

Effect of Temperature on Standard Transformed Gibbs Energies of Formation of Reactants at Specified pH and Ionic Strength and Apparent Equilibrium Constants of Biochemical Reactions

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Received: April 9, 2001; In Final Form: May 30, 2001

When standard Gibbs energies of formation and standard enthalpies of formation are known for the species of a biochemical reactant at 298.15 K, it is possible to calculate the standard transformed Gibbs energy of formation and standard transformed enthalpy of formation of the reactant at any desired pH and low ionic strengths at 298.15 K. This article is concerned with the estimation of standard transformed Gibbs energies of formation of reactants, standard transformed Gibbs energies of reaction, and apparent equilibrium constants K' of biochemical reactions at specified pH and ionic strength in the temperature range 283.15–313.15 K on the basis of the assumption that the standard enthalpies of formation of the species are independent of temperature in this range. The standard transformed Gibbs energies of formation and standard transformed enthalpies of formation are given for 14 reactants as a function of pH and ionic strength at temperatures of 283.15, 298.15, and 313.15 K, and standard transformed Gibbs energies of reaction, standard transformed enthalpies of reaction, and apparent equilibrium constants are given for six biochemical reactions over this temperature range at pH 6, 7, and 8. When the limiting Debye–Hückel slope for the contribution of ionic strength to the transformed Gibbs energy of a species is expressed by a quadratic in temperature, it is possible to estimate the standard transformed properties of reactants and reactions at any temperature in the range 273.15–313.15 K, provided the standard enthalpies of formation of the species involved are known.

For some time, the tables of standard formation properties of species involved in biochemical reactions have been prepared^{1–5} for 298.15 K. However, experimental determinations of apparent equilibrium constants K' and heats of biochemical reactions, which are increasingly the source of these values,⁶ have been carried out over a range of temperatures, and applications may involve other temperatures. Ideally, apparent equilibrium constants and heats of reaction at specified pH and ionic strength should be measured over a range of temperatures with sufficient accuracy that enthalpies and changes in heat capacities at constant pressure can be calculated, but experimental data of this type is extremely limited. However, standard Gibbs energies of formation and standard enthalpies of formation are currently known⁷ for the species of about 70 reactants that have to be considered in the pH range 5–9. This article is about the estimation of standard transformed Gibbs energies of formation and standard transformed enthalpies of formation of reactants in the temperature range 273.15–313.15 K on the basis of the assumption that the standard enthalpies of formation of the species of the reactant are independent of temperature. Fortunately, the range of temperatures of interest for biochemical reactions is usually not very broad.

The criterion for spontaneous change and equilibrium in aqueous solutions at specified pH is provided by the transformed Gibbs energy G' that is defined by the Legendre transform^{8–10}

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

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

$\sum N_{\text{H}}(i)n_i$, where $N_{\text{H}}(i)$ is the number of hydrogen atoms in species i and n_i is the amount of species i . Substitution of this relation and $G = \sum \mu_i n_i$ in eq 1 yields

$$G' = \sum [\mu_i - N_{\text{H}}(i)\mu(\text{H}^+)]n_i = \sum \mu'_i n_i \quad (2)$$

where the transformed chemical potential μ'_i of species i is given by

$$\mu'_i = \mu_i - N_{\text{H}}(i)\mu(\text{H}^+) \quad (3)$$

Making this Legendre transform leads to a transformed enthalpy H' and a transformed entropy S' for the system and also other transformed properties of species.

Calculation of Standard Transformed Thermodynamic Properties of Species at the Desired Temperature, pH, and Ionic Strength from Their Standard Formation Properties at 298.15 K

In calculating apparent equilibrium constants and heats of biochemical reactions, it is convenient to use thermodynamic properties that are functions of ionic strength because this avoids the direct use of activity coefficients and their temperature derivatives. The way this is done is illustrated by considering the calculation of the Gibbs energy of formation $\Delta_f G(B_i, I)$ of species B_i at ionic strength I

$$\begin{aligned} \Delta_f G(B_i, I) &= \Delta_f G^\circ(B_i, I=0) + RT \ln(\gamma_i [B_i]) \\ &= \Delta_f G^\circ(B_i, I) + RT \ln[B_i] \end{aligned} \quad (4)$$

where γ_i is the activity coefficient of B_i and $\Delta_f G^\circ(B_i, I) = \Delta_f G^\circ -$

$(B_i I=0) + RT \ln \gamma_i$ is the standard Gibbs energy of formation of species B_i at ionic strength I . Note that the superscript zeros in eq 4 indicate unit activity of B_i in the first case and 1 mol kg^{-1} ideal solution in the second case. Since biochemical reactions are generally studied at low ionic strengths, it is convenient to represent the activity coefficient using the extended Debye–Hückel equation in the form

$$\ln \gamma_i = -\alpha z_i^2 I^{1/2} / (1 + B I^{1/2}) \quad (5)$$

where α is the Debye–Hückel constant ($1.17582 \text{ kg}^{1/2} \text{ mol}^{-1/2}$ at 298.15 K), z_i is the charge number, and $B = 1.6 \text{ kg}^{1/2} \text{ mol}^{-1/2}$ is an empirical constant.⁵ Thus, the standard Gibbs energy of formation of species i at ionic strength I is given by

$$\Delta_f G_i^\circ(I) = \Delta_f G_i^\circ(I=0) - RT\alpha z_i^2 I^{1/2} / (1 + B I^{1/2}) \quad (6)$$

It should be noted that α is a function of temperature.

Equation 3 in the form $\mu_i^\circ = \mu_i^\circ - N_{\text{H}}(i)\mu(\text{H}^+)$ can be written in terms of standard Gibbs energies of formation that are functions of the ionic strength:

$$\begin{aligned} \Delta_f G_i'^\circ &= \Delta_f G_i^\circ - N_{\text{H}}(i)\Delta_f G(\text{H}^+) \\ &= \Delta_f G_i^\circ - N_{\text{H}}(i)[\Delta_f G^\circ(\text{H}^+, I) + RT \ln 10^{-\text{pH}}] \end{aligned} \quad (7)$$

where eq 4 has been used for the hydrogen ion and $\text{pH} = -\log[\text{H}^+]$. More specifically, this pH can be referred to as pH_c to differentiate it from pH_a measured with a pH meter. The differences $\text{pH}_a - \text{pH}_c$ are given as a function of temperature and ionic strength in the Appendix. Substituting eq 6 for species i and for the hydrogen ion yields the following expression for the standard transformed Gibbs energy of formation $\Delta_f G_i'^\circ$ of species i at a specified pH and ionic strength:

$$\Delta_f G_i'^\circ(\text{pH}, I) = \Delta_f G_i^\circ(I=0) - N_{\text{H}}(i)RT \ln 10^{-\text{pH}} - RT\alpha(z_i^2 - N_{\text{H}}(i))I^{1/2} / (1 + B I^{1/2}) \quad (8)$$

It should be noted that $\Delta_f G_i'^\circ(\text{pH}, I)$ for a species is independent of pH only if $N_{\text{H}}(i) = 0$ and is independent of ionic strength only if $z_i = 0$ and $N_{\text{H}}(i) = 0$ or $z_i^2 - N_{\text{H}}(i) = 0$.

The corresponding expression for the standard transformed enthalpy of formation $\Delta_f H_i'^\circ$ of a species can be obtained by using the Gibbs–Helmholtz relation $\Delta_f H_i'^\circ = -T^2(\partial(\Delta_f G_i'^\circ / T) / \partial T)_P$. This yields

$$\Delta_f H_i'^\circ(I) = \Delta_f H_i^\circ(I=0) + RT^2(\partial\alpha/\partial T)_P(z_i^2 - N_{\text{H}}(i))I^{1/2} / (1 + B I^{1/2}) \quad (9)$$

assuming that the empirical constant B is independent of temperature. Note that the standard transformed enthalpy of a species does not depend on pH, even when the species contains hydrogen atoms. The standard transformed entropy of formation $\Delta_f S_i'^\circ(\text{pH}, I)$ is given by $\Delta_f S_i'^\circ = (\Delta_f H_i'^\circ - \Delta_f G_i'^\circ) / T$.

The corresponding expression for the standard transformed molar heat capacity at constant pressure $C_{P_i}'^\circ$ of a species can be obtained by taking the derivative of eq 9 with respect to temperature at constant pressure

$$\begin{aligned} C_{P_i}'^\circ(I) &= C_{P_i}^\circ(I=0) + RT[2(\partial\alpha/\partial T)_P + \\ &\quad T(\partial^2\alpha/\partial T^2)_P](z_i^2 - N_{\text{H}}(i))I^{1/2} / (1 + B I^{1/2}) \end{aligned} \quad (10)$$

This equation has been written in terms of heat capacities, rather

than heat capacities of formation, because the heat capacities of elements appear on both sides of eq 10 and cancel.

When a reactant consists of a single species in the pH range of interest, eqs 8, 9, and 10 yield the standard transformed formation properties of the reactant. When a reactant consists of two or more species in the pH range of interest, using the Legendre transform makes species that differ only with respect to the number of hydrogen atoms they contain pseudoisomers; that is, their equilibrium distribution is independent of the extents of other reactions. Since there are pseudoisomers, the standard transformed Gibbs energy of formation of the reactant (sum of species) is calculated using

$$\Delta_f G'^\circ(\text{iso}) = -RT \ln \left\{ \sum_{i=1}^{N_{\text{iso}}} \exp[-\Delta_f G_i'^\circ / RT] \right\} \quad (11)$$

where N_{iso} is the number of pseudoisomers in the reactant. The equilibrium mole fraction r_i of the i th pseudoisomer in the reactant is given by

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

The standard transformed enthalpy of formation of the reactant is a mole fraction weighted average that is given by

$$\Delta_f H'^\circ(\text{iso}) = \sum_{i=1}^{N_{\text{iso}}} r_i \Delta_f H_i'^\circ \quad (13)$$

Note that although $\Delta_f H'^\circ$ values for species are independent of pH, that is not true for $\Delta_f H'^\circ$ values of reactants consisting of two or more species because the r_i are functions of pH. The pseudoisomer group has a corresponding standard transformed entropy of formation given by

$$\Delta_f S'^\circ(\text{iso}) = (\Delta_f H'^\circ(\text{iso}) - \Delta_f G'^\circ(\text{iso})) / T \quad (14)$$

Differentiating eq 13 with respect to temperature at constant pressure yields the following expression for the heat capacity $C_{P_i}'^\circ(\text{iso})$ of the pseudoisomer group:¹¹

$$\begin{aligned} C_{P_i}'^\circ(\text{iso}) &= \sum_{i=1}^{N_{\text{iso}}} r_i C_{P_i}^\circ(I) + (1/RT) \left\{ \sum_{i=1}^{N_{\text{iso}}} r_i (\Delta_f H_i'^\circ)^2 - \right. \\ &\quad \left. (\Delta_f H'^\circ(\text{iso}))^2 \right\} \end{aligned} \quad (15)$$

The second term in this equation is always positive because the weighted average of the squares of the individual standard transformed enthalpies of formation is always greater than the square of the weighted average enthalpy of formation. This is in accord with Châtelier's principle that as the temperature is raised the equilibrium shifts in the direction that absorbs heat. The contributions of temperature-dependent chemical equilibrium to heat capacities of ideal gases and ideal association reactions have been discussed by Mains, Larson, and Hepler.¹²

Calculation of the Temperature Dependencies of the Coefficients of the Ionic Strength Terms in Equations 8–10

Clarke and Glew¹³ have calculated the Debye–Hückel slopes for water between 0 and 150 °C. They were primarily concerned with electrostatic deviations from ideality of the solvent osmotic coefficient ϕ , and so they used the Debye–Hückel limiting law in the form $\ln \gamma = -3Am^{1/2}$. Thus, their constant A is equal to $\alpha/3$. They tabulated limiting slopes A , A_L , and A_J , where A_L is

TABLE 1: Debye–Hückel Constant and Limiting Slopes as Functions of Temperature

$T/^{\circ}\text{C}$	$\alpha/\text{kg}^{1/2}\text{mol}^{-1/2}$	$RT\alpha/\text{kJ mol}^{-3/2}\text{kg}^{1/2}$	$RT^2(\partial\alpha/\partial T)_p/\text{kJ mol}^{-3/2}\text{kg}^{1/2}$	$RT[2(\partial\alpha/\partial T)_p + T(\partial^2\alpha/\partial T^2)_p]/\text{J mol}^{-3/2}\text{kg}^{1/2}\text{K}^{-1}$
0	1.129 38	2.564 94	1.075	13.255
10	1.147 17	2.700 73	1.213	15.41
20	1.165 98	2.841 96	1.3845	17.90
25	1.175 82	2.914 82	1.4775	19.27
30	1.185 99	2.989 34	1.5775	20.725
40	1.207 32	3.143 49	1.800	23.885

the limiting slope for the solute enthalpy and A_J is the limiting slope for the solute heat capacity at constant pressure. The later two quantities were calculated by taking derivatives of A with respect to temperature

$$A_L = 6RT^2(\partial A/\partial T)_p \quad (16)$$

$$A_J = 6RT[2(\partial A/\partial T)_p + T(\partial^2 A/\partial T^2)_p] \quad (17)$$

Substituting $A = \alpha/3$ yields

$$A_L = 2RT^2(\partial\alpha/\partial T)_p \quad (18)$$

$$A_J = RT[4(\partial\alpha/\partial T)_p + 2T(\partial^2\alpha/\partial T^2)_p] \quad (19)$$

The quantities needed in biochemical thermodynamics (see the coefficients in eq 8, 9, and 10) can be calculated from A , A_L , and A_J using

$$RT\alpha = 3RTA \quad (20)$$

$$RT^2(\partial\alpha/\partial T)_p = A_L/2 \quad (21)$$

$$RT[2(\partial\alpha/\partial T)_p + T(\partial^2\alpha/\partial T^2)_p] = A_J/2 \quad (22)$$

The first of these coefficients is needed to calculate $\Delta_f G_i^\circ$ at the desired ionic strength, the second is needed to calculate $\Delta_f H_i^\circ$, and the third is needed to calculate C_{Pi}° . Table 1 gives the values of these limiting slopes, which have been calculated from the values of A , A_L , and A_J given by Clarke and Glew.¹³

In discussing the effect of temperature, it is more convenient to use the molality because the molality does not change with the temperature when there are no reactions in the system. The effect of pressure on these limiting slopes has been studied by Bradley and Pitzer.¹⁴

Calculation of Standard Transformed Thermodynamic Properties of Biochemical Reactants at Temperatures Other Than 298.15 K

There is not enough data on C_P° for species in dilute aqueous solution to be able to adjust $\Delta_f H^\circ$ of all the species discussed here to other temperatures. Therefore, the following calculations are based on the assumption that $\Delta_f C_P^\circ$ is equal to zero for reactions between species. When $\Delta_f C_P^\circ$ is equal to zero for a chemical reaction, $\Delta_f H^\circ$ and $\Delta_f S^\circ$ are both independent of temperature. In that case, $\Delta_f H_i^\circ$ and $\Delta_f S_i^\circ$ for the formation of species in the reaction can be considered to be independent of temperature. Under these conditions, the values of $\Delta_f G_i^\circ$ at other temperatures in the neighborhood of 298.15 K can be estimated using

$$\Delta_f G_i^\circ(T) = \Delta_f H_i^\circ(298.15\text{ K}) - T\Delta_f S_i^\circ(298.15\text{ K}) \quad (23)$$

This equation can be written in terms of $\Delta_f G_i^\circ(298.15\text{ K})$ and

TABLE 2: Standard Gibbs Energies of Formation of Species in Dilute Aqueous Solutions at 283.15, 298.15, and 313.15 K, Standard Enthalpies of Formation at 298.15 K, Charge Numbers, and Numbers of Hydrogen Atoms

species	$\Delta_f G^\circ/\text{kJ mol}^{-1}$			$\Delta_f H^\circ/\text{kJ mol}^{-1}$	z_i	$N_{\text{H}}(i)$
	283.15 K	298.15 K	313.15 K			
acetaldehyde	-142.68	-139.00	-135.32	-212.23	0	4
ADP ³⁻	-1726.33	-1711.55	-1696.77	-2005.24	-3	12
HADP ²⁻	-1764.96	-1752.53	-1740.10	-1999.64	-2	13
H ₂ ADP ¹⁻	-1789.49	-1777.42	-1765.35	-2017.24	-1	14
ATP ⁴⁻	-2594.84	-2573.49	-2552.14	-2997.91	-4	12
HATP ³⁻	-2635.72	-2616.87	-2598.02	-2991.61	-3	13
H ₂ ATP ²⁻	-2661.84	-2643.58	-2625.32	-3006.61	-2	14
CO ₂ (g)	-394.32	-394.36	-394.40	-393.50	0	0
CO ₃ ²⁻	-535.32	-527.81	-520.30	-677.14	-2	0
HCO ₃ ⁻	-592.06	-586.77	-581.48	-691.99	-1	1
H ₂ CO ₃	-626.96	-623.11	-619.26	-699.63	0	2
ethanol	-187.01	-181.64	-176.27	-288.30	0	6
formate	-354.75	-351.00	-347.25	-425.55	-1	1
glucose	-933.32	-915.90	-898.48	-1262.19	0	12
glucose 6-phosphate ²⁻	-1789.72	-1763.94	-1738.16	-2276.44	-2	11
glucose 6-phosphate ⁻	-1824.44	-1800.59	-1776.74	-2274.64	-1	12
H ₂ O	-239.64	-237.19	-234.74	-285.83	0	2
NAD ⁻	0	0	0	0	-1	26
NADH ²⁻	19.90	22.65	25.40	-31.94	-2	27
HPO ₄ ²⁻	-1106.31	-1096.10	-1085.89	-1299.00	-2	1
H ₂ PO ₄ ⁻	-1145.62	-1137.30	-1128.98	-1302.60	-1	2
pyruvate ⁻	-478.51	-472.27	-466.03	-596.22	-1	3

$\Delta_f H_i^\circ(298.15\text{ K})$ by substituting the expression for the entropy of formation of the species

$$\Delta_f G_i^\circ(T) = (T/298.15)\Delta_f G_i^\circ(298.15\text{ K}) + (1 - T/298.15)\Delta_f H_i^\circ(298.15\text{ K}) \quad (24)$$

Values of $\Delta_f G_i^\circ$ and $\Delta_f H_i^\circ$ at 298.15 K have been compiled from various sources for about 70 species involved in biochemical reactions.^{15,16} Table 2 gives $\Delta_f G_i^\circ$ and $\Delta_f H_i^\circ$ for 22 species at 298.15 K that are considered here; the specific references to the data on these species are given in the previous publications. These are limiting values at zero ionic strength. In addition, Table 2 gives values of $\Delta_f G_i^\circ$ at 283.15 and 313.25 K calculated using eq 24. It also gives the charge number z_i of the species and the number $N_{\text{H}}(i)$ of hydrogen atoms in the species because these properties are needed to calculate standard transformed formation properties.

The data in Table 2 make it possible to calculate the standard transformed Gibbs energies of formation and standard transformed enthalpies of formation at 283.15, 298.15, and 313.15 K of the 14 reactants that contain these species. The first step is to calculate $\Delta_f G_i^\circ$ and $\Delta_f H_i^\circ$ for all of the species at 283.15, 298.15, and 313.25 K using eqs 8 and 9 with the Debye–Hückel limiting slopes given in Table 1. When a reactant consists of two or more species, the second step is to calculate $\Delta_f G_i^\circ$ and $\Delta_f H_i^\circ$ for the pseudoisomer groups using eqs 11–13. The values of $\Delta_f G_i^\circ$ and $\Delta_f H_i^\circ$ for the 14 reactants containing the species in Table 2 are given in Table 3.

Note that although $\Delta_f H_i^\circ$ values of species have been assumed to be independent of temperature in this range, the $\Delta_f H_i^\circ$ values for reactants do depend on temperature. There are two reasons for this; first, the temperature-dependent coefficients in eqs 8 and 9 are involved, and second, when two or more species are involved in a reactant, the distribution of species may change with temperature, as indicated by eq 13. Another way of looking at this is that there is an electrostatic contribution to C_{Pi}° even when $C_{Pi}^\circ = 0$.

TABLE 3: Standard Transformed Gibbs Energies of Formation and Standard Transformed Enthalpies of Formation of Reactants at Three Temperatures, pH 7, and Ionic Strength 0.25 Mol kg⁻¹

species	$\Delta_f G'^{\circ}/\text{kJ mol}^{-1}$			$\Delta_f H'^{\circ}/\text{kJ mol}^{-1}$		
	283.15 K	298.15 K	313.25 K	283.15 K	298.15 K	313.25 K
acetaldehyde	12.09	24.06	36.04	-213.58	-213.87	-214.23
ADP	-1269.15	-1230.12	-1191.09	-2005.66	-2005.92	-2006.26
ATP	-2143.05	-2097.89	-2052.72	-2995.79	-2995.59	-2995.37
CO ₂ (g)	-394.32	-394.36	-394.40	-393.50	-393.50	-393.50
CO ₂ tot	-554.44	-547.10	-539.77	-693.02	-692.88	-692.76
ethanol	45.16	62.96	80.75	-290.32	-290.76	-291.30
formate	-316.81	-311.04	-305.28	-425.55	-425.55	-425.55
glucose	-468.99	-426.71	-384.43	-1266.23	-1267.12	-1268.19
glucose 6-phosphate	-1367.24	-1318.92	-1270.61	-2278.77	-2279.30	-2279.95
H ₂ O	-162.25	-155.66	-149.07	-286.50	-286.65	-286.83
NAD _{ox}	1005.31	1059.11	1112.91	-8.431	-10.26	-12.50
NAD _{red}	1061.66	1120.09	1178.53	-39.69	-41.38	-43.44
P _i	-1071.56	-1059.49	-1047.42	-1299.64	-1299.39	-1299.11
pyruvate	-363.17	-350.78	-338.39	-596.89	-597.04	-597.22

Calculation of Standard Transformed Thermodynamic Properties of Biochemical Reactions at Three Temperatures

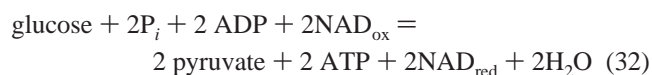
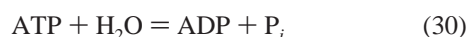
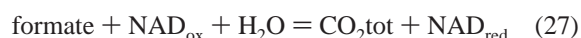
The values of $\Delta_f G'^{\circ}$ and $\Delta_f H'^{\circ}$ in Table 3 can be used to calculate $\Delta_r G'^{\circ}$ and $\Delta_r H'^{\circ}$ for any biochemical reaction between these reactants at pH 7, ionic strength 0.25 mol kg⁻¹, and these three temperatures using

$$\Delta_r G'^{\circ} = \sum \nu_i' \Delta_f G'^{\circ}_i = -RT \ln K' \quad (25)$$

$$\Delta_r H'^{\circ} = \sum \nu_i' \Delta_f H'^{\circ}_i \quad (26)$$

The stoichiometric numbers ν_i' for the biochemical reactions have primes to distinguish them from the stoichiometric numbers of the underlying chemical reactions. These reaction properties and the apparent equilibrium constant K' can also be calculated at any pH in the range 5–9 and ionic strength in the range 0 to about 0.35 mol kg⁻¹ using eqs 8, 9, 11, 12, 13, and 24.

The following enzyme-catalyzed reactions are considered here:



The last reaction is the net reaction for glycolysis, but it can be treated in the same way as the individual enzyme-catalyzed reactions.

The standard transformed Gibbs energies of reaction and standard transformed enthalpies of reaction for these six reactions have been calculated using eqs 25 and 26 at three temperatures at pH 6, 7, 8, and 0.25 mol kg⁻¹ ionic strength. These calculated values are given in Table 4.

Note that $\Delta_r G'^{\circ}$ for reaction 28 does not depend on pH because there is no change in the binding of hydrogen atoms in the reaction. Also note that $\Delta_r H'^{\circ}$ for reactions 28 and 29 do

not depend on pH or temperature because each reactant consists of a single species. The $\Delta_r H'^{\circ}$ values for these reactions at 298.15 K are in good agreement with calorimetric measurements that have been made because the $\Delta_f H'^{\circ}$ values of species are based on these experimental values. There are just two reactions for which K' has been measured as a function of temperature so that $\Delta_r H'^{\circ}$ can be calculated from $d \ln K'/dT$. For reaction 29, Burton¹⁷ obtained 40 kJ mol⁻¹ above pH 7.6. The calculations presented here yield 46 kJ mol⁻¹. For reaction 31, Tewari et al.¹⁸ obtained 0.91 kJ mol⁻¹ for the chemical reference reaction that predominates at high pH, and the calculations presented here yield 0.86 kJ mol⁻¹.

It is of special interest to see how the apparent equilibrium constants for these biochemical reactions depend on temperature and pH, and so apparent equilibrium constants calculated using eq 25 are given in Table 5.

The values of K' are given to three significant figures because an uncertainty of 0.01 kJ mol⁻¹ corresponds with approximately a 1% uncertainty in K' in this temperature range. The effect of temperature and pH on these reactions can be considered from a different point of view. Suppose that K' for a particular reaction is obtained experimentally at a certain T , pH, and ionic strength, and $\Delta_r H'^{\circ}$ is determined calorimetrically under the same conditions. The apparent equilibrium constant at another temperature in the range 283.15–313.25 K can be estimated by assuming that $\Delta_r H'^{\circ}$ is independent of temperature. If $\Delta_r H'^{\circ}$ is independent of temperature, $\Delta_r S'^{\circ}$ is independent of temperature, and $\Delta_r G'^{\circ}(T)$ for the biochemical reaction can be estimated using

$$\Delta_r G'^{\circ}(T) = \Delta_r G'^{\circ}(298.15 \text{ K}) - T\Delta_r S'^{\circ}(298.15 \text{ K}) \quad (33)$$

Substituting the expression for the standard transformed entropy of reaction yields

$$\begin{aligned} \Delta_r G'^{\circ}_i(T) = (T/298.15)\Delta_r G'^{\circ}_i(298.15 \text{ K}) + \\ (1 - T/298.15)\Delta_r H'^{\circ}_i(298.15 \text{ K}) \end{aligned} \quad (34)$$

Calculations with eq 34 have been made by treating values in Table 4 at 298.15 K as experimental data. The results of assuming that the standard transformed enthalpy of reaction is independent of temperature can be compared with the results obtained in Table 5, which are based on the assumption that $\Delta_f H'^{\circ}$ for species are independent of temperature. This has been done, and the values of K' are a little different from the values in Table 5, but only in a couple of cases are these differences greater than 1%. Table 4 shows that when $\Delta_f H'^{\circ}$ values of

TABLE 4: Standard Transformed Gibbs Energies and Standard Transformed Enthalpies of Six Biochemical Reactions at Three Temperatures, pH 6, 7, 9, and $I = 0.25$ Mol kg⁻¹

		$\Delta_r G'^{\circ}/\text{kJ mol}^{-1}$			$\Delta_r H'^{\circ}/\text{kJ mol}^{-1}$		
		283.15 K	298.15 K	313.15 K	283.15 K	298.15 K	313.15 K
Rx 27	pH 6	-15.41	-15.38	-15.39	-16.12	-15.54	-14.92
	pH 7	-19.04	-19.41	-19.81	-12.24	-11.80	-11.32
	pH 8	-24.20	-24.91	-25.63	-11.13	-10.73	-10.25
Rx 28	pH 6	-21.16	-22.33	-23.50	0.78	0.93	1.11
	pH 7	-21.16	-22.33	-23.50	0.78	0.93	1.11
	pH 8	-21.16	-22.33	-23.50	0.78	0.93	1.11
Rx 29	pH 6	28.70	27.80	26.89	45.48	45.77	46.13
	pH 7	23.28	22.10	20.90	45.48	45.77	46.13
	pH 8	17.86	16.39	14.90	45.48	45.77	46.13
Rx 30	pH 6	-32.87	-33.25	-33.63	-25.55	-25.71	-25.91
	pH 7	-35.41	-36.07	-36.72	-23.00	-23.07	-23.17
	pH 8	-40.12	-41.10	-42.08	-21.44	-21.57	-21.73
Rx 31	pH 6	-13.22	-13.75	-14.28	-3.23	-3.27	-3.32
	pH 7	-11.06	-11.62	-12.17	-0.60	-0.55	-0.52
	pH 8	-10.37	-10.96	-11.56	0.84	0.86	0.87
Rx 32	pH 6	57.25	-63.54	-59.90	61.01	62.22	63.70
	pH 7	-73.85	-80.75	-87.70	55.91	56.94	58.21
	pH 8	-86.12	-93.51	-100.96	52.78	53.93	55.33

TABLE 5: Apparent Equilibrium Constants of Six Biochemical Reactions at Three Temperatures, pH 6, 7, and 8, and $I = 0.25$ Mol kg⁻¹ Calculated Using Data from Table 4

		K'		
		283.15 K	298.15 K	313.15 K
Rx 27	pH 6	0.70×10^3	0.50×10^3	0.37×10^3
	pH 7	3.25×10^3	2.52×10^3	2.01×10^3
	pH 8	2.92×10^3	23.1×10^3	18.8×10^3
Rx 28	pH 6	8.02×10^3	8.16×10^3	8.33×10^3
	pH 7	8.02×10^3	8.16×10^3	8.33×10^3
	pH 8	8.02×10^3	8.16×10^3	8.33×10^3
Rx 29	pH 6	5.08×10^{-6}	1.35×10^{-5}	3.27×10^{-5}
	pH 7	5.08×10^{-5}	1.35×10^{-4}	3.27×10^{-4}
	pH 8	5.08×10^{-4}	1.35×10^{-3}	3.27×10^{-3}
Rx 30	pH 6	1.16×10^6	0.67×10^6	0.41×10^6
	pH 7	3.41×10^6	2.08×10^6	1.33×10^6
	pH 8	25.2×10^6	15.9×10^6	10.5×10^6
Rx 31	pH 6	2.75×10^3	2.56×10^2	2.40×10^2
	pH 7	1.10×10^2	1.08×10^2	1.07×10^2
	pH 8	0.82×10^3	0.83×10^2	0.85×10^2
Rx 32	pH 6	3.63×10^{10}	1.36×10^{11}	4.57×10^{11}
	pH 7	4.20×10^{13}	1.40×10^{14}	4.26×10^{14}
	pH 8	7.70×10^{15}	2.41×10^{16}	6.71×10^{16}

species are assumed to be independent of temperature, $\Delta_r H'^{\circ}$ values do depend on temperature. However, the changes in $\Delta_r H'^{\circ}$ are not great, and so the assumption that $\Delta_r H'^{\circ}$ is independent of temperature gives a very good estimate of K' at temperatures in the range 283.15–313.15 K.

Calculation of the Standard Transformed Thermodynamic Properties of Biochemical Reactants at Any Temperature in the Range 273.15–313.15 K

The values of the limiting slopes given in Table 1 make it possible to calculate standard transformed thermodynamic properties of biochemical reactants at these specific temperatures, but fitting the Debye–Hückel constant with a polynomial in temperature makes it possible to calculate the properties of species at any temperature in the range. It is only necessary to fit α because the other limiting slopes can be calculated by differentiation. The use of Fit in Mathematica^R yields¹⁹

$$\alpha = 1.10708 - 1.54508 \times 10^{-3}T + 5.95584 \times 10^{-6}T^2 \quad (35)$$

The coefficient $RT\alpha$ in eq 8 for the standard transformed Gibbs energy of formation of a species is given by

$$RT\alpha = 9.20483 \times 10^{-3}T - 1.28467 \times 10^{-5}T^2 + 4.95199 \times 10^{-8}T^3 \quad (36)$$

This equation reproduced the second column of Table 1 to 0.1% accuracy. The coefficient $RT^2(\partial\alpha/\partial T)_P$ in eq 9 for the standard transformed enthalpy of a species is given by

$$RT^2(\partial\alpha/\partial T)_P = -1.28466 \times 10^{-5}T^2 + 9.90399 \times 10^{-8}T^3 \quad (37)$$

This equation reproduces the third column of Table 1 to 1% accuracy.

The Mathematica^R program (calcdGHT) given in the Appendix was written to calculate the functions of T , pH, and ionic strength that give $\Delta_r G'^{\circ}$ and $\Delta_r H'^{\circ}$ for a reactant at any temperature in the range 273.15–313.15 K, any pH in the range 5–9, and any ionic strength in the range 0–0.35 mol kg⁻¹. The input is a matrix that gives the standard Gibbs energy of formation at 298.15 K, the standard enthalpy of formation at 298.15 K, the electric charge, and the number of hydrogen atoms in each species. There is a row in the matrix for each species in the reactant. Note that the program calcdGHT uses eqs 8 and 11 but does not use eqs 9 and 13 because all the thermodynamic information on a reactant is in eqs 8 and 11. The function for $\Delta_r H'^{\circ}$ for the reactant is obtained using the Gibbs–Helmholtz equation in the form

$$\Delta_r H'_i{}^{\circ}(\text{iso}) = -T^2(\partial(\Delta_r G'_i{}^{\circ}(\text{iso})/T)/\partial T)_P \quad (38)$$

This program has been used with the assignment operator (/.) to recalculate all the values in Tables 2–5, which were calculated as described in the preceding section. There were a few differences of 0.01 kJ mol⁻¹ and even fewer differences of 0.02 kJ mol⁻¹. Thus, the representation of the temperature dependence of α with a quadratic is adequate for these calculations.

Discussion

The standard Gibbs energies of formation of 22 species in dilute aqueous solutions have been estimated at 283.15 and 313.15 K on the basis of the assumption that the standard

TABLE 6: $\text{pH}_a - \text{pH}_c$ as a Function of Ionic Strength and Temperature

$I/(\text{mol kg}^{-1})$	10 °C	25 °C	40 °C
0	0	0	0
0.05	0.082	0.084	0.086
0.1	0.105	0.107	0.110
0.15	0.119	0.122	0.125
0.2	0.130	0.133	0.137
0.25	0.138	0.142	0.146

enthalpies of formation of species are independent of temperature in this range. Since the Debye–Hückel constant and its temperature derivatives are known in this temperature range, the standard transformed Gibbs energies of formation and standard transformed enthalpies of formation of the 14 reactants containing these 22 species have been calculated. This makes it possible to estimate the standard transformed Gibbs energy of reaction, standard transformed enthalpy of reaction, and the apparent equilibrium constant for any biochemical reaction between these 14 reactants at 283.15 and 313.25 K. This has been illustrated with six biochemical reactions.

The thermodynamic properties of these six reactions have also been considered from an experimental point of view. If measurements of K' and $\Delta_r H'^\circ$ are available at 298.15 K for a biochemical reaction at specified pH and ionic strength, values of K' can alternatively be calculated on the basis of the assumption that $\Delta_r H'^\circ$ is independent of temperature. The apparent equilibrium constants for the six reactions at pH 6, 7, and 8 and temperatures of 293.15 and 313.15 K calculated in this way are in good agreement with values calculated on the basis of the assumption that $\Delta_r H'^\circ$ for reactions between species are independent of temperature.

To make it possible to estimate standard transformed thermodynamic properties at any temperature in the range 273.15–313.15 K, the Debye–Hückel constant has been fit to a quadratic in temperature. A program has been written in Mathematica^R to produce the functions of T , pH, and ionic strength that represent $\Delta_r G'^\circ$ and $\Delta_r H'^\circ$ in this range of temperature, any pH in the range 5–9, and any ionic strength in the range 0–0.35 mol kg^{-1} .

Appendix

In biochemical thermodynamics, it is convenient to deal with concentrations of reactants, and so it is convenient to deal with concentrations of hydrogen ions. pH_a is defined as $-\log(\gamma(\text{H}^+)/[\text{H}^+]) = -\log \gamma(\text{H}^+) + \text{pH}_c$, where $\text{pH}_c = -\log[\text{H}^+]$. When the extended Debye–Hückel equation (eq 5) is used

$$\text{pH}_a - \text{pH}_c = \frac{\alpha}{\ln(10)} \frac{I^{1/2}}{1 + BI^{1/2}} \quad (39)$$

Table 6 has been calculated using values of α from Table 1.

These are the adjustments to be subtracted from pH_a obtained with a pH meter to obtain pH_c , which is used in the equations in this article. pH_c is lower than pH_a because the ion atmosphere of H^+ reduces its activity.

calcdGHT[speciesmat_] :=
Module[{dGzero, dGzeroT, dHzero, zi, nH, gibbscoeff, pHterm,
isterm, gpfns, dGfn, dHfn}; (*This program produces the

function of T (in Kelvin), pH and ionic strength (is) that gives the standard transformed Gibbs energy of formation of a reactant (sum of species) and the standard transformed enthalpy. The input speciesmat is a matrix that gives the standard Gibbs energy of formation at 298.15 K, the standard enthalpy of formation at 298.15 K, 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. gpfns is a list of the functions for the transformed Gibbs energies of the species. The output is in the form {dGfn,dHfn}, and energies are expressed in kJ mol⁻¹. The values of the standard transformed Gibbs energy of formation and the standard transformed enthalpy of formation can be calculated at any temperature in the range 273.15 K to 313.15 K, any pH in the range 5 to 9, and any ionic strength in the range 0 to 0.35 m by use of the assignment operator(/.*)
dGzero = speciesmat[[All,1]];
dHzero = speciesmat[[All,2]];
zi = speciesmat[[All,3]];
nH = speciesmat[[All,4]];
gibbscoeff = 9.20483*10⁻³*t-1.284668*10⁻⁵*t² +
4.95199*10⁻⁸*t³;
dGzeroT = dGzero*t/298.15 + dHzero*(1-t/298.15);
pHterm = nH*8.31451*(t/1000)*Log[10^{-pH}];
istermG = gibbscoeff*((zi²) - nH)*(is^{0.5})/(1 + 1.6*is^{0.5});
gpfns = dGzeroT-pHterm-istermG;
dGfn = -8.31451*(t/1000)*Log[Apply[Plus,Exp[-1*gpfns/
(8.31451*(t/1000))]]];
dHfn = -t²*D[dGfn/t,t];
{dGfn,dHfn}

Acknowledgment. I am indebted to Dr. Robert N. Goldberg (NIST) for many helpful discussions. I am indebted to NIH for support of this research by Award 5-R01-GM48358-05.

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