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Calculation of transformed thermodynamic properties of biochemical reactants at specified pH and pMg

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Abstract

The fundamental equation of thermodynamics for the transformed Gibbs energy G' at specified pH and pMg is used to derive the expressions for the standard transformed Gibbs energy of formation $\Delta_f G_i'^0$ of a reactant, its standard transformed enthalpy of formation $\Delta_f H_i'^0$, and its standard transformed entropy of formation $\Delta_f S_i'^0$. The standard transformed Gibbs energy of reaction $\Delta_r G'^0$, standard transformed enthalpy of reaction $\Delta_r H'^0$, and standard transformed entropy of reaction $\Delta_r S'^0$ can be written as functions of pH and pMg in terms of the standard Gibbs energies of formation $\Delta_f G_i^0$, standard enthalpies of formation $\Delta_f H_i^0$, and standard entropies of formation $\Delta_f S_i^0$ of the species involved. The expressions for $\Delta_r G'^0$, $\Delta_r H'^0$, and $\Delta_r S'^0$ can be rearranged to the usual forms involving a reference reaction. The standard transformed formation properties are calculated for glucose 6-phosphate (G6P) at 298.15 K, 1 bar, pH 7, pMg 3, and $I = 0.25$ *M*.

Keywords: Fundamental equation of thermodynamics; Legendre transform; Standard transformed Gibbs energy of formation; Standard transformed enthalpy of formation; Glucose 6-phosphate

1. Introduction

The Gibbs energy G provides the criterion for equilibrium at specified T and P since $(\mathrm{d}G)_{T,P} \leq 0$. However, other choices are possible for independent variables for a system involving biochemical reactions; for example, the concentration of a reactant can be specified at equilibrium. Alberty and Oppenheim [1] showed how a transformed Gibbs energy G' can be defined for the alkylation of benzene with ethylene at a specified partial pressure of ethylene. In the biochemical situation, where H^+ and Mg^{2+} are reactants, Alberty [2] showed that a transformed Gibbs energy G' can be defined by

$$G' = G - \tilde{n}_{H^+}\mu_{H^+} - \tilde{n}_{Mg^{2+}}\mu_{Mg^{2+}} \tag{1}$$

where μ_{H^+} and $\mu_{Mg^{2+}}$ are the specified chemical potentials of H^+ and Mg^{2+} at equilibrium and $\tilde{n}_{H^+}$ and $\tilde{n}_{Mg^{2+}}$ are the total amounts of these ions (free and bound) in the system. When pH and pMg are

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specified, the transformed Gibbs energy G' provides the criterion of equilibrium at specified T, P, μ_{H^+}, and $\mu_{Mg^{2+}}$ or T, P, pH, and pMg: $(dG')_{T,P,pH,pMg} \leq 0$. This is the justification for the use of the apparent equilibrium constant K' at specified pH and pMg that is written in terms of sums of species at each reactant [3]. The example considered here is the hydrolysis of glucose 6-phosphate (G6P) to glucose (Glu) and inorganic phosphate (P_i), which is catalyzed by glucose-6-phosphatase (EC 3.1.3.9) and alkaline phosphatase (EC 3.1.3.1).

$$\mathrm{G6P} + H_2O = \mathrm{Glu} + P_i \qquad K' = [\mathrm{Glu}][P_i]/[\mathrm{G6P}] \tag{2}$$

$$\Delta_r G'^0 = -RT \ln K' = \Delta_r H'^0 - T\,\Delta_r S'^0 \tag{3}$$

where the brackets represent molar concentrations. The apparent equilibrium constant K' should be dimensionless, and so there should be a $c^0 = 1$ M in the denominator, but this factor will be left out here and in other equilibrium constant expressions here to simplify the notation. The apparent equilibrium constant K' is dependent on T, P, pH, pMg, and I (ionic strength), and so these should be specified. The pressure does not need to be specified if it is approximately 1 bar. The standard transformed Gibbs energy of reaction $\Delta_r G'^0$, the standard transformed enthalpy of reaction $\Delta_r H'^0$, and the standard transformed entropy of reaction $\Delta_r S'^0$ are written with the prime before the superscript zero (indicating that the changes are for the separated reactants in standard states of 1 M going to separated products in standard states) to emphasize that G', H', and S' are new thermodynamic properties. Since they are new, quite different symbols could have been used. Waldram [4] emphasized this by using Φ_G for the transformed Gibbs energy and Φ_H for the transformed enthalpy; specifically, $\Phi_G = G - \mu n$ and $\Phi_H = H - \mu n$, where n is amount.

The standard transformed reaction quantities can be written in terms of standard transformed formation properties of the reactants.

$$\Delta_r G'^0 = \Sigma \nu_i \,\Delta_f G_i'^0 \tag{4}$$

$$\Delta_r H'^0 = \Sigma \nu_i \,\Delta_f H_i'^0 \tag{5}$$

$$\Delta_r S'^0 = \Sigma \nu_i \,\Delta_f S_i'^0 \tag{6}$$

The summations are carried out over reactants, such as G6P, H_2O, Glu, and P_i in reaction (2), rather than ionic and neutral species. The stoichiometric numbers ν_i are taken as positive for products and negative for reactants. Thermodynamic information on biochemical reactions is currently stored as $\Delta_r G'^0$ and $\Delta_r H'^0$ values at specified T, P, pH, pMg, and I. The corresponding values of $\Delta_r S'^0$ can be calculated from $\Delta_r S'^0 = (\Delta_r H'^0 - \Delta_r G'^0)/T$. However, there are advantages in tabulating the formation properties $\Delta_f G_i'^0$ and $\Delta_f H_i'^0$ for individual reactants, like G6P, at specified T, P, pH, pMg, and I so that $\Delta_r G'^0$, $\Delta_r H'^0$, and $\Delta_r S'^0$ can be calculated by looking up reactants in a table.

The previous paper [2] used glycolysis to show how standard transformed Gibbs energies of formation of biochemical reactants at specified pH and pMg can be obtained by assigning some zeros to reference reactants as necessary because of lack of data to connect their properties with those of the elements. However, when enough information is available on the acid dissociations and complex ion dissociations of a reactant and $\Delta_r G'^0$ and $\Delta_r H'^0$ are available for its production from reactants with known properties, the formation properties of the reactant can be obtained without assigning any zeros. The standard transformed formation properties of inorganic phosphate (P_i) at pH 7 and pMg 3 were calculated in the previous paper. Since the formation properties for glucose in dilute aqueous solution are known, and since $\Delta_r G'^0$ for reaction (2) has been determined by Lawson and Veech [5] and $\Delta_r H'^0$ for reaction 2 has been determined by Tewari et al. [6], the formation properties are calculated here for G6P at pH 7, pMg 3, $I = 0.25$ M, and 298.15 K. All concentrations are on the molar (mol L^{-1}) scale.

The previous paper dealt with the transformed Gibbs energy, but here the discussion is extended to include the transformed entropy S' and the transformed enthalpy H'.

2. Calculation of the transformed entropy S' and transformed enthalpy H' of a reaction system at specified pH and pMg

In the previous paper [2], the fundamental equation of thermodynamics for G' was written without the terms $-S\mathrm{d}T + V\mathrm{d}P$, but now we are interested in calculating the transformed entropy, and so the usual entropy term has to be included. The complete fundamental equation for the transformed Gibbs energy G' is

$$\mathrm{d}G' = -S\,\mathrm{d}T + V\,\mathrm{d}P - \bar{n}_{\mathrm{H}^+}\,\mathrm{d}\mu_{\mathrm{H}^+} - \bar{n}_{\mathrm{Mg}^{2+}}\,\mathrm{d}\mu_{\mathrm{Mg}^{2+}} + \Sigma\tilde{\mu}_i\,\mathrm{d}n_i \tag{7}$$

In this equation, $\tilde{\mu}_i$ is the transformed chemical potential of reactant i at the specified T, P, pH, pMg, and I. This is a very important equation because it provides the means for calculating the equilibrium composition and the transformed entropy S' (defined below) and the transformed enthalpy H' (defined below) of the system at a specified pH and pMg. The summation in eq. (7) contains a term for each species in the system, except H^+ and Mg^{2+}. In the previous paper this equation was applied to the hydrolysis of ATP, and it was pointed out that at specified pH and pMg, the species ATP^{4-}, $HATP^{3-}$, and $MgATP^{-2}$ are *pseudoisomers*. These three terms in the summation for ATP can be added together since the pseudoisomers have the same transformed chemical potential at equilibrium. Here we want to consider that in more detail so that we can see how standard thermodynamic properties can be calculated for the pseudoisomer group when $\Delta_f G^0$ and $\Delta_f H^0$ of the individual species are known from previous research, and when they are not. But first we want to see how the transformed entropy S' and transformed enthalpy H' are to be calculated at specified pH and pMg.

Equation (7) is written in terms of the equilibrium chemical potentials μ of H^+ and Mg^{2+}, but is more useful to write it in terms of pH and pMg, which are defined here as $-\log[H^+]$ and $-\log[Mg^{2+}]$, respectively. (See Appendix A for a discussion of the effect of using the definition $\mathrm{pH} = -\log a_{\mathrm{H}^+}$.) IN general the chemical potential of a species has to be written in terms of a concentration multiplied by an activity coefficient, but here we will assume that the ionic strength is held constant and that the solutions are ideal so that the chemical potential of species B_i is given by

$$\mu_i = \mu_i^0 + RT\,\ln[B_i] \tag{8}$$

where μ_i^0 is the standard chemical potential of B_i at the specified ionic strength and $[B_i]$ is its molar concentration. In order for eq. (8) to be as good an approximation as possible, the concentrations of biochemical reactants are kept low. In order to discuss chemical equilibrium at specified pH and pMg, we have to take into account the fact that μ_{H^+} and $\mu_{\mathrm{Mg}^{2+}}$ are functions of T and the concentrations of these ions according to eq. (8). The process for doing this is described by Alberty and Oppenheim [7]. For H^+,

$$\mathrm{d}\mu_{\mathrm{H}^+} = \{\partial\mu_{\mathrm{H}^+}/\partial T\}_{[\mathrm{H}^+]}\mathrm{d}T + \{\partial\mu_{\mathrm{H}^+}/\partial[\mathrm{H}^+]\}_T\,\mathrm{d}[\mathrm{H}^+] \tag{9}$$

The effect of pressure is ignored here because pressure is little used as an independent variable in biochemistry. A similar equation can be written for Mg^{2+}. Taking the indicated derivatives of eq. (8) yields

$$\mathrm{d}\mu_{\mathrm{H}^+} = -\{\bar{S}^0_{\mathrm{H}^+} - R\,\ln[\mathrm{H}^+]\}\,\mathrm{d}T - 2.303\,RT\,\mathrm{d\,pH} \tag{10}$$

where $\bar{S}^0_{H^+} - R\ \ln[H^+]$ is the molar entropy $\bar{S}$ of hydrogen ion at the specified pH. Substituting this equation and the corresponding equation for Mg^{2+} into eq. (7) yields

$$dG' = -S'\ dT + V\ dP + 2.303\bar{n}_{H^+}RT\ d\ pH + 2.303\bar{n}_{Mg^{2+}}RT\ d\ pMg + \Sigma\tilde{\mu}_i\ dn_i \tag{11}$$

where

$$S' = S - \bar{n}_{H^+}\{\bar{S}^0_{H^+} - R\ \ln[H^+]\} - \bar{n}(Mg^{2+})\{\bar{S}^0(Mg^{2+}) - R\ \ln[Mg^{2+}]\} \tag{12}$$

Thus the transformed entropy S' of the system is equal to the usual entropy S of the system (which can be written in terms of the standard molar entropies of the species and their equilibrium mole fractions) minus adjustments for the entropy of H^+ at its specified concentration and for the entropy of Mg^{2+} at its specified concentration. These adjustments involve the total amount $\bar{n}(H^+)$ of dissociable H^+ (free and bound) and the total amount $\bar{n}(Mg^{2+})$ of dissociable Mg^{2+} (free and bound) in the system.

At specified pH and pMg, eq. (11) becomes

$$(dG')_{pH,pMg} = -S'\ dT + V\ dP + \Sigma\tilde{\mu}_i\ dn_i \tag{13}$$

This equation is written in terms of species i. It can be applied to a system with many species and many enzyme-catalyzed reactions. The number of terms can be reduced by combining the terms for the various species of each reactant. For example, the terms for $G6P^{2-}$, $HG6P^-$, and MgG6P can be combined at equilibrium since these species are pseudoisomers at specified pH and pMg; that is, their relative concentrations are a function of only temperature under these conditions. As shown earlier [2], the transformed chemical potentials of these species are equal at equilibrium and can be represented by $\tilde{\mu}$(G6P), the chemical potential of the reactant glucose 6-phosphate. Thus eq. (13) can be written

$$(dG')_{pH,pMg} = -S'\ dT + V\ dP + \Sigma\tilde{\mu}_i\ d\tilde{n}_i \tag{14}$$

This equation is written in terms of reactants like G6P, and $\tilde{n}_i$ is the sum of the amounts of species of reactant i. This summation includes H_2O when it is a reactant.

When a system contains many enzyme-catalyzed reactions, extents of reaction for an independent set of reactions can be substituted in eq. (14). Rather than discussing a complicated system here, we will choose a single reaction like reaction (2). When the extent ξ of this reaction at constant T, P, pH, and pMg is introduced into eq. (14), it can be written

$$(dG'/d\xi)_{T,P,pH,pMg} = \Delta_r G' = \Sigma\nu_i\tilde{\mu}_i \tag{15}$$

Substituting

$$\tilde{\mu}_i = \tilde{\mu}^0_i + RT\ \ln[B_i] \tag{16}$$

where B_i is a reactant (sum of species), yields

$$\Delta_r G' = \Delta_r G'^0 + RT\ \ln\ \Pi[B_i]^{\nu_i} \tag{17}$$

where Π indicates a product and

$$\Delta_r G'^0 = \Sigma\nu_i\tilde{\mu}^0_i \tag{18}$$

which is equivalent to eq. (4). When the system is in chemical equilibrium, $\Delta_r G' = 0$, and

$$\Delta_r G'^0 = -RT\ \ln\ K' \tag{19}$$

where

$$K' = \Pi[B_i]^{\nu_i} \tag{20}$$

This is the justification for the expression for the apparent equilibrium constant K'. Equation (18) is the basis for defining the standard transformed Gibbs energies of formation $\Delta_f G_i'^0$ for reactant i since absolute chemical potentials cannot be determined.

Equation (14) shows us how to calculate the transformed entropy S' of the system, its volume, and the transformed chemical potential $\tilde{\mu}_i$ of a reactant.

$$S' = -(\partial G'/\partial T)_{P,\tilde{n}_i,\mathrm{pH},\mathrm{pMg}} \tag{21}$$

$$V = (\partial G'/\partial P)_{T,\tilde{n}_i,\mathrm{pH},\mathrm{pMg}} \tag{22}$$

$$\tilde{\mu}_i = (\partial G'/\partial \tilde{n}_i)_{T,P,\tilde{n}_j,\mathrm{pH},\mathrm{pMg}} \tag{23}$$

where $\tilde{n}_j$ indicates that the amounts of all of the reactants except i are held constant. A prime could be put on the V, but since the effect of pressure on biochemical reactions is not discussed here, the prime is omitted and V is the usual total volume.

The transformed enthalpy H' of the system is defined by $H' = G' + TS'$. It can be calculated directly from G' by use of the Gibbs-Helmholtz equation.

$$H' = [\partial(G'/T)/\partial(1/T)]_{P,\mathrm{pH},\mathrm{pMg},\tilde{n}_i} \tag{24}$$

Equations (21)-(24) will be applied to a system containing a single reactant, that is a pseudoisomer group like G6P.

3. Calculation of $\Delta_f G_i'^0$ and $\Delta_f H_i'^0$ for reactant i at specified pH and pMg

For a single reactant, the summation in eq. (13) may have several terms; for G6P, there are terms in $G6P^{2-}$, $HG6P^-$, and MgG6P. As a simplification, let us a system with a single biochemical reactant that exists as a weak acid HA and the corresponding anion A^- at equilibrium at a specified pH and I. The fundamental equation (eq. 13) for G' for this system can be written

$$(\mathrm{d}G')_{\mathrm{pH}} = -S'\,\mathrm{d}T + \tilde{\mu}_{A^-}\,\mathrm{d}n_{A^-} + \tilde{\mu}_{HA}\,\mathrm{d}n_{HA} \tag{25}$$

The $V\mathrm{d}P$ term has been omitted because we are concerned only with experiments at a total pressure of about 1 bar. Equation (25) can be integrated at constant T, pH, and I to

$$\begin{aligned} G' &= n_{A^-}\tilde{\mu}_{A^-} + n_{HA}\tilde{\mu}_{HA} \\ &= n_t[r_A\tilde{\mu}_{A^-} + r_{HA}\tilde{\mu}_{HA}] \end{aligned} \tag{26}$$

where n_t is the total amount of the reactant in the system and r_A and r_{HA} are the equilibrium mole fractions of A^- and HA in the HA,A^- pseudoisomer group.

As described in the preceding paper [2], the transformed chemical potentials of the species are obtained by subtracting μ_{H^+} if a species has a dissociable proton. Thus

$$\tilde{\mu}_{A^-} = \mu_{A^-} \tag{27}$$

$$\tilde{\mu}_{HA} = \mu_{HA} - \mu_{H^+} = \mu_{HA} - \mu^0_{H^+} - RT\ \ln[H^+] \tag{28}$$

Thus eq. (26) can be written in terms of chemical potentials rather than transformed chemical potentials.

$$G' = n_t[r_{A^-}\mu_{A^-} + r_{HA}\{\mu_{HA} - \mu^0_{H^+} - RT\ \ln[H^+]\}] \tag{29}$$

The equilibrium chemical potentials of A^- and HA are replaced using $\mu_{A^-} = \mu^0_{A^-} + RT \ln r_{A^-}[A]$ and $\mu_{HA} = \mu^0_{HA} + RT \ln r_{HA}[A]$, where $[A] = [A^-] + [HA]$, to obtain

$$\begin{aligned} G' &= n_t\{\mu^0_{A^-} r_{A^-} + \{\mu^0_{HA} - \mu^0_{H^+} - RT \ln[H^+]\} r_{HA} + RT[r_{A^-} \ln r_{A^-} + r_{HA} \ln r_{HA}] + RT \ln[A]\} \\ &= n_t\{\tilde{\mu}^0_A + RT \ln[A]\} = n_t \tilde{\mu}_A \end{aligned} \tag{30}$$

where

$$\tilde{\mu}^0_A = \mu^0_{A^-} r_{A^-} + \{\mu^0_{HA} - \mu^0_{H^+} - RT \ln[H^+]\} r_{HA} + RT[r_{A^-} \ln r_{A^-} + r_{HA} \ln r_{HA}] \tag{31}$$

This equation is a symbolic representation of the standard transformed chemical potential of the HA,A^- pseudoisomer group, but we need to be able to calculate the equilibrium mole fractions of A^- and HA. This form of equation arises in the consideration of the thermodynamics of isomer groups, and so it is known [8,9] that this equation can be written in terms of the standard transformed chemical potentials of A^- and HA by

$$\tilde{\mu}^0_A = -RT \ln\{\exp[-\mu^0_{A^-}/RT] + \exp[-\{\mu^0_{HA} - \mu^0_{H^+} - RT \ln[H^+]\}/RT]\} \tag{32}$$

The equilibrium mole fractions of A^- and HA can be calculated using

$$r_{A^-} = \exp\{[\tilde{\mu}^0_A - \mu^0_{A^-}]/RT\} \tag{33}$$

$$r_{HA} = \exp[\{\tilde{\mu}^0_A - \mu^0_{HA} + \mu^0_{H^+} + RT \ln[H^+]\}/RT] \tag{34}$$

Now we are in a position to calculate the transformed entropy S' of the system. Application of eq. (21) to eq. (30) yields

$$S' = n_t\{\bar{S}'^0_A - R \ln[A]\} \tag{35}$$

where

$$\bar{S}'^0_A = \bar{S}^0_{A^-} r_{A^-} + \{\bar{S}^0_{HA} - \bar{S}^0_{H^+} + R \ln[H^+]\} r_{HA} - R[r_{A^-} \ln r_{A^-} + r_{HA} \ln r_{HA}] \tag{36}$$

The standard transformed molar enthalpy $\bar{H}'^0$ of A at specified pH can be calculated from $\tilde{\mu}^0_A + T\bar{S}'^0_A$.

$$\bar{H}'^0_A = r_A \bar{H}^0_A + r_{HA}[\bar{H}^0_{HA} - \bar{H}^0_{H^+}] \tag{37}$$

It is convenient to carry out derivations using the chemical potential μ, the molar entropy $\bar{S}$, and the molar enthalpy $\bar{H}$, but in making calculations using experimental data it is convenient to use the corresponding formation quantities. The symbols for molar thermodynamic quantities are summarized in Table 1. The first column gives the symbols used in derivations at specified T and P and at specified T,

Table 1

Symbols for molar thermodynamic properties

Species i at specified T, P, I		
μ_i	μ^0_i	$\Delta_f G^0_i$
$\bar{S}_i$	$\bar{S}^0_i$	$\Delta_f S^0_i$
$\bar{H}_i$	$\bar{H}^0_i$	$\Delta_f H^0_i$
Species i or reactants i at specified T, P, pH, pMg, I		
$\tilde{\mu}_i$	$\tilde{\mu}^0_i$	$\Delta_f G'^0_i$
$\bar{S}'_i$	$\bar{S}'^0_i$	$\Delta_f S'^0_i$
$\bar{H}'_i$	$\bar{H}'^0_i$	$\Delta_f H'^0_i$

P, pH, and pMg. The second column gives the symbols for the standard values, and the third column gives the experimental quantities that can be substituted for the quantities in the second column in a calculation. These quantities are all functions of the ionic strength, and so the ionic strength must be specified.

4. Calculation of standard transformed formation properties of reactants at specified T, pH, pMg, and I

Now we return to a system involving an enzyme-catalyzed reaction like reaction (2). When eq. (14) is applied to the system represented by reaction (2) at specified pH and pMg in the neutral pH range, there are eight species and four components. The four components are H_2O, glucose, the three species making up P_i, and the three species making up G6P. Equation (18) for this system becomes

$$\Delta_r G'^0 = \Delta_f G'^0_{\mathrm{Glu}} + \Delta_f G'^0_{\mathrm{P_i}} - \Delta_f G'^0_{\mathrm{G6P}} - \Delta_f G'^0_{\mathrm{H_2O}} \tag{38}$$

There are, of course, corresponding equations for $\Delta_r H'^0$ and $\Delta_r S'^0$. We will be concerned with $\Delta_r H'^0$, but not with $\Delta_r S'^0$ because it can always be calculated from $\Delta_r S'^0 = (\Delta_r H'^0 - \Delta_r G'^0)/T$. In general the formation properties of these four reactants are functions of T, P, pH, pMg, and I. Glucose and water do not form complexes with Mg^{2+}, but all of the reactants contain hydrogen, and so this raises the question of the adjustment of the formation properties to pH 7, or other specified value. The safest way to do this is to make adjustments for all the hydrogen atoms in a species. We may not think of all of the H atoms in glucose as dissociable, but in some sense all of them are because glucose can be oxidized to CO_2 enzymatically. If an adjustment is made for a hydrogen atom that is not dissociated in a particular reaction, a similar adjustment in a product will cancel it so that the adjustment on that hydrogen will not affect the calculation of $\Delta_f G'^0$ or $\Delta_f H'^0$. Thus we adopt the convention that an adjustment to the desired pH is to be made for all of the hydrogens in all of the species of all of the reactants.

This adjustment is discussed in detail later in this section, but first we want to consider how the formation properties at a specified T, pH, pMg, and I are to be calculated for reactants like P_i or G6P, which exist in a mixture of forms at equilibrium. We will see in the next section that there is enough information to calculate standard transformed formation properties of the ionized and unionized species of these reactants at 298.15 K, pH 7, pMg 3, and $I = 0.25$ M, and the standard transformed formation properties of these reactants are calculated in Section 6. Since the formation reactions are simpler for inorganic phosphate, the equations are written out for it, but calculations are made in the same way for G6P. At specified pH and pMg, HPO_4^{2-}, $H_2PO_4^-$, and $MgHPO_4$ are pseudoisomers. Therefore, eq. (32) can be written

$$\begin{aligned}\Delta_f G'^0(\mathrm{P_i}) = -RT \ln\Big\{&\exp\left[-\Delta_f G'^0(\mathrm{HPO_4^{2-}})/RT\right] + \exp\left[-\Delta_f G'^0(\mathrm{H_2PO_4^-})/RT\right]\\ &+\exp\left[-\Delta_f G'^0_{\mathrm{MgHPO_4}}/RT\right]\Big\}\end{aligned} \tag{39}$$

where the transformed Gibbs energies of formation are at a specified T, P, pH, pMg, and I. This is the equation that makes it possible to calculate the standard transformed Gibbs energy of formation of a reactant as a whole.

The three standard transformed Gibbs energies of formation of species can be calculated from the standard Gibbs energies of formation of HPO_4^-, $H_2PO_4^{2-}$, and $MgHPO_4$ given in the literature ($I = 0$), but first they have to be adjusted to $I = 0.25$ M. The standard Gibbs energies of formation and standard enthalpies of formation of ionic species at 298.15 K are adjusted to the specified ionic strength I using the extended Debye-Hückel theory [10,11].

$$\Delta_f G^0_{i(I)} = \Delta_f G^0_{i(I=0)} - 2.91482 z_i^2 I^{1/2}/(1 + BI^{1/2}) \tag{40}$$

$$\Delta_f H^0_{i(I)} = \Delta_f H^0_{i(I=0)} + 1.4775 z_i^2 I^{1/2}/(1 + BI^{1/2}) \tag{41}$$

where kJ mol^{-1} units are used, and z_i is the charge on ion i, $B = 1.6$ L$^{-1/2}$ mol$^{-1/2}$.

In order to adjust $\Delta_f G^0$ and $\Delta_f H^0$ of a species to a specified pH and pMg to obtain $\Delta_f G'^0$ and $\Delta_f H'^0$, it is necessary to include $\mathrm{H}^+_{(10^{-\mathrm{pH}}M)}$ and $\mathrm{Mg}^{2+}_{(10^{-\mathrm{pMg}}M)}$ in the formation reaction, as indicated by the Legendre transform. Since HPO_4^{2-} contains a hydrogen atom, its $\Delta_f G^0$ and $\Delta_f H^0$ values at the desired ionic strength are adjusted to a specified pH as follows:

$$\begin{array}{lll}
\frac{1}{2}\mathrm{H}_{2(g)} + \mathrm{P}_{(s)} + 2\mathrm{O}_{2(g)} + 2e^- = \mathrm{HPO}^{2-}_{4(ao)} & \Delta_f G^0_{\mathrm{HPO}_4^{2-}} & (42) \\
 & \Delta_f H^0_{\mathrm{HPO}_4^{2-}} & \\
\mathrm{H}^+_{(10^{-\mathrm{pH}}M)} + e^- = \frac{1}{2}\mathrm{H}_{2(g)} & -\left[\Delta_f G^0_{\mathrm{H}^+} + RT \ln 10^{-\mathrm{pH}}\right] & (43) \\
 & -\Delta_f H^0_{H^+} & \\
\cline{1-1}
\mathrm{P}_{(s)} + 2\mathrm{O}_{2(g)} + \mathrm{H}^+_{(10^{-\mathrm{pH}}M)} + 3e^- = \mathrm{HPO}^{2-}_{4(ao)} & & (44) \\
 & \Delta_f G'^0_{\mathrm{HPO}_4^{-2}} = \Delta_f G^0_{\mathrm{HPO}_4^{2-}} - \left[\Delta_f G^0_{\mathrm{H}^+} + RT \ln 10^{-\mathrm{pH}}\right] & \\
 & \Delta_f H'^0_{\mathrm{HPO}_4^{2-}} = \Delta_f H^0_{\mathrm{HPO}_4^{2-}} - \Delta_f H^0_{\mathrm{H}^+} &
\end{array}$$

The 'ao'indicates that an ion or molecule as in its standard state in aqueous solution. The standard Gibbs energy of formation of H$^+$ and the standard enthalpy of formation of H$^+$ are represented by $\Delta_f G^0_{\mathrm{H}^+}$ and $\Delta_f H^0_{\mathrm{H}^+}$ because they are not equal to zero at finite ionic strengths.

Since $\mathrm{H_2PO_4^-}$ contains two hydrogen atoms, its $\Delta_f G^0$ and $\Delta_f H^0$ values at the desired ionic strength are adjusted to a specified pH as follows:

$$\begin{array}{lll}
\mathrm{H}_{2(g)} + \mathrm{P}_{(s)} + 2\mathrm{O}_{2(g)} + e^- = \mathrm{H_2PO}^-_{4(ao)} & \Delta_f G^0_{\mathrm{H_2PO_4^-}} & (45) \\
 & \Delta_f H^0_{\mathrm{H_2PO_4^-}} & \\
2\mathrm{H}^+_{(10^{-\mathrm{pH}}M)} + 2e^- = \mathrm{H}_{2(g)} & -2\left[\Delta_f G^0_{\mathrm{H}^+} + RT \ln 10^{-\mathrm{pH}}\right] & (46) \\
 & -2\Delta_f H^0_{\mathrm{H}^+} & \\
\cline{1-1}
\mathrm{P}_{(s)} + 2\mathrm{O}_{2(g)} + 2\mathrm{H}^+_{(10^{-\mathrm{pH}}M)} + 3e^- = \mathrm{H_2PO}^-_{4(ao)} & & (47) \\
 & \Delta_f G'^0_{\mathrm{H_2PO_4^-}} = \Delta_f G^0_{\mathrm{H_2PO_4^-}} - 2\left[\Delta_f G^0_{\mathrm{H}^+} + RT \ln 10^{-\mathrm{pH}}\right] & \\
 & \Delta_f H'^0_{\mathrm{H_2PO_4^-}} = \Delta_f H^0_{\mathrm{H_2PO_4^-}} - 2\ \Delta_f H^0_{\mathrm{H}^+} &
\end{array}$$

Since $\mathrm{MgHPO_4}$ contains a Mg atom and a hydrogen atom, its $\Delta_f G^0$ and $\Delta_f H^0$ values at the desired ionic strength are adjusted to a specified pMg as follows:

$$\begin{array}{lll}
\mathrm{Mg}_{(s)} + \frac{1}{2}\mathrm{H}_{2(g)} + \mathrm{P}_{(s)} + 2\mathrm{O}_{2(g)} = \mathrm{MgHPO}_{4(ao)} & \Delta_f G^0(\mathrm{MgHPO_4}) & (48) \\
 & \Delta_f H^0(\mathrm{MgHPO_4}) & \\
\mathrm{Mg}^{2+}_{(10^{-\mathrm{pMg}}M)} + 2e^- = \mathrm{Mg}_{(s)} & -\Delta_f G^0(\mathrm{Mg}^{2+}) - RT \ln 10^{-\mathrm{pM}} & (49) \\
 & -\Delta_f H^0(Mg^{2+}) &
\end{array}$$

$$\mathrm{H}^{+}_{(10^{-\mathrm{pH}}M)} + e^{-} = \tfrac{1}{2}\mathrm{H}_{2(\mathrm{g})} \qquad -\left[\Delta_{\mathrm{f}}G^{0}(\mathrm{H}^{+}) + RT\ \ln 10^{-\mathrm{pH}}\right] \qquad (50)$$

$$\underline{\qquad\qquad\qquad\qquad\qquad\qquad} \qquad -\Delta_{f}H^{0}(H^{+})$$

$$\mathrm{P}_{(\mathrm{s})} + 2\mathrm{O}_{2(\mathrm{g})} + \mathrm{Mg}^{2+}_{(10^{-\mathrm{pMg}}M)} + \mathrm{H}^{+}_{(10^{-\mathrm{pH}}M)} + 3e^{-} = \mathrm{MgHPO}_{4(\mathrm{ao})} \qquad (51)$$

$$\begin{aligned}\Delta_{\mathrm{f}}G'^{0}(\mathrm{MgHPO}_4) &= \Delta_{\mathrm{f}}G^{0}_{\mathrm{MgHPO}_4} \\ &\quad -\left[\Delta_{\mathrm{f}}G^{0}(\mathrm{Mg}^{2+}) + RT\ \ln 10^{-\mathrm{pMg}}\right] \\ &\quad -\left[\Delta_{\mathrm{f}}G^{0}_{\mathrm{H}^+} + RT\ \ln 10^{-\mathrm{pH}}\right] \\ \Delta_{\mathrm{f}}H'^{0}(\mathrm{MgHPO}_4) &= \Delta_{\mathrm{f}}H^{0}_{\mathrm{MgHPO}_4^-} - \Delta_{\mathrm{f}}H^{0}(\mathrm{Mg}^{2+}) \\ &\quad -\Delta_{\mathrm{f}}H^{0}_{\mathrm{H}^+}\end{aligned}$$

The important point about eqs. (44), (47) and (51) is that H^+ and Mg^{2+} have been introduced on the left hand side of the formation reaction.

Substituting the expressions for standard transformed Gibbs energies of formation from eqs. (44), (47), and (51) into eq. (39) and rearranging yields

$$\Delta_{\mathrm{f}}G'^{0}_{\mathrm{P_i}} = \Delta_{\mathrm{f}}G^{0}_{\mathrm{HPO}_4^{2-}} - RT\ \ln\left\{1 + 10^{-\mathrm{pH}}/K_{\mathrm{H_2PO_4^-}} + 10^{-\mathrm{pMg}}/K_{\mathrm{MgHPO_4}}\right\} \qquad (52)$$

where

$$\begin{aligned}K_{\mathrm{H_2PO_4^-}} &= [\mathrm{H}^+][\mathrm{HPO}_4^{2-}]/[\mathrm{H_2PO_4^-}] \\ &= \exp\left\{-\left[\Delta_{\mathrm{f}}G^{0}(\mathrm{H}^+) + \Delta_{\mathrm{f}}G(\mathrm{HPO}_4^{2-}) - \Delta_{\mathrm{f}}G^{0}(\mathrm{H_2PO_4^-})\right]/RT\right\} \qquad (53) \\ K_{\mathrm{MgHPO_4}} &= [\mathrm{Mg}^{2+}][\mathrm{HPO}_4^{2-}]/[\mathrm{MgHPO_4}] \\ &= \exp\left\{-\left[\Delta_{\mathrm{f}}G^{0}(\mathrm{Mg}^{2+}) + \Delta_{\mathrm{f}}G(\mathrm{HPO}_4^{2-}) - \Delta_{\mathrm{f}}G^{0}(\mathrm{MgHPO_4})\right]/RT\right\} \qquad (54)\end{aligned}$$

The standard formation properties and equilibrium constants are for the desired ionic strength.

Analogous formation reactions can be written for each of the three species of G6P except that $\mathrm{G6P}^{2-}$ is adjusted for 11 hydrogens, $\mathrm{HG6P}^-$ is adjusted for 12 hydrogen, and MgG6P is adjusted for 11 hydrogens. The formation properties of $\mathrm{H_2O}$ are adjusted for two hydrogens, and the formation properties of glucose are adjusted for 12 hydrogens.

The standard transformed enthalpy of formation $\Delta_{\mathrm{f}}H_i'^{0}$ of a reactant at a specified pH and pMg can be calculated with an analog of eq. (37). For $\mathrm{P_i}$,

$$\Delta_{\mathrm{f}}H'^{0}_{\mathrm{P_i}} = r_{\mathrm{HPO}_4^{2-}}\ \Delta_{\mathrm{f}}H'^{0}_{\mathrm{HPO}_4^{2-}} + r_{\mathrm{H_2PO_4^-}}\ \Delta_{\mathrm{f}}H'^{0}_{\mathrm{H_2PO_4^-}} + r_{\mathrm{MgHPO_4}}\ \Delta_{\mathrm{f}}H'^{0}_{\mathrm{MgHPO}_4^{2-}} \qquad (55)$$

This equation has the advantage of focussing attention on the standard transformed enthalpies of formation of the ionic species. The equilibrium mole fractions are calculated with analogs of eqs. (33) and (34).

The standard transformed enthalpy of reaction $\Delta_{\mathrm{r}}H'^{0}$ is given by

$$\Delta_{\mathrm{r}}H'^{0} = \Delta_{\mathrm{f}}H'^{0}_{\mathrm{Glu}} + \Delta_{\mathrm{f}}H'^{0}_{\mathrm{P_i}} - \Delta_{\mathrm{f}}H'^{0}_{\mathrm{G6P}} - \Delta_{\mathrm{f}}H'^{0}_{\mathrm{H_2O}} \qquad (56)$$

In Section 7 it is shown that this gives exactly the same result as the equations that are usually written for $\Delta_{\mathrm{r}}H'^{0}$ in terms of the enthalpies of acid dissociation and complex ion dissociations and the enthalpy of the reference reaction. If $\Delta_{\mathrm{r}}H'^{0}$ is independent of T, $\Delta_{\mathrm{r}}S'^{0}$ will be independent of temperature, and $\ln K_1' = -\Delta_{\mathrm{r}}H'^{0}/RT_1 + \Delta_{\mathrm{r}}S'^{0}/R$ and $\ln K_2' = -\Delta_{\mathrm{r}}H'^{0}/RT_2 + \Delta_{\mathrm{r}}S'^{0}/R$ so that

$$\Delta_{\mathrm{r}}H'^{0} = \left[RT_1T_2/(T_2 - T_1)\right]\ \ln(K_2'/K_1') \qquad (57)$$

Table 2

Standard formation properties of species in kJ mol^{-1} at 298.15 K

Species	$I = 0$		$I = 0.25$ M	
	$\Delta_f H^0$	$\Delta_f G^0$	$\Delta_f H^0$	$\Delta_f G^0$
$H_2O_{(l)}$	−285.83	−237.19	−285.83	−237.19
$H^+_{(ao)}$	0	0	0.41	−0.81
$Mg^{2+}_{(ao)}$	−467.00	−455.30	−465.36	−458.54
$HPO^{2-}_{4(ao)}$	−1299.0	−1096.10	−1297.36	−1099.34
$H_2PO^{-1}_{4(ao)}$	-1302.6	−1137.3	−1302.19	−1138.11
$MgHPO_{4(ao)}$	−1753.80	−1566.87	−1753.80	−1566.87
$Glucose_{(ao)}$	−1262.19	−915.90	−1262.19	−915.90
$G6P^{2-}_{(ao)}$	−2276.44	−1763.94	−2274.80	−1767.18
$HG6P^-_{(ao)}$	−2274.64	−1800.59	−2274.23	−1801.40
$MgG6P_{(ao)}$	−2732.04	−2234.08	−2732.04	−2234.08

Thus it is the standard transformed enthalpy of reaction $\Delta_r H'^0$ that determines the effect of temperature on K' at a specified pH and pMg.

5. Calculation of the standard formation properties of the species of inorganic phosphate and of glucose 6-phosphate at specified T, P, and I

There is enough information to calculate the standard thermodynamic properties of the species of G6P at $I = 0.25$ M, and 298.15 K. The standard thermodynamic properties of $G6P^{2-}$ and $HG6P^-$ at $I = 0$ and 298.15 K have been calculated by Goldberg and Tewari [12]. These calculations can be extended to include MgG6P. The standard transformed formation properties of inorganic phosphate at pH 7, pMg 3, $I = 0.25$ M, and 298.1 K were calculated in the previous paper [2] using data from the NBS Tables, but those values are recalculated here using data from the CODATA Tables [13], since they have been further improved. The values in both of these tables are on the molal scale, but here we are using the molar (mol L^{-1}) scale. The adjustments of standard thermodynamic properties from one scale to another are understood [14,15], but they are negligible here.

Table 2 gives the standard formation properties for H_2O, H^+, Mg^{2+}, HPO_4^{2-}, and HPO_4^- from CODATA [13]. The values for $MgHPO_4$ were calculated with information from Clarke et al. [16].

Glucose 6-phosphate (G6P) was chosen as an example for the calculation of the standard transformed Gibbs energy of formation and the standard transformed enthalpy of formation of a biological phosphate compound at pH 7, pMg 3, $I = 0.25$ M, and 298.15 K because of the experimental work that has been done. Lawson and Veech [5] found the equilibrium constant for reaction 2 at 38°C, $I = 0.25$ M, and pH 6.99 to be $1.10\ 10^2$ at $[Mg^{2+}] = 0$ and 10^{-3} M. Tewari, Steckler, and Goldberg [6] measured the enthalpy of hydrolysis calorimetrically and calculated $\Delta_r H^0 = 0.91$ kJ mol^{-1} at $I = 0$ and 25°C for the reference reaction

$$G6P^{2+} + H_2O = Glu + HPO_4^{2-} \qquad K_{ref} = [Glu][HPO_4^{2-}]/[G6P^{2-}] \tag{58}$$

Goldberg and Tewari [12] used Lawson and Veech's $\Delta_r G'^0$ and their $\Delta_r H^0_{ref}$ to calculate $\Delta_r G^0_{ref} = -10.8$ kJ mol^{-1} [12] for reaction 58 at 25°C and $I = 0$. Since the $\Delta_f G^0$ and $\Delta_f H^0$ of glucose are known in dilute aqueous solution, this made it possible for Goldberg and Tewari to calculate the $\Delta_f G^0$ and $\Delta_f H^0$ of $G6P^{2-}$ at $I = 0$ and 25°C. The second acid dissociation constant of G6P was measured by Bunton and Chaimovich [17] and Degani and Halmann [18]. The $\Delta_f G^0$ and $\Delta_f H^0$ values for glucose and $G6P^{2-}$ and

Table 3

Standard transformed formation properties of species in kJ mol^{-1} at 298.15 K, pH 7, pMg 3, and $I = 0.25$ *M*

Species	$\Delta_f H'^0$	$\Delta_f G'^0$
H_2O	−286.65	−155.66
HPO_4^{2-}	−1297.77	−1058.57
$H_2PO_4^-$	−1303.01	−1056.58
$MgHPO_4$	−1288.85	−1050.44
Glucose	−1267.12	−426.70
$G6P^{2-}$	−2279.31	−1318.75
$HG6P^-$	−2279.15	−1312.20
MgG6P	−2271.19	−1309.98

the $\Delta_f G^0$ for $HG6P^-$ from Goldberg and Tewari [12] are given in Table 2. The value for $\Delta_f H^0$ for $HG6P^-$ has been calculated from data of Bunton and Chaimovich. In the absence of data on the dissociation of MgG6P, its p*K* at 25°C and $I = 0$ is expected to be quite similar to $MgAMP^-$ (pK = 2.81 and $\Delta H^0 = -11.4$ kJ mol^{-1} at $I = 0$ [19]), $MgF6P^-$ (fructose 6-phosphate) ($pK = 2.47$ at $I = 0$), and $MgR5P^-$ (ribose 5-phosphate) ($pK = 2.40$ at $I = 0$). Therefore, $pK = 2.6$ and $\Delta H^0 = -11.4$ kJ mol^{-1} are used at $I = 0$ for the dissociation of MgG6P.

The values of $\Delta_f G^0$ and $\Delta_f H^0$ at $I = 0.25$ *M* in Table 2 have been obtained using eqs. (40) and (41).

6. Calculation of the standard transformed formation properties of inorganic phosphate and glucose 6-phosphate at *T*, *P*, pH, pMg, and *I*

Section 4 has shown how the standard transformed formation properties of species are calculated by making adjustments for the numbers of protons and magnesium ions. These properties of species at 298.15 K, 1 bar, pH 7, and pMg 3 are shown in Table 3.

The next step is to calculated the standard transformed Gibbs energy of formation $\Delta_f G'^0$ of a reactant (pseudoisomer group) using an analog of eq. (39). The calculation of the standard transformed enthalpy $\Delta_f H'^0$ of a reactant (pseudoisomer group) uses an analog of eq. (55). The equilibrium mole fractions of the various species within a pseudoisomer group are calculated using analogs of eqs. (33) and (34). The standard transformed formation properties of the four reactants in the glucose-6-phosphatase reaction are shown in Table 4. The calculation of Table 4 has been one of the primary objectives of this paper.

Table 4 indicates that $\Delta_r G'^0 = -11.61$ kJ mol^{-1}, $\Delta_r H'^0 = -0.50$ kJ mol^{-1}, and $K' = 108.1$ for reaction (1). If the concentrations of the reactants are in a steady-state with $[G6P] = 2\ 10^{-5}$ *M*, $[Glu] = [P_i] = 5\ 10^{-2}$ *M*,

$$\Delta_r G' = \Delta_r G'^0 + RT \ln Q' = -11.61 + RT \ln([Glu][P_i]/[G6P]) = 0.36 \text{ kJ mol}^{-1} \qquad (59)$$

Table 4

Standard transformed formation properties of biochemical reactants in kJ mol^{-1} at 298.15 K, pH 7, pMg 3, and $I = 0.25$ *M*

Species	$\Delta_f H'^0$	$\Delta_f G'^0$
$H_2O_{(l)}$	−286.65	−155.66
$Glucose_{(ao)}$	−1267.12	−426.70
P_i	−1299.12	−1059.55
G6P	−2279.09	−1318.98

where Q' is the reaction quotient. Thus the hydrolysis of glucose 6-phosphate cannot occur under these conditions. The transformed enthalpy of reaction (2) under these conditions is $\Delta_r H' = \Delta_r H'^0 = -0.50$ kJ mol^{-1} because the enthalpy of reaction is not affected by the concentrations of the reactants. The transformed entropy of reaction (2) under these conditions is given by

$$\Delta_r S' = \Delta_r S'^0 - R \ln Q' = 37.26 - R \ln([\mathrm{Glu}][\mathrm{P_i}]/[\mathrm{G6P}]) = -2.89 \text{ J K}^{-1}\text{ mol}^{-1} \tag{60}$$

in agreement with $\Delta_r G' = \Delta_r H' - T \Delta_r S'$.

A computer program has been written in Mathematica (Wolfram Research, Inc., Champaign, IL 61820-7237) to carry out these calculations. An input matrix giving $\Delta_f H^0_{(I=0)}$, $\Delta_f G^0_{(I=0)}$, electric charge, number of hydrogen atoms, and number of magnesium atoms for the species of a reactant is prepared, and the program calculates $\Delta_f H'^0$ and $\Delta_f G'^0$ for the reactant at the specified T, pH, pMg, and I.

7. Two ways of writing expressions for $\Delta_r G'^0$ and $\Delta_r H'^0$ for the hydrolysis of glucose 6-phosphate

In the past the expressions for the standard transformed Gibbs energy of reaction and the standard transformed enthalpy of reaction have been written in terms of the thermodynamic parameters for the H^+ and Mg^{2+} dissociation reactions and a reference reaction. However, $\Delta_r G'^0$ and $\Delta_r H'^0$ can be written in terms of the standard transformed Gibbs energies of formation and the standard transformed enthalpies of formation for the reactants, as represented by eqs. (4) and (5). This can be illustrated by use of the glucose-6-phosphatase reaction. Considering only the acid dissociations, the usual derivations yield the following expressions for $\Delta_r G'^0$ and $\Delta_r H'^0$.

$$\begin{aligned}\Delta_r G'^0 = {} & \Delta_r G'^0_{\mathrm{ref}} - RT \ln\left\{1 + [\mathrm{H^+}]/\exp\left[-\Delta_r G^0_{\mathrm{H_2PO_4^-}}/RT\right]\right\} \\ & + RT \ln\left\{1 + [\mathrm{H^+}]/\exp\left[-\Delta_r G^0_{\mathrm{HG6P^-}}/RT\right]\right\}\end{aligned} \tag{61}$$

$$\begin{aligned}\Delta_r H'^0 = {} & \Delta_r H^0_{\mathrm{ref}} - \frac{[\mathrm{H^+}]/\exp\left[-\Delta_r G^0_{\mathrm{H_2PO_4^-}}/RT\right]}{1 + [\mathrm{H^+}]/\exp\left[-\Delta_r G^0_{\mathrm{H_2PO_4^-}}/RT\right]} \Delta_r H^0_{\mathrm{H_2PO_4^-}} \\ & + \frac{[\mathrm{H^+}]/\exp\left[-\Delta_r G^0_{\mathrm{HG6P^-}}/RT\right]}{1 + [\mathrm{H^+}]/\exp\left[-\Delta_r G^0_{\mathrm{HG6P^-}}/RT\right]} \Delta_r H^0_{\mathrm{HG6P^-}}\end{aligned} \tag{62}$$

$\Delta_r G^0_{\mathrm{H_2PO_4^-}}$ is the standard Gibbs energy of acid dissociation of $H_2PO_4^-$, $\Delta_r H^0_{\mathrm{H_2PO_4^-}}$ is the standard enthalpy of acid dissociation of $H_2PO_4^-$, and subscript$_{\mathrm{ref}}$ refers to reference reaction (58). Note that these two equations involve six parameters.

When eqs. (4) and (5) are applied to the glucose-6-phosphotase reaction, again considering acid dissociations, the standard transformed thermodynamic properties are given by

$$\begin{aligned}\Delta_r G'^0 = {} & -RT \ln\left\{\exp\left[-\Delta_f G'^0_{\mathrm{HPO^{2-}}}/RT\right] + \exp\left[-\Delta_f G'^0_{\mathrm{H_2PO_4^{2-}}}/RT\right]\right\} + \Delta_f G'^0_{\mathrm{glu}} \\ & -RT \ln\left\{\exp\left[-\Delta_f G'^0_{\mathrm{G6P^{2-}}}/RT\right] + \exp\left[-\Delta_f G'^0_{\mathrm{HG6P^-}}/RT\right]\right\} - \Delta_f G'^0_{\mathrm{H_2O}}\end{aligned} \tag{63}$$

$$\begin{aligned}\Delta_r H'^0 = {} & \left[r_{\mathrm{HPO_4^{2-}}}\ \Delta_f H'^0_{\mathrm{HPO_4^{2-}}} + r_{\mathrm{H_2PO_4^-}}\ \Delta_f H'^0_{\mathrm{H_2PO_4^-}}\right] + \Delta_f H'^0_{\mathrm{Glu}} - \left[r_{\mathrm{G6P^{2-}}}\ \Delta_f H'^0_{\mathrm{G6P^{2-}}} + r_{\mathrm{HG6P^-}}\ \Delta_f H'^0_{\mathrm{HG6P^-}}\right] \\ & - \Delta_f H'^0_{\mathrm{H_2O}}\end{aligned} \tag{64}$$

where

$$r_{\mathrm{HPO_4^{2-}}} = \exp\left\{\left[\Delta_\mathrm{f} G_{\mathrm{P_i}}^{\prime 0} - \Delta_\mathrm{f} G_{\mathrm{HPO_4^{2-}}}^{\prime 0}\right]/RT\right\} \tag{65}$$

$$r_{\mathrm{H_2PO_4^-}} = \exp\left[\left\{\Delta_\mathrm{f} G_{\mathrm{P_i}}^{\prime 0} - \Delta_\mathrm{f} G_{\mathrm{H_2PO_4^-}}^{\prime 0}\right\}/RT\right] \tag{66}$$

with similar equations for $r_{\mathrm{G6P^{2-}}}$ and $r_{\mathrm{HG6P^-}}$. Equations (63) and (64) are equivalent to eqs. (61) and (62), and can be rearranged to the same form as eqs. (61) and (62). However, eqs. (63) and (64) have the advantage that they are each made up of four terms for the four reactants. This is a benefit that comes from having tables of standard formation properties of reactants at specified pH and pMg. Equations (4) and (5) are more convenient to use because they are linear in the reactants, and it is not necessary to write out or derive equations like (61) and (62) for each new reaction studied.

A similar equation can be written for $\Delta_\mathrm{r} S'^0$ in terms of formation properties of the reactants.

8. Discussion

When pH and pMg are specified for a biochemical reaction system, a new set of thermodynamic properties, the transformed properties indicated by a prime, come into play. These properties at a specified pH and pMg can be calculated directly from measurements of chemical equilibrium and calorimetric measurements at the specified pH and pMg, but it may be necessary to assign some zero. In the case of glucose-6-phosphatase reaction, no zeros have to be assigned because the standard formation properties of glucose, water, and $\mathrm{HPO_4^{2-}}$, $\mathrm{H_2PO_4^-}$, and $\mathrm{MgHPO_4}$ are known. Standard transformed formation properties of reactants in the glucose 6-phosphatase reaction are given in Table 4 for 298.15, pH 7, pMg 3, and $I = 0.25$ M, but these properties can be calculated over a range of these independent variables. This table can be extended indefinitely by adding biochemical reactants. Standard thermodynamic properties of reactants that exist at pH 7 and pMg 3 in a single ionized form or uncharged form given in other tables can be added directly to this table after adjustment to pH 7, pMg 3, and $I = 0.25$ M.

The specific values in Tables 3 and 4 depend on the convention that adjustments to pH 7 and pMg 3 are made for all of the H atoms and all of the Mg atoms in the species. The production and use of these tables would be much less confusing if everybody adopted this convention.

These discussions show that the equations for $\Delta_\mathrm{r} G'^0$ and $\Delta_\mathrm{r} H'^0$ for a biochemical reaction can be written in terms of the properties of a reference reaction and acid and magnesium dissociation reactions or in terms of $\Delta_\mathrm{f} G_i^0$ and $\Delta_\mathrm{f} H_i^0$ values for the desired T, pH, pMg, and I. By considering the various species of a reactant as pseudoisomers, the formation properties of the reactants at a specified pH and pMg can be calculated and tabulated. Then apparent equilibrium constants and transformed enthalpies of reaction can be calculated at that pH and pMg. This is close to the system used in the rest of chemistry, but pseudoisomer groups replace species in Table 4 at specified pH 7 and pMg 3. These transformed thermodynamic properties follow the same types of equations that are usually used for species. These new equations can be rearranged to exactly the same form as the equations that have been used for approximately the last 25 years, but there are good reasons to adopt this new point of view.

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Appendix A

The derivations have been based on the simplifications that $\mathrm{pH} = -\log[\mathrm{H}^+]$ and $\mathrm{pMg} = -\log[\mathrm{Mg}^{2+}]$ and the application of ionic strength corrections to all ionic species, including H^+ and Mg^{2+}. This has the advantage of simplifying the equations and of facilitating calculations of equilibrium compositions multi-enzyme systems. However, it is not necessary to make these two assumptions, and as more accurate measurements are made, it may be desirable to use $\mathrm{pH} = -\log a_{\mathrm{H}^+}$. Since pMg is calculated, it may be desirable to retain $\mathrm{pMg} = -\log[\mathrm{Mg}^{2+}]$. When it is assumed that the glass electrode measures the hydrogen ion activity, the acid dissociation constant $K_{\mathrm{H_2PO_4^-}}$ at a specified ionic strength has to be written

$$K_{\mathrm{H_2PO_4^-}} = a_{\mathrm{H}^+}[\mathrm{HPO_4^{2-}}]\gamma_{-2}/[\mathrm{H_2PO_4^-}]\gamma_{-1} \tag{A.1}$$

The standard Gibbs energy of reaction that yields this $K_{\mathrm{H_2PO_4^-}}$ at a specified ionic strength can be calculated from $\Delta_f G^0_{\mathrm{HPO_4^{2-}}}$ and $\Delta_f G^0_{\mathrm{H_2PO_4^-}}$ at zero ionic strength without applying an ionic strength correction to $\Delta_f G^0(\mathrm{H}^+)$. Since $\Delta_f G^0_{\mathrm{H}^+} = -0.81$ at $I = 0.25$ *M*, shifting to $\mathrm{pH} = -\log[\mathrm{H}^+]$ at 25°C changes $\mathrm{p}K_{\mathrm{H_2PO_4^-}}$ by $\log[\exp(0.81/RT)] = 0.14$. Experimental errors are usually larger than this for other weak acids in biochemical systems, but this difference in p*K* could be significant.

Notation

G	extensive Gibbs energy of a system (kJ)
$\bar{G}_i^0$	standard molar Gibbs energy of i at specified T, P, and I (kJ mol^{-1}) [equal to $\mu^0(i)$ and can be replaced by $\Delta_f G_i^0$]
G'	extensive transformed Gibbs energy of a system (kJ)
$\bar{G}_i'^0$	standard molar transformed Gibbs energy of species i or reactant i (sum of species) at specified T, P, pH, pMg, and I (kJ mol^{-1}) [equal to $\tilde{\mu}_i^0$ and can be replaced by $\Delta_f G_i'^0$]
$\Delta_r G^0$	standard Gibbs energy of reaction of a specified reaction in terms of ionic species at specified T, P, and I (kJ mol^{-1})
$\Delta_r G'$	transformed Gibbs energy of reaction of a specified reaction in terms of reactants (sums of species) for specified concentrations of reactants and products at specified T, P, pH, pMg, and I (kJ mol^{-1})
$\Delta_r G'^0$	standard transformed Gibbs energy of a specified reaction in terms of reactants (sums of species) at specified T, P, pH, pMg and I (kJ mol^{-1})
$\Delta_f G_i^0$	standard Gibbs energy of formation of species i at specified T, P, and I (kJ mol^{-1})
$\Delta_f G_i'^0$	standard transformed Gibbs energy of formation of species i or reactant i (sum of species) at specified T, P, pH, pMg, and I (kJ mol^{-1})
H	extensive enthalpy of a system (kJ)
$\bar{H}_i^0$	standard molar enthalpy of species i at specified T, P, and I (kJ mol^{-1}) [can be replaced by $\Delta_f H_i^0$]
H'	extensive transformed enthalpy of a system (kJ)
$\bar{H}_i'^0$	standard molar transformed enthalpy of species i or reactant i (sum of species) at specified T, P, pH, pMg, and I (kJ mol^{-1}) [can be replaced by $\Delta_f H_i'^0$]
$\Delta_r H^0$	standard enthalpy of reaction of a specified reaction in terms of ionic species at specified T, P, and I (kJ mol^{-1})

$\Delta_r H'$	transformed enthalpy of reaction of a specified reaction in terms of reactants (sums of species) for specified concentrations of reactants and products at specified T, P, pH, pMg, and I (kJ mol^{-1})
$\Delta_r H'^0$	standard transformed enthalpy of a specified reaction in terms of reactants (sums of species) at specified T, P, pH, pMg and I (kJ mol^{-1})
$\Delta_f H_i^0$	standard enthalpy of formation of species i at specified T, P, and I (kJ mol^{-1})
$\Delta_f H_i'^0$	standard transformed enthalpy of formation of species i or reactant i (sum of species) at specified T, P, pH, pMg, and I (kJ mol^{-1})
I	ionic strength calculated with ionic concentrations in mol L^{-1} (M)
K	equilibrium constant for a specified reaction written in terms of concentrations of species at specified T, P, and I (omitting H_2O when it is a reactant) (dimensionless)
K'	apparent equilibrium constant for a specified reaction written in terms of concentrations of reactants (sums of species) at specified T, P, pH, pMg, and I (omitting H_2O when it is a reactant) (dimensionless)
n	amount in the system (mol)
n_t	total amount of a reactant (that is, sum of species) in the system (mol)
n_i	amount of species i (mol)
$\tilde{n}_i$	amount of reactant i (that is, sum of species) (mol)
P	pressure (bar)
Q'	apparent reaction quotient of specified concentrations of reactants and products in the same form as the equilibrium constant expression (dimensionless)
R	gas constant (J K^{-1} mol^{-1})
r_i	equilibrium mole fraction of i within a specified class of molecules (dimensionless)
S	extensive entropy of a system (J K^{-1})
$\bar{S}_i^o$	standard molar entropy of i at specified T, P, and I (kJ mol^{-1}) [can be replaced by $\Delta_f S_i^0$]
S'	extensive transformed entropy of a system (kJ)
$\bar{S}_i'^0$	molar transformed entropy of species i or reactant i at specified T, P, pH, pMg, and I (kJ mol^{-1}) [can be replaced by $\Delta_f S_i'^0$]
$\Delta_r S^0$	standard entropy of reaction of a specified reaction in terms of ionic species at specified T, P, and I (kJ mol^{-1})
$\Delta_r S'$	transformed entropy of reaction of a specified reaction in terms of reactants (sums of species) for specified concentrations of reactants and products at specified T, P, pH, pMg, and I (J K^{-1} mol^{-1})
$\Delta_r S'^0$	standard transformed entropy of a specified reaction in terms of sums of species at specified T, P, pH, pMg and I (kJ mol^{-1})
$\Delta_f S_i^0$	standard entropy of formation of species i at specified T, P, and I (kJ mol^{-1})
$\Delta_f S_i'^0$	standard transformed entropy of formation of species i or reactant i (sum of species) at specified T, P, pH, pMg, and I (kJ mol^{-1})
T	temperature (K)
V	volume (L)
z_i	charge of ion i (dimensionless)
μ_i^0	standard chemical potential of species i at specified T, P, and I (kJ mol^{-1}) [can be replaced by $\Delta_f G_i^0$]
$\tilde{\mu}_i^0$	standard transformed chemical potential of species i or reactant i at specified T, P, pH, pMg, and I (kJ mol^{-1}) [can be replaced by $\Delta_f G_i'^0$]
ν_i	stoichiometric number of reactant i in a specified chemical reaction (dimensionless)

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