Levels of Thermodynamic Treatment of Biochemical Reaction Systems

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ABSTRACT Equilibrium calculations on biochemical reaction systems can be made at three levels. Level 1 is the usual chemical calculation with species at specified temperature and pressure using standard Gibbs energies of formation of species or equilibrium constants K. Level 2 utilizes reactants such as ATP (a sum of species) at specified T, P, pH, and pMg with standard transformed Gibbs energies of formation of reactants or apparent equilibrium constants K'. Calculations at this level can also be made on the enzymatic mechanism for a biochemical reaction. Level 3 utilizes reactants at specified T, P, pH, and pMg, but the equilibrium concentrations of certain reactants are also specified. The fundamental equation of thermodynamics is derived here for Level 3. Equilibrium calculations at this level use standard transformed Gibbs energies of formation of reactants at specified concentrations of certain reactants or apparent equilibrium constants K''. Level 3 is useful in calculating equilibrium concentrations of reactants that can be reached in a living cell when some of the reactants are available at steady-state concentrations. Calculations at all three levels are facilitated by the use of conservation matrices and stoichiometric number matrices for systems. Three cases involving glucokinase, glucose-6-phosphatase, and ATPase are discussed.

INTRODUCTION

When a biochemical reaction system contains many species, the calculation of the equilibrium composition in terms of species involves many equilibrium expressions and many conservation equations. There are advantages in making calculations with a smaller number of reactants (sums of species), and this can be done by specifying the concentrations of one or more species like H^+ and Mg^{2+}. The number of reactants that have to be considered can be further reduced if it is possible to specify the steady state concentrations of some reactants like ATP and ADP. Thus equilibrium calculations can be made at three levels:

Level 1: When the initial composition is specified in terms of species, the needed dissociation constants and complex ion dissociation constants are known, and the equilibrium constant K for a chemical reference reaction for each enzyme-catalyzed reaction is known, the equilibrium composition in terms of species can be calculated by solving the simultaneous equilibrium and conservation equations (Alberty, 1991). This approach yields the pH and the concentrations of other ions, such as Mg^{2+}, as well. The equilibrium composition in terms of species can also be calculated without using equilibrium constants if standard Gibbs energies of formation are known for all of the species, at the desired temperature and ionic strength, and the conservation matrix $\underline{A}$ for the system is known.

Level 2: When the pH and pMg (or equilibrium concentrations of other ions) are specified, the equilibrium composition can be calculated in terms of reactants (sums of species), like ATP, provided that the apparent equilibrium constants K' are known at the desired pH and pMg for each enzyme-catalyzed reaction, and the conservation relations are correctly formulated. Alternatively, the equilibrium composition can be calculated if the standard transformed Gibbs energies of formation are known for the reactants at the desired pH and pMg and the conservation matrix $\underline{A}'$ for the system is known (Alberty, 1992a, 1992b). The initial conditions are stated in terms of concentrations of reactants. Calculations can also be made on the enzymatic mechanism of a reaction. The $\underline{A}'$ matrix for the mechanism reduces to the $\underline{A}'$ matrix for the biochemical reaction when the concentration of the enzyme approaches zero.

Level 3: When pH and pMg are specified and, in addition, steady-state concentrations are known for certain reactants like ATP, ADP, P_i, NAD_{ox}, and NAD_{red}, the equilibrium concentrations of the other reactants can be calculated using apparent equilibrium constants K'' at the specified concentration of ATP, etc., or by using standard transformed Gibbs energies of formation for the reactants at the specified pH, pMg, [ATP], etc. (Alberty, 1992a). This is useful in considering a systems of reactions, such as glycolysis, in a living cell where reactants such as ATP, ADP, P_i, NAD_{ox}, and NAD_{red} are available at steady-state concentrations.

At Level 1, the Gibbs energy G is minimized and the equilibrium composition in terms of species can be calculated if there is enough information. At Level 2 (where pH is specified, for example), the Gibbs energy G is not minimized, but the transformed Gibbs energy G' is and the equilibrium composition in terms of reactants can be calculated if you have enough information on reactants. At Level 2 there is a whole new world of thermodynamics; there is also a transformed enthalpy H', a transformed entropy S', a new way of writing reactions (biochemical reactions rather than chemical reactions), and a new way of counting degrees of freedom. The transformed Gibbs energy G' can be calculated if you have enough information about species, but generally there is not enough information (take glycolysis for example), and so you determine $\Delta_r G'^\circ$ by measuring apparent equilibrium constants K' at a specified pH without knowing all of the acid dissociations constants. At Level 3 (where the concentrations

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of ATP and ADP are specified, for example), the transformed Gibbs energy G' is not minimized, but G'' is. At Level 3 there is in another whole new world of thermodynamics; there is G'', H'', S'', etc. On the other hand, if a system can be described quantitatively at Level 2, the new thermodynamic properties can be calculated at Level 3.

Equilibrium calculations at higher levels involve fewer reactants, it is not necessary to know the standard thermodynamic properties of individual species, and the equilibrium calculations are simpler. However, it is necessary to know apparent equilibrium constants at the pH and pMg of interest or standard transformed Gibbs energies of formation that have been calculated from data on other reactions. These methods are illustrated here with small molecules, but they also apply to macromolecules.

Calculations at Level 2 are made with standard transformed Gibbs energies of formation $\Delta_f G'^\circ$ for reactants at specified T, P, pH, pMg, and I (ionic strength). These transformed properties are based on the use of the transformed Gibbs energy G' defined by the Legendre transform (Alberty, 1992a) as,

$$G' = G - n'(H^+)\mu(H^+), \tag{1}$$

where $n'(H^+)$ is the total amount of H^+ in the system (bound and unbound) and $\mu(H^+)$ is the equilibrium chemical potential of H^+. This leads to a new fundamental equation of thermodynamics, a new Gibbs-Duhem equation, a transformed entropy, a transformed enthalpy, and a reconceptualization of the equilibrium calculation (Alberty, 1992c). This brings in the apparent equilibrium constant K', the apparent number N' of reactants (sums of species), the number C' of apparent components, the number R' of independent biochemical reactions, and the number F' of apparent degrees of freedom. A term can be added to Eq. 1 for Mg^{2+}, or other ions that are bound by reactants, but we will not consider pMg as an independent variable here in order to simplify the treatment.

EQUATIONS FOR LEVEL 1

In making equilibrium calculations on biochemical reaction systems at all three levels, it is convenient to use matrices. The conservation matrix $\underline{A}$ for Level 1 is made up of the numbers of atoms of the various elements in the species in the system and numbers to provide for constraints that are implicit in the mechanism of chemical change (Smith and Missen, 1982). Thus the $\underline{A}$ matrix gives the coefficients of the conservation equations for the system. The stoichiometric number matrix $\underline{\nu}$ is made up of the stoichiometric numbers in a set of independent chemical equations that represent the chemical changes in the system. These two types of matrices are related by the matrix equation

$$\underline{A}\,\underline{\nu} = \underline{0}. \tag{2}$$

The $\underline{\nu}$ matrix is the null space (Strang, 1988) of the $\underline{A}$ matrix, and so it can be calculated from the $\underline{A}$ matrix. The $\underline{\nu}$ matrix is not unique, and, for that matter, the $\underline{A}$ matrix is not unique either because the conservation equations can be written in different ways. However, the row-reduced forms of these matrices are unique for a given order of species.

The equilibrium composition of a system involving chemical reactions can be calculated by starting with the $\underline{A}$ matrix and minimizing G subject to the conservation equations. Alternatively, the equilibrium composition can be calculated by starting with the $\underline{\nu}$ matrix and solving the simultaneous equilibrium expressions subject to the conservation relations.

The $\underline{\nu}$ matrix for a system can be used to calculate the $\underline{A}$ matrix by using

$$\underline{\nu}^T\underline{A}^T = \underline{0}, \tag{3}$$

where the T's indicate transposes. The number C of components in a system at Level 1 is given by

$$C = \text{rank}\,\underline{A}. \tag{4}$$

The components of a system is a subset of the species, or in some cases simply the elements, in terms of which the composition can be described completely. The equilibrium concentrations of the other species, referred to as noncomponents, can be expressed in terms of components by use of equilibrium expressions. The number R of independent reactions between species is given by

$$R = \text{rank}\,\underline{\nu}. \tag{5}$$

The ranks of $\underline{A}$ and $\underline{\nu}$ are related by

$$N = R + C, \tag{6}$$

where N is the number of species in the system. The number F of degrees of freedom (number of independent intensive variables required to specify the intensive state of the system) is given by the phase rule

$$F = C - p + 2, \tag{7}$$

where p is the number of phases. Note that for a one phase system, $F = C + 1$, so that $C - 1$ independent intensive variables have to be specified in addition to T and P to describe the state of the system.

The phase rule really comes from the fundamental equation of thermodynamics for G which is

$$dG = -SdT + VdP + \sum_{i=1}^{N} \mu_i dn_i, \tag{8}$$

where S is the entropy of the system, μ_i is the chemical potential of species i, and n_i is the amount of species i. The fundamental equation can be used to derive the expression for the equilibrium constant K, which is written in terms of species, with the assumption that the solution is ideal so that the chemical potential of species i is given by

$$\mu(i) = \mu^\circ(i) + RT\ln([i]/c^\circ), \tag{9}$$

where c° is the standard state concentration of 1 M. The

standard Gibbs energy of reaction is related to the equilibrium constant K by

$$\Delta_r G^\circ = -RT \ln K = \sum \nu_i \Delta_f G_i^\circ. \quad (10)$$

Here both $\Delta_r G^\circ$ and K are functions of T, P, and I, where I is ionic strength, but we will not specifically refer to I each time because it is a different type of variable than T and P. Specifying the ionic strength is like specifying the solvent; if the solvent contains, 10% by weight ethanol, for example, that needs to be stated explicitly. The second part of Eq. 10 indicates that $\Delta_r G^\circ$ can be expressed by a summation of standard Gibbs energies of formation $\Delta_f G_i^\circ$ of species. Since the measurement of K gives information on G by Eq. 10, S can be calculated by means of Eq. 8, as well as the enthalpy H, which is defined by $H = G + TS$.

Equations 2–10 apply at Level 1, but it has been shown (Alberty, 1992a, 1992b, 1992c) that, when primes are added, they also apply at Level 2, except for the fundamental equation (Eq. 8), which has additional terms at Levels 2. We will see here that when double primes are added, Eqs. 2–10 also apply at Level 3, except for the fundamental equation (Eq. 8), which has additional terms at Level 3. Primes are not placed on R and T in Eq. 10 and p in Eq. 7.

EQUATIONS FOR LEVEL 2

When the pH is specified, the conservation equation for the element hydrogen does not apply because it is as if the enzyme-catalyzed reaction is carried out in a reaction chamber connected to a reservoir of H^+ at the desired pH by a semipermeable membrane through which only H^+ and a counter ion can flow. Thus at Level 2, when the row for hydrogen in the $\underline{A}$ matrix is deleted, some columns become redundant, and the $\underline{A}$ matrix becomes the apparent conservation matrix $\underline{A}'$, as illustrated below. The apparent conservation matrix $\underline{A}'$ has a row for each element that is conserved and a column for each reactant (sum of species); it can also have rows for constraints in addition to element balances. Constraints in the $\underline{A}$ matrix that are implicit in the mechanism of chemical change are simply passed on to the $\underline{A}'$ matrix.

The $\underline{A}'$ matrix can be used in Eq. 2 with primes to calculate the apparent stoichiometric matrix ν', which is made up of the apparent stoichiometric numbers of a set of independent biochemical equations for the system, that is, reactions written in terms of reactants (sums of species). Alternatively, the known ν' matrix for a system at specified pH can be used to calculate the $\underline{A}'$ matrix with the primed version of Eq. 3. The number C' of apparent components at Level 2 is given by the primed version of Eq. 4. The number R' of biochemical reactions between reactants (sums of species) is given by the primed version of Eq. 5. The number N' of reactants in the system is given by the primed version of Eq. 6. The number F' of apparent degrees of freedom after specification of the pH is given by the primed version of Eq. 7.

At Level 2, the fundamental equation of thermodynamics in terms of the transformed Gibbs energy, defined in Eq. 1, is (Alberty, 1992b)

$$dG' = -S'dT + VdP + 2.303n'(H^+)RTd\text{pH} + \sum_{i=1}^{N'} \mu_i' dn_i', \quad (11)$$

where S' is the transformed entropy for the system [$S' = S - n'(H^+)\bar{S}(H^+)$], $n'(H^+)$ is the total amount of hydrogen (bound and free), μ_i' is the transformed chemical potential of reactant i at a specified pH, and dn_i' is the amount of reactant i (sum of species). The transformed chemical potential of a reactant in an ideal solution is related to its concentration by Eq. 9 with primes on the chemical potentials. Equation 11 can be used to derive the expression for the apparent equilibrium constant K'. The standard transformed Gibbs energy $\Delta_r G'^\circ$ of a biochemical reaction is calculated from the apparent equilibrium constant K' by the primed version of Eq. 10. Thus when the pH is specified, the thermodynamic treatment of a system is quite parallel to that when the pH is not specified, but different nomenclature and symbols with different meanings have to be used. Since the experimental value of K' yields information on G', S' can be calculated using Eq. 11, as well as the transformed enthalpy H', which is defined by $H' = G' + TS'$.

In order to describe the equations that apply at Level 3, we will consider a system in which the glucokinase reaction (EC 2.7.1.2) (Webb, 1992) occurs at specified pH[1]:

$$\text{ATP} + \text{Glc} = \text{GlcP} + \text{ADP}. \quad (12)$$

Equation 11 for a system in which the glucokinase reaction occurs is

$$dG' = -S'dT + VdP + 2.303n'(H^+)RTd\text{pH} + \mu'(\text{ATP})dn'(\text{ATP}) + \mu'(\text{Glc})dn'(\text{Glc}) + \mu'(\text{ADP})dn'(\text{ADP}) + \mu'(\text{GlcP})dn'(\text{GlcP}), \quad (13)$$

where $n'(\text{ATP})$ is the sum of the amounts of the species of ATP. The H_2O term is omitted here, because it is not involved in reaction 12, but it is counted as a species later in this paper. The H_2O term is not required explicitly in Eq. 13 because $dn(H_2O) = 0$ when reaction 12 occurs. When the glucokinase reaction occurs at constant T, P, pH, and I, $dn'(\text{ATP}) = dn'(\text{GlcP}) = -dn'(\text{ADP}) = -dn'(\text{GlcP})$. Equation 13 shows that at equilibrium

$$\mu'(\text{ATP}) + \mu'(\text{Glc}) = \mu'(\text{ADP}) + \mu'(\text{GlcP}). \quad (14)$$

When Eq. 9 with primes is used, Eq. 10 with primes can be derived, where the apparent equilibrium constant is given by

$$K' = \frac{[\text{GlcP}][\text{ADP}]}{[\text{ATP}][\text{Glc}]}. \quad (15)$$

[1]The reactants in the glucokinase reaction are represented by Glc for D-glucose and GlcP for D-glucose 6-phosphate.

Equation 13 is written in terms of reactants, but at chemical equilibrium it can be written in terms of apparent components. The C' apparent components are entities in terms of which the composition of the system at equilibrium can be expressed. Equation 13 can be expressed in terms of apparent components by using Eq. 14 to eliminate $\mu'(\mathrm{GlcP})$. This yields the fundamental equation in terms of three apparent components instead of four reactants:

$$\begin{aligned} dG' = &-S'dT + VdP + 2.303n'(\mathrm{H}^+)RTd\mathrm{pH} \\ &+ \mu'(\mathrm{ATP})[dn'(\mathrm{ATP}) + dn'(\mathrm{GlcP})] \\ &+ \mu'(\mathrm{ADP})[dn'(\mathrm{ADP}) - dn'(\mathrm{GlcP})] \\ &+ \mu'(\mathrm{Glc})[dn'(\mathrm{Glc}) + dn'(\mathrm{GlcP})]. \end{aligned} \quad (16)$$

The differential amounts multiplied by the three transformed chemical potentials μ' are the differential amounts dn″ of the three apparent components so that Eq. 16 becomes

$$\begin{aligned} dG' = &-S'dT + VdP + 2.303n'(\mathrm{H}^+)RTd\mathrm{pH} \\ &+ \mu'(\mathrm{ATP})dn''(\mathrm{ATP}) + \mu'(\mathrm{ADP})dn''(\mathrm{ADP}) \\ &+ \mu'(\mathrm{Glc})dn''(\mathrm{Glc}), \end{aligned} \quad (17)$$

where $n''(\mathrm{ATP})$ is the amount of the ATP component, that is the amount of ATP plus the amount of GlcP. This equation is an illustration of Beattie and Oppenheim's statement (Beattie and Oppenheim, 1979) that the chemical potential of a constituent of a phase when considered to be a species is equal to its chemical potential when considered to be a component. Equation 17 is used in the next section.

EQUATIONS FOR LEVEL 3

Level 3 is used when calculations are made at specified concentrations of reactants like ATP, ADP, $\mathrm{NAD_{ox}}$, and $\mathrm{NAD_{red}}$, which are produced by other reactions in a living cell. Thus, it is as if the reaction chamber for which calculations are made is in contact with reservoirs of these other reactants at specified concentrations through semipermeable membranes, as well as a heat reservoir, pressure reservoir, and pH reservoir. To make the discussion of Level 3 as concrete as possible, we will consider a system in which the glucokinase reaction occurs at specified T, P, pH, [ATP], [ADP], and I. Since concentrations of additional reactants are being held constant, the criterion for equilibrium cannot be expressed in terms of G'. It is necessary to make another Legendre transform to take explicit terms in ATP and ADP out of the fundamental equation, in this case, to obtain a new thermodynamic potential G''. The Legendre transform that does this is

$$G'' = G' - n''(\mathrm{ATP})\mu'(\mathrm{ATP}) - n''(\mathrm{ADP})\mu'(\mathrm{ADP}), \quad (18)$$

where $n''(\mathrm{ATP})$ is the amount of the ATP component and $n''(\mathrm{ADP})$ is the amount of the ADP component. We will refer to G'' as a transformed Gibbs energy, just like G', rather than using a different name. Thus, whenever the term "transformed thermodynamic property" is used, it is necessary to be clear what species and reactant concentrations are being held constant. The following derivation parallels the derivation (Alberty, 1992b, 1992c) for Level 2.

The differential of the Legendre transform defined by Eq. 18 is

$$\begin{aligned} dG'' = &\, dG' - n''(\mathrm{ATP})d\mu'(\mathrm{ATP}) - \mu'(\mathrm{ATP})dn''(\mathrm{ATP}) \\ &- n''(\mathrm{ADP})d\mu'(\mathrm{ADP}) - \mu'(\mathrm{ADP})dn''(\mathrm{ADP}). \end{aligned} \quad (19)$$

Substituting Eq. 17 for dG' yields

$$\begin{aligned} dG'' = &-S'dT + VdP + 2.303n'(\mathrm{H}^+)RTd\mathrm{pH} \\ &- n''(\mathrm{ATP})d\mu'(\mathrm{ATP}) - n''(\mathrm{ADP})d\mu'(\mathrm{ADP}) \\ &+ \mu'(\mathrm{Glc})dn''(\mathrm{Glc}). \end{aligned} \quad (20)$$

Thus when $\mu'(\mathrm{ATP})$ and $\mu'(\mathrm{ADP})$ are specified, the terms in ATP and ADP in Eq. 20 disappear, and it can be shown that the criterion for equilibrium at specified T, P, pH, $\mu'(\mathrm{ATP})$, and $\mu'(\mathrm{ADP})$ is $dG'' \leq 0$.

Now let us consider the last term of Eq. 20 in some detail. Since $dn''(\mathrm{Glc}) = dn'(\mathrm{Glc}) + dn'(\mathrm{GlcP})$, we see that

$$\begin{aligned} &\mu'(\mathrm{Glc})dn''(\mathrm{Glc}) \\ &\quad = \mu'(\mathrm{Glc})dn'(\mathrm{Glc}) + \mu'(\mathrm{Glc})dn'(\mathrm{GlcP}). \end{aligned} \quad (21)$$

Using Eq. 14 to eliminate $\mu'(\mathrm{Glc})$ from the second term on the right yields

$$\begin{aligned} &\mu'(\mathrm{Glc})dn''(\mathrm{Glc}) \\ &\quad = \mu'(\mathrm{Glc})dn'(\mathrm{Glc}) \\ &\qquad + [\mu'(\mathrm{GlcP}) + \mu'(\mathrm{ADP}) - \mu'(\mathrm{ATP})]dn'(\mathrm{GlcP}) \\ &\quad = \mu''(\mathrm{Glc})dn'(\mathrm{Glc}) + \mu''(\mathrm{GlcP})dn'(\mathrm{GlcP}), \end{aligned} \quad (22)$$

where

$$\mu''(\mathrm{Glc}) = \mu'(\mathrm{Glc}) \quad (23)$$

and

$$\mu''(\mathrm{GlcP}) = \mu'(\mathrm{GlcP}) + \mu'(\mathrm{ADP}) - \mu'(\mathrm{ATP}) \quad (24)$$

are transformed chemical potentials of Glc and GlcP at specified concentrations of ATP and ADP. Equation 22 contains two terms, and so it is similar to the chemical terms for the reaction of two isomers. When chemical change takes place, $dn'(\mathrm{Glc}) = -dn'(\mathrm{GlcP})$, and Eq. 22 becomes

$$\mu'(\mathrm{Glc})dn''(\mathrm{Glc}) = [\mu''(\mathrm{Glc}) - \mu''(\mathrm{GlcP})]dn'(\mathrm{Glc}). \quad (25)$$

When we put this term back into Eq. 20, we can see that at chemical equilibrium at specified T, P, pH, $\mu'(\mathrm{ATP})$, and $\mu'(\mathrm{ADP})$, the transformed chemical potentials of glucose, $\mu''(\mathrm{Glc})$, and glucose phosphate, $\mu''(\mathrm{GlcP})$, are equal. This is the basis for the expression for the apparent equilibrium

constant K'' for the reaction

$$\text{Glc} = \text{GlcP}, \tag{26}$$

$$K'' = \frac{[\text{GlcP}]}{[\text{Glc}]}. \tag{27}$$

The value of K'' is given by Eq. 10 with double primes. Reaction 26 balances glucose, but not phosphate, which is supplied by ATP at a specified concentration. When these five independent variables are held constant, the system behaves like a one-component system. However, there is a second component, H_2O, that is not involved in the reaction, but is counted as a component later in the paper, because it is a component of the system. Thus the mixture of glucose and glucose phosphate behaves like a pseudoisomer group when glucokinase is present and μ'(ATP) and μ'(ADP) are specified.

However, μ'(ATP) and μ'(ADP) in Eq. 20 are not convenient independent variables, because they depend on temperature as well as concentration. To eliminate $d\mu'$(ATP) and $d\mu'$(ADP) from Eq. 20, the following equations are used:

$$d\mu'(\text{ATP}) = \left[\frac{\partial\mu'(\text{ATP})}{\partial T}\right]_{P,\,\text{pH},\,[\text{ATP}]} dT + \left[\frac{\partial\mu'(\text{ATP})}{\partial[\text{ATP}]}\right]_{T,\,P,\,\text{pH}} d[\text{ATP}], \tag{28}$$

$$d\mu'(\text{ADP}) = \left[\frac{\partial\mu'(\text{ADP})}{\partial T}\right]_{P,\,\text{pH},\,[\text{ADP}]} dT + \left[\frac{\partial\mu'(\text{ADP})}{\partial[\text{ADP}]}\right]_{T,\,P,\,\text{pH}} d[\text{ADP}]. \tag{29}$$

The derivatives in the first terms of these equations are $-\underline{S}'$(ATP) and $-\bar{S}'$(ADP), and the derivatives in the second terms are calculated using the primed version of Eq. 9. Thus,

$$d\mu'(\text{ATP}) = -\bar{S}'(\text{ATP})dT + RTd\ln[\text{ATP}], \tag{30}$$

$$d\mu'(\text{ADP}) = -\bar{S}'(\text{ADP})dT + RTd\ln[\text{ADP}]. \tag{31}$$

Substituting these equations in Eq. 20 yields

$$dG'' = -S''dT + VdP + 2.303n'(\text{H}^+)RTd\text{pH} - n''(\text{ATP})RTd\ln[\text{ATP}] - n''(\text{ADP})RTd\ln[\text{ADP}] + \mu''(\text{Glc})dn''(\text{Glc}), \tag{32}$$

where $S'' = S' - n''(\text{ATP})\,\bar{S}'(\text{ATP}) - n''(\text{ADP})\bar{S}'(\text{ADP})$. A double prime is used on the chemical potential in the last term to indicate that it is the transformed chemical potential at specified [ATP] and [ADP] as well as pH (see Eq. 23). It can be shown that when T, P, pH, [ATP], and [ADP] are specified, $dG'' \leq 0$ is the criterion of equilibrium.

The conservation matrix $\underline{A}''$ is obtained by deleting the rows for ATP and ADP from the $\underline{A}'$ matrix (see examples given below). The constraints in the $\underline{A}'$ matrix in addition to atom balance, if any, are simply passed on to the $\underline{A}''$ matrix. The $\underline{A}''$ matrix can be used in Eq. 2 with double primes to calculate the apparent stoichiometric matrix $\underline{\nu}''$, which is made up of the apparent stoichiometric numbers in equations like Eq. 26. Alternatively, a $\underline{\nu}''$ matrix for a system at specified [ATP] and [ADP], for example, can be used to calculate the corresponding $\underline{A}''$ matrix with the double-primed version of Eq. 3. The number C'' of apparent components at Level 3 is given by the double-primed version of Eq. 4. The number R'' of independent reactions between reactants (sums of species) is given by the double-primed version of Eq. 5. The number N'' of reactants in the system is given by the double-primed version of Eq. 6. The number F'' of apparent degrees of freedom after specification of pH, [ATP], and [ADP], in this example, is given by the double-primed version of Eq. 7.

Thermodynamic equations are usually derived using the chemical potential, but, in calculations, chemical potentials are replaced with Gibbs energies of formation. The standard transformed Gibbs energy of reaction for reaction 26 is given by

$$\Delta_r G''^{\circ} = \Delta_f G''^{\circ}(\text{GlcP}) - \Delta_f G''^{\circ}(\text{Glc}). \tag{33}$$

$\Delta_f G''^{\circ}$ for Glc and GlcP can be obtained from by Eqs. 23 and 24, which become

$$\Delta_f G''^{\circ}(\text{Glc}) = \Delta_f G'^{\circ}(\text{Glc}) \tag{34}$$

and

$$\Delta_f G''^{\circ}(\text{GlcP}) = \Delta_f G'^{\circ}(\text{GlcP}) + \Delta_f G'^{\circ}(\text{ADP}) + RT\ln([\text{ADP}]/c^{\circ}) - \{\Delta_f G'^{\circ}(\text{ATP}) + RT\ln([\text{ATP}]/c^{\circ})\}, \tag{35}$$

where the concentrations of ATP and ADP are the steady state concentrations in the cell.

When enough reactants have been specified so that the apparent reactions become pseudoisomerizations, the equilibrium composition can be calculated by use of isomer group thermodynamics (Smith and Missen, 1983; Alberty, 1983).

EQUILIBRIUM CALCULATIONS ON SYSTEMS OF REACTIONS

Although there are two ways to make equilibrium calculations on complicated systems (Smith and Missen, 1982), the equilibrium constant approach is especially useful in biochemistry, because there may be constraints in a biochemical reaction beyond element balances. These constraints are generally referred to as coupling. This article will deal with coupling in the glucokinase reaction. Constraints of this type can arise in the mechanism of an enzyme-catalyzed reaction. The constraints can be represented by conservation equations, just like the element balance constraints. It is sometimes hard to know how these constraints are to be represented in the $\underline{A}$, $\underline{A}'$, or $\underline{A}''$ matrices. These constraints are implicit in the $\underline{\nu}$, $\underline{\nu}'$, or $\underline{\nu}''$ matrices. The $\underline{A}$, $\underline{A}'$, and $\underline{A}''$ matrices are needed because they

show how many components there are and what has to be done to specify initial concentration conditions for equilibrium calculations for a given system.

The calculation of equilibrium compositions at Levels 1, 2, and 3 is illustrated by considering three cases:

Case 1

Glucokinase (EC 2.7.1.2) is present in a system that contains ATP, ADP, Glc, GlcP, and H_2O. Since P_i is not included in the calculation, there is no provision for the hydrolysis of ATP and ADP. This insures that the following two hydrolysis reactions,

$$GlcP + H_2O = Glc + P_i \quad (36)$$

$$ATP + H_2O = ADP + P_i, \quad (37)$$

are coupled and do not occur separately. The term "coupling" is used in many ways in biochemistry, but here it is used to indicate that two reactions are coupled through a common enzyme-substrate complex. This kind of coupling can be referred to as stoichiometric coupling, because it ensures an integer relationship between the changes in amounts of reactants in the two biochemical reactions that are coupled together.

Case 2

Glucose-6-phosphatase (EC 3.1.3.9) and ATPase (EC 3.6.1.3) are both present in the system that contains ATP, ADP, Glc, GlcP, P_i, and H_2O. Reactions 36 and 37 are not coupled (even though they share two reactants) because they do not share a common enzyme-substrate complex.

Case 3

Glucokinase is in a system containing phosphate, and so in equilibrium calculations the coupling must be represented in the $\underline{A}$, $\underline{A}'$, and $\underline{A}''$ matrices. Case 3 is a reconsideration of Case 1 with orthophosphate present. In this case the calculation includes a constraint to exclude hydrolysis of ATP and GlcP. It is important to be able to do this so the glucokinase reaction can be considered in phosphate buffers and to show how coupling can be handled in more complicated systems.

CASE 1: GLUCOKINASE IS PRESENT

The biochemical equation for the glucokinase reaction is given in Eq. 12.

Level 1

As a simplification only one acid dissociation is considered for ATP, ADP, and GlcP. The conservation matrix $\underline{A}$ is written with columns for the species and rows for the elements.

$$\underline{A} = \begin{array}{c} \\ C \\ H \\ O \\ N \\ P \end{array} \begin{array}{c} \begin{array}{ccccccccc} ATP^{4-} & ADP^{3-} & Glc & H_2O & H^+ & HATP^{3-} & HADP^{2-} & GlcP^{2-} & HGlcP^- \end{array} \\ \begin{bmatrix} 10 & 10 & 6 & 0 & 0 & 10 & 10 & 6 & 6 \\ 12 & 12 & 12 & 2 & 1 & 13 & 13 & 11 & 12 \\ 13 & 10 & 6 & 1 & 0 & 13 & 10 & 9 & 9 \\ 5 & 5 & 0 & 0 & 0 & 5 & 5 & 0 & 0 \\ 3 & 2 & 0 & 0 & 0 & 3 & 2 & 1 & 1 \end{bmatrix} \end{array} \quad (38)$$

The orders of components in the rows and of species (and later reactants) in the columns are arbitrary, but it is advantageous to represent all of the elements in the early columns so that the row-echelon form is obtained by row reduction. The row-reduced form of the $\underline{A}$ matrix obtained with *Mathematica* (Wolfram Research, Inc., Champaign, IL) is

$$\underline{A} = \begin{array}{c} \\ ATP^{4-} \\ ADP^{3-} \\ Glc \\ H_2O \\ H^+ \end{array} \begin{array}{c} \begin{array}{ccccccccc} ATP^{4-} & ADP^{3-} & Glc & H_2O & H^+ & HATP^{3-} & HADP^{2-} & GlcP^{2-} & HGlcP^- \end{array} \\ \begin{bmatrix} 1 & 0 & 0 & 0 & 0 & 1 & 0 & 1 & 1 \\ 0 & 1 & 0 & 0 & 0 & 0 & 1 & -1 & -1 \\ 0 & 0 & 1 & 0 & 0 & 0 & 0 & 1 & 1 \\ 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 1 & 1 & -1 & 0 \end{bmatrix} \end{array} \quad (39)$$

The rows have been relabeled with the first five species because the matrix is in row-echelon form; thus the first five species are taken as components and the last four species are noncomponents. The same result can be obtained by multiplying by a transformation matrix to shift to this new set of components (Alberty, 1993). Matrices 38 and 39 are equivalent. When the $\underline{A}$ matrix is in row-echelon form, the columns for the noncomponents give information on the

TABLE 1 Level 1 calculation of degrees of freedom (systems described in terms of species at *T*, *P*, and *C* – 1 initial concentrations of species)

	N	*R*	*C*	*F*	*C* – 1 degrees of freedom				
Case 1	9	4	5	6	$[ATP^{4-}]_0$	$[ADP^{3-}]_0$	$[Glc]_0$	$[H^+]_0$	
Case 2	11	6	5	6	$[ATP^{4-}]_0$	$[ADP^{3-}]_0$	$[Glc]_0$	$[H^+]_0$	
Case 3	11	5	6	7	$[ATP^{4-}]_0$	$[ADP^{3-}]_0$	$[Glc]_0$	$[H^+]_0$	$[HPO_4^{2-}]^0$

stoichiometric numbers for a set of independent reactions for the system (Smith and Missen, 1982), but here the use of Eq. 2 to determine the stoichiometric numbers will be emphasized.

Since there are nine species and five components (C, H, O, N, P, or ATP^{4-}, ADP^{3-}, Glc, H_2O, H^+), $R = N - C$ (Eq. 6) indicates that four independent reactions are required to represent the changes in composition that are possible. These reactions can be taken to be the three acid dissociation reactions and the chemical reference reaction

$$ATP^{4-} + Glc = ADP^{3-} + GlcP^{2-} + H^+. \qquad (40)$$

The stoichiometric numbers in these four chemical equations can be used to construct the $\underline{\nu}$ matrix for this system. According to Eq. 3, the null space of $\underline{\nu}^T$ is $\underline{A}^T$, and this is an alternative way to obtain Eq. 39.

The calculation of the number *F* of degrees of freedom is summarized in Table 1. To describe the system at Level 1, it is necessary to specify *T*, *P*, and $C - 1$ intensive variables involving amounts of components, which can be taken to be the initial concentrations of ATP^{4-}, ADP^{3-}, Glc, and H^+. This choice is not unique. The equilibrium composition can be calculated using EQUCALC (Krambeck, 1978, 1991) by specifying these initial concentrations. Standard Gibbs energies of formation of species are required for the equilibrium calculation, and they are available (Alberty and Goldberg, 1992). Alternatively, the four equilibrium expressions can be solved simultaneously with the four conservation equations using *Mathematica*. At Level 1 the pH is obtained from the calculation.

Level 2

It is usually of more interest in biochemistry to make equilibrium calculations on such a system at a specified pH. At a specified pH, the hydrogen row and column in the $\underline{A}$ matrix are deleted and redundant columns are dropped (Alberty, 1992d) to obtain the apparent conservation matrix $\underline{A}'$. This can be done with Eqs. 38 or 39, but it is more interesting to use the row-reduced form in Eq. 39. When the row for H^+ is deleted, the columns for ATP^{4-} and $HATP^{3-}$ become identical, and so one is dropped and the remaining column is labeled ATP; the same applies to the ADP and GlcP columns. The resulting apparent conservation matrix is as follows:

$$\underline{A}' = \begin{array}{c} \\ ATP \\ ADP \\ Glc \\ H_2O \end{array} \begin{array}{c} \begin{array}{ccccc} ATP & ADP & Glc & H_2O & GlcP \end{array} \\ \begin{bmatrix} 1 & 0 & 0 & 0 & 1 \\ 0 & 1 & 0 & 0 & -1 \\ 0 & 0 & 1 & 0 & 1 \\ 0 & 0 & 0 & 1 & 0 \end{bmatrix} \end{array} \qquad (41)$$

This apparent conservation matrix shows that $N' = 5$, $C' = 4$ (ATP, ADP, Glc, and H_2O), and $R' = 1$. The single reaction can be taken to be reaction 12. As indicated in Table 2, $F' = 5$, and the state of the system can be specified by giving *T*, *P*, $[ATP]_0$, $[ADP]_0$, and $[Glc]_0$; note that pHwas specified before calculating the number of apparent degrees of freedom *F'*. The calculation of the $\underline{\nu}'$ matrix from $\underline{A}'$ matrix 41 using Eq. 2 yields a result that is consistent with reaction 12. The calculation of the equilibrium composition at Level 2 is much simpler than Level 1, because only one apparent equilibrium constant expression has to be satisfied. The row-reduced A' matrix (Eq. 41) can also be calculated from the $\underline{\nu}'$ matrix for reaction 12 by use of Eq. 3.

The A' matrix at specified pH can also be written in terms of moities (adenosine = A, phosphate, glucose, and H_2O), rather than atoms.

$$\underline{A}' = \begin{array}{c} \\ A \\ Phos \\ Glc \\ H_2O \end{array} \begin{array}{c} \begin{array}{ccccc} ATP & ADP & Glc & H_2O & GlcP \end{array} \\ \begin{bmatrix} 1 & 1 & 0 & 0 & 0 \\ 3 & 2 & 0 & 0 & 1 \\ 0 & 0 & 1 & 0 & 1 \\ 0 & 0 & 0 & 1 & 0 \end{bmatrix} \end{array} \qquad (42)$$

This matrix has the same row-reduced form as matrix 41. It is easier to construct matrix 42 directly, rather than starting with Eq. 38, because it is not necessary to count all of the atoms.

TABLE 2 Level 2 calculation of apparent degrees of freedom (systems described in terms of reactants at *T*, *P*, and *C'* – 1 initial concentrations of reactants)

	N'	*R'*	*C'*	*F'*	*C'* – 1 degrees of freedom				
Case 1	5	1	4	5	$[ATP]_0$	$[ADP]_0$	$[Glc]_0$		
Case 2	6	2	4	5	$[ATP]_0$	$[ADP]_0$	$[Glc]_0$		
Case 3	6	1	5	6	$[ATP]_0$	$[ADP]_0$	$[Glc]_0$	$[Pi]_0$	
Enz. Mech.	8	2	6	7	$[ATP]_0$	$[ADP]_0$	$[Glc]_0$	$[Pi]_0$	$[E]_0$

Note that pH was specified before calculating the number of apparent degrees of freedom.

Level 3

If [ATP] and [ADP] in a living cell are known, the concentrations of glucose and glucose phosphate that can be reached at equilibrium can be calculated by using an $\underline{A}''$ matrix, which is obtained by deleting the rows and columns for ATP and ADP in matrix 41. This yields

$$\underline{A}'' = \begin{array}{c} \\ \text{Glc} \\ \text{H}_2\text{O} \end{array} \begin{array}{c} \begin{array}{ccc} \text{Glc} & \text{H}_2\text{O} & \text{GlcP} \end{array} \\ \begin{bmatrix} 1 & 0 & 1 \\ 0 & 1 & 0 \end{bmatrix} \end{array} \tag{43}$$

which corresponds with reaction 26. The last column indicates that H_2O is not involved in the reaction.

Matrix 43 shows that $N'' = 3$, $C'' = 2$ (Glc, H_2O), $R'' = 1$, and $F'' = 3$, as indicated in Table 3. This system is described by specifying T, P, $[\text{Glc}]_0$ in addition to pH, [ATP], and [ADP], which are specified in advance. Since reaction 26 has the form of an isomerization, isomer group thermodynamics can be used to calculate the equilibrium composition (Smith and Missen, 1982; Alberty, 1983).

CASE 2: GLUCOSE-6-PHOSPHATASE AND ATPASE ARE PRESENT

Biochemical reactions 36 and 37 are catalyzed.

Level 1

The species list for Case 1 has to be extended to include HPO_4^{2-} and $H_2PO_4^-$. Thus the $\underline{A}$ matrix can be written

$$\underline{A} = \begin{array}{c} \\ \text{C} \\ \text{H} \\ \text{O} \\ \text{N} \\ \text{P} \end{array} \begin{array}{c} \begin{array}{ccccccccccc} \text{ATP}^{4-} & \text{ADP}^{3-} & \text{Glc} & \text{H}_2\text{O} & \text{H}^+ & \text{HPO}_4^{2-} & \text{HATP}^{3-} & \text{HADP}^{2-} & \text{GlcP}^{2-} & \text{HGlcP}^- & \text{H}_2\text{PO}_4^- \end{array} \\ \begin{bmatrix} 10 & 10 & 6 & 0 & 0 & 0 & 10 & 10 & 6 & 6 & 0 \\ 12 & 12 & 12 & 2 & 1 & 1 & 13 & 13 & 11 & 12 & 2 \\ 13 & 10 & 6 & 1 & 0 & 4 & 13 & 10 & 9 & 9 & 4 \\ 5 & 5 & 0 & 0 & 0 & 0 & 5 & 5 & 0 & 0 & 0 \\ 3 & 2 & 0 & 0 & 0 & 1 & 3 & 2 & 1 & 1 & 1 \end{bmatrix} \end{array} \tag{1}$$

The row-reduced form of the $\underline{A}$ matrix is

$$\underline{A} = \begin{array}{c} \\ \text{ATP}^{4-} \\ \text{ADP}^{3-} \\ \text{Glc} \\ \text{H}_2\text{O} \\ \text{H}^+ \end{array} \begin{array}{c} \begin{array}{ccccccccccc} \text{ATP}^{4-} & \text{ADP}^{3-} & \text{Glc} & \text{H}_2\text{O} & \text{H}^+ & \text{HPO}_4^{2-} & \text{HATP}^{3-} & \text{HADP}^{2-} & \text{GlcP}^{2-} & \text{HGlcP}^- & \text{H}_2\text{PO}_4^- \end{array} \\ \begin{bmatrix} 1 & 0 & 0 & 0 & 0 & 1 & 1 & 0 & 1 & 1 & 1 \\ 0 & 1 & 0 & 0 & 0 & -1 & 0 & 1 & -1 & -1 & -1 \\ 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 0 \\ 0 & 0 & 0 & 1 & 0 & 1 & 0 & 0 & 0 & 0 & 1 \\ 0 & 0 & 0 & 0 & 1 & -1 & 1 & 1 & -1 & 0 & 0 \end{bmatrix} \end{array} \tag{2}$$

Thus $N = 11$, $C = 5$ (C, H, O, N, P, or ATP^{4-}, ADP^{3-}, Glc, H_2O, H^+), and $R = 6$. These six reactions can be taken to be the four acid dissociation reactions and the two chemical reference reactions

$$\text{GlcP}^{2-} + \text{H}_2\text{O} = \text{Glc} + \text{HPO}_4^{2-}, \tag{46}$$

$$\text{ATP}^{4-} + \text{H}_2\text{O} = \text{ADP}^{3-} + \text{HPO}_4^{2-} + \text{H}^+. \tag{47}$$

These statements can be confirmed by use of Eqs. 2–6.

As indicated in Table 1, we can take the degrees of freedom to be T, P, $[\text{ATP}^{4-}]_0$, $[\text{ADP}^{3-}]_0$, $[\text{Glc}]_0$, and $[\text{H}^+]_0$, if the phosphate is initially all in ATP and ADP, and use EQUCALC to calculate the equilibrium composition.

Level 2

When the pH is specified, the H^+ row and column in matrix 45 are deleted to obtain the apparent conservation matrix

$$\underline{A}' = \begin{array}{c} \\ \text{ATP} \\ \text{ADP} \\ \text{Glc} \\ \text{H}_2\text{O} \end{array} \begin{array}{c} \begin{array}{cccccc} \text{ATP} & \text{ADP} & \text{Glc} & \text{H}_2\text{O} & \text{P}_\text{i} & \text{GlcP} \end{array} \\ \begin{bmatrix} 1 & 0 & 0 & 0 & 1 & 1 \\ 0 & 1 & 0 & 0 & -1 & -1 \\ 0 & 0 & 1 & 0 & 0 & 1 \\ 0 & 0 & 0 & 1 & 1 & 0 \end{bmatrix} \end{array} \tag{48}$$

Now $N' = 6$, $C' = 4$ (ATP, ADP, Glc, and H_2O), and $R' = 2$. The biochemical reactions can be taken to be Eqs. 36 and 37. (Alternatively, we can start with $\underline{\nu}$ and calculate Eq. 48.) The equilibrium composition can be calculated using EQUCALC and standard transformed Gibbs energies of formation of the reactants at the specified pH.

TABLE 3 Level 3 calculation of apparent degrees of freedom (systems described in terms of reactants at *T*, *P*, and *C*″ − 1 initial concentrations of reactants) Note that pH, [ATP], and [ADP] were specified before calculating the number of apparent degrees of freedom.

	N″	*R*″	*C*″	*F*″	*C*″ − 1 degrees of freedom	
Case 1	3	1	2	3	$[\text{Glc}]_0$	
Case 2	4	2	2	3	$[\text{Glc}]_0$	
Case 3	4	1	3	4	$[\text{Glc}]_0$	$[\text{Pi}]_0$

Alternatively, the two equilibrium constant expressions can be solved simultaneously with the conservation equations. When the initial solution contains 1 mM ATP and 1 mM GlcP at 298.15 K, pH 7, pMg 3, and I = 0.25 M, the equilibrium solution contains 4.06×10^{-9} mM ATP, 1 mM ADP, 2 mM P_i, 1 mM Glc, and 1.84×10^{-5} mM GlcP. These values are calculated with the apparent equilibrium-constants (Alberty and Goldberg,1992) for the hydrolysis of ATP, 4.93×10^5, and, for glucose 6-phosphate, 108.5, under these conditions.

Level 3

If [ATP] and [ADP] are specified, their rows and columns are deleted in Eq. 48 to obtain

$$\underline{A}'' \text{ - } \begin{matrix} \\ \text{Glc} \\ \text{H}_2\text{O} \end{matrix} \begin{matrix} \begin{matrix} \text{Glc} & \text{H}_2\text{O} & \text{P}_i & \text{GlcP} \end{matrix} \\ \begin{bmatrix} 1 & 0 & 0 & 1 \\ 0 & 1 & 1 & 0 \end{bmatrix} \end{matrix} \tag{49}$$

This leads to the reactions $H_2O = P_i$ and Glc = GlcP, which can be viewed as the effect of specifying [ATP] and [ADP] on Eqs. 36 and 37. Thus $N'' = 4$, $C'' = 2$ (Glc and H_2O), and $R'' = 2$. We can calculate $[P_i]$ at equilibrium from the specified concentrations of ATP and ADP. Using this value of $[P_i]$, we can calculate [GlcP]/[Glc], because the two biochemical reactions involved are separately at equilibrium. Only the initial concentration of glucose has to be specified in order to calculate the equilibrium composition of the system under these conditions. The use of Level 3 here is not so interesting, because the two biochemical equations are not coupled. If [ATP] = [ADP] = 1 mM, $[P_i]$ would have to build up to about 10^5 M to produce a significant concentration of GlcP. A more interesting use of Level 3 is given in discussing Case 3, where reactions 36 and 37 are coupled.

CASE 3: GLUCOKINASE IS PRESENT IN A PHOSPHATE BUFFER

This is a reconsideration of Case 2 because the only reaction considered is reaction 12, but orthophosphate is present.

Level 1

If we simply proceed as above, we will not obtain the correct $\underline{A}$ matrix. One way to obtain the correct $\underline{A}$ matrix is to use the stoichiometric number matrix $\underline{\nu}$ in Eq. 3 to calculate the $\underline{A}$ matrix. The $\underline{\nu}$ matrix is made up of reference reaction 40 and four acid dissociation reactions.

$$\underline{\nu} = \begin{matrix} \begin{matrix} \text{ATP}^{4-} & \text{ADP}^{3-} & \text{Glc} & \text{H}_2\text{O} & \text{H}^+ & \text{HPO}_4^{2-} & \text{HATP}^{3-} & \text{HADP}^{2-} & \text{GlcP}^{2-} & \text{HGlcP}^- & \text{H}_2\text{PO}_4^- \end{matrix} \\ \begin{bmatrix} -1 & 1 & -1 & 0 & 1 & 0 & 0 & 0 & 1 & 0 & 0 \\ 1 & 0 & 0 & 0 & 1 & 0 & -1 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 1 & 0 & 0 & -1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 1 & -1 & 0 \\ 0 & 0 & 0 & 0 & 1 & 1 & 0 & 0 & 0 & 0 & -1 \end{bmatrix} \end{matrix} . \tag{50}$$

Using Eq. 50 in Eq. 3 to obtain matrix $\underline{A}$, and row reducing it yields

$$\underline{A} = \begin{matrix} \\ \text{ATP}^{4-} \\ \text{ADP}^{3-} \\ \text{Glc} \\ \text{H}_2\text{O} \\ \text{H}^+ \\ \text{HPO}_4^{2-} \end{matrix} \begin{matrix} \begin{matrix} \text{ATP}^{4-} & \text{ADP}^{3-} & \text{Glc} & \text{H}_2\text{O} & \text{H}^+ & \text{HPO}_4^{2-} & \text{HATP}^{3-} & \text{HADP}^{2-} & \text{GlcP}^{2-} & \text{HGlcP}^- & \text{H}_2\text{PO}_4^- \end{matrix} \\ \begin{bmatrix} 1 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 1 & 1 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & -1 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 & 1 & 1 & -1 & 0 & 1 \\ 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 1 \end{bmatrix} \end{matrix} . \tag{51}$$

Thus according to Eq. 4 there are six components, but only five elements are involved. This means that there is an additional constraint, which can be stated in different ways.

One way is that ATP can only be converted to GlcP so that $[ATP^{4-}] + [HATP^{3-}] + [GlcP^{2-}] + [HGlcP^-]$ does not change during the reaction. This constraint (represented by the row labeled con) is included in the following $\underline{A}$ matrix, in which the first five rows are for the elements.

$$\underline{A} = \begin{matrix} \\ \text{C} \\ \text{H} \\ \text{O} \\ \text{N} \\ \text{P} \\ \text{con} \end{matrix} \begin{matrix} \begin{matrix} \text{ATP}^{4-} & \text{ADP}^{3-} & \text{Glc} & \text{H}_2\text{O} & \text{H}^+ & \text{HPO}_4^{2-} & \text{HATP}^{3-} & \text{HADP}^{2-} & \text{GlcP}^{2-} & \text{HGlcP}^- & \text{H}_2\text{PO}_4^- \end{matrix} \\ \begin{bmatrix} 10 & 10 & 6 & 0 & 0 & 0 & 10 & 10 & 6 & 6 & 0 \\ 12 & 12 & 12 & 2 & 1 & 1 & 13 & 13 & 11 & 12 & 2 \\ 13 & 10 & 6 & 1 & 0 & 4 & 13 & 10 & 9 & 9 & 4 \\ 5 & 5 & 0 & 0 & 0 & 0 & 5 & 5 & 0 & 0 & 0 \\ 3 & 2 & 0 & 0 & 0 & 1 & 3 & 2 & 1 & 1 & 1 \\ 1 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 1 & 1 & 0 \end{bmatrix} \end{matrix} \tag{52}$$

This matrix is the same as matrix 44, except for the last row. This matrix includes all of the other matrices in this article, except matrices 57 and 58 for the enzymatic mechanism. The row-reduced form of matrix 52 is the same as matrix 51, and so they are equivalent. If we compare these matrices with those for Case 1: Level 1 we see that we have added two phosphate species and a constraint, the acid dissociation for phosphate, and one degree of freedom; in short, $N = 11$, $C = 6$, $R = 5$, and $F = 7$. Thus in specifying the intensive state of the equilibrium system we have to specify the concentration of one of the phosphate ions.

The constraint in the last row of matrix 52 couples reactions 46 and 47 (or reactions 36 and 37). Physically, this coupling is due to the fact that the two reactions have a common enzyme-substrate intermediate.

Level 2

When the pH is specified, the row and column for hydrogen in matrix $\underline{A}$ in Eq. 51 are deleted, and the following $\underline{A}'$matrix is obtained.

$$\underline{A}' = \begin{array}{c} \\ \text{ATP} \\ \text{ADP} \\ \text{Glc} \\ \text{H}_2\text{O} \\ \text{P}_\text{i} \end{array} \begin{array}{c} \begin{array}{cccccc} \text{ATP} & \text{ADP} & \text{Glc} & \text{H}_2\text{O} & \text{P}_\text{i} & \text{GlcP} \end{array} \\ \begin{bmatrix} 1 & 0 & 0 & 0 & 0 & 1 \\ 0 & 1 & 0 & 0 & 0 & -1 \\ 0 & 0 & 1 & 0 & 0 & 1 \\ 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 \end{bmatrix} \end{array} \tag{53}$$

Thus, $N' = 6$, $C' = 5$, and $R' = 1$, where this reaction can be taken to be reaction 12.

The constraint due to coupling in matrix 52 is still in this matrix because $C' = 5$, although only four elements are conserved. The difference from Case 1: Level 2 is that we have one more component (P_i), had to add a constraint to prevent the hydrolysis of ATP and GlcP, and have to specify one more initial concentration, namely, $[P_i]$. In matrix 53, note that H_2O and P_i are not involved in the single reaction that occurs; this is indicated in the last column.

Calculations at Level 2 can also be made on mechanisms of enzyme-catalyzed reactions. Level 1 calculations on enzymatic mechanisms are difficult because of the lack of detailed information on ionizations of the enzyme and its reactions with specific ions. At Level 2 the free enzymatic sites can simply be represented by E. Here we assume that the glucokinase reaction is catalyzed by a two-step mechanism; phosphate is transferred from ATP to E in the first step, and phosphate is transferred from EP to glucose in the second step. This is a mechanism of the coupling that is represented by the last row of matrix 52:

$$\text{ATP} + \text{E} = \text{EP} + \text{ADP}, \tag{54}$$

$$\text{EP} + \text{Glc} = \text{GlcP} + \text{E}. \tag{55}$$

The A' matrix corresponding with these two biochemical equations can be calculated from the apparent stoichiometric number matrix of these two biochemical reactions, which is given by

$$\underline{\nu} = \begin{array}{c} \begin{array}{cccccccc} \text{ATP} & \text{ADP} & \text{Glc} & \text{H}_2\text{O} & \text{P}_\text{i} & \text{E} & \text{GlcP} & \text{EP} \end{array} \\ \begin{bmatrix} -1 & 1 & 0 & 0 & 0 & -1 & 0 & 1 \\ 0 & 0 & -1 & 0 & 0 & 1 & 1 & -1 \end{bmatrix} \end{array} \tag{56}$$

The row-reduced form of $\underline{A}'$ calculated from $\underline{\nu}'$ with Eq. 3 using *Mathematica*, is

$$\underline{A}' = \begin{array}{c} \\ \text{ATP} \\ \text{ADP} \\ \text{Glc} \\ \text{H}_2\text{O} \\ \text{P}_\text{i} \\ \text{E} \end{array} \begin{array}{c} \begin{array}{cccccccc} \text{ATP} & \text{ADP} & \text{Glc} & \text{H}_2\text{O} & \text{P}_\text{i} & \text{E} & \text{GlcP} & \text{EP} \end{array} \\ \begin{bmatrix} 1 & 0 & 0 & 0 & 0 & 0 & 1 & 1 \\ 0 & 1 & 0 & 0 & 0 & 0 & -1 & -1 \\ 0 & 0 & 1 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & 0 & 1 \end{bmatrix} \end{array} \tag{57}$$

This matrix can also be obtained by conserving A (adenosine), Glc, P, H_2O, E, and con by use of the following matrix:

$$\underline{A}' = \begin{array}{c} \\ \text{A} \\ \text{Glc} \\ \text{P} \\ \text{H}_2\text{O} \\ \text{E} \\ \text{con} \end{array} \begin{array}{c} \begin{array}{cccccccc} \text{ATP} & \text{ADP} & \text{Glc} & \text{H}_2\text{O} & \text{P}_\text{i} & \text{E} & \text{GlcP} & \text{EP} \end{array} \\ \begin{bmatrix} 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 & 1 & 1 \\ 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & 0 & 1 \\ 1 & 0 & 0 & 0 & 0 & 0 & 1 & 1 \end{bmatrix} \end{array} \tag{58}$$

The row-reduced form of this matrix is identical with Eq. 57. The ν' matrix obtained from the last two columns corresponds with reactions 54 and 55.

Since the concentrations of E and EP are arbitrary, they can be made arbitrarily small, and in the limit they do not make any contribution to the conservation equations. The E row and column and the EP column of matrix 58 are deleted to obtain matrix 53.

If the initial solution contains [ATP] = [Glc] = 1 mM, [ADP] = [GlcP] = 0 at 298.15 K, pH 7, pMg 3, I = 0.25 M, the equilibrium solution will contain [ATP] = [Glc] = 0.0146 mM, and [ADP] = [GlcP] = 0.985 mM.

Level 3

When [ATP] and [ADP] are specified at equilibrium, matrix 53 becomes

$$\underline{A}'' = \begin{array}{c} \\ \text{Glc} \\ \text{H}_2\text{O} \\ \text{P}_\text{i} \end{array} \begin{array}{c} \begin{array}{cccc} \text{Glc} & \text{H}_2\text{O} & \text{P}_\text{i} & \text{GlcP} \end{array} \\ \begin{bmatrix} 1 & 0 & 0 & 1 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & 1 & 0 \end{bmatrix} \end{array} \tag{59}$$

$N'' = 4$, $C'' = 3$ (Glc, H_2O, P_i), $R'' = 1$, and $F'' = 4$, which can be taken to be T, P, $[Glc]_0$, and $[P_i]_0$. Note that P_i and H_2O are not involved in the reaction. As in the discussion of Case 1: Level 3, the only reaction is a pseudoisomerization,

and the equilibrium composition is readily calculated. If the initial solution contains 1 mM Glc at 298.15 K, pH 7, pMg 3, [ATP] = 1 mM, [ADP] = 1 mM, and I = 0.25 M, the equilibrium solution will contain 1 mM GlcP and 2.2×10^{-4} mM Glc.

DISCUSSION

In calculating the equilibrium composition that can be reached by a system of biochemical reactions, the calculation can be made in terms of species, if there is enough information, or it can be made in terms of reactants (sums of species), if apparent equilibrium constants K' are known at the desired pH. In calculating compositions that can be reached in a living cell at specified steady state concentrations of reactants like ATP and ADP, the calculations can be made by adjusting the standard transformed Gibbs energy of formation of the nonspecified reactants at the desired pH to the specified concentrations of ATP and ADP. This is especially useful for large systems. Going to higher levels of thermodynamic calculation has the effect of simplifying the equilibrium calculation. One of the problems in such calculations is the difficulty in identifying the conservation equations that have to be satisfied at each level. The conservation matrices $\underline{A}$, $\underline{A}'$, and $\underline{A}''$ indicate the number of conservation relations required. The number of conservation equations required will be greater than the number required to conserve the elements when of coupling of biochemical reactions is involved. This coupling arises in the mechanism of the enzyme-catalyzed reaction and leads to an additional row in the $\underline{A}$, $\underline{A}'$, and $\underline{A}''$ matrices. The $\underline{A}'$ matrix can be obtained from the $\underline{A}$ matrix by deleting the row and column for hydrogen, or the $\underline{A}'$ matrix can be constructed directly without counting atoms. The $\underline{A}''$ matrix can be obtained from the $\underline{A}'$ matrix by deleting rows and columns for reactants at specified concentrations.

It is important to be able to calculate the number R' of independent reactions in a reaction system at specified pH, and in principle this can be done using $R' = N' - C'$. In a complex system it is difficult to determine the number C' of apparent components because some elements may be redundant and because each coupling increases C' by one. A coupling is equivalent to an additional apparent component, because it introduces an additional conservation equation. Another way of saying this is that in a series of reactions like glycolysis, there are missing reactions. In glycolysis, there are three missing reactions because $R' = 17 - 4 = 13$, if the only constraints at specified pH are the conservation equations for C, O, N, and P. For example, although glycolysis involves ATP, ADP, and P_i, these reactants are not equilibrated because they are only involved in coupled reactions.

These methods can be applied to enzymatic mechanisms at Level 2 as well as to biochemical reactions. Coupling arises in the details of the enzymatic mechanism. A single biochemical reaction is discussed here, but these considerations become more important when larger systems are investigated.

This description of the system at equilibrium provides a framework for the discussion of kinetics that will give the correct equilibrium composition at infinite time. In other words, thermodynamic analysis at Level 2 is consistent with rate equations written in terms of reactants like Glc, GlcP, P_i, ATP, and ADP.

This treatment is readily extended to include enthalpy calculations (Alberty and Goldberg, in press). The enthalpy of a system before and after adding an enzyme can be calculated, and the difference gives the enthalpy change for this change in state.

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NOMENCLATURE[2]

$\underline{A}$	conservation matrix ($C \times N$) (dimensionless)
$\underline{A}'$	apparent conservation matrix at specified pH ($C' \times N'$) (dimensionless)
$\underline{A}''$	apparent conservation matrix at specified pH and concentrations of certain reactants ($C'' \times N''$) (dimensionless)
C	number of components (dimensionless)
C'	number of apparent components at specified pH (pseudocomponents) (dimensionless)
C''	number of apparent components at specified pH and concentrations of certain reactants (pseudocomponents) (dimensionless)
c°	standard state concentration (1 M)
F	number of degrees of freedom (dimensionless)
F'	number of apparent degrees of freedom at specified pH (dimensionless)
F''	number of apparent degrees of freedom at specified pH and concentrations of certain reactants (dimensionless)
G	Gibbs energy of a system (kJ)
G'	transformed Gibbs energy of a system at specified pH (kJ)
G''	transformed Gibbs energy of a system at specified pH and concentrations of certain reactants (kJ)
$\Delta_r G^\circ$	standard Gibbs energy of reaction (kJ mol^{-1})
$\Delta_r G'^\circ$	standard transformed Gibbs energy of reaction at specified pH (kJ mol^{-1})
$\Delta_r G''^\circ$	standard transformed Gibbs energy of reaction at specified pH and concentrations of certain reactants (kJ mol^{-1})
$\Delta_f G^\circ(i)$	standard Gibbs energy of formation of i (kJ mol^{-1})
$\Delta_f G'^\circ(i)$	standard transformed Gibbs energy of formation of i at specified pH (kJ mol^{-1})
$\Delta_f G''^\circ(i)$	standard transformed Gibbs energy of formation of i at specified pH and concentrations of certain reactants (kJ mol^{-1})
H	enthalpy of a system (J)
H'	transformed enthalpy of a system at specified pH (J)
I	ionic strength (mol/liter^{-1})

[2]Usual dimensions are shown in parentheses.

K	equilibrium constant at specified T, P, and I (dimensionless)
K'	apparent equilibrium constant at specified T, P, pH, pMg, and I (dimensionless)
K''	apparent equilibrium constant at specified T, P, pH, pMg, I, and concentrations of certain reactants (dimensionless)
n_i	amount of species i (mol)
$n'(i)$	amount of reactant i (sum of species) (mol)
$n''(i)$	total amount of apparent component i in the system (mol)
N	number of species (dimensionless)
N'	number of reactants (pseudospecies) at specified pH (dimensionless)
N''	number of reactants (pseudospecies) at specified pH and concentrations of certain reactants (dimensionless)
p	number of phases (dimensionless)
pH	$-\log([H^+]/c^o)$ (dimensionless)
pMg	$-\log([Mg^{+2}]/c^o)$ (dimensionless)
P	pressure (bar)
R	gas constant (8.3145 J K^{-1} mol^{-1})
R	number of independent reactions in a system (dimensionless)
R'	number of independent biochemical reactions in a system at specified pH (dimensionless)
R''	number of independent biochemical reactions in a system at specified pH and concentrations of certain reactants (dimensionless)
S'	transformed entropy of a system at specified pH (J K^{-1})
S''	transformed entropy of a system at specified pH, [ATP], and [ADP] (J K^{-1})
$\bar{S}(i)$	molar entropy of species i (J K^{-1} mol^{-1})
$\bar{S}'(i)$	molar transformed entropy of reactant i (J K^{-1} mol^{-1})
T	temperature (K)
μ_i	chemical potential of species i (kJ mol^{-1})
μ_i'	transformed chemical potential of reactant i (kJ mol^{-1})
$\underline{\nu}$	stoichiometric number matrix ($N \times R$) (dimensionless)
$\underline{\nu}'$	apparent stoichiometric number matrix at specified pH ($N' \times R'$) (dimensionless)
$\underline{\nu}''$	apparent stoichiometric number matrix at specified pH and concentrations of certain reactants ($N'' \times R''$) (dimensionless)

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