Calculation of Thermodynamic Properties of Species of Biochemical Reactants Using the Inverse Legendre Transform

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The determination of apparent equilibrium constants and heats of enzyme-catalyzed reactions provides a way to determine $\Delta_f G°$ and $\Delta_f H°$ of species of biochemical reactants. These calculations are more difficult than the calculation of transformed thermodynamic properties from species properties, and they are an application of the inverse Legendre transform. The $\Delta_f G°$ values of species of a reactant can be calculated from an apparent equilibrium constant if the $\Delta_f G°$ values are known for all the species of all the other reactants and the p*K*s of the reactant of interest are known. The $\Delta_f H°$ of species of a reactant can be calculated from the heat of reaction if the $\Delta_f H°$ values are known for all species of the other reactants and $\Delta_f G°$ values are known for all species in the reaction. The standard enthalpies of acid dissociation of the reactant of interest are also needed. The inverse Legendre transformation is accomplished by using computer programs to set up the simultaneous equations that involve the $\Delta_f H°$ of the species and solving them. Thirty two new species matrixes providing $\Delta_f G°$ values and eight new species matrixes providing $\Delta_f H°$ values are calculated. It is the specificity and speed of enzyme-catalyzed reactions that make it possible to determine standard thermodynamic properties of complicated species in aqueous solution that could never have been obtained classically.

The equilibrium state of an enzyme-catalyzed reaction can be discussed in terms of chemical thermodynamic properties of species or in terms of transformed thermodynamic properties of reactants (sums of species).[1−4] The transformed properties have the advantage that they provide a more global view since adenosine triphosphate, for example, can be considered as a reactant made up of the three species: ATP^{4-}, $HATP^{3-}$, and H_2ATP^{2-} when the pH is specified in the range 5−9. It is relatively straightforward to calculate standard transformed thermodynamic properties ($\Delta_f G_i'°$, $\Delta_f H_i'°$, ...) of reactant *i* from standard thermodynamic properties ($\Delta_f G_j°$, $\Delta_f H_j°$, ...) of species, but it is more difficult to calculate species properties from experimental values of apparent equilibrium constants K' and standard transformed enthalpies $\Delta_r H'°$ of enzyme-catalyzed reactions. The reason is that transformed thermodynamic properties are composites of species properties. As is always true in thermodynamics, various paths can be used for calculations. The path that has often been used is to calculate $\Delta_f G_j°$ for a species from the equilibrium constant K of a chemical reference reaction for the enzyme-catalyzed reaction when the standard Gibbs energies of formation are known for all the other species in the chemical reaction using

$$\Delta_r G° = \sum_{j=1}^{N} \nu_j \Delta_f G_j° = -RT \ln K \qquad (1)$$

where N is the number of species involved in the reference reaction and ν_j is the stoichiometric number of the *j*th species. The equilibrium constant K for a chemical reference reaction can be calculated from the apparent equilibrium constant K' for the enzyme-catalyzed reaction at specified T, pH, and I by use of

$$K' = K[H^+]^{-\nu(H^+)} \prod_{i=1}^{N'} P_i^{\nu_i} \qquad (2)$$

$\nu(H^+)$ is the stoichiometric number of the hydrogen ion in the chemical reference reaction, the P_i are the binding polynomials for the various reactants (sums of species), and N' is the number of reactants (sums of species) in the enzyme-catalyzed reaction, excluding H^+. Binding polynomials have the form[5]

$$P_i = 1 + 10^{pK_1 - pH} + 10^{pK_1 + pK_2 - 2pH} + ... \qquad (3)$$

The calculation of $\Delta_f G_j°$ of the species of a reactant from the apparent equilibrium constant involves a number of steps. (1) First, it is necessary to have K' for an enzyme-catalyzed reaction at a specified pH and ionic strength. (2) The p*K*s in eq 3 at the experimental temperature and ionic strength are needed for all of the reactants. (3) The binding polynomials P_i are calculated for all the reactants. (4) Equation 2 is used to calculate K for the reference chemical reaction. (5) If the standard Gibbs energies of formation are known for all the species but one, eq 1 can be used to calculate $\Delta_f G°$ for that one species at the experimental ionic strength. (6) This $\Delta_f G°$ needs to be adjusted to zero ionic strength so that the $\Delta_f G°$ of the other species of the reactant can be calculated using the p*K*s at zero ionic strength.

Since this calculation involves six steps, other paths for this calculation have been explored. A preceding article[6] has shown how $\Delta_f G°$ (298.15 K, $I = 0$) values of species can be calculated from measurements of K' (298.15 K, pH, I) by use of computer programs on the basis of the inverse Legendre transform discussed by Callen.[7]

1. Use of Computer Programs to Calculate $\Delta_f G°$ (298.15 K, $I = 0$) of Species On the Basis of the Inverse Legendre Transform. When the pH is specified, the criteria for spontaneous change and equilibrium are provided by the transformed Gibbs energy G' defined by the Legendre transform[1,2]

$$G' = G - n_c(H)\mu(H^+) \qquad (4)$$

where $n_c(H)$ is the amount of the hydrogen component (total amount of hydrogen atoms in the system) and $\mu(H^+)$ is the chemical potential of hydrogen ions that corresponds with the

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specified pH. If the $\Delta_f G_i'^\circ$ values of the other reactants are known, the standard transformed Gibbs energy of formation $\Delta_f G_i'^\circ$ of one of the reactants can be calculated from the experimental value of K' using

$$\Delta_r G'^\circ = \sum_{i=1}^{N'} \nu_i' \Delta_f G_i'^\circ = -RT \ln K' \quad (5)$$

The stoichiometric numbers ν_i' of the reactants have a prime to distinguish them from the stoichiometric numbers of the underlying chemical reactions.

If a reactant consists of a single species, the standard transformed Gibbs energy $\Delta_f G_j'^\circ$ (pH, I) of this species at 298.15 K and zero ionic strength can be calculated using[8]

$$\Delta_f G_j'^\circ (\text{pH}, I) = \Delta_f G_j^\circ (I=0) + N_H(j) RT \ln(10) \text{pH} - 2.91482(z_j^2 - N_H(j)) I^{1/2}/(1 + 1.6 I^{1/2}) \quad (6)$$

where $N_H(j)$ is the number of hydrogen atoms in the species, z_i is its charge number, and the extended Debye−Hückel equation has been used. The pH in this and other calculations is defined[9] by $\text{pH} = -\log[H^+]$, rather than $\text{pH}_a = -\log\{a(H^+)\}$ where $a(H^+)$ is the activity of hydrogen ions. This is done so that molar concentrations can be used for all species. The calculation of $\Delta_f G_j^\circ (I = 0)$ when the reactant consists of a single species is relatively simple; a Mathematica program[10] calcGef1sp for doing this is given in the Appendix.

When a reactant consists of two or more species, more complicated programs are required. The writing of these programs was guided by the concept of the inverse Legendre transform[6,7] that shows how to calculate G from G'.

$$G = G' + n_c(H)\mu(H^+) \quad (7)$$

The most efficient way to store thermodynamic information on enzyme-catalyzed reactions is to store data on the species of a reactant in the form of a small matrix:[11,12]

$$\text{namesp} = \{\{\Delta_f G_1^\circ, \Delta_f H_1^\circ, z_1, N_{H1}\}, \{\Delta_f G_2^\circ, \Delta_f H_2^\circ, z_2, N_{H2}\}, ...\} \quad (8)$$

where z_i is the charge number for species i and N_{Hi} is the number of hydrogen atoms in species i. The first row is for the species with the fewest hydrogen atoms. The thermodynamic properties are for 298.15 K and zero ionic strength. With this information, it is possible to calculate apparent equilibrium constants K' and enthalpies of biochemical reactions at desired temperatures, pHs, and ionic strengths I. The database on the web contains data on 131 biochemical reactants.[11]

When a reactant consists of two or more species with different numbers of hydrogen atoms, the standard transformed Gibbs energy of formation of the reactant is given by

$$\Delta_f G'^\circ = -RT \ln \sum_{i=1}^{N'} \exp(-\Delta_f G_i'^\circ/RT) \quad (9)$$

This equation can be used to calculate $\Delta_f G_1'^\circ$ for the most basic species of the reactant from $\Delta_f G_i'^\circ$ by rearranging it to[12]

$$\Delta_f G'^\circ = \Delta_f G_1'^\circ - RT \ln(1 + 10^{pK_1 - pH} + 10^{pK_1 + pK_2 - 2pH} + ...) \quad (10)$$

The relation between pK_1 (I) and pK_1 ($I = 0$) for a weak acid at 298.15 K is given by[6]

$$pK_1 (I) = pK_1 (I=0) + 0.510651(\sum \nu_j z_j^2) I^{1/2}/(1 + 1.6 I^{1/2}) \quad (11)$$

The standard Gibbs energy of formation of species 1 at zero ionic strength, $\Delta_f G_1^\circ$ ($I = 0$), can be calculated from $\Delta_f G_1'^\circ$ (I) using eq 6. After $\Delta_f G_1^\circ$ ($I = 0$) has been calculated, $\Delta_f G_2^\circ$ ($I = 0$) can be calculated using

$$\Delta_f G_2^\circ (I=0) = \Delta_f G_1^\circ (I=0) - RT \ln(10) pK_1 (I=0) \quad (12)$$

and higher pKs can be calculated in a similar way. The computer programs calcGef2ps and calcGef3sp for calculating $\Delta_f G^\circ$ values were published earlier.[6,12]

2. Use of Computer Programs to Calculate $\Delta_f H^\circ$ (298.15 K, $I = 0$) of Species On the Basis of the Inverse Legendre Transform. The calculation of $\Delta_f H^\circ$ (298.15 K, $I = 0$) of species from the experimental heat of reaction ($\Delta_r H'^\circ$) at specified temperature, pH, and ionic strength is a two-step process in the sense that $\Delta_f G^\circ$ of the species of the reactant have to be obtained first.

It is assumed here that the heat of reaction is measured at 298.15 K and that $\Delta_f G^\circ$ values are known for all the species in the reaction at 298.15 K and zero ionic strength. It is also assumed that the $\Delta_f H^\circ$ are known for all the species in the reaction except for the species of one reactant. If the enzyme-catalyzed reaction produces or consumes hydrogen ions, the calorimetric heat of reaction needs to be corrected by the heat of neutralization of these hydrogen ions.[13] This correction is proportional to the change in binding of hydrogen ions $\Delta_r N_H$. The standard transformed enthalpy of the enzyme-catalyzed reaction is given by

$$\Delta_r H'^\circ = \sum_{i=1}^{N'} \nu_i' \Delta_f H_i'^\circ \quad (13)$$

This equation makes it possible to calculate $\Delta_f H_i'^\circ$ for the reactant of interest. If the reactant consists of a single species, the standard transformed enthalpy of formation of the single species can be calculated using[8]

$$\Delta_f H_j'^\circ (298.15\text{ K}, \text{pH}, I) = \Delta_f H_j^\circ (298.15\text{ K}, I=0) + 1.4775(z_j^2 - N_H(j)) I^{1/2}/(1 + 1.6 I^{1/2}) \quad (14)$$

The Mathematica program calcHf1sp for calculating $\Delta_f H_j^\circ (I = 0)$ when the reactant consists of a single species is given in the Appendix.

If the reactant of interest consists of two species, the calculation is more complicated because the pK and the standard enthalpy of dissociation $\Delta_{diss} H^\circ$ of the weak acid are involved. The objective of the calculation is to obtain the values of $\Delta_f H_j^\circ$ of the various species. The standard transformed enthalpy of formation of the reactant of interest at the pH and ionic strength of the calorimetric experiment is given by

$$\Delta_f H'^\circ = \sum_{j=1}^{N'} r_j \Delta_f H_j'^\circ \quad (15)$$

where $\Delta_f H_j'^\circ$ of the species are given by eq 14. The equilibrium mole fractions r_j of the species at the pH and ionic strength of the calorimetric experiment are calculated using

$$r_j = \exp[(\Delta_f G_i'^\circ (\text{pH}, I) - \Delta_f G_j'^\circ (\text{pH}, I))/RT] \quad (16)$$

Substitution of eqs 14 and 16 into eq 15 yields a linear relation between the $\Delta_f H_j^\circ$ (298.15 K, $I = 0$) of the species. Additional

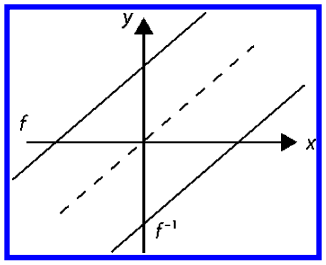

Figure 1. Plots of the functions f and f^{-1} versus x. The plots that are shown by solid lines are reflections across the dashed line.

relations are provided by the standard enthalpies of dissociation for the acid dissociations.

$$HA^{z+1} = H^+ + A^z \quad (17)$$

At zero ionic strength, the standard enthalpy of dissociation is given by

$$\Delta_{diss}H^\circ\,(I=0) = \Delta_f H^\circ\,(A^z, I=0) - \Delta_f H^\circ\,(HA^{z+1}, I=0) \quad (18)$$

The symbolic capabilities of Mathematica are used to express the simultaneous equations to make it possible to use Solve to calculate the $\Delta_f H_j^\circ$ (298.15 K, $I = 0$) of the species. The programs calcHf1sp, calcHf2sp, and calcHf3sp are given in the Appendix. If the temperature of the calorimetric experiment is not 298.15 K, the calculation becomes more complicated. The output from these programs can be added to the database[11] in BasicBiochemData2.

3. The Transformed Gibbs Energy as the Inverse of the Gibbs Energy. The standard transformed Gibbs energy of formation and the standard Gibbs energy of the species are inverses. When the effect of ionic strength is ignored, eq 6 shows that the standard transformed Gibbs energy of formation of a reactant is given by

$$\Delta_f G'^\circ = \Delta_f G^\circ + N_H RT\ln(10)\text{pH} \quad (19)$$

To compare this equation with the mathematical literature,[14,15] this equation will be used in the form

$$y = x + N_H RT\ln(10)\text{pH} = f(y) \quad (20)$$

where $f(y)$ means the function y. This equation can be solved for x to obtain the inverse $f^{-1}(y)$ of the function y. This -1 is not an exponent, but means the inverse of y. Since x and y are inverses, $x = f^{-1}(y)$. To compare these two functions, it is useful to express f and f^{-1} in terms of the same variable, and so

$$f^{-1}(x) = x - N_H RT\ln(10)\text{pH} \quad (21)$$

To verify that f and f^{-1} are in fact inverses, it is readily shown that $f(f^{-1}(x)) = f^{-1}(f(x)) = x$. Mathematically these functions are referred to being "one-to-one." The two functions f and f^{-1} are plotted in Figure 1. This reflection across the dashed line $y = x$ is characteristic of inverse functions, as, for example, e^x and $\ln x$.

When a reactant consists of two species, the standard transformed Gibbs energy of the reactant is given by

$$\Delta_f G'^\circ = \Delta_f G_1^\circ + N_{H1}RT\ln(10)\text{pH} - RT\ln(1+10^{pK_1-pH}) \quad (22)$$

where $\Delta_f G_1^\circ$ is the standard Gibbs energy of formation of the more basic species, N_{H1} is the number of hydrogen atoms in this species, and pK1 is the pK of the acidic species. This equation will be used in the form

$$y = x + N_{H1}RT\ln(10)\text{pH} - RT\ln(1+10^{pK_1-pH}) = f(y) \quad (23)$$

This equation can be solved for x to obtain the inverse $f^{-1}(y)$ of the function y. Following the steps after eq 20, we obtain

$$f^{-1}(x) = x - N_{H1}RT\ln(10)\text{pH} + RT\ln(1+10^{pK_1-pH}) \quad (24)$$

Figure 1 also represents the two functions f and f^{-1}. There is a difference this time because of the $RT\ln(1+10^{pK_1-pH})$ term. The term $N_{H1}RT\ln(10)$pH is always positive, but the term $RT\ln(1+10^{pK_1-pH})$ can be of either sign and may dominate so that the identities of the solid lines in the figure can be reversed.

4. Calculation of $\Delta_f G^\circ$ for Species of Thirty-Two Reactants at 298.15 K and Zero Ionic Strength. The data compilations of Goldberg and Tewari[16−21] were surveyed for apparent equilibrium constants and heats of reactions that can yield $\Delta_f G^\circ$ and $\Delta_f H^\circ$ for species of reactants that are not yet included in BasicBiochemData2. The reactions selected were those considered to be the most likely to lead to species matrixes that will later lead to species matrixes for more reactants. The calculation of $\Delta_f G^\circ$ of species are discussed in this section, and the calculation of $\Delta_f H^\circ$ of species are discussed in the next section.

Enzymes are assigned names by a committee of IUBMB,[22] and these names are accompanied by EC numbers. Structures of reactants are given in the EC−PDB database.[23−56] In this paper, EC numbers, with A, B,... added when the EC number applies to a group of reactions, are used here to refer to particular reactions written in a specific direction. When the first number is 1, the enzymes are referred to as oxidoreductases; when it is 2, transferases; when it is 3, hydrolases; when it is 4, lyases, and when it is 5, isomerases.

The biochemical reactions selected are the following, where the reactants for which $\Delta_f G^\circ$ values to be calculated are underlined:

EC 1.1.1.48 D-galactose + NAD_{ox} = <u>D-galactono-1,4-lactone</u> + NAD_{red} (25)

EC 1.1.1.95 3-phosphoglycerate + NAD_{ox} = <u>3-phosphohydroxypyruvate</u> + NAD_{red} (26)

EC 1.1.99.2 <u>(*S*)-hydroxyglutarate</u> + NAD_{ox} = ketoglutarate + NAD_{red} (27)

EC 1.4.1.9 L-leucine + NAD_{ox} + H_2O = <u>4-methyl-2-oxopentanoate</u> + NAD_{red} + ammonia (28)

EC 2.1.2.1 glycine + acetaldehyde = <u>L-threonine</u> (29)

EC 2.1.3.1 oxaloacetate + <u>propanoyl-CoA</u> = pyruvate + (*S*)-methylmalonyl-CoA (30)

EC 2.2.1.2 D-fructose + D-glyceraldehyde-3-phosphate = D-fructose 6-phosphate + <u>D-glyceraldehyde</u> (31)

EC 2.3.1.9 2 acetyl-CoA = CoA + <u>acetoacetyl-CoA</u> (32)

EC 2.4.1.20 <u>cellobiose</u> + phosphate = D-glucose + D-glucose 6-phosphate (33)

EC 2.6.1.15 L-glutamine + pyruvate = <u>ketoglutaramate</u> + L-alanine (34)

EC 2.6.1.51 L-alanine + <u>3-hydroxypyruvate</u> = L-serine + pyruvate (35)

EC 2.7.6.1 ATP + D-ribose 5-phosphate = AMP + 5-phospho-D-ribose 1-diphosphate (36)

EC 2.7.7.1 ATP + nicotinamide ribonucleotide = NAD_{ox} + diphosphate (37)

EC 2.7.7.4 ATP + sulfate = adenosine 5′-phosphosulfate + diphosphate (38)

EC 2.8.2.1 succinyl-CoA + acetoacetate = succinate + acetoacetate-CoA (39)

EC 3.1.3.1A D-galactose 6-phosphate + H_2O = D-galactose + phosphate (40)

EC 3.1.3.1B D-mannose 6-phosphate + H_2O = D-mannose + phosphate (41)

EC 3.1.3.1C 3-phospho-D-glycerate + H_2O = (*R*)-glycerate + phosphate (42)

EC 3.1.3.1D L-O-phosphoserine + H_2O = L-serine + phosphate (43)

EC 3.2.1.2 α,α-trehalose + H_2O = 2 D-glucose (44)

EC 3.2.1.10 isomaltose + H_2O = 2 D-glucose (45)

EC 3.2.1.23 lactulose + H_2O = D-galactose + D-fructose (46)

EC 4.1.3.16 4-hydroxy-2-oxyglutarate = pyruvate + glyoxylate (47)

EC 4.1.3.22 (*R*)-2-methylmalate = pyruvate + acetate (48)

EC 4.2.1.31 (*R*)-malate = maleate + H_2O (49)

EC 4.2.1.35 (*R*)-2-methylmalate = methylmaleate + H_2O (50)

EC 4.2.1.48 D-glutamate = 5-oxy-D-proline + H_2O (51)

EC 4.3.1.5 phenylalanine = *trans*-cinnamate + ammonia (52)

EC 4.6.1.1 ATP = 3′,5′-cyclic AMP + diphosphate (53)

EC 5.3.1.6 D-ribose 5-phosphate = D-ribulose 5-phosphate (54)

EC 5.3.1.13 D-arabinose 5-phosphate = D-ribulose 5-phosphate (55)

EC 5.4.2.8 D-mannose 1-phosphate = D-mannose 6-phosphate (56)

EC 1.1.1.37 (*S*)-malate + NAD_{ox} = oxaloacetate + NAD_{red} (57)

The experimental data on these reactions that are used to calculate $\Delta_f G°$ values at 298.15 K and zero ionic strength are summarized in Table 1.

The p*K*s at 298.15 K and zero ionic strength are from BasicBiochemData2 or have been estimated from p*K*s of related reactants.

Mathematica computer programs[6] were published earlier to calculate species properties for reactants for which two or three species have to be considered in the pH range 5−9. When there are two species, one p*K* is required, and when there are three species, two p*K*s are required. The simpler program calcGef1sp that can be used when the reactant consists of a single species is given in the Appendix of this paper. These programs require the charge number and number of hydrogen atoms for the most basic species. The programs used to calculate species properties actually produce species matrixes (see eq 8). These calculations were verified by using calckprime[6] to calculate K' at the experimental pH and ionic strength.

The calculation of $\Delta_f G°$ of the species of the underlined reactants using calcdGf1sp, calcdGf2sp, or calcdGf3sp requires values of z and N_H that are given in Table 2. This table provides the values of $\Delta_f G°$ (298.15 K, $I = 0$) that were calculated for the species that have significant concentrations in the pH range 5−9.

Data in Table 2 can be added to BasicBiochemData2.

5. Calculation of $\Delta_f H°$ for Species of Eight Reactants at 298.15 K and Zero Ionic Strength. The calculation of $\Delta_f H°$ for species is a two-step process because $\Delta_f G°$ values for the reactant of interest are needed first. The calculation of $\Delta_f H°$ values of species of reactants from heats of reaction are only undertaken here for reactions in which $\Delta_f H°$ values are known for all reactants but one. When there are more than one species, the successive standard enthalpies of dissociation also have to be known. The reactions selected were those considered to be the most likely to lead to species matrixes that will later lead to species matrixes for more reactants. Some of the calculations in this section depend on new $\Delta_f G°$ values in Table 2.

The calculation of $\Delta_f H°$ values also involves the concept of inverse Legendre transforms because the experimental data are $\Delta_f H'°$ values. Programs calcHf1sp, calcHf2sp, and calcHf3sp (see Appendix) have to be written to extract the species values. These calculations were verified by calculating the heat of reaction at the experimental pH and ionic strength.

The reactions selected are the following, where the reactants for which $\Delta_f H°$ values for species are sought are underlined:

EC 1.4.1.3 L-glutamate + $NADP_{ox}$ + H_2O = ketoglutarate + $NADP_{red}$ + ammonia (58)

EC 2.7.1.11 ATP + fructose 6-phosphate = ADP + D-fructose-1,6-bisphosphate (59)

EC 2.7.1.30 ATP + glycerol = ADP + glycerol-3-phosphate (60)

EC 2.7.1.40 ATP + pyruvate = ADP + phosphoenolpyruvate (61)

EC 3.1.3.1B D-mannose 6-phosphate + H_2O = D-mannose + phosphate (62)

EC 3.1.3.1E D-fructose 6-phosphate + H_2O = D-fructose + phosphate (63)

EC 3.2.1.10 isomaltose + H_2O = 2 D-glucose (64)

EC 4.2.1.20 indole + L-serine = tryptophan + H_2O (65)

The data selected for calculating $\Delta_f H°$ values of species are given in Table 3.

The standard enthalpies of acid dissociation $\Delta_{diss} H°$ ($I = 0$) were obtained from glucose 6-phosphate.[12] Data on $\Delta_f G°$ of mannose 6-phosphate and isomaltose were obtained earlier in this paper.

The calculation of $\Delta_f H°$ of species of the underlined reactants with calcHf1sp, calcHf2sp, and calcHf3sp use the z and N_H values in Table 4. The experimental data of Table 3 make it possible to calculate the $\Delta_f H°$ values in Table 4.

TABLE 1: Measurements of Apparent Equilibrium Constants that Are Used to Calculate $\Delta_f G°$ of the Species of a Reactant at 298.15 K and Zero Ionic Strength

EC	reactant	pH	I/M	K'	pK ($I = 0$)	ref
1.1.1.48	D-galactono-1,4-lactone	6.8	0.15	390		24
1.1.1.95	3-phosphohydroxypyruvate	7.3	0.25	2.3×10^{-6}		25
1.1.99.2	(*S*)-hydroxyglutarate	7.0	0.10	1.47×10^{-5}		26
1.4.1.9	4-methyl-2-oxopentanoate	11.1	0.10	1.11×10^{-2}		27
2.1.2.1	L-threonine	7.6	0.002	56		28
2.1.3.1	propanoyl-CoA	6.5	0.025	1.9		29
2.2.1.2	D-glyceraldehyde	7.6	0.02	0.27		30
2.3.1.9	acetoacetyl-CoA	7.0	0.10	1.56×10^{-5}		31
2.4.1.20	cellobiose	7.0	0.01	0.23		32
2.6.1.15	ketoglutaramate	8.4	0.05	340		33
2.6.1.51	3-hydroxypyruvate	7.0	0.025	4.26		34
2.7.6.1	5-phospho-D-ribose 1-diphosphate	7.4	0.25	64.5	7.18, 6.69	35
2.7.7.1	nicotinamide ribonucleotide	7.4	0.05	0.40	6.44	36
2.7.7.4	adenosine 5′-phosphosulfate	7.2	0.06	1.8×10^{-8}		37
2.8.2.1	acetoacetate	7.0	0.1	2.8×10^{-3}		38
3.1.3.1A	D-galactose 6-phosphate	8.5	0.10	87	6.44	39
3.1.3.1B	D-mannose 6-phosphate	8.5	0.10	39	6.44	39
3.1.3.1C	(*R*)-glycerate	7.0	0.25	294		40
3.1.3.1D	L-O-phosphoserine	7.0	0.10	56		41
3.2.1.2	α,α-trehalose	5.65	0.10	119		42
3.2.1.10	isomaltose	5.65	0.10	16.2		42
3.2.1.23	lactulose	5.65	0.10	128		42
4.1.3.16	4-hydroxy-2-oxyglutarate	8.4	0.01	0.010		43
4.1.3.22	(*R*)-2-methylmalate	7.4	0.21	0.211	7.4	26
4.2.1.31	maleate	7.0	0.10	4.88×10^{-3}		44
4.2.1.35	methylmaleate	7.0	0.10	0.0962	6.27	44
4.2.1.48	5-oxy-D-proline	7.9	0.15	35.8		45
4.3.1.5	trans-cinnamate	7.69	0.10	2.47	7.69	46
4.6.1.1	3′,5′-cyclic AMP	7.0	0.06	0.065		47
5.3.1.6	D-ribulose 5-phosphate	7.0	0.25	0.83	6.69	48
5.3.1.13	D-arabinose 5-phosphate	8.0	0.003	0.295	6.69	49
5.4.2.8	D-mannose 1-phosphate	7.0	0.02	8.5	6.44	50

The number of new species matrixes calculated in the preceding section is 32, and the number of new entries in this section is 9. Some of these reactants are in BasicBiochemData2 without $\Delta_f H°$ values. Others are in Table 2 without $\Delta_f H°$ values. In both cases, the final values are those in Table 4. The data in Tables 2 and 4 can be added to those published earlier in BasicBiochemData2. This will make it possible to calculate standard transformed thermodynamic properties of many enzyme-catalyzed reactions that could not have been calculated before.

Discussion

Because applications of these species properties are not discussed here, it is important to summarize the calculations that can be made on the thermodynamics of enzyme-catalyzed reactions. When $\Delta_f G°$ values are available for all the species in a biochemical reaction at 298.15 K, the equilibrium constants can be calculated for the chemical reactions involved at ionic strengths in the range 0 to about 0.35 M. The apparent equilibrium constants and changes in binding of hydrogen ions in enzyme-catalyzed reactions can be calculated at 298.15 K, ionic strengths in the range approximately 0−0.35 M, and pHs in the range 5−9. When $\Delta_f H°$ values are available for all the species in a biochemical reaction, the equilibrium constants, standard Gibbs energy of reaction, standard enthalpies of reaction, and standard entropies of reaction can be calculated for the chemical reactions involved over a range of temperatures and desired ionic strengths on the assumption that the $\Delta_f H°$ and $\Delta_f S°$ are independent of temperature.

When $\Delta_f G°$ are available for all species in a biochemical reaction at 298.15 K, apparent equilibrium constants, standard transformed Gibbs energies of reaction, and changes in the binding of hydrogen ions can be calculated. When $\Delta_f H°$ values are available for all species in a biochemical reaction at 298.15 K, the apparent equilibrium constants, changes in standard transformed Gibbs energies of reaction, changes in standard transformed enthalpies of reaction, changes in transformed entropies of reaction, and changes in binding of hydrogen ions in enzyme-catalyzed reactions can be calculated over a range of temperatures, pHs, and ionic strengths.

These calculations of species properties have been restricted to reactions for which standard formation properties are known for all the reactants but one. However, similar calculations can be made on reactions in which standard formation properties are known for all the reactants but two. This was first done when the assignment of $\Delta_f G° (I = 0) = 0$ and $\Delta_f H° (I = 0) = 0$ was applied to adenosine[4] so that transformed thermodynamic properties could be calculated for other members of the ATP series. Later, Boeiro-Goates and co-workers[57] determined the third law entropy of adenosine, and since $\Delta_f H°$ was known, it was possible for them to adjust the previous values of $\Delta_f G° (I = 0)$ and $\Delta_f H° (I = 0)$ for members of the ATP series so that they are with respect to the elements in reference states. However, this change did not affect calculations of apparent equilibrium constants and heats of reaction that had been made earlier.

In none of the cases discussed in this article was it necessary to assign $\Delta_f G° (I = 0) = 0$ and $\Delta_f H° (I = 0) = 0$. That is done in BasicBiochemData2 for half a dozen pairs of reactants. The disadvantage of using the convention that $\Delta_f G° (I = 0) = 0$ and $\Delta_f H° (I = 0) = 0$ for a species is that these entries are not useful in exploring the syntheses of these reactants.

This article has shown the applicability of the concept of the inverse Legendre transform and has shown how computer programs can be used to calculate $\Delta_f G° (I = 0)$ of species properties in a single step. When the standard transformed enthalpy of an enzyme-catalyzed reaction has been determined,

TABLE 2: Species Data on $\Delta_f G^\circ$ at 298.15 K and Zero Ionic Strength in Aqueous Solutions

EC	reactant	$\Delta_f G^\circ$/kJ mol^{-1}	z	N_H
1.1.1.48	D-galactono-1,4-lactone	−905.34	0	10
1.1.1.95	3-phosphohydroxypyruvate	−1448.67	−3	2
1.1.99.2	(*S*)-hydroxyglutarate	−840.18	−2	6
1.4.1.9	4-methyl-2-oxopentanoate	−445.14	−1	9
2.1.2.1	L-threonine	−528.89	0	9
2.1.3.1	propanoyl-CoA	−179.14	0	5
2.2.1.2	D-glyceraldehyde	−440.02	0	6
2.3.1.9	acetoacetyl-CoA	−285.32	0	5
2.4.1.20	cellobiose	−1585.94	0	22
2.6.1.15	ketoglutaramate	−595.88	−1	5
2.6.1.51	3-hydroxypyruvate	−608.55	−1	3
2.7.6.1	5-phospho-D-ribose 1-diphosphate	−3284.25	−5	8
		−3325.23	−4	9
		−3363.42	−3	10
2.7.7.1	nicotinamide ribonucleotide	840.08	−2	14
		803.32	−1	15
2.7.7.4	adenosine 5′-phosphosulfate	−1541.98	−1	12
2.8.2.1	acetoacetate	−482.49	0	5
3.1.3.1A	D-galactose 6-phosphate	−1756.81	−2	11
		−1793.57	−1	12
3.1.3.1B	D-mannose 6-phosphate	−1759.87	−2	11
		−1796.63	−1	12
3.1.3.1C	(*R*)-glycerate	−661.01	−1	5
3.1.3.1D	L−O-phosphoserine	−1360.75	−2	6
		−1397.51	−1	7
3.2.1.2	α,α-trehalose	−1582.76	0	22
3.2.1.10	isomaltose	−1587.71	0	22
3.2.1.23	lactulose	−1575.22	0	22
4.1.3.16	4-hydroxy-2-oxyglutarate	−951.78	−2	4
4.1.3.22	(*R*)−2-methylmalate	−843.90	−2	6
4.2.1.31	maleate	−592.09	−2	2
			−1	3
4.2.1.35	methylmaleate	−600.7	−2	4
		−636.49	−1	5
4.2.1.48	5-oxy-D-proline	−469.15	−1	6
4.3.1.5	trans-cinnamate	−128.75	−1	7
4.6.1.1	3′,5′-cyclic AMP	−629.68	−1	7
5.3.1.6	D-ribulose 5-phosphate	−1595.32	−2	9
		−1633.50	−1	10
5.3.1.13	D-arabinose 5-phosphate	−1598.34	−2	9
		−1636.53	−1	10
5.4.2.8	D-mannose 1-phosphate	−1754.56	−2	11
		−1791.32	−1	12

a second step can be used to calculate $\Delta_f H^\circ$ ($I = 0$) of species with computer programs given in the Appendix. By use of enzyme-catalyzed reactions, it is possible to obtain thermodynamic information on complicated organic molecules that could never have been obtained classically.

Species matrixes on reactants make it possible to make sensitivity analyses on reactions to determine the sensitivities to errors in temperature, pH, and ionic strength. There are two limitations on these calculations: (1) When Mg^{2+} is present, there is not enough information to take this effect into account, but apparent equilibrium constants and heats at the lowest concentrations are always used. (2) Some experimental measurements were not made at 298.15 K, but small differences in temperature are ignored.

Appendix

calcGef1sp[equat_,pHc_,ionstr_,z1_,nH1_]:=Module[{energy,trGereactant},(*This program uses $\sum \nu_i \Delta_f G_i'^\circ = -RT \ln K'$ to calculate the standard Gibbs energy of formation of the species of a reactant that does not have a pK in the range 4−10. The 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 is. The reactant has charge number z_1 and hydrogen atom number N_{H1}. The output is the species vector without the standard enthalpy of formation.*)

energy =Solve[equat,x]/.pH->pHc/.is->ionstr;

trGereactant=energy[[1,1,2]];

gef1=trGereactant-nH1*8.31451*Log[10]*pHc+(2.91482*-(z1∧2-nH1)*ionstr∧.5)/(1+1.6*ionstr∧.5);

{{gef1,_,z1,nH1}}]

calcHf1sp[equat_,spmat_,pHc_,ionstr_]:=Module[{energy,trHreactant,enthf1,gef1,dHzero1,z1,nH1},(*This program uses $\sum \nu i \Delta_f H_i'^\circ = \Delta_r H^{\circ\prime}$ (298.15 K) to calculate the standard enthalpy of formation ($I = 0$) of the single species of a reactant for which the species matrix (spmat) contains $\Delta_f G^\circ$ at zero ionic strength. The reaction equation (equat) is of the form x + nadredh-malateh-nadoxh = 89.5, where 89.5 kJ mol^{-1} is the heat of reaction and x is oxaloacetate. The species matrix (spmat) is that for oxaloacetate. The calorimetric experiment is at pHc and ionic strength ionstr. The reactant has charge number z_1 and hydrogen atom number N_{H1}. The output is the complete species matrix for *x*. 11-21-04*)

TABLE 3: Measurements of Standard Transformed Enthalpies of Reaction that Are Used to Calculate $\Delta_f H^\circ$ of the Species of Reactants at 298.15 K and Zero Ionic Strength

EC	reactant	pH	I/M	$\Delta_f H'^\circ$/kJ mol^{-1}	$\Delta_{diss} H^\circ$/kJ mol^{-1}	ref
1.1.3.7	oxaloacetate	7.3	0.20	89.5		51
1.4.1.3	ketoglutarate	7.5	0.10	64.6		52
2.7.1.11	D-fructose-1,6-bisphosphate	7.0	0.10	−84.2	−1.8,−1.8	53
2.7.1.30	glycerol-3-phosphate	9.0	0.10	−56.0	−1.8	54
2.7.1.40	phosphoenolpyruvate	8.5	0.10	−31.9		54
3.1.3.1B	D-mannose 6-phosphate	8.5	0.10	1.78	−1.8	55
3.1.3.1E	D-fructose 6-phosphate	8.75	0.10	−7.43	−1.8	55
3.2.1.10	isomaltose	5.65	0.10	5.93		56

TABLE 4: Species Data on $\Delta_f H$ at 298.15 K and Zero Ionic Strength in Aqueous Solutions

EC	reactant	$\Delta_f G^\circ$/kJ mol^{-1}	$\Delta_f H^\circ$/kJ mol^{-1}	z	N_H
1.1.3.7	oxaloacetate	−793.29	−959.90	−2	2
1.4.1.3	ketoglutarate	−793.41	−1044.06	−2	4
2.7.1.11	D-fructose-1,6-bisphosphate	−2601.40	−3343.25	−4	10
		−2639.36	−3341.45	−3	11
		−2673.89	−3339.65	−2	12
2.7.1.30	glycerol-3-phosphate	−1358.96	−1724.59	−2	7
		−1397.04	−1722.79	−2	3
2.7.1.40	phosphoenolpyruvate	−1263.65	−1621.38	−3	2
		−1303.61	−1619.58	−2	3
3.1.3.1B	D-mannose 6-phosphate	−1759.87	−2273.71	−2	11
		−1796.63	−2271.90	−1	12
3.1.3.1E	D-fructose 6-phosphate	−1760.80	−2265.17	−2	11
		−1796.60	−2263.37	−1	12
3.2.1.10	isomaltose	−1587.71	−2244.48	0	22

```
{gef1,dHzero1,z1,nH1}=Transpose[spmat];
energy=Solve[equat,x]/.pH->pHc/.is->ionstr;
trHreactant=energy[[1,1,2]];
enthf1=trHreactant-1.4775*(z1∧2-nH1)*ionstr∧.5/(1+1.6*ionstr∧.5);
Flatten[{{gef1,enthf1,z1,nH1}}]]
calcHf2sp[equat_,spmat_,pHc_,ionstr_,dHdisszero_]:=Module[{dGzero,dHzero,zi,nHi,pHterm,isterm,gpfnsp,energy,trHreactant,stdtrGerx,r1,r2,solution,dH1zero,dH2zero,dH1,dH2},(*This program uses ∑νiΔfHi°° = ΔrH°′ (298.15 K) to calculate the standard enthalpy of formation (I = 0) of the two species of a reactant for which the species matrix (spmat) contains ΔfG° at zero ionic strength for the two species of the reactant. The reaction equation (equat) is of the form mannoseh + pih-xh2oh = 1, 7, where 1.7 kJ mol−1 is the heat of reaction and z is mannose 6-phosh. The species matrix (spmat) is that for mannonese 6-phos. The calorimetric experiment is at pHc and ionic strength ionstr. The first step in the calculation is to use the information on the standard Gibbs energies of formation of the species of the reactant of interest to calculate the equilibrium mole fractions r1 (base form) and r2 (acid form) of the two species of the reactant of interest. The final output is the complete species matrix for x.*)
.{dGzero,dHzero,zi,nHi}=Transpose[spmat];
pHterm=nHi*8.31452*.29815*Log[10∧-pH]/.pH->pHc;
isterm=2.91482*((zi∧2)-nHi)*(is∧.5)/(1+1.6*is∧.5)/.is->ionstr;
gpfnsp=dGzero-pHterm-isterm;
stdtrGereactant=-8.31451*.29815*Log[Apply[Plus,Exp[1*gpfnsp/(8.31451*.29815)]];
r1=Exp[(stdGereactant-gpfsp[[1]]/(8.31451*.29815)];
r2=Exp[(stdGereactant-gpfsp[[2]]/(8.31451*.29815)];
(*Now calculate dfH′° (reactant) from drH′° (298.15 K) for the reaction.*)
energy=Solve[equat,x]/.pH->pHc/.is->ionstr;
trHreactant=energy[[1,1,2]];
(*dH1zero is given by the following equation. dH2zero is calculated from the equation for the enthalpy of dissociation.*)
solution=Solve[trHreactant==r1*(dH1zero+1.4775*(zi-[[1]]∧2-nHi[[1]])*ionstr∧.5/(1+1.6*ionstr∧.5))+r2*(dH1zero-dHdisszero+1.4775*(zi[[2]]∧2-nHi[[2]])*ionstr∧.5/(1+1.6*ionstr∧.5)),dH1zero];
dH1=solution[[1,1,2]];
dH2=dH1-dHdisszero;
Transpose[[dGzero,{dH1,dH2},zi,nHi}]]
calcHf3sp[equat_,spmat_,pHc_,ionstr_,dHdisszero1_,-dHdisszero2_]:=Module[{dGzero,dHzero,zi,nHi,pHterm,gpfnsp,-energy,trHreactant,stdtrGereactant,r1,r2,r3,solution,dH1zero,-dH2zero,dH3zero,dH1expt,dH2expt,dH3expt,dH1,dH2,dH3},
(*This program uses ∑ νiΔfHi°° = ΔrH°′ (298.15 K) to calculate the standard enthalpy of formation (I = 0) of the three species of a reactant for which the species matrix (spmat) contains ΔfG° at zero ionic strength for the three species of the reactant. The reaction equation (equat) is of the form adph + x-fructose 6phosh-atph = −84.2, where −84.2 kJ mol−1 is the heat of reaction and x is fructose 16-phosh. The species matrix (spmat) is that for fructose 16-phos. The calorimetric experiment is at pHc and ionic strength ionstr. The first step in the calculation is to use the information on the standard Gibbs energies of formation of the species of the reactant of interest to calculate the equilibrium mole fractions r1 (base form), r2 (intermediate form), and r3 (acid form) of the three species of the reactant of interest. The final output is the complete species matrix for x.*),
{dGzero,dHzero,zi,nHi}=Transpose[spmat];
pHterm=nHi*8.31452*.29815*Log[10∧-pH]/.pH->pHc;
isterm=2.91482*((zi∧2)-nHi)*(is∧.5)/(1+1.6*is∧.5)/.is->ionstr;
gpfnsp=dGzero-pHterm-isterm;
stdtrGereactant=-8.31451*.29815*Log[Apply[Plus,Exp[1*gpfnsp/(8.31451*.29815)]];
r1=Exp[(stdGereactant-gpfsp[[1]]/(8.31451*.29815)];
r2=Exp[(stdGereactant-gpfsp[[2]]/(8.31451*.29815)];
r3=Exp[(stdGereactant-gpfsp[[3]]/(8.31451*.29815)];
(*Now calculate dfH′° (reactant) from drH′° (298.15 K) for the reaction.*)
energy=Solve[equat,x]/.pH->pHc/.is->ionstr;
trHreactant=energy[[1,1,2]];
(*The standard transformed enthalpies of formation of the three species are given by the following six equations. dH1zero, dH2zero, and dH3zero are also related by the equations for the enthalpy of dissociation.*)
solution=Solve[dH1expt== dH1zero+1.4775*(zi[[1]]∧2-nHi[[1]])*ionstr∧.5/(1+1.6*ionstr∧.5),dH2expt==dH2zero+1.4775*(zi[[2]]∧2-nHi[[2]])*ionstr∧.5/(1+1.6*ionstr∧.5),-dH3expt==+1.4775*(zi[[3]]∧2-nHi[[3]])*ionstr∧.5/(1+1.6*ionstr∧.5),
trHreactant==r1*dH1expt+r2*dH2expt+r3*dH3expt,-dH2zero==dH1zero-dHdisszero1,dH3zero==dH2zero-dHdisszero2),{dH1zero,dH2zero,dH3zero},{dH1expt,dH2expt,-dH3expt}];
dHzerocalc={solution[[1,1,2]],solution[[1,2,2]],solution-[[1,3,2]]};
Transpose[{dGzero,dHzerocalc,zi,nHi}]]
```

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Supporting Information Available: A *Mathematica* notebook containing calculations with each of these programs is available free of charge via the Internet at http://pubs.acs.org.

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