Inverse Legendre Transform in Biochemical Thermodynamics: Illustrated with the Last Five Reactions of Glycolysis

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Legendre transforms are needed to introduce intensive variables into the description of thermodynamic systems. But when experimental thermodynamic data involve an inconveniently large number of intensive variables, inverse Legendre transforms are needed to reduce the number of independent intensive variables. This is illustrated by the elimination of pH in the calculation of standard Gibbs energies of formation of species from apparent equilibrium constants of enzyme-catalyzed reactions. Apparent equilibrium constants have been determined for about 500 enzyme-catalyzed reactions involving about 1000 different reactants at various pHs and ionic strengths. However, these apparent equilibrium constants cannot be compared directly with each other because of differences in pH and ionic strength. More than one pathway can be used to calculate standard Gibbs energies of formation of the species involved. This discussion of inverse Legendre transforms provides guidance on how to write computer programs to eliminate pH as an independent variable and obtain standard Gibbs energies of formation of the species involved. This is illustrated by calculation of standard Gibbs energies of formation of species of phospho*enol*pyruvate, 2-phospho-D-glycerate, 3-phospho-D-glycerate, and 3-phospho-D-glyceroyl phosphate at zero ionic strength and 298.15 K. These calculations show that the reactions in a series such as glycolysis do not necessarily provide the best route for the calculation of standard transformed Gibbs energies of reaction, even though values of apparent equilibrium constants of these reactions have been reported.

There are two ways to make quantitative calculations on the thermodynamics of biochemical reactions in dilute aqueous solutions: (1) The reactions can be discussed in terms of species so that the thermodynamic properties are related to the Gibbs energy G, the enthalpy H, the entropy S, and the heat capacity at constant pressure C_P. (2) The reactions can be discussed in terms of reactants, that is sums of species such as ATP, at a specified pH so that the thermodynamic properties are related to the transformed Gibbs energy G', transformed enthalpy H', transformed entropy S', and transformed heat capacity C_P'. Both approaches have their uses. The advantage of the second approach is that a more global view is obtained. Therefore, it is used in discussing metabolism. The two approaches are related by the Legendre transform[1−3]

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

where $n_c(\mathrm{H})$ is the amount of the hydrogen compoment (total amount of hydrogen atoms) in the system and $\mu(\mathrm{H}^+)$ is the chemical potential of hydrogen ions that corresponds with the specified pH. In dilute aqueous solutions thermodynamic properties can be taken to be functions of the ionic strength so that equations can be written in terms of concentrations of species or reactants. Therefore, G', G, and $\mu(\mathrm{H}^+)$ are taken to be functions of the ionic strength. The effects of ionic interactions are usually calculated using the extended Debye−Hückel equation, but more complicated equations can be used if their coefficients are known. Equations and computer programs have been developed to calculate the transformed thermodynamic properties of reactants (like ATP) at a specified pH and ionic strength from those of species.[4,5]

Legendre transforms are needed to introduce pH and other intensive variables into the description of thermodynamic systems. But when experimental thermodynamic data involve an inconveniently large number of intensive variables, inverse Legendre transforms are needed to reduce the number of independent intensive variables. This is illustrated by the elimination of pH in the calculation of standard Gibbs energies of formation of species from apparent equilibrium constants of enzyme-catalyzed reactions at specified pH and ionic strength.

1. Fundamental Equation of Thermodynamics for the Transformed Gibbs Energy at Specified pH

The apparent equilibrium constant K' written in terms of concentrations of reactants (sums of species), with H^+ not treated as a reactant, and the transformed enthalpy of reaction $\Delta_r H'$ can be determined for an enzyme-catalyzed reaction at a series of pHs, ionic strengths, and temperatures. The treatment of these thermodynamic data is based on the fundamental equation[2,3] for the transformed Gibbs energy G':

$$\mathrm{d}G' = -S'\,\mathrm{d}T + V\,\mathrm{d}P + \sum_{i=1}^{N'} \mu_i'\,\mathrm{d}n_i' + n_c(\mathrm{H})RT\ln(10)\,\mathrm{dpH} \tag{2}$$

where N' is the number of reactants, μ_i' is the transformed chemical potential of reactant i, n_i' is the amount of reactant i, and $n_c(\mathrm{H})$ is the amount of the hydrogen component in the system, that is the total amount of hydrogen atoms. S' is the transformed entropy of the system:

$$S' = S - n_c(\mathrm{H})\,S_m(\mathrm{H}^+) \tag{3}$$

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where $S_m(H^+)$ is the molar entropy of the hydrogen ion at the specified pH and ionic strength. An apparent equilibrium constant K' can be used to calculate the standard transformed Gibbs energy of reaction:

$$\Delta_r G'^\circ = -RT \ln K' \tag{4}$$

The standard transformed Gibbs energies of formation of reactants $\Delta_f G_i'^\circ$ can be calculated by using

$$\Delta_r G'^\circ = \sum_{i=1}^{N'} \nu_i' \Delta_f G_i'^\circ \tag{5}$$

The stoichiometric numbers ν_i' of the reactants have a prime to distinguish them from the stoichiometric numbers of the underlying chemical reactions. Similarly, standard transformed enthalpies of formation $\Delta_f H_i'^\circ$ of the reactants can be calculated from the standard transformed enthalpy of reaction by use of

$$\Delta_r H'^\circ = \sum_{i=1}^{N'} \nu_i' \Delta_f H_i'^\circ \tag{6}$$

Thermodynamic properties such as K', $\Delta_r G'^\circ$, $\Delta_f G_i'^\circ$, $\Delta_r H'^\circ$, and $\Delta_f H_i'^\circ$ are taken to be functions of ionic strength as well as temperature and pH.

The Maxwell equation between the first term in the summation in eq 2 and the term in dpH is

$$\left(\frac{\partial \mu_1'}{\partial \mathrm{pH}}\right)_{T,P,\{n_i'\}} = RT \ln(10) \left(\frac{\partial n_c(\mathrm{H})}{\partial n_1'}\right)_{T,P,\mathrm{pH},\{n_i' \neq 1\}} \tag{7}$$

where $\{n_i'\}$ is the set of amounts of reactants. Equation 7 can be interpreted as[6]

$$\bar{N}_{H1} = \frac{1}{RT \ln(10)} \left(\frac{\partial \mu_1'}{\partial \mathrm{pH}}\right)_{T,P,\{n_i'\}} = \frac{1}{RT \ln(10)} \left(\frac{\partial \Delta_f G_1'^\circ}{\partial \mathrm{pH}}\right)_{T,P,\{n_i'\}} \tag{8}$$

where $\bar{N}_{H1}$ is the average number of hydrogen atoms bound by a molecule of reactant 1. When the Gibbs energy of reaction $\Delta_r G'^\circ$ is used in eq 8 rather than $\Delta_f G_i'^\circ$, this relation gives the change in binding of hydrogen ions $\Delta_r N_H$ in the reaction. This type of equation traces back to Wyman.[7] There are other Maxwell equations and a Gibbs−Duhem equation of eq 2 that are not discussed here. Integration of eq 2 at constant T, P, and pH yields

$$G' = \sum_{i=1}^{N'} n_i' \mu_i' \tag{9}$$

and so the transformed Gibbs energy of the system is additive in the transformed chemical potentials of the reactants at specified T, P, pH, and ionic strength.

Equation 2 provides a complete thermodynamic description of the reaction system in terms of reactants, but now we want to divide the various properties of reactants into contributions by species.

2. Dividing Properties of Reactants into Properties of Species

It is important to be able to do this because there is a lot of experimental data on K' and $\Delta_r H'^\circ$ of enzyme-catalyzed reactions, but these properties have been determined at various pHs and ionic strengths. To calculate K' and $\Delta_r H'^\circ$ at various pHs and ionic strengths, it is necessary to know $\Delta_f G_j^\circ(I=0)$ and $\Delta_f H_j^\circ(I=0)$ for all the species involved in a reaction. These values can be obtained by calculating $\Delta_r G^\circ(I=0)$ and $\Delta_r H^\circ(I=0)$ for a reference chemical reaction.[4] However, this paper is based on the use of an inverse Legendre transform. Callen[8] discussed the Legendre transform to go from a function of (X,Y) to a function of (P,ϕ) and pointed out that "the relationship between (X,Y) and (P,ϕ) is symmetrical with its inverse except for a change in sign in the equation for the Legendre transform." The inverse Legendre transform used here is the definition of the Gibbs energy G in terms of the transformed Gibbs energy G'.

$$G = G' + n_c(\mathrm{H})\, \mu(\mathrm{H}^+) \tag{10}$$

The following derivation illustrates the fact that no thermodynamic information is lost in making a Legendre transform. This derivation provides guidance in writing computer programs to calculate standard Gibbs energies of formation and standard enthalpies of formation of species in dilute aqueous solutions from K' and $\Delta_r H'$ values for enzyme-catalyzed reactions. Enzyme-catalyzed reactions provide the best way to obtain this information because these reactions are usually clean cut and fast enough for accurate measurements.

The first step is to see how S' and n_i' can be divided into contributions of species. The partial derivative of the transformed Gibbs energy with respect to temperature is equal to $-S'$, and so eq 3 shows that

$$\left(\frac{\partial G'}{\partial T}\right)_{P,\{n_i'\},\mathrm{pH}} = -S' = -S - n_c(\mathrm{H}) \left(\frac{\partial \mu(\mathrm{H}^+)}{\partial T}\right)_{P,\{n_i'\},\mathrm{pH}} \tag{11}$$

Thus the fundamental equation for the transformed Gibbs energy (eq 2) can be written

$$\begin{aligned} \mathrm{d}G' &= -S\,\mathrm{d}T + V\,\mathrm{d}P + \sum_{i=1}^{N'} \mu_i'\,\mathrm{d}n_i' - \\ &\qquad n_c(\mathrm{H}) \left(\left(\frac{\partial \mu(\mathrm{H}^+)}{\partial T}\right)_{P,\{n_i'\},\mathrm{pH}} \mathrm{d}T - RT \ln(10)\,\mathrm{dpH} \right) \\ &= -S\,\mathrm{d}T + V\,\mathrm{d}P + \sum_{i=1}^{N'} \mu_i'\,\mathrm{d}n_i' - n_c(\mathrm{H})\,\mathrm{d}\mu(\mathrm{H}^+) \end{aligned} \tag{12}$$

where the term in parentheses is the total differential of the chemical potential of hydrogen ions.

The summation in eq 12 can be written in terms of species exclusive of the hydrogen ion because when species in a pseudoisomer group are in equilibrium at a specified pH, these species have the same transformed chemical potential:

$$\mathrm{d}G' = -S\,\mathrm{d}T + V\,\mathrm{d}P + \sum_{j=1}^{N_s-1} \mu_i'\,\mathrm{d}n_j - n_c(\mathrm{H})\,\mathrm{d}\mu(\mathrm{H}^+) \tag{13}$$

where N_s is the number of different species.

Now the inverse Legendre transform given in eq 10 is needed. The differential of the Gibbs energy is given by

$$\mathrm{d}G = \mathrm{d}G' + n_c(\mathrm{H})\,\mathrm{d}\mu(\mathrm{H}^+) + \mu(\mathrm{H}^+)\,\mathrm{d}n_c(\mathrm{H}) \tag{14}$$

Substituting eq 13 into this equation yields

$$dG = -S\,dT + V\,dP + \sum_{j=1}^{N_s-1} \mu_j'\,dn_j + \mu(H^+)\,dn_c(H) \quad (15)$$

The amount of the hydrogen component $n_c(H)$ in the system is given by

$$n_c(H) = \sum_{j=1}^{N_s} N_H(j) n_j \quad (16)$$

where $N_H(j)$ is the number of hydrogen atoms in species j. Substituting this equation in eq 15 yields

$$dG = -S\,dT + V\,dP + \sum_{j=1}^{N_s} \{\mu_j' + N_H(j)\,\mu(H^+)\}\,dn_j \quad (17)$$

The term in braces is the chemical potential of ion j:

$$\mu_j = \mu_j' + N_H(j)\,\mu(H^+) \quad (18)$$

and so

$$dG = -S\,dT + V\,dP + \sum_{j=1}^{N_s} \mu_j\,dn_j \quad (19)$$

This is the fundamental equation that describes the reaction system in terms of species. Integration of this equation at specified T and P yields

$$G = \sum_{j=1}^{N_s} \mu_j n_j \quad (20)$$

Thus the Gibbs energy of the system is additive in the chemical potentials of the species, as expected.

3. Calculations of Standard Thermodynamic Properties of Species from Apparent Equilibrium Constants of Enzyme-Catalyzed Reactions

If the apparent equilibrium constant K' has been determined at 298.15 K for an enzyme-catalyzed reaction for which $\Delta_f G'^\circ$ values can be calculated from known functions of pH and ionic strength at the experimental pH and ionic strength for all the reactants but one, the $\Delta_f G'^\circ$ of the reactant under these conditions can be calculated using

$$-RT \ln K' = \sum_{i=1}^{N'} \nu_i' \Delta_f G_i'^\circ \quad (21)$$

So far functions of pH and ionic strength that yield $\Delta_f G'^\circ$ have been published for 119 reactants[5] at 298.15 K.

If the reactant of interest consists of a single species, $\Delta_f G^\circ(I=0)$ for this species at 298.15 K can be calculated using eq 18 in the form[9]

$$\Delta_f G_j^\circ(I=0) = \Delta_f G'^\circ(\mathrm{pH},I) + N_H(j)\,RT \ln(10)\mathrm{pH} + 2.91482(z_j^2 - N_H(j))I^{1/2}/(1 + 1.6I^{1/2}) \quad (22)$$

where z_i is the charge number and the extended Debye−Hückel equation has been used. The pH in this and other calculations is defined[10] by $\mathrm{pH} = -\log[H^+]$, rather than $\mathrm{pH_a} = -\log\{a(H^+)\}$, where $a(H^+)$ is the activity of hydrogen ions. This is done so that molar concentrations can be used for all species.

If a reactant consists of two species with different numbers of hydrogen atoms, the pK of the weak acid is needed to calculate $\Delta_f G'^\circ(I=0)$ of the two species, and the calculation is more complicated. The standard transformed Gibbs energy of formation of a pseudoisomer group containing two species is given by

$$\Delta_f G'^\circ = -RT \ln\{\exp(-\Delta_f G_1'^\circ/RT) + \exp(-\Delta_f G_2'^\circ/RT)\} \quad (23)$$

The value of $\Delta_f G_1'^\circ$ at the experimental pH and ionic strength can be calculated using[10]

$$\Delta_f G'^\circ = \Delta_f G_1'^\circ - RT \ln(1 + 10^{\mathrm{p}K_1 - \mathrm{pH}}) \quad (24)$$

where pK_1 is the value at the experimental ionic strength. The relation between p$K_1(I)$ and p$K_1(I=0)$ for a weak acid at 298.15 K is given by

$$\mathrm{p}K_1(I) = \mathrm{p}K_1(I=0) + 0.510651(\Sigma \nu_i z_i^2)I^{1/2}/(1 + 1.6I^{1/2}) \quad (25)$$

This value of p$K_1(I)$ has to be used in eq 24 to obtain $\Delta_f G_1'^\circ(I)$.

The next step is to adjust $\Delta_f G_1^\circ(I)$ to $\Delta_f G_1^\circ(I=0)$ using eq 22. After $\Delta_f G_1^\circ(I=0)$ has been calculated, $\Delta_f G_2^\circ(I=0)$ can be calculated using

$$\Delta_f G_2^\circ(I=0) = \Delta_f G_1^\circ(I=0) - RT \ln(10)\mathrm{p}K_1(I=0) \quad (26)$$

In the database BasicBiochemData[5] the data on species of a reactant is in the form

$$\mathrm{namesp} = \{\{\Delta_f G_1^\circ(I=0), \Delta_f H_1^\circ(I=0), z_1, N_{H1}\}, \{\Delta_f G_2^\circ(I=0), \Delta_f H_2^\circ(I=0), z_2, N_{H2}\}, \ldots\} \quad (27)$$

A *Mathematica* program calcGef2sp, which is given in the Appendix, has been written to produce the output in the form of eq 27 for a reactant made of two species. This output can be added to the database in BasicBiochemData and can be used to calculate $\Delta_f G'^\circ$ of the reactant at 298.15 K, pH 5−9, and ionic strengths 0−0.35 M.

When the reactant consists of three species with different numbers of hydrogen atoms, eq 24 becomes

$$\Delta_f G'^\circ = \Delta_f G_1'^\circ - RT \ln(1 + 10^{\mathrm{p}K_1 - \mathrm{pH}} + 10^{\mathrm{p}K_1 + \mathrm{p}K_2 - 2\mathrm{pH}}) \quad (28)$$

$\Delta_f G_1'^\circ(I=0)$ can then be calculated by using eq 22. Equation 26 can be used to calculate $\Delta_f G_2'^\circ(I=0)$, and $\Delta_f G_3'^\circ(I=0)$ can be calculated using

$$\Delta_f G_3^\circ(I=0) = \Delta_f G_2^\circ(I=0) - RT \ln(10)\mathrm{p}K_2(I=0) \quad (29)$$

A *Mathematica* program calcGef3sp, which is given in the Appendix, has been written to produce output in the form of eq 27. This output can be added to the database in BasicBiochemData.

The species matrix for a reactant can be verified by use of the programs calcdGmat and calckprime, which are also given in the Appendix. The program calcdGmat yields the function of pH and ionic strength for $\Delta_r G'^\circ$ of the reactant. The program calckprime can then be used to calculate K' for the reaction used at the experimental pH and ionic strength. This procedure was used to verify the species matrices reported here and to construct tables later in this paper. It is evident from these programs that it is easier to go from a species matrix for a

TABLE 1: Properties of the Most Basic Species of the Four Reactants

reactant	z_j	$N_H(j)$	pK(298.15 K,I=0)
PEP	−3	2	7.00 (ref 18)
PG2	−3	4	7.64 (ref 19)
PG3	−3	4	7.53 (ref 20)
BPG	−4	4	7.96, 7.04 (ref 21)

reactant to its contribution to K' than to go from K' to the species matrix of a reactant.

4. Calculation of $\Delta_f G^\circ$ ($I = 0$) for Species in the Last Five Reactions of Glycolysis

Glycolysis consists of 10 enzyme-catalyzed reactions involving 16 reactants, and the standard Gibbs energies of formation of all the species involved in 12 of these reactants are included in BasicBiochemData.[5] The standard Gibbs energies of formation of the species of phospho*enol*phosphate, 3-phospho-D-glycerate, 2-phospho-D-glycerate, amd 3-phospho-D-glyceroyl phosphate have not been calculated previously. These reactants are involved in the last five reactions in glycolysis that are

EC 1.2.1.12 $$\mathrm{GAP + P_i + NAD_{ox} = BPG + NAD_{red}} \quad (30)$$

EC 2.7.2.3 $$\mathrm{BPG + ADP = PG3 + ATP} \quad (31)$$

EC 5.4.2.1 $$\mathrm{PG3 = PG2} \quad (32)$$

EC 4.2.1.11 $$\mathrm{GP2 = PEP + H_2O} \quad (33)$$

EC 2.7.1.40 $$\mathrm{PEP + ADP = pyruvate + ATP} \quad (34)$$

where GAP is D-glyceraldehyde 3-phosphate, BPG is 1,3-bisphosphoglycerate (3-phospho-D-glyceroyl phosphate), PG3 is 3-phospho-D-glycerate, PG2 is 2-phospho-D-glycerate, and PEP is phospho*enol*pyruvate. EC numbers[11] are given, and these reactions will be referred to by EC number in the discussion of experimental data. The apparent equilibrium constant has also been measured for the following reaction involving PEP.

EC 4.1.1.32

$$\mathrm{ATP + oxaloacetate + H_2O = ADP + PEP + CO_2tot} \quad (35)$$

CO_2tot is the sum of the species of carbon dioxide in aqueous solution.

The critical compilations of Goldberg and Tewari[12−17] of apparent equilibrium constants and transformed enthalpies of reaction of enzyme-catalyzed reactions have been very helpful in identifying the best literature values for these reactions. Other properties of the most basic species of the four reactants that are needed for calculations are given in Table 1.

The pK's of the first three reactants are available in the literature, but the two pK's of BPG had to be estimated from measurements on 1,3-diphosphoglycerate and 2-phospho-D-glycerate. More measurements of pK's of biochemical reactants are needed. Note that the pHs in this section are $-\log[H^+]$ and that they have been calculated[10] from pH_a using the extended Debye−Hückel equation.

Species of Phospho*enol*pyruvate. The apparent equilibrium constant for reaction EC 2.7.1.40 has been measured by Krimsky,[22] who obtained $K' = 2.0 \times 10^3$ at pH 7.63 at $I =$ 0.07 M, and by McQuate and Utter,[23] who obtained 6.5×10^3 at pH 7.32 and $I = 0.05$ M. However, there is a serious question as to the reliability of these values because of the analytical difficulties in determining K' values this large. Fortunately, the value of K' for reaction EC 4.1.1.32 has been measured by Wood, Davis, and Lochmüller,[24] who obtained 0.50 at pH 6.95 and $I = 0.25$ M. They actually used ITP and IDP, but there is no reason to believe that ATP and ADP would have yielded different results. Use of the program calcGef2sp yields the following data matrix for PEP.

$$\mathrm{pepsp} = \{\{-1263.65,\_,-3,2\},\{-1303.61,\_,-2,3\}\} \quad (36)$$

These values can be verified by using pepsp to calculate K' for reaction EC 4.1.1.32 at the experimental conditions. Equation 36 can also be used to calculate the value for pK(I=0) for PEP in Table 1. When pepsp is used to calculate K' for EC 2.7.1.40 at pH 7.63 and $I = 0.07$ M, $K' = 2.7 \times 10^4$ is obtained in comparison with 2.0×10^3 obtained by Krimsky. When pepsp is used to calculate K' for EC 2.7.1.40 at pH 7.32 and $I = 0.05$ M, $K' = 6.0 \times 10^4$ is obtained in comparison with 6.5×10^3 obtained by McQuate and Utter. The Wood, Davis, and Lochmüller value can hardly be off this much since they approached the equilibrium from both sides and were not faced by such serious analytical difficulties.

Species of 2-Phospho-D-glycerate. Since $\Delta_f G'^\circ$ for PEP can now be calculated at desired pHs and ionic strengths, reaction EC 4.2.1.11 can be used to obtain species data on 2-phospho-D-glycerate. Wold and Ballou[25] have reported $K' = 5.0$ at pH 7.22 and ionic strength 0.4 M. Use of the program calcGef2sp yields the following data matrix for PG2:

$$\mathrm{pg2sp} = \{\{-1496.38,\_,-3,4\},\{-1539.99,\_,-2,5\}\} \quad (37)$$

Species of 3-Phospho-D-glycerate. Now reaction EC 5.4.2.1 can be used to obtain species data on PG3. Guynn[26] has reported $K' = 0.94$ at pH 6.75 and ionic strength 0.25 M. This leads to the following data matrix for PG3:

$$\mathrm{pg3sp} = \{\{-1502.54,\_,-3,4\},\{-1545.52,\_,-2,5\}\} \quad (38)$$

for the two species of PG3.

Species of 3-Phosphoglyceroyl Phosphate. The standard Gibbs energies of formation of the three species can be calculated from both reactions EC 1.2.1.12 and EC 2.7.2.3 because there are experimental determinations of K'. However, K' for reaction EC 2.7.2.3 is about 240 at pH 7.0 and $I = 0.25$ M according to Cornel, Ledbetter, and Veech,[27] and so the analytical difficulties are pretty serious. These authors have also reported that K' for reaction EC 1.2.1.12 is $K' = 0.58$ at pH 6.94 and ionic strength 0.25 M. The program calcGef3sp yields

$$\mathrm{bpgsp} = \{\{-2356.14,\_,-4,4\},\{-2401.58,\_,-3,5\},\{-2441.76,\_,-2,6\}\} \quad (39)$$

These values for the three species of BPG can be used to calculate the apparent equilibrium constant of reaction 2.7.2.3 at pH 7 and 0.25 M ionic strength; this yields 25 in comparison with the approximate 240 mentioned above.

5. Tables Calculated from Species Data

The apparent equilibrium constants for the last five reactions of glycolysis and EC 4.1.1.32 (eq 35) at 298.15 K, pHs 5, 6, 7, 8, and 9, and ionic strength 0.25 M calculated with data files recommended here are given in Table 2. These K' values have been calculated using the program calckprime, which is also given in the Appendix. The corresponding standard transformed

TABLE 2: Apparent Equilibrium Constants as a Function of pH at 298.15 K and 0.25 M Ionic Strength

EC no.	pH 5	pH 6	pH 7	pH 8	pH 9
1.2.1.12	0.054	0.156	0.65	5.5	54
2.7.2.3	1.70	11.3	25.0	27.8	28.1
5.4.2.1	0.107	0.103	0.091	0.084	0.083
4.2.1.11	1.46	2.04	4.27	5.77	6.02
2.7.1.40	10.5×10^5	6.1×10^5	1.09×10^5	0.116×10^5	0.0116×10^5
4.1.1.32	0.65	0.191	0.54	4.7	53

TABLE 3: Apparent Standard Transformed Gibbs Energies (kJ mol^{-1}) of Reaction as a Function of pH at 298.15 K and 0.25 M Ionic Strength

EC no.	pH 5	pH 6	pH 7	pH 8	pH 9
1.2.1.12	7.20	4.61	1.07	−4.22	−9.88
2.7.2.3	−1.32	−6.02	−7.98	−8.25	−8.27
5.4.2.1	5.54	5.63	5.94	6.13	6.16
4.2.1.11	−0.94	−1.76	−3.60	−4.35	−4.45
2.7.1.40	−34.38	−33.01	−28.75	−23.19	−17.50
4.1.1.32	1.06	4.10	1.51	−3.82	−9.84

TABLE 4: Changes in Binding of Hydrogen Ions as a Function of pH at 298.15 K and 0.25 M Ionic Strength

EC no.	pH 5	pH 6	pH 7	pH 8	pH 9
1.2.1.12	−0.62	−0.42	−0.83	−0.98	−1.00
2.7.2.3	−0.94	−0.63	−0.12	−0.01	0.00
5.4.2.1	0.01	0.03	0.06	0.01	0.00
4.2.1.11	−0.05	−0.28	−0.26	−0.04	−0.01
2.7.1.40	0.08	0.48	0.93	0.99	1.00
4.1.1.32	0.84	0.07	−0.82	−1.00	−1.15

Gibbs energies of reaction are given in Table 3. The changes in binding of hydrogen ions calculated using eq 8 are given in Table 4.

Discussion

The inverse Legendre transform (eq 10) makes it possible to interchange the roles of the chemical potential of hydrogen ions $\mu(H^+)$ and the amount of the hydrogen component $n_c(H)$ in the system in the fundamental equation for G' (eq 2). This has been demonstrated by converting the fundamental equation for the transformed Gibbs energy G' to the fundamental equation for the Gibbs energy G and by calculating the standard Gibbs energies of formation $\Delta_f G_j^\circ$ of the species of four reactants at zero ionic strength from experimental measurements of apparent equilibrium constants at various specified pHs and ionic strengths. These calculations require information on pK's of reactants in approximately the range pH 4−10. These thermodynamic properties of species make it possible to calculate functions of pH and ionic strength that represent $\Delta_f G'^\circ$(298.15 K) for PEP, PG2, PG3, and BPG. These functions can be used to calculate apparent equilibrium constants of biochemical reactions involving these four reactants at desired pHs and ionic strength.

These calculations of $\Delta_f G^\circ$ of species have been greatly assisted by the critical compilations of thermodynamic measurements on biochemical reactions by Goldberg and Tewari.[12−17] Their surveys show that apparent equilibrium constants have been determined for about 500 enzyme-catalyzed reactions at various pHs and ionic strengths. By using species data that can be calculated from these experimental apparent equilibrium constants, apparent equilibrium constants can be calculated for many more reactions. The best value of K' for a given reaction may be obtained from other pathways, rather than direct determination. This is illustrated by consideration of the last five reactions of glycolysis because the apparent equilibrium constants for two of these reactions that are recommended here are based on other reactions, although apparent equilibrium constants have been determined for all five reactions. This analysis emphasizes the difficulties in direct measurement of very large apparent equilibrium constants. However, when apparent equilibrium constants are large, their values can be obtained from tables constructed using the smaller equilibrium constants for reactions in alternate pathways.

This paper has discussed reactions where $\Delta_f G'^\circ$ values are known for all reactants but one. However, these same calculations can be made when $\Delta_f G'^\circ$ values are known for all reactants but two. If the two reactants are related (for example, NAD_{ox} and NAD_{red}) and there seems to be no current way to obtain $\Delta_f G_j^\circ$ of species of either one, $\Delta_f G^\circ$ and $\Delta_f H^\circ$ can each be taken as zero at each temperature for one of the species of one of the reactants. This becomes a convention of the thermodynamic table.

These data analyses depend on more than experimental apparent equilibrium constants because acid dissociation constants are also required. If more data were available on the free concentrations of magnesium and other metal ions that are bound and on the dissociation constants of metal ion complexes of reactant species, metal ion binding could be included in the Legendre transform. In the present analyses data at the lowest experimental concentrations of metal ions are used with the expectation that metal ion effects will largely cancel between reactants and products.

The same type of analyses can be made with experimental enthalpies of biochemical reaction, but one difference is that heats of enzyme-catalyzed reactions can be measured for reactions with large values of K'. However, there is less calorimetric data than equilibrium data, and the dependence of K' on temperature has been determined for only a few enzyme-catalyzed reactions. Calculations of $\Delta_f H^\circ(I=0)$ of species also requires information on $\Delta_f H^\circ$ of acid dissociations if the reactant has pK's in the range 5−9.

The computer programs calcGef2sp and calcGef3sp included in the Appendix make it possible to go from experimental values of K' to standard Gibbs energies of formation of species in one step. An advantage of these programs is that it is convenient to test the sensitivity of the final results to experimental errors by changing the input values of K', pH, ionic strength, and pK's, one at a time. The programs given in the Appendix help make it clear that it is much easier to go from basic data on species to apparent equilibrium constants at specified pH and ionic strength than to go from experimental values of K' to $\Delta_f G_j^\circ$ values of species.

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Appendix

calcGef2sp[equat_,pHc_,ionstr_,z1_,nH1_,pK0_]:=Module-[{energy,trGereactant,pKe,trgefpHis,gef1,gef2},(*This program uses ∑viΔfGi′o=-RTlnK′ to calculate the standard Gibbs energies of formation of the two species of a reactant for which the pK at zero ionic strength is pK0. The biochemical equation is of the form pyruvate+atp-x-adp=-8.31451*.29815*Log[K′], where K′ is the apparent equilibrium constant at 298.15 K, pHc, and ionic strength (ionstr). The more basic form of the reactant has charge number z1 and hydrogen atom number nH1. The output is the species matrix without the standard enthalpies of formation of the two species.*)

```
energy=Solve[(equat),x]/.pH->pHc/.is->ionstr;
trGereactant=energy[[1,1,2]];
(*Calculate the pK of the weak acid at the experimental ionic strength.*)
pKe=pK0+(0.510651*ionstr∧.5)*2*z1/(1+1.6*ionstr∧.5);
(*Calculate the standard transformed Gibbs energy of formation of the more basic species at the experimental conditions.*)
trgefpHis=trGereactant+8.31451*.29815*Log[1+10∧(pKe-pHc)];
(*Calculate the standard Gibbs energy of formation of the more basic species at zero ionic strength.*)
gef1=trgefpHis-nH1*8.31451*.29815*Log[10]*pHc+2.91482*-(z1∧2-nH1)*(ionstr∧.5)/(1+1.6*ionstr∧.5);
(*Calculate the standard Gibbs energy of formation of the acidic form of the reactant.*)
gef2=gef1+8.31451*.29815*Log[10∧-pK0];
{{gef1,_,z1,nH1},{gef2,_,z1+1,nH1+1}}]
```

```
calcGef3sp[equat_,pHc_,ionstr_,z1_,nH1_,pK10_,pK20_]:=Module[{energy,trGereactant,pKe,trgefpHis,gef1,gef2,-gef3,pK1e,pK2e},(*This program uses ∑viΔfGi′o=-RTlnK′ to calculate the standard Gibbs energies of formation of the three species of a reactant for which the pK's at zero ionic strength are pK10 and pK20. The biochemical equation is of the form pyruvate+atp-x-adp==-8.31451*.29815*Log[K′], where K′ is the apparent equilibrium constant at 298.15 K, pHc, and ionic strength (ionstr). The more basic form of the reactant has charge number z1 and hydrogen atom number nH1. The output is the species matrix without the standard enthalpies of formation of the three species.*)
energy=Solve[(equat),x]/.pH->pHc/.is->ionstr;
trGereactant=energy[[1,1,2]];
(*Calculate the pK's of the weak acid at the experimental ionic strength.*)
pK1e=pK10+(0.510651*ionstr∧.5)*2*z1/(1+1.6*ionstr∧.5);
pK2e=pK20+(0.510651*ionstr∧.5)*(2*z1+2)/(1+1.6*ionstr∧.5);
(*Calculate the standard transformed Gibbs energy of formation of the more basic species (species 1) at the experimental conditions.*)
trgefpHis=trGereactant+8.31451*.29815*Log[1+10∧(pK1e-pHc)+10∧(pK1e+pK2e-2*pHc)];
(*Calculate the standard Gibbs energy of formation of the more basic species (species 1) at zero ionic strength.*)
gef1=trgefpHis-nH1*8.31451*.29815*Log[10]*pHc+2.91482*-(z1∧2-nH1)*(ionstr∧.5)/(1+1.6*ionstr∧.5);
(*Calculate the standard Gibbs energy of formation of the second species of the reactant.*)
gef2=gef1+8.31451*.29815*Log[10∧-pK10];
(*Calculate the standard Gibbs energy of formation of the third species of the reactant.*)
gef3=gef2+8.31451*.29815*Log[10∧-pK20];
{{gef1,_,z1,nH1},{gef2,_,z1+1,nH1+1},{gef3,_,z1+2,-nH1+2}}]
```

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

```
calckprime[eq_,pHlist_,islist_]:=Module[{energy,dG},-(*Calculates the apparent equilibrium constant K′ at specified pHs and ionic strengths for a biochemical equation typed in the form atp+h2o+de==adp+pi. The names of the reactants call the appropriate functions of pH and ionic strength. pHlist and islist can be entered as lists.*)
energy=Solve[eq,de];
dG=energy[[1,1,2]]/.pH->pHlist/.is->islist;
Exp[-dG/(8.31451*.29815)]]
```

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