln[\mathrm{A}]_{\mathrm{eq}} \tag{28}$$

and so the expression for the apparent equilibrium constant is

$$K' = [\mathrm{B}]^2_{\mathrm{eq}}/[\mathrm{A}]_{\mathrm{eq}} \tag{29}$$

This treatment can be extended to systems containing many enzyme-catalyzed reactions.

3.2. Use of the semigrand partition function Γ'

The semigrand partition function for a system containing two pseudoisomer groups is given by

$$\begin{aligned}\Gamma'(T,P,\mathrm{pH},N'_{\mathrm{isoA}},N'_{\mathrm{isoB}}) &= \{\exp(-\beta\mu'_{\mathrm{isoA}})\}^{N'_{\mathrm{isoA}}} \times \{\exp(-\beta\mu'_{\mathrm{isoB}})\}^{N'_{\mathrm{isoB}}}\end{aligned} \tag{30}$$

Substituting this in $G' = -kT\ln\Gamma'$ yields

$$\begin{aligned}G' &= -N'_{\mathrm{isoA}}kT\ln\left[\{\exp(-\beta\mu'_{\mathrm{isoA}})\}^{N'_{\mathrm{isoA}}} \times \{\exp(-\beta\mu'_{\mathrm{isoB}})\}^{N'_{\mathrm{isoB}}}\right]\\ &= N'_{\mathrm{isoA}}\mu'_{\mathrm{isoA}} + N'_{\mathrm{isoB}}\mu'_{\mathrm{isoB}}\end{aligned} \tag{31}$$

which is equivalent to Eq. (25). This treatment can be extended to multi-reaction systems.

4. System of biochemical reactions at specified T, P, pH, [ATP], and [ADP]

4.1. Use of the further transformed Gibbs energy G'' at specified [ATP] and [ADP]

In considering systems of biochemical reactions like glycolysis, it is important to seek ways to obtain a more global view of thermodynamic equilibrium because of the complexity of the reaction system. One way to do this is to study the

effects of the concentrations of coenzymes like ATP, ADP, NAD_{ox}, and NAD_{red} on the equilibria. Coenzymes are often in a steady state in a living system because they are produced and consumed by so many different reactions. When the concentrations of ATP and ADP are specified, the criterion for spontaneous change and equilibrium is stated in terms of the further transformed Gibbs energy G'' of the reaction system that is defined by the Legendre transform [3,4,15]

$$G'' = G' - n_c(\mathrm{ATP})\mu'(\mathrm{ATP}) - n_c(\mathrm{ADP})\mu'(\mathrm{ADP}) \quad (32)$$

where $n_c(\mathrm{ATP})$ is the amount of the ATP component and $\mu'(\mathrm{ATP})$ is the transformed chemical potential of ATP at the specified pH. This leads to the following equation for the further transformed chemical potential of reactant i:

$$\mu''_i = \mu'_i - N_{\mathrm{ATP}}(i)\mu'(\mathrm{ATP}) - N_{\mathrm{ADP}}(i)\mu'(\mathrm{ADP}) \quad (33)$$

where $N_{\mathrm{ATP}}(i)$ and $N_{\mathrm{ADP}}(i)$ are the numbers of ATP and ADP molecules required to make up the ith reactant. These numbers can be either positive or negative and are most easily determined for complex systems by use of matrix operations [15]. The fundamental equation for the further transformed Gibbs energy is given by

$$\mathrm{d}G'' = -S''\mathrm{d}T + V\mathrm{d}P + \sum\mu''_i\mathrm{d}n''_i + n_c(\mathrm{H})RT\ln(10)\mathrm{dpH} - n_c(\mathrm{ATP})RTd\ln[\mathrm{ATP}] - n_c(\mathrm{ADP})RTd\ln[\mathrm{ADP}] \quad (34)$$

where n''_i is the amount of a pseudoisomer groups after the specification of [ATP] and [ADP]. In the preceding article [6] this fundamental equation was used to treat the second and third steps in glycolysis, and so the summation consisted of a single term because these reactants are pseudoisomers when [ATP] and [ADP] are specified. In the current article the first five reactions in glycolysis are considered. They are

$$\mathrm{Glc} + \mathrm{ATP} = \mathrm{G6P} + \mathrm{ADP} \quad (35)$$

$$\mathrm{G6P} = \mathrm{F6P} \quad (36)$$

$$\mathrm{F6P} + \mathrm{ATP} = \mathrm{FBP} + \mathrm{ADP} \quad (37)$$

$$\mathrm{FBP} = \mathrm{GlycP} + \mathrm{GAP} \quad (38)$$

$$\mathrm{GAP} = \mathrm{GlycP} \quad (39)$$

where Glc is glucose, G6P is glucose 6-phosphate, F6P is fructose 6-phosphate, FBP is fructose 1,6-biphosphate, GAP is glyceraldehyde phosphate, and GlycP is glycerone phosphate. When [ATP] and [ADP] are specified, the system consists of two pseudoisomer groups, and this series of reactions is effectively reduced to

$$\mathrm{C_6} = 2\mathrm{C_3} \quad (40)$$

where $\mathrm{C_6}$ is the sum of Glc, G6P, F6P, and FBP, and $\mathrm{C_3}$ is the sum of GlycP and GAP. The fundamental equation for G'' for this system when there is equilibrium within the pseudoisomer groups is

$$\mathrm{d}G'' = -S''\mathrm{d}T + V\mathrm{d}P + \mu''_6\mathrm{d}n''_6 + \mu''_3\mathrm{d}n''_3 + n_c(\mathrm{H})RT\ln(10)\mathrm{dpH} - n_c(\mathrm{ATP})RT\mathrm{dln}[\mathrm{ATP}] - n_c(\mathrm{ADP})RT\mathrm{dln}[\mathrm{ADP}] \quad (41)$$

When the extent of Reaction 40 is introduced, this equation becomes

$$\mathrm{d}G'' = -S''\mathrm{d}T + V\mathrm{d}P + \Delta_r G''\mathrm{d}\xi'' + n_c(\mathrm{H})RT\ln(10)\mathrm{dpH} - n_c(\mathrm{ATP})RT\mathrm{dln}[\mathrm{ATP}] - n_c(\mathrm{ADP})RT\mathrm{dln}[\mathrm{ADP}] \quad (42)$$

where $\Delta_r G'' = 2\mu''_3 - \mu''_6$. This fundamental equation has some interesting Maxwell equations that are not discussed here.

At constant values of the intensive variables, Eq. (41) can be integrated to obtain the following expression for the further transformed Gibbs energy of the system:

$$G'' = \mu''_6 n''_6 + \mu''_3 n''_3 \quad (43)$$

4.2. Use of the semigrand partition function Γ''

The thermodynamic treatment in the preceding section of a system involving Reactions 35–39 corresponds with the use of a semigrand partition function Γ'' at specified concentrations of ATP and ADP.

$$\begin{aligned}\Gamma''(T,P,\mathrm{pH},[\mathrm{ATP}],[\mathrm{ADP}],N''_6,N''_3) &= \{\exp(-\beta\mu''(\mathrm{Glc})) \\ &+ \exp(-\beta\mu''(\mathrm{G6P})) \\ &+ \exp(-\beta\mu''(\mathrm{F6P})) \\ &+ \exp(-\beta\mu''(\mathrm{FBP}))\}^{N''_6}\{\exp \\ &(-\beta\mu''(\mathrm{GlyP})) + \exp \\ &(-\beta\mu''(\mathrm{GAP}))\}^{N''_3} \quad (44)\end{aligned}$$

This can be written as

$$\begin{aligned}\Gamma''(T,P,\mathrm{pH},[\mathrm{ATP}],[\mathrm{ADP}],N''_6,N''_3) &= \{\exp(-\beta\mu''_6)\}^{N''_6}\{\exp(-\beta\mu''_3)\}^{N''_3} \quad (45)\end{aligned}$$

The further transformed Gibbs energy is given by $G'' = -kT\ln\Gamma''$:

$$G'' = -kT\ln\left[\{\exp(-\beta\mu''_6)\}^{N''_6} \times \{\exp(-\beta\mu''_3)\}^{N''_3}\right] = \mu''_6 N''_6 + \mu''_3 N''_3 \quad (46)$$

which is equivalent to Eq. (43).

5. Calculation of the equilibrium composition for the first five reactions of glycolysis at specified concentrations of ATP and ADP

5.1. Calculation of $\Delta_f G'^\circ$ values for all of the reactants

To calculate the equilibrium composition for the first five reactions of glycolysis at 298.15 K, pH 7, ionic strength 0.25 M, and specified concentrations of ATP and ADP, the first requirement is the values of $\Delta_f G'^\circ$ of the eight reactants at 298.15 K, pH 7, and ionic strength 0.25 M. The values for Glc, G6P, and F6P have already been published [16]. The standard Gibbs energies of formation of the species of FBP can be calculated from the experimental apparent equilibrium constant for the reaction

$$\mathrm{FBP} + \mathrm{H_2O} = \mathrm{F6P} + \mathrm{P}_i \quad (47)$$

that was determined by Lawson et al. [17], who obtained $K' = 174$ at pH 6.99, $I = 0.25$ M, and pMg 3.05 at 311.15 K. This experimental value of K' makes it possible to calculate $\Delta_f G'^\circ$ of FBP under the desired conditions using $\Delta_r G'^\circ = \Sigma\nu'_i\Delta_f G'^\circ_i$ because the $\Delta_f G'^\circ$ of the other three reactants are known [16]. This calculation is based on the assumption that the effect of temperature and the presence of magnesium ions are negligible. $\Delta_f G'^\circ$ (FBP^{4-}) can be calculated using Eq. (14) when K_1 and K_2 are known. Since these values are not known, it was necessary to estimate the pK values of FBP, and this was done in the following way: The pK values of G6P and F6P at 298.15 K and zero ionic strength are 6.27 and 6.42. Therefore, it was assumed that the phosphate groups in FBP are identical and independent and have an intrinsic pK of 6.35. This makes it possible to calculate K_1 and K_2 because $K_1 = K/2$ and $K_2 = 2K$. Adjustments for ionic strength are made with the extended Debye–Hückel equation [10]. The $\Delta_f G^\circ$ values for the three species of FBP are given in Table 1.

The apparent equilibrium constants for Reac-

Table 1
Basic thermodynamic properties of species at 298.15 K and zero ionic strength

Species	$\Delta_f G'^\circ$ (kJ mol^{-1})	z_i	$N_H(i)$
FBP^{4-}	−2601.40	−4	10
HFBP^{3-}	−2639.36	−3	11
$\mathrm{H_2FBP}^{2-}$	−2673.89	−2	12
GAP^{2-}	−1288.60	−2	5
HGAP^{-}	−1321.14	−1	6
GlycP^{2-}	−1296.26	−2	5
HGlycP^{-}	−1328.80	−1	6

Table 2
Standard transformed Gibbs energies of formation at 298.15 K, pH 7, and 0.25 M ionic strength, standard further transformed Gibbs energies of formation at $[ATP] = 10^{-4}$ M and $[ADP] = 10^{-2}$ M, and standard further transformed Gibbs energies of formation at $[ATP] = 10^{-2}$ M and $[ADP] = 10^{-2}$ M

	$\Delta_f G'^\circ$ (kJ mol^{-1})	$\Delta_f G''^\circ$ (kJ mol^{-1})	$\Delta_f G''^\circ$ (kJ mol^{-1})
Glc	−426.71	−426.71	−416.71
G6P	−1318.92	−439.74	−451.16
F6P	−1315.74	−436.56	−447.97
FBP	−2206.78	−448.42	−471.25
GAP	−1088.04	−208.86	−220.28
GlycP	−1095.70	−216.52	−227.94
ATP	−2097.89		
ADP	−1230.12		

tions 38 and 39 have been determined by Veech et al. [18] at 311.15 K, pH 7, and 0.25 M ionic strength; these apparent equilibrium constants are $K' = 9.9 \times 10^{-5}$ and $K' = 22$, respectively. This yields two linear equations for $\Delta_f G'^\circ$(GAP) and $\Delta_f G'^\circ$(GlycP) at pH 7 and 0.25 M ionic strength. These values have been used to calculate the values for the species of GAP and GlycP given in Table 2 on the assumption that the acid dissociation constants of GAP and GlycP are the same as for F6P.

Since these calculations are complicated, it is really necessary to use a personal computer with a mathematical application, and Mathematica® [19] has been found to be very convenient for this purpose. Data for 116 reactants like that given in Table 1 has been put on the web [20].

5.2. Calculation of the further transformed Gibbs energies at specified [ATP] and [ADP] and the equilibrium composition for the $C_6 = 2C_3$ reaction

The standard transformed Gibbs energies of formation of the eight reactants involved in Reactions 35–39 at 298.15 K, pH 7, and ionic strength 0.25 M calculated using Eqs. (11) and (12) are given in the first column of Table 2.

The third column of Table 2 gives the further transformed Gibbs energies of formation of the first six reactants at $[ATP] = 10^{-4}$ M and $[ADP] = 10^{-2}$ M. The fourth column gives the values at $[ATP] = 10^{-2}$ M and $[ADP] = 10^{-2}$ M. These values were calculated using Eq. (33). There is no adjustment for glucose, and the adjustment for G6P is given by

$$\Delta_f G''^\circ(\mathrm{G6P}) = \Delta_f G'^\circ(\mathrm{G6P}) - \{\Delta_f G'^\circ(\mathrm{ATP}) + RT\ln[\mathrm{ATP}]\} + \{\Delta_f G'^\circ(\mathrm{ADP}) + RT\ln[\mathrm{ADP}]\} \quad (48)$$

The same adjustment is applied to the other reactants, except for FBP where the adjustment terms are both multiplied by a factor of 2 because it contains two phosphate groups.

As mentioned earlier, when [ATP] and [ADP] are specified, the four reactants with six carbons become pseudoisomers and the two reactants with three carbons become pseudoisomers. The values of $\Delta_f G''^\circ(C_6)$ and $\Delta_f G''^\circ(C_3)$ are calculated with an equation like Eq. (12). The values of these standard further transformed Gibbs energies of formation of pseudoisomer groups are given in Table 3 along with the apparent equilibrium constant K'' and the equilibrium extent of reaction for an initial concentration of glucose of 0.01 M.

The equilibrium extent of reaction ξ'' is readily calculated by use of the quadratic formula, and this leads to the equilibrium concentrations of C_6 and C_3. The equilibrium mole fractions within the two pseudoisomer groups are readily calculated

Table 3
Standard further transformed Gibbs energies of formation of C_6 and C_3 at pH 7 ionic strength 0.25 M for different specified concentrations of ATP

	$[ATP] = 10^{-4}$ M $[ADP] = 10^{-2}$ M	$[ATP] = 10^{-2}$ M $[ADP] = 10^{-2}$ M
$\Delta_f G''^\circ(C_6)$ (kJ mol^{-1})	−448.51	−471.25
$\Delta_f G''^\circ(C_3)$ (kJ mol^{-1})	−216.63	−228.05
$\Delta_r G''^\circ$(rx 37)/kJ mol^{-1} (kJ mol^{-1})	15.25	15.15
K''	0.00213	0.00221
ξ''	0.00205	0.00209

Table 4
Equilibrium compositions for the first five reactions of glycolysis at 298.15 K, pH 7, ionic strength 0.25 M and specified concentrations of ATP and ADP

	$[ATP] = 10^{-4}$ M $[ADP] = 10^{-2}$ M	$[ATP] = 10^{-2}$ M $[ADP] = 10^{-2}$ M
[Glc]/M	1.20×10^{-6}	1.24×10^{-10}
[G6P]/M	2.31×10^{-4}	2.39×10^{-6}
[F6P]/M	6.39×10^{-5}	6.61×10^{-7}
[FBP]/M	7.64×10^{-3}	7.90×10^{-3}
[GAP]M	1.79×10^{-4}	1.82×10^{-4}
[GlycP]M	3.94×10^{-3}	4.00×10^{-3}

using Eq. (13). The equilibrium compositions for the first five reactions in glycolysis at specified [ATP] and [ADP] are given in Table 4.

These equilibrium concentrations have been tested in two ways: (1) carbon is conserved; and (2) these concentrations yield correct values for the five apparent equilibrium constants. It is perhaps surprising that raising the concentration of ATP by a factor of 100 makes so little difference, but of course it does make a big difference for the first three reactants. The concentration of fructose 1,6-biphosphate cannot increase very much because it already dominates, and that limits the effects on GAP and GlycP. These calculations can be applied to larger systems and can include the specification of the concentrations of other coenzymes like NAD_{ox} and NAD_{red}.

6. Discussion

The use of semigrand partition functions is a way to emphasize the importance of specifying intensive variables, such as the pH and concentrations of coenzymes like ATP and ADP in studying the thermodynamics of systems of enzyme-catalyzed reactions. Callen [7] has pointed out that 'The choice of variables in terms of which a given problem is formulated, while a seemingly innocuous step, is often the most critical step in the solution'. There is just one way to introduce intensive variables, and that is to use a Legendre transform. There is no loss in information when a new thermodynamic potential is defined by use of a Legendre transform, and the fundamental equation of thermodynamics for the new thermodynamic potential shows how all the other thermodynamic properties of the system can be calculated. Enzyme-catalyzed reactions can be discussed in terms of species, but when this is done the concentration of hydrogen ions has to be calculated from the equilibrium constants and the constraint equations that apply to the system. From the point of view of a semigrand partition function, a system can be considered to be in contact with a large reservoir of hydrogen ions at a specified pH. The semigrand partition function Γ' contains all the thermodynamic information on the system, and the transformed Gibbs energy G' of the system can be calculated using $G' = -kT\ln\Gamma'$. The fundamental equation for G' shows how all the other thermodynamic properties of the system can be calculated; they can be calculated from G' or Γ'.

In this paper this process has been carried a step further in considering that the system can be considered to be in contact with a large reservoir of ATP at a specified concentration and a large reservoir of ADP at a specified concentration. The semigrand partition function Γ'' contains all the thermodynamic information on the system, and the further transformed Gibbs energy G'' of the system can be calculated using $G'' = -kT\ln\Gamma''$. The fundamental equation for G'' shows how all the other thermodynamic properties of the system can be calculated.

These methods can be extended to larger systems and to specifying more independent variables like $[NAD_{ox}]$ and $[NAD_{red}]$. However, there is a maximum number of intensive variables that can be specified because one component must remain. In this treatment of part of glycolysis, the remaining component is carbon. The complete Legendre transform for a system yields the Gibbs–Duhem equation, which shows that the intensive variables for a system are not all independent. There are many mathematical relations between the thermodynamic properties that are not explored here. Since the calculations are complicated, it is really necessary to use a personal computer with a mathematical application. Some

programs in Mathematica® [19] are available on the web [20].

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