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Equilibrium calculations on systems of biochemical reactions at specified pH and pMg

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Abstract

The use of G' in discussing the thermodynamics of biochemical reactions at a specified pH and pMg is justified by use of a Legendre transform of the Gibbs energy G. When several enzymatic reactions occur simultaneously in a system, the standard transformed Gibbs energies of reaction $\Delta_r G'^0$ can be used in a computer program to calculate the equilibrium composition that minimizes the transformed Gibbs energy at the specified pH and pMg. The calculation of standard transformed Gibbs energies of formation of reactants at pH 7 and pMg 3 is described. In addition a method for calculating the equilibrium concentrations of reactants is illustrated for a system with steady state concentrations of some reactants like ATP and NAD.

Keywords: Reaction Gibbs energy; Transformed Gibbs energy; Equilibrium composition; Legendre transform; Glycolysis; Systems of reactions

1. Introduction

Expressions for the apparent equilibrium constant K' for biochemical reaction at specified pH and pMg [1], which is expressed in terms of sums of concentrations of ionic species of reactants, have been derived [2–7] by expressing it in terms of ionic species and deriving its dependence on pH and pMg. This apparent equilibrium constant is also a function of the ionic strength and composition of the solvent. However, a more fundamental justification for this type of equilibrium constant is that the standard Gibbs energy of reaction $\Delta_r G'^0 = -RT \ln K'$ calculated from K' is a new thermodynamic potential G', the transformed Gibbs energy, that is minimized at equilibrium at specified T, P, pH, and pMg. The usual Gibbs energy G is a Legendre transform of the internal energy that is useful because $(\mathrm{d}G)_{T,P} \leqslant 0$, where the equality applies to equilibrium. It will be shown here that the thermodynamic potential G' is a Legendre transform of the Gibbs energy G and provides a criterion of equilibrium that is useful in biochemistry because $(\mathrm{d}G')_{T,P,\mathrm{pH},\mathrm{pMg}} \leqslant 0$, where the equality applies to equilibrium. Before discussing the Legendre

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transform, the application of the fundamental equation of thermodynamics to the hydrolysis of ATP is considered.

The apparent equilibrium constant K' for the reaction

$$ATP + H_2O \rightleftharpoons ADP + P_i \tag{1}$$

is calculated with

$$K' = [ADP][P_i]/[ATP]c^0 \tag{2}$$

where ATP, ADP, and P_i represent sums of concentrations of species and c^0 is the standard state concentration of 1 mol L^{-1} ($c^0 = 1$ M). In the neutral pH range and at low magnesium ion concentrations this reaction system contains the following eleven species ATP^{4-}, $HATP^{3-}$, $MgATP^{2-}$, ADP^{3-}, $HADP^{2-}$, $MgADP^{-}$, HPO_4^{2-}, $H_2PO_4^{-1}$, $MgHPO_4$, H^+, and Mg^{2+}. (OH^- is omitted here because its equilibrium concentration can be calculated from $[H^+]$ and the ion product for water) The choice of independent reactions for the calculation of the equilibrium composition is arbitrary, but the following set is generally used

$$ATP^{4-} + H_2O \rightleftharpoons ADP^{3-} + HPO_4^{2-} + H^+, \quad K \tag{3}$$

$$HATP^{3-} \rightleftharpoons H^+ + ATP^{4-}, \quad K_{HATP^{3-}} \tag{4}$$

$$MgATP^{2-} \rightleftharpoons Mg^{2+} + ATP^{4-}, \quad K_{MgATP^{2-}} \tag{5}$$

$$HADP^{2-} \rightleftharpoons H^+ + ADP^{3-}, \quad K_{HADP^{2-}} \tag{6}$$

$$MgADP^{-} \rightleftharpoons Mg^{2+} + ADP^{3-}, \quad K_{MgADP_4^-} \tag{7}$$

$$H_2PO_4^- \rightleftharpoons H^+ + HPO_4^{2-}, \quad K_{H_2PO_4^-} \tag{8}$$

$$MgHPO_4 \rightleftharpoons Mg^{2+} + HPO_4^{2-}, \quad K_{MgHPO_4} \tag{9}$$

where reaction (3), which requires ATPase, is referred to as a reference reaction. The reactions in a set are independent if no reaction in the set can be obtained by adding and subtracting other reactions in the set. These seven equilibrium constants for reactions (3)–(9) depend on the ionic strength because they are written in terms of concentrations and have activity coefficients incorporated in them. If pH and pMg are specified ($pH = -\log([H^-]/c^0)$, and $pMg = -\log([Mg^{2+}]/c^0)$), the apparent equilibrium constant K' for reaction (1) is expressed in terms of the equilibrium constants of reactions (3)–(9) by

$$\begin{aligned} K' &= \left([ADP^{3-}] + [HADP^{2-}] + [MgADP^{-}]\right) \\ &\quad \times \left([HPO_4^{2-}] + [H_2PO_4^-] \right. \\ &\quad \left. + [MgHPO_4]\right) / \left([ATP^{4-}] + [HATP^{3-}] \right. \\ &\quad \left. + [MgATP^{2-}]\right) c^0 \\ &= K\left\{1 + 10^{-pH}/K_{HADP^{2-}} \right. \\ &\quad \left. + 10^{-pMg}/K_{MgADP^-}\right\} \\ &\quad \times \left\{1 + 10^{-pH}/K_{H_2PO_4^-} \right. \\ &\quad \left. + 10^{\;PMg}/K_{MgHPO_4}\right\} \\ &\quad / \left\{1 + 10^{-pH}/K_{HATP^{-3}} \right. \\ &\quad \left. + 10^{-pMg}/K_{MgATP^{2-}}\right\} 10^{-pH} \end{aligned} \tag{10}$$

For many biochemical reactions this information on acid dissociation constants and complex ion dissociation constants is not available, but the experimental determination of K' at specified pH and pMg is not dependent on knowing these constants. Equation (10) can be used to derive expressions for $\Delta_r G'^0$, $\Delta_r H'^0$, and $\Delta_r S'^0$ in terms of the thermodynamic parameters for reactions 3–9 [8]. Given the value of K' and the initial concentration, c_0 of ATP, the equilibrium extent of reaction ξ (expressed in mol L^{-1}) at specified pH and pMg can be calculated with

$$K' = \xi^2/(c_0 - \xi)c^0 \tag{11}$$

and so [ATP], [ADP], and [P_i] at equilibrium are readily calculated.

The concept of an equilibrium constant at specified T and P and the basis for calculating the equilibrium composition of a system containing many species is the fundamental equation of thermodynamics in terms of the Gibbs energy G, which has the following form at a specified temperature and pressure.

$$(dG)_{T,P} = \sum_{i=1}^{N} \mu_i \, dn_i \tag{12}$$

The number of species in the system is represented by N, μ_i is the chemical potential of species i, and n_i is its amount. In applying this equation to biochemical reactions, it is convenient to make the same approximations that are involved in deriving eq. (10). These include the assumptions that the solutions are dilute, there are no volume changes on reaction, the activity of water is constant, and the electrolyte (other than the reactants) does not have to be represented by additional terms in the fundamental equation. It is understood here that aqueous solutions of constant ionic strength are being considered, and so the chemical potentials do depend upon the ionic strength I.

The fundamental equation for the ATP system discussed above has eleven terms. In principle, seven extents of reaction can be introduced for reactions 3–9, and the eleven terms can be arranged in seven groups, each yielding an equilibrium constant expression. Equation (12) shows that the seven independent reactions must simultaneously be in equilibrium in order for the system to have its minimum Gibbs energy. The basic method for calculating the equilibrium composition of a closed system with a number of independent reactions is to minimize the Gibbs energy G of the system, using Lagrange multipliers to bring in the conservation relations that have to be obeyed. The set of simultaneous nonlinear equations is solved by the Newton–Raphson method, which involves an iteration carried out with a computer [9]. This method requires standard Gibbs energies of formation for reactants and a matrix **A** of the coefficients in the conservation equations for the system. An excellent example of a general equilibrium program is EQUCALC, written in APL (a programming language) in 17 lines by Krambeck and published in 1978 [10]. The conservation matrix **A** can be calculated from the stoichiometric numbers for a set of independent reactions that represent possible chemical changes in the system or can be written down from the conservation equations [11]. Smith has taken advantage of the fact that **A** can be calculated from the stoichiometric numbers by writing a computer program EQS in FORTRAN that calculates the equilibrium composition using the stoichiometric numbers for a set of independent chemical reactions and the standard reaction Gibbs energies. This program can also use the **A** matrix and standard Gibbs energies of formation. The calculation of the equilibrium composition for the ATP system by use of the computer programs EQUCALC and EQS has been discussed [12]. When the initial composition of the solution is expressed in terms of species, these calculations yield the $[H^+]$ and $[Mg^{2+}]$ of the solution at equilibrium, as well as the concentrations of the nine biochemical species.

For the hydrolysis of ATP, the fundamental equation is pretty complicated if all of the ionic species are considered, but if pH and pMg at equilibrium are specified the fundamental equation can be simplified to the form expected for a single reaction (reaction 1), as shown in the next section. This is important because the objective of this article is to discuss the chemical equilibrium for systems of biochemical reactions, each as complicated in itself as the hydrolysis of ATP.

2. Use of a Legendre transform to derive the fundamental equation in terms of the thermodynamic potential G'

To obtain a thermodynamic potential G' that is a minimum at specified T, P, pH, and pMg, terms in the chemical potentials of H^+ and Mg^{2+} need to be subtracted from G. The new thermodynamic potential G' is at a minimum at equilibrium because eq. (12) will be used to show that $(dG')_{T,P,\mathrm{pH},\mathrm{pMg}} \leqslant 0$. Alberty and Oppenheim [13] showed how a transformed Gibbs energy can be derived for the alkylation of benzene with ethylene at a specified partial pressure of ethylene. Wyman and Gill [14] have described the use of transformed Gibbs energies in describing macromolecular components.

The transformed Gibbs energy G' of a system at specified chemical potentials of hydrogen ion and magnesium ion is defined by

$$G' = G - \tilde{n}_{H^+}\mu_{H^+} - \tilde{n}_{Mg^{2+}}\mu_{Mg^{2+}} \qquad (13)$$

where the specified chemical potentials of H^+ and Mg^{+2} are represented by μ_{H^+} and $\mu_{Mg^{2+}}$. In this definition, $\tilde{n}_{H^+}$ and $\tilde{n}_{Mg^{2+}}$ are the total amounts of dissociable H^+ and Mg^{2+} (bound and free) in the system.

The next step is to define the transformed chemical potentials of the nine biochemical species present at the specified pH and pMg as follows:

$$\tilde{\mu}_{ATP^{4-}} = \mu_{ATP^{4-}} \tag{14}$$

$$\tilde{\mu}_{HATP^{3-}} = \mu_{HATP^{3-}} - \mu_{H^+} \tag{15}$$

$$\tilde{\mu}_{MgATP^{2-}} = \mu_{MgATP^{2-}} - \mu_{Mg^{2+}} \tag{16}$$

$$\tilde{\mu}_{ADP^{3-}} = \mu_{ADP^{3-}} \tag{17}$$

$$\tilde{\mu}_{HADP^{2-}} = \mu_{HADP^{2-}} - \mu_{H^+} \tag{18}$$

$$\tilde{\mu}_{MgADP^-} = \mu_{MgADP^-} - \mu_{Mg^{2+}} \tag{19}$$

$$\tilde{\mu}_{HPO_4^{2-}} = \mu_{HPO_4^{2-}} \tag{20}$$

$$\tilde{\mu}_{H_2PO_4^-} = \mu_{H_2PO_4^-} - \mu_{H^+} \tag{21}$$

$$\tilde{\mu}_{MgHPO_4} = \mu_{MgHPO_4} - \mu_{Mg^{2+}} \tag{22}$$

In other words, the chemical potentials of species containing dissociable H^+ (in the pH range considered) are adjusted by subtracting $\nu_{H^+}\mu_{H^+}$ and the chemical potentials of species containing dissociable Mg^{2+} are adjusted by subtracting $\nu_{Mg^{2+}}\mu_{Mg^{2+}}$, where ν_{H^+} and $\nu_{Mg^{2+}}$ are the stoichiometric numbers for hydrogen ion and magnesium ion in the dissociation reactions for the species. The equations where nothing is subtracted are unnecessary in a way, but they emphasize the fact that when pH and pMg are specified, the chemical potential variable that will be used will be $\tilde{\mu}$ for each biochemical species in the system, whether it contains dissociable hydrogen or magnesium or not.

The chemical potentials of the nine biochemical species in eq. (12) can be eliminated with eqs. (14) to (22) to obtain

$$\begin{aligned}(\mathrm{d}G)_{T,P} = {} & \mu_{H^+}\left[\mathrm{d}n_{H^+} + \mathrm{d}n_{HATP^{3-}} + \mathrm{d}n_{HADP^{2-}} \right. \\ & \left. + \mathrm{d}n_{H_2PO_4^-}\right] \\ & + \mu_{Mg^{2+}}\left[\mathrm{d}n_{Mg^{2+}} + \mathrm{d}n_{MgATP^{2+}} \right. \\ & \left. + \mathrm{d}n_{MgADP^-} + \mathrm{d}n_{MgHPO_4}\right] + \Sigma\tilde{\mu}_i\,\mathrm{d}n_i \end{aligned} \tag{23}$$

where the summation contains nine terms. This equation can be simplified by introducing the symbols

$$\mathrm{d}\tilde{n}_{H^+} = \mathrm{d}n_{H^+} + \mathrm{d}n_{HATP^{3-}} + \mathrm{d}n_{HADP^{2-}} + \mathrm{d}n_{H_2PO_4^-} \tag{24}$$

$$\begin{aligned}\mathrm{d}\tilde{n}_{Mg^{2+}} = {} & \mathrm{d}n_{Mg^{2+}} + \mathrm{d}n_{MgATP^{2-}} + \mathrm{d}n_{MgADP^-} \\ & + \mathrm{d}n_{MgHPO_4}\end{aligned} \tag{25}$$

Thus eq. (23) can be written

$$(\mathrm{d}G)_{T,P} = \mu_{H^+}\,\mathrm{d}\tilde{n}_{H^+} + \mu_{Mg^{2+}}\,\mathrm{d}\tilde{n}_{Mg^{2+}} + \Sigma\tilde{\mu}_i\,\mathrm{d}n_i \tag{26}$$

The differential of eq. (13) at constant T and P is

$$\begin{aligned}(\mathrm{d}G')_{T,P} = {} & (\mathrm{d}G)_{T,P} - \tilde{n}_{H^+}\,\mathrm{d}\mu_{H^+} - \mu_{H^+}\,\mathrm{d}\tilde{n}_{H^+} \\ & - \tilde{n}_{Mg^{2+}}\,\mathrm{d}\mu_{Mg^{2+}} - \mu_{Mg^{2+}}\,\mathrm{d}\tilde{n}_{Mg^{2+}}\end{aligned} \tag{27}$$

If pH and pMg are constant, this equation becomes

$$\begin{aligned}(\mathrm{d}G')_{T,P,\mathrm{pH},\mathrm{pMg}} = {} & (\mathrm{d}G)_{T,P} - \mu_{H^+}\,\mathrm{d}\tilde{n}_{H^+} \\ & - \mu_{Mg^{2+}}\,\mathrm{d}\tilde{n}_{Mg^{2+}}\end{aligned} \tag{28}$$

For a closed system, $\mathrm{d}\tilde{n}_{H^+} = 0$ and $\mathrm{d}\tilde{n}_{Mg^{2+}} = 0$ so that

$$(\mathrm{d}G')_{T,P,\mathrm{pH},\mathrm{pMg}} = (\mathrm{d}G)_{T,P} \leqslant 0 \tag{29}$$

Thus the transformed Gibbs energy G' is at a minimum at equilibrium at specified T, P, pH, and pMg, just like the Gibbs energy G is at a minimum at equilibrium at specified T and P.

Substituting eq. (26) in eq. (28) yields

$$(\mathrm{d}G')_{T,P,\mathrm{pH},\mathrm{pMg}} = \Sigma\tilde{\mu}_i\,\mathrm{d}n_i \tag{30}$$

The summation has nine terms for the ATP example. Hydrogen ions and magnesium ions are *implicitly* involved on the right side, but they are not *explicitly* involved. This equation can be further simplified by recognizing that at a specified pH and pMg, the species ATP^{4-}, $HATP^{3-}$, and $MgATP^{2-}$ have become *pseudoisomers*, and so have ADP^{3-}, $HADP^{2-}$, and $MgADP^-$ and HPO_4^{2-}, $H_2PO_4^-$, and $MgHPO_4$. In other words, the concentration ratios within these three groups have become functions of temperature only [9,15]. At equilibrium, the transformed chemical potentials of pseudoisomers are equal, and so

$$\tilde{\mu}_{ATP^{4-}} = \tilde{\mu}_{HATP^{3-}} = \tilde{\mu}_{MgATP^{2-}} = \tilde{\mu}_{ATP} \qquad (31)$$

$$\tilde{\mu}_{ADP^{3-}} = \tilde{\mu}_{HADP^{2-}} = \tilde{\mu}_{MgADP^{-}} = \tilde{\mu}_{ADP} \qquad (32)$$

$$\tilde{\mu}_{HPO_4^{2-}} = \tilde{\mu}_{H_2PO_4^{-}} = \tilde{\mu}_{MgHPO_4} = \tilde{\mu}_{P_i} \qquad (33)$$

The new symbols $\tilde{\mu}_{ATP}$, $\tilde{\mu}_{ADP}$, and $\tilde{\mu}_{P_i}$ represent the transformed chemical potentials of ATP, ADP, and P_i as a whole at specified T, P, pH, and pMg. Since $n_{ATP^{4-}} + n_{HATP^{3-}} + n_{MgATP^{2-}} = \tilde{n}_{ATP}$, $n_{ADP^{3-}} + n_{HADP^{2-}} + n_{MgADP^{-}} = \tilde{n}_{ADP}$, and $n_{HPO_4^{2-}} + n_{H_2PO_4^{-}} + n_{MgHPO_4} = \tilde{n}_{P_i}$ eq. (30) can be written as

$$(\mathrm{d}G')_{T,P,\mathrm{pH},\mathrm{pMg}} = \tilde{\mu}_{ADP}\,\mathrm{d}\tilde{n}_{ADP} + \tilde{\mu}_{ATP}\,\mathrm{d}\tilde{n}_{ATP} + \tilde{\mu}_{P_i}\,\mathrm{d}\tilde{n}_{P_i} \qquad (34)$$

Equation (34) has only three terms on the right and corresponds with reaction (1), which is written in terms of sums of concentrations and has a single extent of reaction. This is the fundamental equation for the hydrolysis of ATP. The new thermodynamic potential G' is different from G, but it behaves in very much the same way.

Equation (34) can be generalized to apply to a system with N reactants like ATP, in a multi-enzyme system.

$$(\mathrm{d}G')_{T,P,\mathrm{pH},\mathrm{pMg}} = \sum_{i=1}^{N} \tilde{\mu}_i\,\mathrm{d}\tilde{n}_i \qquad (35)$$

This is the fundamental equation at specified T, P, pH, and pMg in terms of the transformed Gibbs energy G', and it is easily confused with eq. (12), the fundamental equation in terms of the Gibbs energy T at specified T and P. The difference is that we are now considering a system at specified pH and pMg, and we are taking reactants like ATP as species, rather than ionic species, such as ATP^{4-}. Thus when pH and pMg are specified for a multi-enzyme system, the reactions are written in terms of sums of species, as in reaction (1). As discussed in connection with eq. (12), eq. (35) shows that the independent reactions written in terms of reactants such as ATP for a multi-enzyme system must be simultaneously in equilibrium for the system to have its minimum G' value. The same computer programs (EQS and EQUCALC) can be used to calculate the equilibrium composition in terms of reactants as were used to calculate the equilibrium composition in terms of ionic species. The EQS program requires $\Delta_r G'^0$ for the enzyme catalyzed reactions (see Section 3). EQUCALC requires $\Delta_f G'^0$, which are calculated in Section 4, and the **A** matrix calculated in Section 5.

3. Calculation of the equilibrium composition of a multi-enzyme system at specified pH and pMg using $\Delta_r G'^0$

Glycolysis is an example of a multi-enzyme system for which the equilibrium composition can be calculated at a specified T, P, pH, pMg, and ionic strength for a given initial composition. Glycolysis involves 16 reactants (17 including water, but we will leave water out) and 10 reactions for which the $\Delta_r G'^0$ are known at pH 7 and 25°C [16]. The first calculation considered here applies to a batch process in which the initial concentrations of the four reactants in the net reaction of glycolysis of glucose to pyruvate

$$\mathrm{GLU} + 2\mathrm{P_i} + 2\mathrm{ADP} + 2\mathrm{NAD} = 2\mathrm{PYR} + 2\mathrm{ATP} + 2\mathrm{NADH} \qquad (36)$$

are specified. Of course, any concentrations of the 16 reactants can be specified initially.

The stoichiometric numbers and $\Delta_{rc}G'^0$ and $\Delta_{rx}G'^0$ values at 25°C and pH 7 for the 10 reaction of glycolysis are given in Table 1. The $\Delta_{rc}G'^0$ values are the usual standard apparent Gibbs energies of reaction on the mol L^{-1} concentration scale, but when concentrations are expressed on the mole fraction scale (see Section 5) the $\Delta_{rx}G'^0$ values will be needed. The equilibrium constants expressed in terms of mole fractions can be calculated from the equilibrium constants expressed in terms of mol L^{-1} by dividing by 55.35^s, where s is the difference between the number of concentration terms in the numerator of the equilibrium constant expression and the number of concentration terms in the denominator. This involves the approximation that the amount of water in a liter of solution is 55.35 mol — the amount in a liter of pure water at 25°C.

The columns in Table 1 correspond with the reactants in the order glucose (GLU), ATP, glucose 6-phosphate (G6P), fructose 6-phosphate (F6P), fructose 1,6-biphosphate (F1,6BP), dihydroxyacetone phosphate (DHAP), NAD, 1,3-bisphosphoglycerate (1,3BPG), 3-phosphoglycerate (3PG), 2-phosphosphoglycerate (2PG), phosphoenolpyruvate (PEP), pyruvate (PYR), ADP, glyceraldehyde 3-phosphate (GAP), NADH, and P_i. The order of entry of reactants into EQS is arbitrary, and the reasons for the order in Table 1 will be clarified in Section 4 on the calculation of the standard transformed Gibbs energies of formation.

The equilibrium composition calculated using EQS is given in Table 2 at pH 7 and 25°C for a batch process starting with 1 10^{-3} *M* GLU, 2 10^{-3} *M* ADP, 2 10^{-3} *M* NAD, and 2 10^{-3} *M* P_i.

Looking at these results from a system point of view, the equilibrium system has an extremely low (8.8 10^{-11} *M*) concentration of 1,3-bisphosphoglycerate. This could be avoided if the glyceraldehyde 3-phosphate dehydrogenase and phosphoglycerate kinase activities were in a single enzyme so that this substrate would not be liberated into

Table 2

Initial concentrations and equilibrium concentrations in mol L^{-1} for the 16 components of the glycolysis system at pH 7 and 25°C calculated with EQS (the last column gives $\Delta_{fc}G'^{0}$/kJ mol^{-1} calculated in Table 3)

Component	Initial Conc. (mol/L)	Equilibrium Conc. (mol/L)	$\Delta_{fc}G'^{0}$ (kJ/mol)
1 Glucose	0.001	1.38 10^{-10}	60.68
2 ATP	0	1.25 10^{-3}	−31.38
3 ADP	0.002	7.54 10^{-4}	0
4 Glucose 6-phosphate	0	1.95 10^{-7}	12.56
5 Fructose 6-phosphate	0	9.94 10^{-8}	14.23
6 Fructose 1,6-biphosphate	0	4.91 10^{-5}	−31.38
7 Dihydroxy-acetone phosphate	0	2.26 10^{-4}	−7.53
8 Glyceraldehyde 3-phosphate	0	1.27 10^{-5}	0
9 NAD	0.002	3.77 10^{-4}	−25.1
10 NADH	0	1.62 10^{-3}	0
11 P_i	0.002	3.77 10^{-4}	0
12 1,3-Bisphospho-glycerate	0	8.87 10^{-11}	−18.82
13 3-Phospho-glycerate	0	1.07 10^{-7}	−6.27
14 2-Phospho-glycerate	0	1.67 10^{-8}	−1.67
15 Phosphoenol-pyruvate	0	8.52 10^{-9}	0
16 Pyruvate	0	1.62 10^{-3}	0

Table 1

Stoichiometric numbers for the 10 reactions of glycolysis and the standard transformed Gibbs energies of reaction for concentrations in mol L^{-1}, ($\Delta_{rc}G'^{0}$/kJ mol^{-1}) and mole fractions ($\Delta_{rx}G'^{0}$/kJ mol^{-1}) at pH 7 and 25°C

Reaction	Component																$\Delta_{rc}G'^{0}$ (kJ/mol)	$\Delta_{rx}G'^{0}$ (kJ/mol)
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16		
1	−1	−1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	−16.74	−16.74
2	0	0	−1	1	0	0	0	0	0	0	0	0	0	0	0	0	1.67	1.67
3	0	−1	0	−1	1	0	0	0	0	0	0	0	1	0	0	0	−14.23	−14.23
4	0	0	0	0	−1	1	0	0	0	0	0	0	0	1	0	0	23.85	33.80
5	0	0	0	0	0	−1	0	0	0	0	0	0	0	1	0	0	7.53	7.53
6	0	0	0	0	0	0	−1	1	0	0	0	0	0	−1	1	−1	6.28	−3.67
7	0	1	0	0	0	0	0	−1	1	0	0	0	−1	0	0	0	−18.83	−18.83
8	0	0	0	0	0	0	0	0	−1	1	0	0	0	0	0	0	4.60	4.60
9	0	0	0	0	0	0	0	0	0	−1	1	0	0	0	0	0	1.67	1.67
10	0	1	0	0	0	0	0	0	0	0	−1	1	−1	0	0	0	−31.38	−31.38

the solution. Cornell et al. [17] actually determined the equilibrium constant directly for the combined glyceraldehyde phosphate dehydrogenase and phosphoglycerate kinase reactions.

Although we can calculate the equilibrium compositions, it should be noted that if we prepare a solution of GLU, P_i, ADP, NAD, and the 10 enzymes, nothing will happen because there is no ATP for the first step. In the cell ATP is available, and a way to make the equilibrium calculation in a way more appropriate for the cell will be described in Section 6.

A simple way to check that the equilibrium composition is in agreement with the 10 equilibrium constant expressions is described in the Appendix A.

4. Calculation of standard transformed Gibbs energies of formation at specified pH and pMg

In order to calculate the equilibrium composition with EQUCALC, it is necessary to have a consistent set of standard transformed Gibbs energies of formation of the reactants. An earlier paper [12] has shown how this can be done for ionic species in biochemical reactions and has proposed the production of a table of standard Gibbs energies of formation and standard enthalpies of formation for ionic species of biochemical reactants. Here the same method is applied, and the production of a large table for reactants as pH 7 and pMg 3 is proposed. The advantage of $\Delta_f G'^0$ values over $\Delta_r G'^0$ values is that $\Delta_r G'^0$ for a biochemical reaction at pH 7 and pMg 3 can be calculated by use of

$$\Delta_r G'^0 = \Sigma \nu_i \Delta_f G_i'^0 \tag{37}$$

where ν_i is the stoichiometric number of reactant i in the reaction considered. It is easier to calculate $\Delta_r G'^0$ for a new reaction using eq. (37) than by adding and subtracting reactions, which is the method currently used in biochemistry. For example, 10 $\Delta_r G'^0$ values have to be added, with some of them multiplied by 2, to obtain $\Delta_r G'^0$ for the net reaction of glycolysis, reaction (36).

The thermodynamic data on glycolysis provides 10 linear equations (see eq. 37) in 16 unknown standard Gibbs energies of formation. This is not enough information to determine the 16 unknown $\Delta_f G'^0$ values. These 10 linear reactions have an infinite number of solutions, but we only need one solution. The procedure for determining a solution is well known [18] and has been applied by Smith and Missen [9] and by Cheluget et al. [19]. A consistent set of $\Delta_f G'^0$ values at the same pH and pMg can be obtained by forming the augmented matrix $[\nu^T, \Delta_r \boldsymbol{G}'^0]$ and carrying out a Gaussian reduction. Here $\boldsymbol{\nu}^T$ represents the transpose of the stoichiometric number matrix ν and $\Delta_r \boldsymbol{G}'^0$ is the column matrix of standard transformed Gibbs energies of reaction. This can be done conveniently with the command row

Table 3

Row reduced form of Table 1. The last two columns give the Gibbs energies at pH 7 and 25°C

Radion	Component																$\Delta_{fc}G'^0$ (kJ/mol)	$\Delta_{fx}G'^0$ (kJ/mol)
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16		
1	1	0	0	0	0	0	0	0	0	0	2	−2	0	−2	0	0	60.68	50.73
2	0	1	0	0	0	0	0	0	0	0	−1	1	−1	0	0	0	−31.38	−31.38
3	0	0	1	0	0	0	0	0	0	0	1	−1	0	−2	0	0	12.56	2.61
4	0	0	0	1	0	0	0	0	0	0	1	−1	0	−2	0	0	14.23	4.28
5	0	0	0	0	1	0	0	0	0	0	0	0	0	−2	0	0	−31.38	−41.33
6	0	0	0	0	0	1	0	0	0	0	0	0	0	−1	0	0	−7.53	−7.53
7	0	0	0	0	0	0	1	0	0	0	−2	1	0	1	−1	1	−25.1	−15.15
8	0	0	0	0	0	0	0	1	0	0	−2	1	0	0	0	0	−18.82	−18.82
9	0	0	0	0	0	0	0	0	1	0	−1	0	0	0	0	0	−6.27	−6.27
10	0	0	0	0	0	0	0	0	0	1	−1	0	0	0	0	0	−1.67	−1.67

Reduce from the program MATHEMATICA [20]. The result of the Gaussian reduction of the matrix in Table 1 is shown in Table 3. It is evident from Table 3 that if the reactants represented by the last six columns are assigned $\Delta_{fc}G'^0 = \Delta_{fx}G'^0 = 0$, the 10 simultaneous equations are satisfied by using the standard apparent Gibbs energies of formation in the last two columns for the remaining 10 reactants. The list of 16 $\Delta_{fc}G'^0$ values, given in Table 2, is called a consistent set, and it contains all of the thermodynamic information in the 10 $\Delta_{rc}G'^0$ values for glycolysis at pH 7.

The six reactants to be assigned zeros can be chosen arbitrarily to some extent, but not completely. The Gaussian reduction tends to assign zeros to the reactants listed in the later columns of the $\boldsymbol{\nu}^T$ matrix, but reactants like ATP and ADP cannot both be assigned zeros. To obtain a table in the form of Table 3 (that is, with a submatrix in row echolon form on the left), it is necessary to rearrange columns.

The consistent set of standard transformed Gibbs energies of formation for the reactants in glycolysis given in Tables 2 and 3 are used in the next section to calculate the equilibrium composition. This process has not taken into account the fact that the standard transformed Gibbs energies of formation of glucose [21] and pyruvate [21,22] are known at pH 7. The $\Delta_f G^0$ and $\Delta_f H^0$ values of inorganic phosphate are known, and the value for P_i at pH 7 and pMg 3 can be calculated (see Appendix B) using data from the NBS Tables [23]. Furthermore, the difference in the standard Gibbs energies of formation of ATP and ADP can be calculated from data on reaction (1). These values could have been used in the calculations shown in Tables 1 and 3 by subtracting these values out before the use of RowReduce. This should be done in preparing larger tables of standard transformed Gibbs energies of formation so that they will be consistent with other tables, but this is not necessary to calculate the equilibrium composition of the glycolysis system.

The $\Delta_f G'^0$ values obtained in Table 3 do not yield the correct K' for the hydrolysis of ATP at pH 7. This is not surprising since ATP, ADP, and P_i are not in equilibrium in this system. However, if $\Delta_r G'^0(\mathrm{ATP} \rightleftharpoons \mathrm{ADP} + P_i)$ is added as an 11th reaction, and $\Delta_f G'^0$ values are recalculated, only two are changed. The value of $\Delta_{fc}G'^0$ for P_i is decreased by 63.38 kJ mol^{-1}, and the $\Delta_{fc}G'^0$ value is increased by 63.38 kJ mol^{-1}. In EQUCALC they give the same equilibrium composition for glycolysis as before. This shows that a consistent set of $\Delta_f G'^0$ values can be extended by adding further independent reactions.

5. Calculation of the equilibrium composition of a multi-enzyme system at specified pH and pMg using $\Delta_f G'^0$

This method requires the conservation matrix **A**. Matrix **A** gives the coefficients of the amounts of the various species (in this case the 16 reactants) in the conservation equations for the system. In the absence of a specific mechanism, this matrix may include a row for each element in the system and electric charge. However, such a matrix may have redundant rows, and they should be eliminated. For example, if two elements in the system always occur together in a certain ratio, the combination can be considered to be a pseudoelement and one row can be eliminated. If a specific mechanism is involved, there may be conservation relations implicit in the mechanism [9,24], and these must be included in the **A** matrix.

There are two methods to obtain the **A** matrix for a system: (1) it can be calculated from the $\boldsymbol{\nu}$ matrix, or (2) it can be written down directly from the conservation relations. The **A** matrix is C × S, where C is the number of components (in this case six).

$$\mathrm{C} = \operatorname{rank} \mathbf{A} \tag{38}$$

and S is the number of species (in this case, 16). The number of components is also given by

$$\mathrm{C} = \mathrm{S} - \mathrm{R} \tag{39}$$

where R is the number of independent reactions (in this case, 10)

$$\mathrm{R} = \operatorname{rank} \boldsymbol{\nu} \tag{40}$$

The **A** matrix can be calculated from the stoichiometric number matrix $\boldsymbol{\nu}$ of Table 1 by use of the following equation [10]

$$\mathbf{A}\boldsymbol{\nu} = \mathbf{0} \tag{41}$$

where **A** is CxS and $\boldsymbol{\nu}$ is S × R, so that **0** is CxR. This equation can also be written in the form

$$\boldsymbol{\nu}^{\mathrm{T}}\mathbf{A}^{\mathrm{T}} = \mathbf{0} \tag{42}$$

where $^{\mathrm{T}}$ indicates the transpose. Note that $\boldsymbol{\nu}^{\mathrm{T}}$ is R × S and $\mathbf{A}^{\mathrm{T}}$ is SxC, so that **0** is RxC. Thus $\mathbf{A}^{\mathrm{T}}$ is the null space of $\boldsymbol{\nu}^{\mathrm{T}}$ and can be readily calculated with MATHEMATICA from the stoichiometric number matrix of the mechanism by use of the command NullSpace.

The **A** matrix for glycolysis calculated from the $\boldsymbol{\nu}$ matrix (Table 1 without the columns of Gibbs energies) using the command NullSpace in MATHEMATICA is given in Table 4 along with its row reduced form calculated with the command RowReduce in MATHEMATICA. The form of the **A** matrix is not unique, but its row reduced form is, for given order of reactant columns.

To obtain the **A** matrix method by the second method, we first consider the conservation of the elements C, H, O, N, and P. Hydrogen is not conserved because because the pH is specified, and we can imagine that hydrogen ions are added to the system to the extent necessary to give the specified pH from a hypothetical reservoir at the specified pH. Oxygen is not conserved in the sense that H_2O is not included in the equilibrium expressions. However, the adenosine of ATP and ADP and the adenosine and nicotinamide of NAD and NADH are conserved separately, and so this yields two conservation equations. Carbon and phosphorous are conserved. Thus, there are four conservation equations of the "element" type. According to the **A** matrix of Table 4 and eq. (38), there are six conservation relations, and so there are two conservation relations that are implicit in the mechanism. There is a conservation equation linking P_i and NADH; the mechanism shows that for each mole of inorganic phosphate that is consumed, one mole of NADH is produced. The second conservation equation implicit in the mechanism comes from the fact that NAD produces 1,3-bisphophoglycerate, which is partially converted to 3-phosphoglycerate, which is partially converted to 2-phosphoglycerate, which is partially converted to phosphoenolpyruvate, which is partially converted to pyruvate. Thus, the sum of these six concentrations is constant. It is interesting to find that reaction (6) is the origin of two non-element conservation relations. There are other conservation equations, but they will be redundant. The **A** matrix obtained in this way is shown in Table 5, along with its row reduced form. Since the row reduced forms in Tables 4 and 5 are the same, methods (1) and (2) give the same result.

The conservation matrix is not unique, and there are other ways that it can be developed.

Table 4

Conservation matrix **A** calculated from $\boldsymbol{\nu}^{\mathrm{T}}$ and its row reduced form

$$\mathbf{A} = \left|\begin{array}{cccccccccccccccc}
0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \\
0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 \\
2 & 0 & 2 & 2 & 2 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 \\
0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 \\
2 & -1 & 1 & 1 & 0 & 0 & -1 & -1 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 \\
-2 & 1 & -1 & -1 & 0 & 0 & 2 & 2 & 1 & 1 & 1 & 0 & 0 & 0 & 0 & 0
\end{array}\right|$$

$$\left|\begin{array}{cccccccccccccccc}
1 & 0 & 0 & 0 & -1 & -1/2 & 0 & 0 & 1 & 1 & 1 & 2 & 1 & -1/2 & 0 & 1/2 \\
0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 \\
0 & 0 & 1 & 1 & 2 & 1 & 0 & 0 & -1 & -1 & -1 & -2 & -1 & 1 & 0 & -1 \\
0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 1 & 1 & 1 & 0 & 0 & 0 & 1 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1
\end{array}\right|$$

However, the row reduced form is unique for a given order for the reactants in the columns. In the past there has not been much attention to the conservation relations introduced by a mechanism, but this is a important feature that should be recognized. Whenever the number of reactions in a mechanism is less than the number R calculated from eq. (4) by taking the number of components equal to the number of elements (four, as derived above), there are constraints implicit in the mechanism. For glycolysis, there are 16 reactants and 4 "element" conservation relations, and so the number of independent reactions required to represent all possible changes in chemical composition permitted by the element balances is $16-4=12$. However, there are only 10 reactions in glycolysis, and so 2 constraints are implicit in the mechanism.

Since EQUCALC used $\Sigma x_i = 0$, the equilibrium constants have to be expressed in terms of mole fractions. Therefore, $[\boldsymbol{\nu}^{\mathrm{T}}, \Delta_{\mathrm{rc}}G'^{0}, \Delta_{\mathrm{rx}}G'^{0}]$ was used in the preceding section. The use of **A** and standard transformed Gibbs energies of formation in EQUCALC gives the same equilibrium composition as given in Table 3. EQUCALC can be used directly by including 55.35 mol of water with a standard Gibbs energy of zero, or the program can be modified to take concentrations directly (Krambeck [10], personal communication). The calculations were made on a personal computer with STSC APL 8 (STSC, Rockville, MD).

6. Calculation of the equilibrium composition of a multi-enzyme system in a steady state at specified pH and pMg

An equilibrium calculation on glycolysis that is more relevant to the situation in a particular cell can be made by taking the steady state concentrations of ATP, ADP, P_i, NAD, and NADH as specified, rather than to be calculated. For example, it is possible to do this with Minakami's data [25] for red cells because there are no mitochondria to complicate the interpretation of the analytical data on the reactants.

The ATP, ADP, P_i, NAD, and NADH involved in glycolysis are produced and consumed by other reaction systems that hold their concentrations rather constant. The simplest way to hold these concentrations constant in an equilibrium calculation on the other reactants is to incorporate these five steady state concentrations into the equilibrium constant expressions for the successive steps of glycolysis, and calculate an adjusted set of standard transformed Gibbs energies of reaction for use in EQS.

In order to find a situation where several successive reactions in a pathway are close to equilibrium, the best place to look is between control points, and the calculations in this section are therefore concerned with reactions (4)–(9) in glycolysis. In fact, Minakami says these reactions are in a state of quasi equilibria in red cells. The

Table 5

Conservation matrix **A** developed from the conservation equations and its row reduced form

$$\mathbf{A} = \begin{vmatrix} 6 & 0 & 6 & 6 & 6 & 3 & 0 & 3 & 3 & 3 & 3 & 3 & 0 & 3 & 0 & 0 \\ 0 & 3 & 1 & 1 & 2 & 1 & 0 & 2 & 1 & 1 & 1 & 0 & 2 & 1 & 0 & 1 \\ 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 1 & 1 & 1 & 1 & 0 & 0 & 0 & 0 \end{vmatrix}$$

$$\begin{vmatrix} 1 & 0 & 0 & 0 & -1 & -1/2 & 0 & 0 & 1 & 1 & 1 & 2 & 1 & -1/2 & 0 & 1/2 \\ 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 1 & 1 & 2 & 1 & 0 & 0 & -1 & -1 & -1 & -2 & -1 & 1 & 0 & -1 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 1 & 1 & 1 & 0 & 0 & 0 & 1 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 \end{vmatrix}$$

equilibrium constants for these reactions at pH 7, pMg 3, and 38°C are taken to be $K_4' = 0.99\ 10^{-4}$ [26], $K_5' = 0.0455$ [26], $K_6' = 0.51$ [17], $K_7' = 3600$ [17], $K_8' = 0.102$ [27], and $K_9' = 4.55$ [27]. Since Minakami calculated the concentration of 1,3B-PG, reactions (6) and (7) have been combined to eliminate 1,3BPG from the calculations here. When reactions (6) and (7) are added together, the equilibrium constant for the combined reaction is 1836. The standard transformed Gibbs of reaction for the reactions involved are given in Table 6. Only reaction (6) + (7) is together affected by the steady state concentrations of [ATP], [ADP], [P_i], [NAD], and [NADH]. Actually, only the ratio [NAD]/[NADH] is required. When Minakami's values for the steady state concentrations are substituted in the equilibrium constant expression for the reaction (6) (7), [3PG]/[GAP] = 177.9 is obtained. The adjusted reaction Gibbs energies $\Delta_r G''^0$ in the last column apply in the steady state. The extra prime indicates that some reactant concentrations are specified.

In calculating the equilibrium concentrations of the six reactants involved in this part of glycolysis at the steady state concentrations of [ATP] = 1.83 mM, [ADP] = 180 μM, [P_i] = 1.0 mM, and [NAD]/[NADH] = 985, any initial composition of the remaining reactants can be chosen, and Table 7 is based on choosing the steady state concentrations determined by Minakami. The equilibrium composition calculated with EQS is given in Table 7. This calculation shows that in general material is shifted forward, but there are a couple of exceptions in 3PG and 2PG. However, this may not be significant in view of the experimental errors in determining the concentrations of reactants and, especially, in determining the equilibrium constants of the five reactions at the correct T, pH, pMg, and ionic strength. The calculation indicates that the experimental composition is quite close to the equilibrium composition; in general the first reactants in the direction of glycolysis are above their equilibrium concentrations and the later reactants are below their equilibrium concentrations, as expected.

Table 6

Standard transformed Gibbs energies of reaction at 38°C, pH 7, pMg 3, and 0.25 ionic strength for reactions (4)–(9) in glycolysis. The last column gives the transformed Gibbs energies of reaction $\Delta_r G''^0$ after adjustment for the steady state concentrations of ATP, ADP, P_i, NAD, and NADH

Reaction	$\Delta_r G'^\circ$ (kJ/mol)	$\Delta_r G''^\circ$ (kJ/mol)
(4) F1,6BP $\rightleftharpoons$ DHAP + GAP	24.84	24.84
(5) DHAP $\rightleftharpoons$ GAP	7.99	7.99
(6 + 7) GAP $\rightleftharpoons$ 3PG	− 19.43 [a]	− 13.40
(8) 3PG $\rightleftharpoons$ 2PG	5.90	5.90
(9) 2PG $\rightleftharpoons$ PEP	−3.92	−3.92

[a] This is the value for GAP + P_i + ADP + NAD $\rightleftharpoons$ 3PG + ATP + NADH.

Table 7

Initial and equilibrium concentrations of six reactants in glycolysis between control points at the steady state concentrations of [ATP], [ADP], [P_i], [NAD], and [NADH] in red blood cells at 38°C, pH 7, pMg 3, and 0.25 mol L^{-1} ionic strength.

Reactant	Concentration (μM)		
	Initial	Equilibrium	Change
F1,6BP	7	0.04	−6.96
DHAP	17	9.7	−7.3
GAP	5.7	0.44	−5.26
3PG	68.5	78.5	10.0
2PG	10	8	−2
PEP	17	36.4	19.4

This type of equilibrium calculation can be used to answer various "what if" questions. What if [P_i] is decreased? what if [NAD]/[NADH] is decreased? What if the initial concentrations of the reactant are doubled? (The equilibrium concentrations will not simply double because of reaction (4) of glycolysis.) What if pH or pMg is changed?

Note that although this is an equilibrium calculation, ATP, ADP, and P_i are not at equilibrium. The system for which the equilibrium is calculated can be described as follows: The reactants for reactions (4) to (9) in glycolysis and the enzymes required are enclosed in a container with semipermeable walls through which ATP, ADP, P_i, NAD, and NADH can enter from reservoirs at the specified concentrations. The reactants F1,6BP, DHAP, GAP, 1,3BPG, 3PG, 3PG, 2PG, and PEP and the enzymes cannot escape from the system, and [F1,6BP], [DHAP], [GAP], [1,3BPG], [3PG], [2PG], and [PEP] reach their equilibrium values. In the calculation here, it is assumed that the amounts bound by the enzymes are negligible.

7. Discussion

The apparent Gibbs energy G' used in biochemistry at specified pH and pMg has been shown to be a Legendre transformed Gibbs energy that is at a minimum at equilibrium at T, P, pH, and pMg. There are corresponding transformed enthalpies and entropies.

The equilibrium concentrations of the 16 reactants in glycolysis can be calculated at a given T, P, pH, pMg, and ionic strength for a batch process starting with given initial concentrations of the reactants using either EQS or EQUCALC. The equilibrium concentrations of the reactants in a system like glycolysis can also be calculated using steady state concentrations of ATP, ADP, P_i, NAD, and NADH. Equilibrium is important in modelling because a model of a system like glycolysis should yield the equilibrium composition at long times and should account for the reverse reaction as well as the forward reaction. In particular, the reactions considered in Table 7 go in the reverse direction in gluconeogenesis.

The table of $\Delta_f G'^0$ values proposed here is related to the table of $\Delta_f G^0$ values proposed earlier [12], but is not completely dependent on it. If all of the acid dissociation constants and magnesium binding constants are known, $\Delta_f G^0$ values can be calculated for all of the species and these values in turn can be used to calculate $\Delta_f G'^0$ for the sum of species at pH 7 and pMg 3. For example, for ADP the $\Delta_f G^0$ value of zero could be assigned to H_2ADP^-, and this will yield a nonzero values for $\Delta_f G'^0$ for ADP and ATP at pH 7 and pMg 3. The consistent set of standard apparent Gibbs energies of formation given in Table 2 can be extended indefinitely by including values from other reactions for other reactants. Values from Miller and Smith-Magowan [22] can be incorporated, but some values there need to be adjusted to pH 7. Goldberg and Tewari [28,29] have summarized $\Delta_r G'^0$ values that can be used to calculate standard apparent Gibbs energies of formation for other reactants. Since there are some choices of reactants to be assigned $\Delta_f G'^0$, international agreement is needed on which reactants should be assigned zeros. The calculation of the standard transformed Gibbs energy of formation and standard transformed enthalpy of formation of inorganic phosphate at pH 7 and pMg 3 is described in Appendix B.

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Appendix A

When a calculation requires a good deal of computer input, it is a good idea to check the calculated results. One way to do that is to consider the calculated concentrations as experimental data and calculate the thermodynamic properties of the reactants from the calculated composition. In this case the $\Delta_f G'^0$ values can be calculated for the 10 reactions. This calculation is represented in terms of matrices by

$$\Delta_r G'^0 = -RT \ln K' = -RT \boldsymbol{\nu}^T \ln \boldsymbol{n} \tag{A1}$$

In words, the logarithm of the column vector n of calculated equilibrium concentrations is calculated and this matrix is multiplied by the transpose of the $\boldsymbol{\nu}$ matrix (Table 1 without the two columns of thermodynamic properties) and is multiplied by $-RT$. The resulting column vector $\Delta_r G'^0$ should give the standard apparent Gibbs energies of reaction used as input.

In a second type of test, the conservation relations are applied to check that the system contains the same amounts of "elements" at equilibrium as it did initially. This test is applied by simply taking the matrix product of **A** and the vector ***n*** initially and at equilibrium.

Appendix B

The values of $\Delta_f G'^0$ and $\Delta_f H'^0$ for P_i at 25°C, pH 7, pMg 3, and ionic strength 0.25 mol L^{-1} with respect to the elements in their standard states can be calculated from the data of The NBs Tables [23] and Clarke et al. [30]. The data from these sources at 25°C and zero ionic strength are given in the first part of Table 8. The correction of standard thermodynamic properties of ions to higher ionic strengths has been discussed in the literature [29–34]. Following Goldberg and Tewari [29], the following formulas are used at 25°C.

$$\Delta_f G_i^0(I) = \Delta_f G_i^0(I=0) + 2.91482 z_i^2 I^{1/2}/(1+BI^{1/2}) \quad \text{(B1)}$$

$$\Delta_f H_i^0(I) = \Delta_f H_i^0(I=0) + 1.4775 z_i^2 I^{1/2}/(1+BI^{1/2}) \quad \text{(B2)}$$

Table 8

Calculation of $\Delta_f G'^0$ and $\Delta_f H'^0$ for P_i at 25°C, pH 7, pMg 3, and $I = 0.25$ mol L^{-1}

Reactant	$\Delta_f H^0$ (kJ/mol)	$\Delta_f G^0$ (kJ/mol)
At I = 0 mol L⁻¹		
HPO_4^{2-}	−1292.14	−1089.15
$H_2PO_4^-$	−1296.29	−1130.28
$MgHPO_4$	−1746.79	−1559.41
Mg^{2+}	−466.85	−454.8
H^+	0	0
At I = 0.25 mol L⁻¹		
HPO_4^{2-}	−1290.50	−1092.31
$H_2PO_4^-$	−1295.88	−1131.09
$MgHPO_4$	−1746.79	−1559.41
Mg^{2+}	−465.21	−458.04
H^+	0.41	−0.81
At I = 0.25, pH 7, pMg 3		
HPO_4^{2-}	−1290.50	−1092.39
$H_2PO_4^-$	−1296.29	−1090.32
$MgHPO_4$	−1281.58	−1084.25
	$\Delta_f H'^0$/kJ mol^{-1}	$\Delta_f G'^0$/kJ mol^{-1}
P_i	−1290.50	−1093.35

where kJ mol^{-1} are used and $B = 1.6$ L$^{-1/2}$ mol$^{-1/2}$. The thermodynamic properties of the ions at $I = 0.25$ mol L^{-1} are given in the second part of the table. In the third part of the table, the value of $\Delta_f G^0$ for $H_2PO_4^-$ is adjusted to pH 7 by subtracting $\Delta_f G^0_{H^+} + RT \ln 10^{-pH}$, and the value of $\Delta_f G^0$ for $MgHPO_4$ is adjusted to pMg 3 by subtracting $\Delta_f G^0(Mg^{2+}) + RT \ln 10^{-pMg}$. Since HPO_4^{2-}, $H_2PO_4^-$, and $MgHPO_4$ are pseudoisomers at pH 7 and pMg 3, the standard transformed Gibbs energy of formation $\Delta_f G'^0$ and the standard transformed enthalpy of formation $\Delta_f H'^0$ of inorganic phosphate at pH 7 and pMg 3 are given by the following equations [15].

$$\Delta_f G'^0_{P_i} = -RT \ln\Big\{\exp\big[-\Delta_f G'^0_{HPO_4^{2-}}/RT\big] + \exp\big[-\Delta_f G'^0_{H_2PO_4^-}/RT\big] + \exp\big[\Delta_f G'^0_{MgHPO_4}/RT\big]\Big\} \quad \text{(B3)}$$

$$\Delta_f H'^0_{P_i} = r_{HPO_4^{2-}} \Delta_f H'^0_{HPO_4^{2-}} + r_{H_2PO_4^-} \Delta_f H'^0_{H_2PO_4^-} + r_{MgHPO_4} \Delta_f H'^0_{MgHPO_4} \quad \text{(B4)}$$

where

$$r_i = \exp\Big\{\big[\Delta_f G'^0_{P_i} - \Delta_f G_i'^0\big]/RT\Big\} \quad \text{(B5)}$$

Note that $\Delta_f G'^0_{P_i}$ is more negative than for any of the pseudoisomers, while $\Delta_f H'^0_{P_i}$ is a mole fraction weighted average.

Notation

A	conservation matrix (C × N) (matrix of coefficients in the C conservation equations for the N species)
c^0	standard state concentration (1 mol L^{-1})
C	number of components (= rank **A**)
G	extensive Gibbs energy
G'	extensive transformed Gibbs energy (The concentrations of certain reactants at equilibrium have to be specified).

$\Delta_r G_j^0$	$= (-RT\ln K_j = \Delta_r H_j^0 - T\Delta_r S_j^0 = \Sigma\nu_i\Delta_f G_i^0)$ standard Gibbs energy of the jth reaction written in terms of ionic species at a specified ionic strength
$\Delta_r G_j'^0$	$(= -RT\ln K_j' = \Delta_r H_j'^0 - T\Delta_r S_j'^0 = \Sigma\nu_i\Delta_f G_i'^0$ standard transformed Gibbs energy of reaction at specified pH, pMg and ionic strength. (The subscripts rc, rx, fc and fx are used to indicate the mol L^{-1} and mole fraction concentration scales.)
$\Delta_r G''^0$	standard transformed Gibbs energy of reaction at specified pH, pMg, ionic strength, and concentrations of certain reactants
$\Delta_f G_i^0$	standard Gibbs energy of formation of i at $I = 0$ mol L^{-1}
$\Delta_f G_{P_i}'^0$	standard transformed Gibbs energy of formation of P_i (sum of species) at specified pH, pMg, and ionic strength (The subscripts fc and fx are used to indicate the mol L^{-1} and mole fraction concentration scales.)
H	extensive enthalpy
H'	extensive transformed enthalpy at specified pH, pMg, and I
I	ionic strength in mol L^{-1}
K_j	equilibrium constant of the jth reaction written in terms of molar concentrations of species (omitting H_2O) at a specified ionic strength
K_j'	apparent equilibrium constant of the jth reaction written in terms of molar concentrations of sums of species (omitting H_2O) at a specified pH, pMg, and I
N	number of species (or isomer groups) in a system ($= C + R$)
$n_{ATP^{-4}}$	amount of species ATP^{4-}
n_{ATP}	amount of ATP at specified pH and pMg
$\bar{n}_{H^+}$	amount of species containing H^+
$\bar{n}_{Mg^{2+}}$	amount of species containing Mg^{2+}
r_i	equilibrium mole fraction of isomer i within the isomer group
P	pressure
R	gas constant
R	number of independent reactions (= rank ν)
S	number of species
T	absolute temperature
x_i	equilibrium mole fraction of species i
ν_i	stoichiometric number of i in a reaction
$\boldsymbol{\nu}$	stoichiometric number matrix (S × R) for a system
$\mu_{MgATP^{2-}}$	chemical potential of $MgATP^{2-}$ at a specified ionic strength
$\tilde{\mu}_{MgATP^{2-}}$	transformed chemical potential of $MgATP^{2-}$ at specified pH, pMg, and I
$\tilde{\mu}_{ATP}$	transformed chemical potential of ATP at specified pH, pMg, and ionic strength
ξ_j	extent of reaction j

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