

Changes in the Standard Transformed Thermodynamic Properties of Enzyme-Catalyzed Reactions with Ionic Strength

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The ionic strength has significant effects on the thermodynamic properties of ionic species and on the transformed thermodynamic properties of biochemical reactants at specified pH values. These effects are discussed for species, reactants, and enzyme-catalyzed reactions. This has led to three new thermodynamic properties: $(z_j^2 - N_H(j))$, $(z_j^2 - N_H)_i$, and $\Delta_r(z_j^2 - N_H)$, which are referred to as ionic strength coefficients. The first of these is a property of a species, the second is a property of a reactant, and the third is the property of an enzyme-catalyzed reaction. The effects of ionic strength on standard thermodynamic properties of species, standard transformed thermodynamic properties of reactants, and standard transformed thermodynamic properties of enzyme-catalyzed reactions are proportional to these new thermodynamic properties.

Introduction

Ionic strength does not have a significant effect on the thermodynamic properties of neutral species in dilute aqueous solutions, but the effect on ionic species is significant and is proportional to z_j^2 , the square of the electric charge. According to the extended Debye–Hückel equation the standard Gibbs energy of formation of species j at ionic strength I is given by¹

$$\Delta_f G_j^\circ(I) = \Delta_f G_j^\circ(I=0) - RT \alpha z_j^2 I^{1/2} / (1 + BI^{1/2}) \quad (1)$$

where α is the constant in the Debye equation $\ln \gamma_j = -\alpha z_j^2 I^{1/2}$ and B is an empirical constant that is taken to be independent of temperature.² As an indication of the importance of ionic strength, note that $\Delta_f G_j^\circ(I) - \Delta_f G_j^\circ(I=0)$ for ATP⁴⁻ at 0, 0.05, 0.15, and 0.25 M ionic strength is 0, −7.68, 11.15, and −12.45 kJ mol^{−1}. The standard enthalpy of formation of species j at ionic strength I is obtained by applying the Gibbs–Helmholtz equation ($H = -T^2 \{ \partial(G/T) / \partial T \}$) to eq 1.

$$\Delta_f H_j^\circ(I) = \Delta_f H_j^\circ(I=0) + RT^2 (\partial \alpha / \partial T) z_j^2 I^{1/2} / (1 + BI^{1/2}) \quad (2)$$

The standard entropy of formation of species j at ionic strength I is obtained by applying $S = -(\partial G / \partial T)$ to eq 1.

$$\Delta_f S_j^\circ(I) = \Delta_f S_j^\circ(I=0) + R (\alpha + T \partial \alpha / \partial T) z_j^2 I^{1/2} / (1 + BI^{1/2}) \quad (3)$$

In this article, $-\Delta_f S_j^\circ(I)$ is used because that makes it easier to see the relative contributions of $\Delta_f H_j^\circ(I)$ and $-\Delta_f S_j^\circ(I)$ to $\Delta_f G_j^\circ(I)$.

$$-\Delta_f S_j^\circ(I) = -\Delta_f S_j^\circ(I=0) - RT(\alpha + T \partial \alpha / \partial T) z_j^2 I^{1/2} / (1 + BI^{1/2}) \quad (4)$$

Adding eqs 2 and 4 yields eq 1.

When the pH is specified, the Gibbs energy does not provide the criterion for spontaneous change and equilibrium, and so it is necessary to define a transformed Gibbs energy using the Legendre transform^{3,4}

$$G' = G - n_c(H) \mu(H^+) \quad (5)$$

where $n_c(H)$ is the total amount of hydrogen atoms in the system and $\mu(H^+)$ is the chemical potential of hydrogen ions, which is related to the pH.

The standard transformed Gibbs energy of formation of a species at a specified pH and ionic strength is given by⁵

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

where $N_H(j)$ is the number of hydrogen atoms in the species. When the pH is specified, two terms are added to eq 1 that give the standard Gibbs energy of a species; these terms are both proportional to $N_H(j)$. The standard transformed enthalpy of formation of a species is given by

$$\Delta_f H_j'^\circ(I) = \Delta_f H_j^\circ(I=0) + RT^2 (\partial \alpha / \partial T) (z_j^2 - N_H(j)) I^{1/2} / (1 + BI^{1/2}) \quad (7)$$

When the pH is specified, only one term is subtracted from the right side of eq 2. The standard transformed entropy of formation of a species is given by⁶

$$\Delta_f S_j'^\circ(I) = \Delta_f S_j^\circ(I=0) - N_H(j) R \ln(10) \text{pH} + R(\alpha + T \partial \alpha / \partial T) (z_j^2 - N_H(j)) I^{1/2} / (1 + BI^{1/2}) \quad (8)$$

When the pH is specified, two terms are subtracted from the right side of eq 3 that give the standard entropy of formation of a species; these terms are both proportional to $N_H(j)$. In this article, $-\Delta_f S_j'^\circ(I)$ is used because that makes it easier to see the relative contributions of $\Delta_f H_j'^\circ(I)$ and $\Delta_f S_j'^\circ(I)$ to $\Delta_f G_j'^\circ(I)$. This property of a species is given by

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$$-T\Delta_f S_j^{\circ}(I) = -T\Delta_f S_j^{\circ}(I=0) + N_H(j)RT \ln(10)pH - RT^2(\alpha + T\partial\alpha/\partial T)(z_j^2 - N_H(j))I^{1/2}/(1 + BI^{1/2}) \quad (9)$$

When a biochemical reactant consists of two or more species, the standard transformed Gibbs energy of formation of the reactant is calculated using

$$\Delta_f G_i^{\circ}(I) = -RT \ln \sum \exp(-\Delta_f G_j^{\circ}/RT) \quad (10)$$

where there is a term in the partition function for each species. The corresponding expressions for $\Delta_f H_j^{\circ}(I)$ and $\Delta_f S_j^{\circ}(I)$ can be obtained by taking partial derivatives of $\Delta_f G_i^{\circ}(I)$.

At 298.15 K, Clarke and Glew² give $\alpha = 1.17582 \text{ kg}^{1/2} \text{ mol}^{-1/2}$, $RT\alpha = 2.91482 \text{ kJ mol}^{-3/2} \text{ kg}^{1/2}$, $RT^2(\partial\alpha/\partial T) = 1.4775 \text{ kJ mol}^{-3/2} \text{ kg}^{1/2}$, and $R(\alpha + \partial\alpha/\partial T) = 14.7319 \text{ J mol}^{-3/2} \text{ kg}^{1/2} \text{ K}^{-1}$. The parameter B is taken as 1.6 kg mol^{-1} independent of temperature.¹

Methods

1. Effects of Ionic Strength on Standard Thermodynamic Properties of Ions. The effects of ionic strength on the standard thermodynamic properties of species in dilute aqueous solution are significant. Figure 1 shows the effects on the standard Gibbs energy of formation $\Delta_f G_j^{\circ}(I)$ at 298.15 K for ionic charges of 0, 1, 2, 3, and 4. The corresponding plots for the standard enthalpy of formation $\Delta_f H_j^{\circ}(I)$ are shown in Figure 2.

The effects on the enthalpy make the species less stable at higher ionic strength. The plots for $-T\Delta_f S_j^{\circ}(I)$ are shown in Figure 3. The effects on the entropy make the species more stable at higher ionic strengths. The entropy effects predominate, and so species are more stable at higher ionic strengths, as shown by Figure 1.

It is of interest to calculate the effects of increasing the ionic strength on various properties when there is a small change in ionic strength; in other words, it is of interest to calculate the partial derivatives $\partial\Delta_f G_j^{\circ}/\partial I$, $\partial\Delta_f H_j^{\circ}/\partial I$, and $-T \partial\Delta_f S_j^{\circ}/\partial I$. These derivatives are all proportional to $\partial(I^{1/2}/(1 + 1.6I^{1/2}))/\partial I$, and so this derivative is plotted with respect I in Figure 4. This derivative was calculated using Mathematica,⁷ and it is given by

$$\frac{\partial(I^{1/2}/(1 + 1.6I^{1/2}))}{\partial I} = -\frac{0.8}{(1 + 1.6I^{1/2})^2} + \frac{0.5}{(1 + 1.6I^{1/2})^2 I^{1/2}} \quad (11)$$

Note that this derivative is infinitely large at $I = 0$.

2. Effects of Ionic Strength on the Standard Gibbs Energy of Formation of Aspartate and the Standard Transformed Gibbs Energy of Formation of Aspartate. In the range pH 5–9, aspartate has a single charge. The standard Gibbs energy of formation of this ion is $-695.88 \text{ kJ mol}^{-1}$ at 298.15 K and zero ionic strength,⁸ and so $\Delta_f G_j^{\circ}(I)$ is readily calculated at higher ionic strengths using eq 1. The rate of change of $\Delta_f G_j^{\circ}(I)$ with ionic strength for aspartate can be obtained by multiplying the ordinate in Figure 4 by $-2.91482 \text{ kg mol}^{-3/2} \text{ kg}^{1/2}$.

When the pH is specified, the transformed Gibbs energy of formation $\Delta_f G^{\circ}$ of a species is given by eq 6. This property is a function of the pH and ionic strength, as shown in Figure 5. The effect of ionic strength on the standard transformed Gibbs energy of formation can be misleading because it contains a contribution proportional to $z_j^2 - N_H(j)$, rather than z_j^2 . A lot of

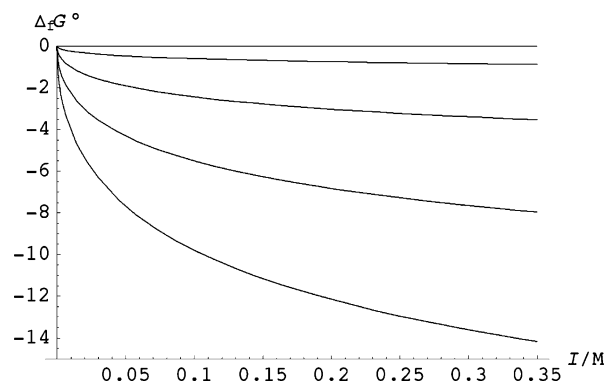


Figure 1. Standard Gibbs energy of formation $\Delta_f G_j^{\circ}(I)$ in kJ mol^{-1} of a species in dilute aqueous solution relative to its value at zero ionic strength at 298.15 K for ionic charges of 0, 1, 2, 3, and 4.

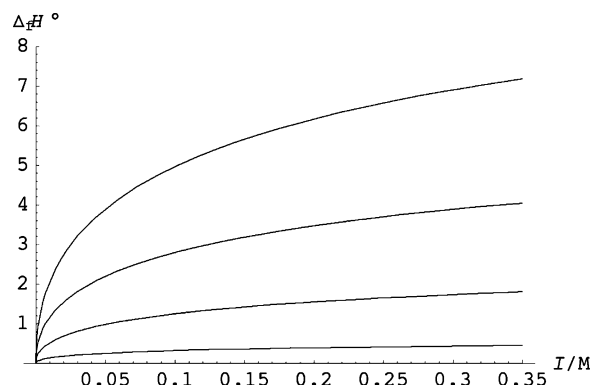


Figure 2. Standard enthalpy of formation $\Delta_f H_j^{\circ}(I)$ in kJ mol^{-1} of a species in dilute aqueous solution relative to its value at zero ionic strength at 298.15 K for ionic charges of 0, 1, 2, 3, and 4.

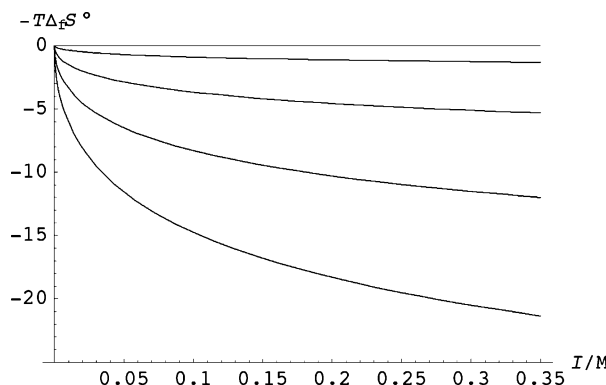


Figure 3. $-T\Delta_f S_j^{\circ}(I)$ in kJ mol^{-1} of a species in dilute aqueous solution relative to its value at zero ionic strength at 298.15 K for ionic charges of 0, 1, 2, 3, and 4.

the contribution of $-N_H(j)$ cancels in an enzyme-catalyzed reaction.

The partial derivative of $\Delta_f G^{\circ}$ with respect to ionic strength (see eq 6) for a species is given by

$$\partial\Delta_f G_j^{\circ}(I)/\partial I = -RT\alpha(z_j^2 - N_H(j)) \partial(I^{1/2}/(1 + 1.6I^{1/2}))/\partial I \quad (12)$$

This derivative is infinite at $I = 0$, and the values at $I = 0.01$, 0.10 , 0.15 , and 0.25 M are 54.15 , 10.16 , 7.17 , and $4.50 \text{ kJ mol}^{-2} \text{ kg}$. If the values of $\Delta_f G_j^{\circ}(I)$ for aspartate ion had been determined experimentally, $z_j^2 - N_H(j)$ could be calculated using

$$z_j^2 - N_H(j) = -\frac{\partial\Delta_f G_j^{\circ}/\partial I}{RT\alpha\partial(I^{1/2}/(1 + 1.6I^{1/2}))/\partial I} \quad (13)$$

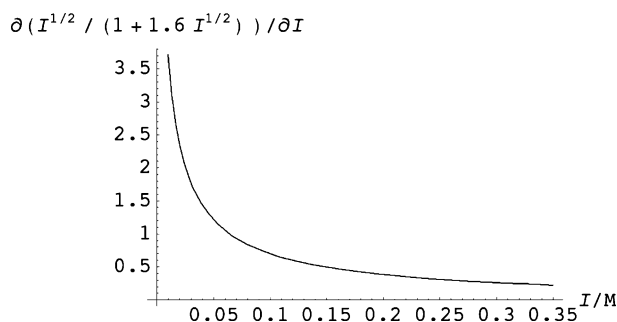


Figure 4. Derivative of $\partial(I^{1/2}/(1 + 1.6I^{1/2}))/\partial I$ with respect to ionic strength.

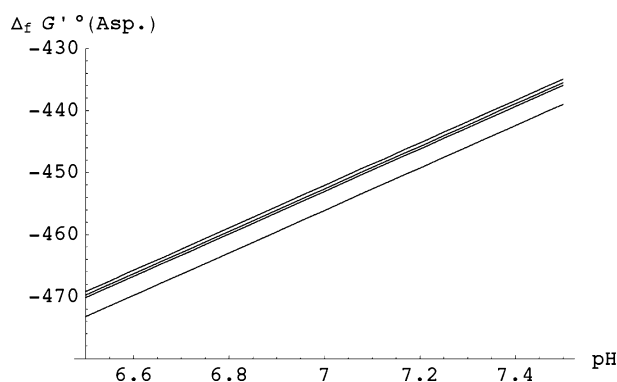


Figure 5. Standard transformed Gibbs energy of aspartate in kJ mol^{-1} at 298.15 K and ionic strengths of 0, 0.10, 0.15, and 0.25 M as a function of pH. The plots are linear in the range pH 5–9.

Calculating the right-hand side of eq 13 for aspartate using Mathematica yields -5 , independent of pH and ionic strength. This is the expected result because $z_j = -1$ and $N_H(j) = 6$. This shows that $z_j^2 - N_H(j)$ is a thermodynamic property of a species in biochemical thermodynamics at a specified pH. It will be referred to as the ionic strength coefficient for species j . The same values of $z_j^2 - N_H(j)$ can be obtained from standard transformed enthalpies of formation (see eq 7) and standard transformed entropies of formation (see eq 8).

The standard transformed Gibbs energy of a species contains a contribution proportional to $z_j^2 - N_H(j)$ so that the calculation of a transformed thermodynamic property of a reaction keeps track of the number of hydrogen atoms in reactants on the two sides of the enzyme-catalyzed reaction. This makes it possible to calculate the change in binding of hydrogen ions $\Delta_r N_H$ in an enzyme-catalyzed reaction. Species with large numbers of hydrogen atoms have large calculated ionic strength effects. When the change in a transformed thermodynamic property of an enzyme-catalyzed reaction is calculated, most of the contributions due to hydrogen atoms cancel. However, when the amounts of hydrogen atoms on the two sides of a biochemical equation do not cancel, the reaction produces or consumes hydrogen ions.

3. Effects of Ionic Strength on the Standard Transformed Gibbs Energy of Formation of Inorganic Phosphate. The standard transformed Gibbs energy of formation of inorganic phosphate at 298.15 K as a function of pH and ionic strength calculated using eq 10 is shown in Figure 6.

Note that the ionic strength effects are larger at pH values above the pK because a doubly charged ion is involved. The partial derivative of $\Delta_f G'^{\circ}(\text{phosphate})$ with respect to the ionic strength is plotted in Figure 7. Calculations at pH values lower than 5 and at pH values higher than 9 show that these plots level off as H_2PO_4^- or HPO_4^{2-} predominate. These limiting values of $\partial\Delta_f G'^{\circ}(I)/\partial I$ can be calculated using eq 12. At

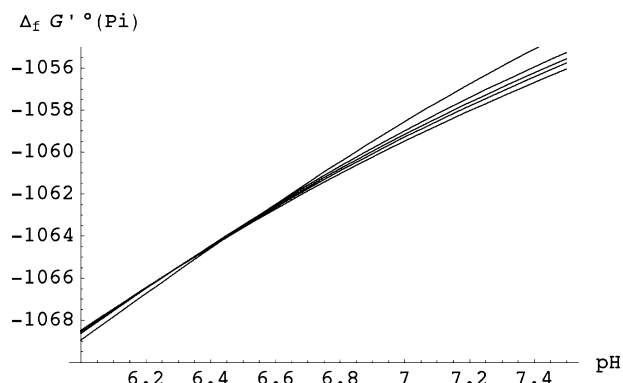


Figure 6. Standard transformed Gibbs energies of formation of inorganic phosphate in kJ mol^{-1} at 298.15 K as a function of pH. Plots are given for $I = 0, 0.05, 0.10, 0.15$, and 0.25 M .

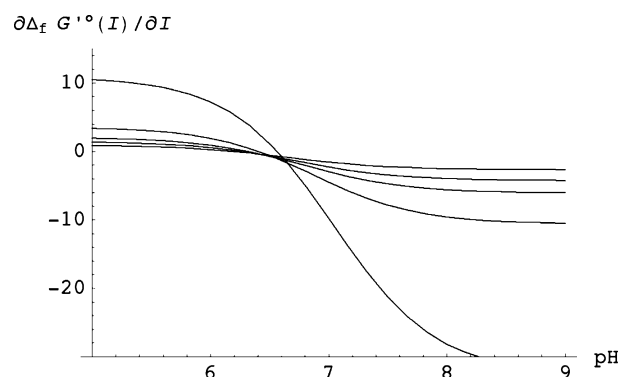


Figure 7. Derivative of $\Delta_f G'^{\circ}(\text{phosphate})$ with respect to the ionic strength as a function of pH at 298.15 K and ionic strengths of 0.01, 0.05, 0.15, and 0.25 M. The units of the derivative are $\text{kJ mol}^{-1} \text{ M}^{-1}$.

intermediate pH values, inorganic phosphate is a mixture of species. The derivative $\partial\Delta_f G'^{\circ}(I)/\partial I$ at $I = 0.25 \text{ M}$ goes from the value $0.90 \text{ kJ mol}^{-1} \text{ M}^{-1}$ at low pH to the value $-2.70 \text{ kJ mol}^{-1} \text{ M}^{-1}$ at high pH. The curves at different ionic strengths intersect in the vicinity of the pK of inorganic phosphate. This pK depends on ionic strength: 7.22 at zero ionic strength, 6.79 at 0.10 M, and 6.65 at 0.25 M.

The derivative of the standard transformed Gibbs energy of formation of inorganic phosphate at 298.15 K and $I = 0.25 \text{ M}$ is given by (see eq 10 and Figure 7)

$$\begin{aligned} \partial\Delta_f G'_i{}^{\circ}(I)/\partial I &= -RT\alpha \sum r_j(z_j^2 - N_H(j))\partial(I^{1/2}/(1 + BI^{1/2}))/\partial I \\ &= -RT\alpha(\overline{z^2 - N_H})_i\partial(I^{1/2}/(1 + BI^{1/2}))/\partial I \end{aligned} \quad (14)$$

where the mole fraction average $(\overline{z^2 - N_H})_i$ for the reactant is referred to as the average ionic strength coefficient for reactant i .

$$(\overline{z^2 - N_H})_i = \sum r_j(z_j^2 - N_H(j)) \quad (15)$$

This is exactly what is to be expected from eq 10. Thus $(\overline{z^2 - N_H})_i$ is a thermodynamic property for a reactant in the same sense that the average number of hydrogen atoms in a reactant, $\overline{N_H}(i)$, is. If $\partial\Delta_f G'_i{}^{\circ}(I)/\partial I$ is determined experimentally, $(\overline{z^2 - N_H})_i$ can be calculated using the following equation:

$$(\overline{z^2 - N_H})_i = -\frac{\partial\Delta_f G'_i{}^{\circ}(I)/\partial I}{RT\alpha\partial(I^{1/2}/(1 + 1.6I^{1/2}))/\partial I} \quad (16)$$

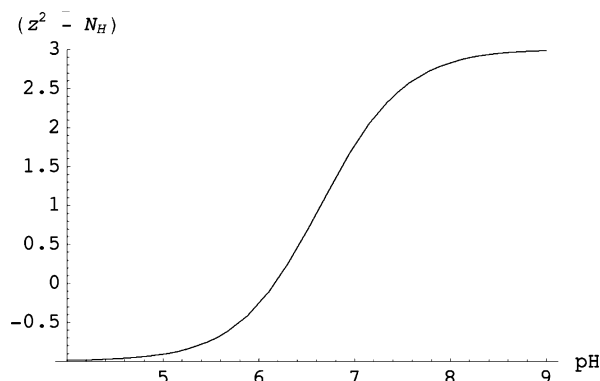


Figure 8. $(z^2 - N_H)$ for inorganic phosphate at 298.15 K and $I = 0.25$ M as a function of pH.

The right-hand side of eq 16 is plotted versus pH in Figure 8 for $I = 0.25$ M.

In the limit of low pH, $(z^2 - N_H) = 1 - 2 = -1$ for H_2PO_4^- and in the limit of high pH, $(z^2 - N_H) = 4 - 1 = 3$ for HPO_4^{2-} . At intermediate pH values, $(z^2 - N_H)$ is a mole fraction weighted average, as required by eq 15.

4. Effects of Ionic Strength on the Standard Transformed Gibbs Energy of the Reaction for $\text{ATP} + \text{H}_2\text{O} = \text{ADP} + \text{P}_i$. The functions of pH and ionic strength that give $\Delta_r G_i'^{\circ}(I)$ at 298.15 K for 199 reactants are given⁸ in BasicBiochemData3. These functions can be added and subtracted to obtain $\Delta_r G'^{\circ}$ for more than 200 enzyme-catalyzed reactions.⁹ These functions for $\Delta_r G'^{\circ}$ can be differentiated with respect to I to obtain the change in the average ionic strength coefficient in an enzyme-catalyzed reaction $\Delta_r(z^2 - N_H)$. This statement is based on the following five equations. The standard transformed Gibbs energy of reaction for an enzyme-catalyzed reaction is given by

$$\Delta_r G'^{\circ} = \sum \nu_i' \Delta_r G_i'^{\circ} \quad (17)$$

where ν_i' is the stoichiometric number for reactant i . The prime is needed to distinguish ν_i' from the stoichiometric numbers of the underlying chemical reactions. Differentiating this equation with respect to ionic strength yields

$$\partial \Delta_r G'^{\circ}(I) / \partial I = \sum \nu_i' \partial \Delta_r G_i'^{\circ}(I) / \partial I \quad (18)$$

Substituting eq 14 yields

$$\begin{aligned} \partial \Delta_r G'^{\circ}(I) / \partial I &= -RT\alpha(\partial(I^{1/2}/(1 + BI^{1/2}))/\partial I) \sum \nu_i' \overline{(z^2 - N_H)}_i \\ &= -RT\alpha(\partial(I^{1/2}/(1 + BI^{1/2}))/\partial I) \Delta_r \overline{(z^2 - N_H)} \end{aligned} \quad (19)$$

where the change in average ionic strength coefficient in an enzyme-catalyzed reaction $\Delta_r(z^2 - N_H)$ is given by

$$\Delta_r \overline{(z^2 - N_H)} = \sum \nu_i' \overline{(z^2 - N_H)}_i \quad (20)$$

Dividing $\partial \Delta_r G'^{\circ}(I) / \partial I$ in eq 19 by $-RT\alpha(\partial(I^{1/2}/(1 + BI^{1/2}))/\partial I)$ yields the change in average ionic strength coefficient in an enzyme-catalyzed reaction.

$$\Delta_r \overline{(z^2 - N_H)} = - \frac{\partial \Delta_r G'^{\circ} / \partial I}{RT\alpha(\partial(I^{1/2}/(1 + BI^{1/2}))/\partial I)} \quad (21)$$

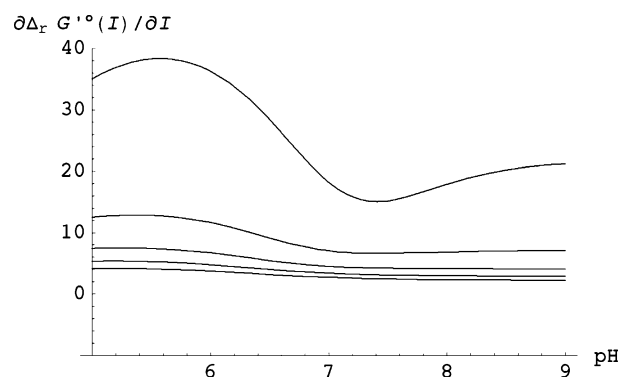


Figure 9. Partial derivative of $\Delta_r G'^{\circ}(\text{ATP} + \text{H}_2\text{O} = \text{ADP} + \text{P}_i)$ with respect to the ionic strength as a function of pH at 298.15 K and ionic strengths of 0.01, 0.05, 0.15, and 0.25 M. The units of the derivative are $\text{kJ mol}^{-1} \text{M}^{-1}$.

TABLE 1: Five Transformed Thermodynamic Properties of the Reaction $\text{ATP} + \text{H}_2\text{O} = \text{ADP} + \text{P}_i$ at 298.15 K, Four Ionic Strengths, and Five pH Values

	pH 5	pH 6	pH 7	pH 9	pH 10
$\Delta_r G'^{\circ} / \text{kJ mol}^{-1}$					
$I = 0$	-35.40	-35.91	-37.60	-42.50	-48.39
$I = 0.05$	-33.77	-34.30	-36.78	-41.74	-47.36
$I = 0.15$	-32.98	-33.59	-36.31	-41.31	-46.93
$I = 0.25$	-32.56	-33.22	-36.04	-41.07	-46.70
K'					
$I = 0$	1.53×10^6	1.96×10^6	3.87×10^6	2.79×10^7	2.89×10^8
$I = 0.05$	8.24×10^5	1.02×10^6	2.77×10^6	2.05×10^7	1.98×10^8
$I = 0.15$	6.00×10^5	7.68×10^5	2.29×10^6	1.72×10^7	1.66×10^8
$I = 0.25$	5.07×10^5	6.66×10^5	2.06×10^6	1.57×10^7	1.52×10^8
$\Delta_r N_H$					
$I = 0$	-0.15	-0.12	-0.58	-1.01	-1.00
$I = 0.05$	-0.06	-0.19	-0.71	-0.96	-1.00
$I = 0.15$	-0.04	-0.23	-0.73	-0.96	-1.00
$I = 0.25$	-0.04	-0.25	-0.74	-0.96	-1.00
$\partial \Delta_r G'^{\circ}(I) / \partial I / \text{kJ mol}^{-1} \text{M}^{-1}$					
$I = 0.01$	34.99	36.29	18.18	17.85	21.16
$I = 0.05$	12.44	11.61	7.00	6.82	7.04
$I = 0.15$	5.30	4.73	3.35	2.93	2.88
$I = 0.25$	3.38	3.03	2.23	1.86	1.81
$\Delta_r(z^2 - N_H)$					
$I = 0.01$	-3.23	-3.35	-1.68	-1.65	-1.95
$I = 0.05$	-3.52	-3.28	-1.98	-1.93	-1.99
$I = 0.15$	-3.70	-3.30	-2.34	-2.04	-2.00
$I = 0.25$	-3.76	-3.37	-2.48	-2.07	-2.00

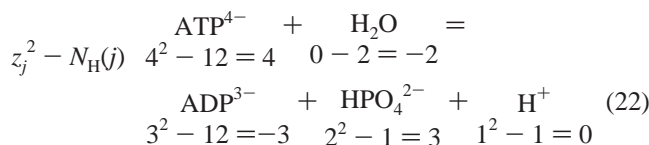
This property can be obtained experimentally by determining the effect of ionic strength on the apparent equilibrium constant K' . It is related to $\overline{(z^2 - N_H)}_i$ of the reactants by eq 20. Thus if $\partial \Delta_r G'^{\circ}(I) / \partial I$ is determined experimentally and $\overline{(z^2 - N_H)}_i$ is known for all the reactants but one, $\overline{(z^2 - N_H)}_i$ of this one reactant can be calculated using eq 20.

Figure 9 gives the partial derivative of $\Delta_r G'^{\circ}$ with respect to ionic strength for the reaction $\text{ATP} + \text{H}_2\text{O} = \text{ADP} + \text{P}_i$ as a function of pH at 298.15 K and ionic strengths of 0.01, 0.05, 0.10, and 0.25 M.

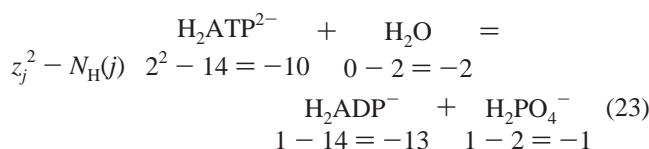
The effects of ionic strength and pH on the transformed thermodynamic properties of the hydrolysis of ATP to ADP calculated⁸ using BasicBiochemData3 are shown in Table 1.

The value of $\partial \Delta_r G'^{\circ}(I) / \partial I = 2.23$ at pH 7 and 0.25 M ionic strength can be used as follows to estimate the effect of an increase in ionic strength of 0.10 M: $\Delta(\Delta_r G'^{\circ}) / \Delta \text{pH} = 2.23 \text{ kJ mol}^{-1}$ so that $\Delta(\Delta_r G'^{\circ}) = 0.22 \text{ kJ mol}^{-1}$. This is an approximate calculation because $\partial \Delta_r G'^{\circ}(I) / \partial I$ changes with ionic strength.

At very high pH values, $\Delta_r(z^2 - N_H)$ of an enzyme-catalyzed reaction has a simple interpretation. In the limit of high pH the value of the change in the average ionic strength coefficient $\Delta_r(z^2 - N_H)$ for the hydrolysis of ATP can be calculated as follows:



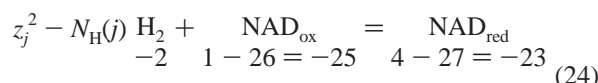
The change in the reaction is $-3 + 3 - (4 - 2) = -2$, as shown in Table 1. In the limit of low pH the value of the change in the average ionic strength coefficient $\Delta_r(z^2 - N_H)$ for the hydrolysis of ATP can be calculated as follows:



The change in the reaction is $-14 - (-12) = -2$. The values of $\Delta_r(z^2 - N_H)$ for $\text{ATP} + \text{H}_2\text{O} = \text{ADP} + \text{P}_i$ at 298.15 K, $I = 0.25$ M, and pH 3, 4, 5, 6, 7, 8, and 9 are -2.22 , -3.11 , -3.76 , -3.37 , -2.48 , -2.07 , and -2.00 were calculated using eq 21.

5. Effects of Ionic Strength on the Standard Transformed Gibbs Energies of a Variety of Enzyme-Catalyzed Reactions. The transformed thermodynamic properties shown in Table 1 for the hydrolysis of ATP can also be calculated at 298.15 K for more than 200 enzyme-catalyzed reactions⁸ on the basis of data in BasicBiochemData3. These properties are given in Table 2 for eight reactions,¹⁰ but only at 0.25 M.

In the nitrite reductase reaction, there is a big consumption of hydrogen ions, and so $\Delta_r G^\circ$ changes rapidly with pH. $\partial \Delta_r G^\circ(I)/\partial I$ and $\Delta_r(z^2 - N_H)$ are about 10 times greater than those for ATP hydrolysis. For the sulfite reductase reaction, the consumption of hydrogen ions is even larger, and $\partial \Delta_r G^\circ(I)/\partial I$ and $\Delta_r(z^2 - N_H)$ are about twice as large as for ATP hydrolysis. For the hydrogen hydrogenase reaction, $\partial \Delta_r G^\circ(I)/\partial I$, $\Delta_r N_H$, and $\Delta_r(z^2 - N_H)$ do not depend on pH. The value of $\Delta_r(z^2 - N_H)$ can be calculated as follows:



The change in the reaction is $\Delta_r(z^2 - N_H) = -23 - (-27) = 4$ independent of pH, as shown in Table 2.

For the pyruvate phosphate dikinase reaction, $\partial \Delta_r G^\circ(I)/\partial I$ changes rapidly with pH. For the fructose-bisphosphate reaction, $\partial \Delta_r G^\circ(I)/\partial I$ is about four times larger than that for ATP hydrolysis. These effects can probably be studied experimentally for EC 2.7.9.1 and EC 3.1.3.11, which do not have very large apparent equilibrium constants.

Discussion

Consideration of the effect of ionic strength on thermodynamic properties of species, reactants (sums of species), and enzyme-catalyzed reactions has led to three new thermodynamic properties: $(z_j^2 - N_{H(j)})$, $(z^2 - N_H)_i$, and $\Delta_r(z^2 - N_H)$. The first of

TABLE 2: Thermodynamic Properties for a Variety of Enzyme-Catalyzed Reactions at 298.15 K and 0.25 M Ionic Strength*

	pH 5	pH 6	pH 7	pH 8	pH 9
EC 1.7.1.4 Nitrite Reductase (NADP) Nitrite + 3NADP _{red} = Ammonia + 3NAD _{ox} + 2H ₂ O					
$\Delta_r G^\circ$	-434.10	-405.60	-377.10	-348.70	-321.10
$\Delta_r N_H$	4.99	5.00	4.99	4.95	4.64
$\partial \Delta_r G^\circ(I)/\partial I$	23.38	23.39	23.39	23.39	23.39
$\Delta_r(z^2 - N_H)$	-25.98	-26.00	-26.00	-26.00	-26.00
EC 1.8.7.1 Sulfite Reductase (ferredoxin) Sulfite + 3ferredoxin _{red} = H ₂ S + 3ferredoxin _{ox} + 3H ₂ O					
$\Delta_r G^\circ$	-122.00	-82.00	-41.92	-1.89	38.07
$\Delta_r N_H$	7.00	7.01	7.02	7.00	7.00
$\partial \Delta_r G^\circ(I)/\partial I$	4.54	4.84	5.77	6.23	6.29
$\Delta_r(z^2 - N_H)$	-505	-5.38	-6.41	-6.92	-6.99
EC 1.12.1.2 Hydrogen Hydrogenase H ₂ + NAD _{ox} = NAD _{red}					
$\Delta_r G^\circ$	-26.73	-32.44	-38.14	-43.85	-49.56
$\Delta_r N_H$	-1.00	-1.00	-1.00	-1.00	-1.00
$\partial \Delta_r G^\circ(I)/\partial I$	-3.60	-3.60	-3.60	-3.60	-3.60
$\Delta_r(z^2 - N_H)$	4.00	4.00	4.00	4.00	4.00
EC 2.7.1.2 Glucokinase ATP + Glucose = ADP + Glucose 6-Phosphate					
$\Delta_r G^\circ$	-17.41	-19.47	-24.42	-30.11	-35.82
$\Delta_r N_H$	-0.14	-0.65	-0.98	-1.00	-1.00
$\partial \Delta_r G^\circ(I)/\partial I$	3.02	1.59	1.36	1.73	1.79
$\Delta_r(z^2 - N_H)$	-3.36	-1.77	-1.51	-1.93	-1.99
EC 2.7.9.1 Pyruvate, Phosphate Dikinase AMP + Phosphoenolpyruvate + PP _i = ATP + Pyruvate + P _i					
$\Delta_r G^\circ$	-27.52	-24.47	-17.54	-10.36	-1.20
$\Delta_r N_H$	0.21	0.97	1.26	1.36	1.83
$\partial \Delta_r G^\circ(I)/\partial I$	-0.63	2.57	3.36	4.30	7.76
$\Delta_r(z^2 - N_H)$	0.70	-2.86	-3.74	-4.78	-8.63
EC 3.1.3.11 Fructose-bisphosphatase Fructose 1,6-Bisphosphate + H ₂ O = Fructose 6-Phosphate + P _i					
$\Delta_r G^\circ$	-20.21	-16.26	-12.79	-11.95	-11.84
$\Delta_r N_H$	0.37	0.84	0.32	0.04	0.00
$\partial \Delta_r G^\circ(I)/\partial I$	4.33	9.17	8.25	7.35	7.21
$\Delta_r(z^2 - N_H)$	-4.81	-10.19	-9.17	-8.17	-8.02
EC 3.5.1.5 Urease Urea + 2H ₂ O = CO _{2tot} + 2Ammonia					
$\Delta_r G^\circ$	-47.67	-37.55	-30.19	-24.51	-21.06
$\Delta_r N_H$	1.92	1.55	1.10	0.89	0.14
$\partial \Delta_r G^\circ(I)/\partial I$	-0.14	-0.81	-1.61	-1.84	-2.33
$\Delta_r(z^2 - N_H)$	0.15	0.91	1.79	2.04	2.59
EC 6.4.1.6 Acetone Carboxylase Acetone + CO _{2tot} + ATP = Acetoacetate + AMP + 2P _i					
$\Delta_r G^\circ$	-207.4	-201.8	-197.80	-196.60	-196.10
$\Delta_r N_H$	0.99	0.93	0.41	0.08	0.15
$\partial \Delta_r G^\circ(I)/\partial I$	6.03	6.14	5.36	4.69	5.04
$\Delta_r(z^2 - N_H)$	-6.71	-6.82	-5.96	-5.22	-5.61

* $\Delta_r G^\circ$ is in kJ mol⁻¹ and $\partial \Delta_r G^\circ(I)/\partial I$ is in kJ mol⁻¹ M⁻¹.

these is a property of a species, the second is a property of a reactant, and the third is the property of an enzyme-catalyzed reaction. The effects of ionic strength on standard thermodynamic properties of species, standard transformed thermodynamic properties of reactants, and standard transformed thermodynamic properties of enzyme-catalyzed reactions are proportional to these new properties. The discussion here has been restricted to the standard Gibbs energy of formation of species, the standard transformed Gibbs energies of reactants at specified pH, and the standard transformed Gibbs energies of enzyme-catalyzed reactions, but they apply as well to the corresponding enthalpy and entropy functions. Similar treatments can be made on the effects of pMg. All the calculations

in this article are for 298.15 K, but for about 50 enzyme-catalyzed reactions,⁹ calculations can be made for any temperature in the range 273.15 K to about 313.15 K.

The extended Debye–Hückel equation involves an empirical function with a single constant that is independent of temperature. More accurate equations have been developed,¹¹ but they depend on empirical constants determined for specific ions that may be present. In general these specific effects become significant at ionic strengths at higher concentrations than the physiological range. However, the calculations described here can be carried out with more complicated equations than eq 1.

These calculations emphasize the need for specification of ionic strength in reporting apparent equilibrium constants of enzyme-catalyzed reactions. In the literature investigators have too often not given enough information to make it possible to calculate the ionic strength.

Glossary

B	coefficient in the extended Debye–Hückel equation
G	Gibbs energy of a system
G'	transformed Gibbs energy of a system
$\Delta_f G_j^\circ$	standard Gibbs energy of formation of species j
$\Delta_f G_i'^\circ$	standard transformed Gibbs energy of formation of reactant i
$\Delta_r G'^\circ$	standard transformed Gibbs energy of reaction
$\Delta_f H_j^\circ$	standard enthalpy of formation of species j
$\Delta_f H_i'^\circ$	standard transformed enthalpy of formation of reactant i
$\Delta_r H'^\circ$	standard transformed enthalpy of reaction
K'	apparent equilibrium constant
$n_c(\text{H})$	amount of the hydrogen component (amount of hydrogen atoms)
$n_c(\text{Mg})$	amount of the magnesium component (amount of magnesium atoms)
$N_{\text{H}}(j)$	number of hydrogen atoms in species j
$\bar{N}_{\text{H}}(i)$	average number of hydrogen atoms in reactant i
$\Delta_r N_{\text{H}}$	change in the binding of hydrogen ions in a biochemical reactant

pH	$-\log[\text{H}^+]$
pMg	$-\log[\text{Mg}^{2+}]$
pK	$-\log(\text{acid dissociation constant})$
r_j	equilibrium mole fraction of species j in a reactant
$\Delta_f S_j^\circ$	standard entropy of formation of species j
$\Delta_f S_i'^\circ$	standard transformed entropy of formation of reactant i
$\Delta_r S'^\circ$	standard transformed entropy of reaction
z_j	electric charge of species j
$(z_j^2 - N_{\text{H}}(j))$	ionic strength coefficient for species j
$(z^2 - \bar{N}_{\text{H}})_i$	average ionic strength coefficient for reactant i
$\Delta_r(z^2 - \bar{N}_{\text{H}})$	change in average ionic strength coefficient in an enzyme-catalyzed reaction
α	coefficient in the Debye equation
ν_i'	stoichiometric number of reactant i in an enzyme-catalyzed reaction

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