The fundamental equation of thermodynamics for biochemical reaction systems

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Abstract - In biochemistry it is useful to consider pH and pMg as independent variables along with T and P. When T, P, pH, and pMg are specified, the Gibbs energy $G$ is not the criterion of equilibrium, but a transformed Gibbs energy $G'$ can be defined by use of a Legendre transform involving the specified chemical potentials of H$^+$ and Mg$^{2+}$. It can be shown that $(dG')_T,P,pH,pMg < 0$ is the criterion of equilibrium. The fundamental equation for $G'$ is the basis for deriving the expression for the apparent equilibrium constant $K'$. The standard transformed Gibbs energy and the standard transformed enthalpy of reaction are calculated using $\Delta G' = -RT\ln K'$ and $\Delta H' = [RT_1T_2/(T_2 - T_1)]\ln(K_2/K_1) = \Sigma v_i\Delta H'_{o}(i)$.

INTRODUCTION

Biochemists usually treat pH as an independent variable along with $T$ and $P$ (ref. 1). Since Mg$^{2+}$ is often present and forms complexes with a number of biochemical species, it is also useful to treat pMg as an independent variable in addition, but there is currently no satisfactory pMg electrode and so pMg has to be calculated from the total amount of magnesium and other components and information on equilibrium constants for a set of independent reactions. The thermodynamics of a biochemical reaction can be discussed in terms of species or in terms of reactants (sums of species). This article is concerned primarily with the use of reactants rather than species. The hydrolysis of adenosine triphosphate to adenosine diphosphate and inorganic phosphate at a particular $T$, $P$, pH, pMg, and ionic strength I is represented by

$$\text{ATP} + \text{H}_2\text{O} = \text{ADP} + \text{Pi}. \quad (1)$$

The expression for the apparent equilibrium constant $K'$ is given by

$$K' = [\text{ADP}][\text{Pi}]/[\text{ATP}]_c \quad (2)$$

where $c_0 = 1$ M. This way of writing the equilibrium constant expression is justified by the fundamental equation of thermodynamics by use of a Legendre transform to define a transformed Gibbs energy $G'$ (ref. 2-5). The standard transformed Gibbs energy of reaction and the standard transformed enthalpy of reaction are calculated using

$$\Delta_r G' = -RT\ln K' = \Sigma v_i\Delta_r G'_{o}(i) \quad (3)$$

$$\Delta_r H' = [RT_1T_2/(T_2 - T_1)]\ln(K_2/K_1) = \Sigma v_i\Delta_r H'_{o}(i) \quad (4)$$

where the calculation of the standard transformed enthalpy of reaction applies when it is independent of temperature in the range considered. The $v_i$ are the stoichiometric numbers for the reactants (positive for products and negative for reactants). $\Delta_r H'_{o}$ can be calculated from calorimetric measurements by subtracting the heat effects due to the production (consumption) of H$^+$ and Mg$^{2+}$ by the enzyme-catalyzed reaction. The standard transformed Gibbs energy and the standard transformed enthalpy of formation of a reactant are defined by equations 3-4 where the summations are over reactants. These properties apply at specified $T$, $P$, pH, pMg, and I.

The above equations can be contrasted with the equations for a chemical reaction that predominates at high pH in the absence of Mg$^{2+}$, which is
ATP$^4 + \text{H}_2\text{O} = \text{ADP}^3 + \text{HPO}_4^{-2} + \text{H}^+$.  

(5)

The expression for the equilibrium constant $K$ that is a function of $T$, $P$, and $I$ is

$$K = [\text{ADP}^3][\text{HPO}_4^{-2}][\text{H}^+]/[\text{ATP}^4](c^0)^2$$

(6)

The standard Gibbs energy of reaction and the standard enthalpy of reaction at the specified $T$, $P$, and $I$ can be calculated using

$$\Delta G^0 = -RT\ln K = \sum v_i \Delta G^0(i)$$

(7)

$$\Delta H^0 = [RT_1 T_2/(T_2-T_1)] \ln (K_2/K_1) = \sum v_i \Delta H^0(i)$$

(8)

These standard thermodynamic properties apply at specified $T$, $P$, and $I$.

THE FUNDAMENTAL EQUATION OF THERMODYNAMICS FOR THE TRANSFORMED GIBBS ENERGY $G'$

It is well known that equations 5-8 are justified by use of the fundamental equation of thermodynamics for the Gibbs energy $G$ for a one-phase system in which only $PV$ work is done.

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

(9)

$N$ is the number of species. This provides the criterion of chemical equilibrium at constant temperature and pressure: $(dG)_{T,P} \leq 0$. When the independent variables are $T$, $P$, pH, and pMg, the Gibbs energy is no longer the criterion of chemical equilibrium, and it is necessary to make a Legendre transform to a new thermodynamic potential $G'$ that is at a minimum at chemical equilibrium (ref. 5-6). This type of thermodynamic potential arises naturally in considering semigrand ensembles in statistical mechanics, and Alberty and Oppenheim (ref. 2) used a Legendre transform of this type to treat the thermodynamics of the alkylation of benzene at specified ethylene partial pressures. Alberty (ref. 3) showed how a biochemical reaction can be treated in terms of the transformed Gibbs energy $G'$ defined with the following Legendre transform.

$$G' = G - n'(\text{H}^+)\mu(\text{H}^+) - n'(\text{Mg}^{2+})\mu(\text{Mg}^{2+})$$

(10)

$n'(\text{H}^+)$ and $n'(\text{Mg}^{2+})$ are the total amounts of these species (bound and unbound) in the system. $G'$ is used as the symbol for the new thermodynamic potential because biochemists have been using this symbol to indicate that the pH at equilibrium is specified (ref. 1), but quite a different symbol could have been used. For example, Waldram (ref. 8) uses $\Omega_G$. The advantage of using the term transformed Gibbs energy is that it emphasizes both the differences from the Gibbs energy and the source of the new potential.

If the differential of equation 10 is taken and equation 9 is substituted, the following new form of the fundamental equation is obtained.

$$dG' = -SdT + VdP - n'(\text{H}^+)d\mu(\text{H}^+) - n'(\text{Mg}^{2+})d\mu(\text{Mg}^{2+}) + \sum_{i=1}^{N-2} \mu_i' dn_i$$

(11)

The transformed chemical potential $\mu_i'$ of species $i$ is calculated by following the form of equation 10, that is

$$\mu_i' = \mu_i - N_i(\text{H}^+)|\mu(\text{H}^+)| - N_i(\text{Mg}^{2+})|\mu(\text{Mg}^{2+})|$$

(12)

where $N_i(\text{H}^+)$ is the number of atoms of H in species $i$ and $N_i(\text{Mg}^{2+})$ is the number of atoms of Mg in species $i$.

When we want to study the effect of temperature on chemical equilibrium, it is better to use pH and pMg as independent variables, rather than $\mu(\text{H}^+)$ and $\mu(\text{Mg}^{2+})$, because it is very hard to hold the chemical potential constant when the temperature is changed. Note that the definitions used here are $\text{pH} = -\log([\text{H}^+]/c^0)$ and $\text{pMg} = -\log ([\text{Mg}^{2+}]/c^0)$. In order to change variables we have to eliminate $d\mu(\text{H}^+)$ and $d\mu(\text{Mg}^{2+})$ from equation 11. The process for doing this is described by Alberty and Oppenheim (ref. 5). For $\text{H}^+$,

$$d\mu(\text{H}^+) = (\partial \mu(\text{H}^+)/\partial T)[\text{H}^+dT + (\partial \mu(\text{H}^+)/\partial [\text{H}^+])Td[\text{H}^+]$$.  

(13)

The effect of pressure is ignored here because the pressure in biochemical experiments is almost always the atmospheric pressure. A similar equation can be written for Mg$^{2+}$. Up to this point the equations have been quite general, but now we assume ideal solutions in the medium of specified ionic strength. Biochemical reactions are usually studied in electrolyte solutions with ionic strengths $I$ in the range 0.15 - 0.25 M. It is
assumed that the species in biochemical reactions form ideal solutions in this supporting electrolyte so that the chemical potential of species $B_i$ at $T$, $P$, and $I$ and concentration $[B_i]$ is given by

$$\mu_i = \mu_i^0 + RT \ln([B_i]/c^0).$$  \hspace{0.5cm} (14)

Taking the derivatives indicated in equation 13 yields

$$d\mu(H^+) = - (S^0(H^+)^0 - R \ln([H^+]/c^0))dT - 2.303RT dpH$$  \hspace{0.5cm} (15)

where $S^0(H^+)^0 = R \ln([H^+]/c^0)$ is the molar entropy $S$ of hydrogen ion at the specified pH and ionic strength. Substituting this equation and the corresponding equation for $Mg^{+2}$ into equation 11 yields

$$dG' = - S'dT + VdP + 2.303n'(H^+)RT dpH + 2.303n'(Mg^{+2})RT dpMg + \sum_{i=1}^{N-2} \mu_i'dn_i$$  \hspace{0.5cm} (16)

where $S'$ is the transformed entropy of the system that is given by

$$S' = S - n'(H^+)\left(S^0(H^+)^0 - R \ln([H^+]/c^0)\right) - n'(Mg^{+2})\left(S^0(Mg^{+2})^0 - R \ln([Mg^{+2}]/c^0)\right).$$  \hspace{0.5cm} (17)

At specified pH and pMg, equation 16 becomes

$$dG'_{pH,pMg} = - S'dT + VdP + \sum_{i=1}^{N'} \mu_i'dn_i.$$  \hspace{0.5cm} (18)

This equation is written in terms of species, but the terms for pseudoisomers can be collected together because the pseudoisomers have the same transformed chemical potential at chemical equilibrium. This chemical potential can be calculated using equations that apply to an isomer group. Collecting the terms for the isomer groups yields

$$dG'_{pH,pMg} = - S'dT + VdP + \sum_{i=1}^{N'} \mu_i'dn_i.$$  \hspace{0.5cm} (19)

$N'$ is the number of reactants (pseudospecies) in the system. The transformed Gibbs energy $G'$ provides a criterion of equilibrium at specified $T$, $P$, pH, and pMg: $(dG')_{T,P,pH,pMg} \leq 0$. Note the similarity of equation 19 to equation 9.

**CALCULATION OF THE STANDARD TRANSFORMED FORMATION PROPERTIES OF BIOCHEMICAL REACTANTS AT SPECIFIED pH AND pMg**

For ideal solutions the standard Gibbs energy of formation of an isomer group $\Delta G^0(iso)$ can be calculated from the standard Gibbs energies of formation $\Delta fG_i^0$ of the various isomers using (ref. 9)

$$\Delta fG^0(iso) = - RT \ln \sum_{i=1}^{N_1} \exp(-\Delta fG_i^0/RT)$$  \hspace{0.5cm} (20)

and the standard enthalpy of formation $\Delta fH^0(iso)$ of the isomer group can be calculated using (ref. 10),

$$\Delta fH^0(iso) = \sum_{i=1}^{N_1} r_i \Delta fH_i^0,$$  \hspace{0.5cm} (21)

where $r_i$ is the equilibrium mole fraction of the $i$th isomer in the group that is given by

$$r_i = \exp\left(\left[\Delta fG^0(iso) - \Delta fG_i^0\right]/RT\right).$$  \hspace{0.5cm} (22)

Extents of reaction for an independent set of reactions (written in terms of pseudospecies) can be introduced into equation 19 to find the expression for the apparent equilibrium constant $K$ that has to be satisfied at equilibrium. Equation 2 is derived in this way. The form of a biochemical equation, like equation 1, is dictated by linear algebra (refs. 11,12).

Fundamental equation 19 shows us how to calculate the transformed entropy $S'$ of the system, its volume, and the transformed chemical potential $\mu_i'$ of a reactant (pseudospecies).

$$S' = - (\partial G'/\partial T)_{P,n_i',pH,pMg}$$  \hspace{0.5cm} (23)

$$V = (\partial G'/\partial P)_{T,n_i',pH,pMg}$$  \hspace{0.5cm} (24)

$$\mu_i' = (\partial G'/\partial n_i')_{T,P,n_j',pH,pMg}$$  \hspace{0.5cm} (25)

The $n_i'$ indicates that the amounts of all of the reactants except $i$ are held constant. The expressions for $S'$, $\mu_i'$ and $H'$ are given in ref. 4. From the standpoint of applications, the important thing is that the standard
transformed properties of species can be calculated using formation reactions and the standard transformed formation properties of reactants (sums of species) can be calculated using the isomer group equations 20-22.

The adjustment of standard formation properties of species to the desired pH and pMg is accomplished by writing the formation reaction for a species with $\text{H}^+(\text{1O-PH M})$ and $\text{Mg}^{2+}(\text{1O-PMg M})$ on the left side, rather than $\text{H}_2(\text{g})$ and $\text{Mg}(\text{s})$. For example, the formation reaction for $\text{MgHPO}_4$ is written

$$\text{P}(\text{s}) + 2\text{O}_2(\text{g}) + \text{H}^+(\text{1O-PH M}) + \text{Mg}^{2+}(\text{1O-PMg M}) + 3\text{e}^- = \text{MgHPO}_4(\text{ao}).$$  \hspace{1cm} (26)

The standard transformed Gibbs energy of formation and standard transformed enthalpy of formation of $\text{MgHPO}_4$ are given by

$$\Delta G^o(\text{MgHPO}_4) = \Delta G^o(\text{MgHPO}_4) - \left\{ \Delta G^o(\text{H}^+) + R\ln([\text{H}^+]_0/c^o) \right\} - \left\{ \Delta G^o(\text{Mg}^{2+}) + R\ln([\text{Mg}^{2+}]_0/c^o) \right\}$$  \hspace{1cm} (27)

$$\Delta H^o(\text{MgHPO}_4) = \Delta H^o(\text{MgHPO}_4) - \Delta H^o(\text{H}^+) - \Delta H^o(\text{Mg}^{2+}).$$  \hspace{1cm} (28)

$\Delta G^o(\text{H}^+)$ and $\Delta H^o(\text{H}^+)$ are not equal to zero at ionic strengths greater than zero. The standard transformed entropy of formation can be calculated using

$$\Delta S^o(i) = [\Delta H^o(i) - \Delta G^o(i)]/T.$$  \hspace{1cm} (29)

When these standard transformed properties are given for a reaction, $T$, $P$, pH, pMg, and $I$ have to be specified. The standard transformed formation properties of species like those of inorganic phosphate, for which the standard formation properties are known at $I = 0$ (ref. 13-14), can be calculated at any desired $T$, $P$, pH, pMg, and $I$. Since biochemical reactions are usually studied at about $I = 0.25 \text{ M}$, adjustments have to be made for the difference in ionic strength (ref. 15-17). The isomer group equations can then be used to calculate the standard transformed formation properties of the reactants. These equations have been used to calculate the transformed formation properties of $\text{Pi}$, glucose 6-phosphate, glucose, and water at 298.15 K, 1 bar, pH = 7, pMg = 3, and $I = 0.25 \text{ M}$ (ref. 4).

When enough information about the standard Gibbs energies and enthalpies of formation of species is available, the standard transformed Gibbs energies of formation and enthalpies of formation of a reactant can be calculated, but this is not the only way to obtain an entry for a table at, for example, pH = 7 and pMg = 3. The more direct way is to measure $K$ for a number of enzyme-catalyzed reactions under these conditions. $\Delta G^o$ values for a number of reactions can be used to calculate $\Delta G^o(i)$ values for the reactants. In the absence of data on $\Delta G^o(i)$ and $\Delta H^o(i)$ for species, these values may have to be assigned zeros by convention, but this does not affect $\Delta S^o(i)$. If $K$ is measured at several temperatures, $\Delta H^o$ values can be obtained using equation 4. Alberty and Goldberg (ref. 18) have calculated $\Delta H^o$ and $\Delta G^o$ at 298.15 K, pH = 7, pMg = 3, and $I = 0$ from data of Larson, Tewaxi, and Goldberg (ref. 19) for adenosine, AMP, ADP, and ATP using the convention that $\Delta H^o = \Delta G^o = 0$ for the neutral species of adenosine.

**DISCUSSION**

We have seen how useful the fundamental equation of thermodynamics is for studies of chemical equilibrium with choices of independent variables that are different from the usual $T$ and $P$. In biochemistry, pH and pMg can have big effects on chemical equilibria, so that it is useful to choose $T$, $P$, pH, and pMg as independent variables. The composition of the solvent and the ionic strength are also very important. The fundamental equation is useful in at least seven ways:

1. A Legendre transform can be used to define a new thermodynamic potential $G'$ and obtain a new form of the fundamental equation.
2. The fundamental equation can be used to show that $(dG')_{T,P,\text{pH},\text{pMg}} \leq 0$ is the criterion of chemical equilibrium when pH and pMg are specified.
3. The fundamental equation can be used to derive the expression for the apparent equilibrium constant $K'$ for a biochemical reaction.
4. The fundamental equation can be used to show what has to be done to calculate the equilibrium composition of a multi-enzyme system.
5. The fundamental equation can be used to derive expressions for $\Delta G^0$, $\Delta H^0$ and $\Delta S^0$. The fundamental equation can be used to calculate the change in binding $\Delta_{\text{N}}(\text{H}^+)$ and $\Delta_{\text{N}}(\text{Mg}^{2+})$ in a biochemical reaction (ref. 20). $\Delta_{\text{N}}(\text{H}^+)$ can be measured using a pHStat.
6. The fundamental equation can be used to obtain linkage equations (ref. 20).

International agreement on symbols and nomenclature is very much to be desired. Furthermore, the development of tables involves conventions, and these must be agreed upon internationally if values from different tables are to be used in a calculation.
Nomenclature (the units are indicated in parentheses)

\[ c^0 \quad \text{standard state concentration (} c^0 = 1 \text{ M} = \text{mol L}^{-1} \) 
\[ G \quad \text{extensive Gibbs energy of a system (kJ)} \]
\[ G' \quad \text{extensive transformed Gibbs energy of a system (kJ)} \]
\[ \Delta G^0 \quad \text{standard Gibbs energy of reaction of a specified reaction in terms of ion species at specified } T, P, \text{ and } I \text{ (kJ mol}^{-1} \) 
\[ \Delta G^0(,) \quad \text{standard transformed Gibbs energy of a specified reaction in terms of reactants (sums of species) at specified } T, P, \text{ pH, pMg and } I \text{ (kJ mol}^{-1} \) 
\[ \Delta G^0(i) \quad \text{standard Gibbs energy of formation of species } i \text{ at specified } T, P, \text{ and } I \text{ (kJ mol}^{-1} \) 
\[ \Delta G^0(,) \quad \text{standard transformed Gibbs energy of formation of species } i \text{ or reactant } i \text{ (sum of species) at specified } T, P, \text{ pH, pMg, and } I \text{ (kJ mol}^{-1} \) 
\[ H' \quad \text{extensive transformed enthalpy of a system (kJ)} \]
\[ \Delta H^0 \quad \text{standard enthalpy of reaction of a specified reaction in terms of ion species at specified } T, P, \text{ and } I \text{ (kJ mol}^{-1} \) 
\[ \Delta H^0(,) \quad \text{standard transformed enthalpy of a specified reaction in terms of reactants (sums of species) at specified } T, P, \text{ pH, pMg and } I \text{ (kJ mol}^{-1} \) 
\[ \Delta H^0(i) \quad \text{standard enthalpy of formation of species } i \text{ at specified } T, P, \text{ pH, pMg and } I \text{ (kJ mol}^{-1} \) 
\[ \Delta H^0(,) \quad \text{standard transformed enthalpy of formation of species } i \text{ or reactant } i \text{ (sum of species) at specified } T, P, \text{ pH, pMg, and } I \text{ (kJ mol}^{-1} \) 
\[ I \quad \text{ionic strength calculated with ionic concentrations in mol L}^{-1} \text{ (M)} \]
\[ K \quad \text{equilibrium constant for a specified reaction written in terms of concentrations of species at specified } T, P, \text{ and } I \text{ (omitting } H_2O \text{ when it is a reactant) (dimensionless)} \]
\[ K' \quad \text{apparent equilibrium constant for a specified reaction written in terms of concentrations of reactants (sums of species) at specified } T, P, \text{ pH, pMg, and } I \text{ (omitting } H_2O \text{ when it is a reactant) (dimensionless)} \]
\[ n_i \text{ or } n(,) \quad \text{amount of species } i \text{ (mol)} \]
\[ n(i) \quad \text{amount of reactant } i \text{ (that is, sum of species) (mol)} \]
\[ N \quad \text{number of species (dimensionless)} \]
\[ N_i \text{ or } N(,) \quad \text{number of isomer groups (dimensionless)} \]
\[ N_i(E) \quad \text{number of reactants (sums of species) (dimensionless)} \]
\[ N(E) \quad \text{number of atoms of type } E \text{ in species } i \text{ (dimensionless)} \]
\[ \Delta n(N(H^+)) \quad \text{change in binding of } H^+ \text{ in a reaction (dimensionless)} \]
\[ P \quad \text{pressure (bar)} \]
\[ pH \quad -\log([H^+]/c^0) \text{ (dimensionless)} \]
\[ pMg \quad -\log([Mg^{2+}]/c^0) \text{ (dimensionless)} \]
\[ R \quad \text{gas constant (} 8.31451 \text{ J K}^{-1} \text{ mol}^{-1} \) 
\[ r_i \quad \text{equilibrium mole fraction of } i \text{ within a specified class of molecules (dimensionless)} \]
\[ S \quad \text{extensive entropy of a system (J K}^{-1} \) 
\[ S(i) \quad \text{molar entropy of species } i \text{ (J K}^{-1} \text{ mol}^{-1} \) 
\[ S^0(i) \quad \text{standard molar entropy of species } i \text{ at specified } T, P, \text{ and } I \text{ (J K}^{-1} \text{ mol}^{-1} \) 
\[ S' \quad \text{extensive transformed entropy of a system (J K}^{-1} \) 
\[ \Delta S^0 \quad \text{standard transformed entropy of formation of species } i \text{ at specified } T, P, \text{ pH, pMg and } I \text{ (J K}^{-1} \text{ mol}^{-1} \) 
\[ T \quad \text{temperature (K)} \]
\[ V \quad \text{volume (L)} \]
\[ \mu_i \quad \text{chemical potential of species } i \text{ (kJ mol}^{-1} \) 
\[ \mu^0(i) \quad \text{standard chemical potential of species } i \text{ at specified } T, P, \text{ and } I \text{ (kJ mol}^{-1} \) 
\[ \mu(i) \quad \text{transformed chemical potential of species } i \text{ or reactant } i \text{ at specified } T, P, \text{ pH, pMg, and } I \text{ (kJ mol}^{-1} \) 
\[ v_i \quad \text{stoichiometric number of reactant } i \text{ in a specified chemical reaction (dimensionless)} \]

REFERENCES