ELSEVIER

Biophysical Chemistry 100 (2003) 183–192

Biophysical Chemistry

www.elsevier.com/locate/bpc

The role of water in the thermodynamics of dilute aqueous solutions

Robert A. Alberty*

Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

Received 27 November 2001; accepted 18 April 2002

Abstract

Water plays a role in the thermodynamics of dilute aqueous solutions that is unusual in two ways. First, knowledge of hydration equilibrium constants of species is not required in calculations of thermodynamic properties of biochemical reactants and reactions at specified pH. Second, since solvent provides an essentially infinite source of oxygen atoms in a reaction system where water is a reactant, oxygen atoms are not conserved in the reaction system in dilute aqueous solutions. This is related to the fact that H_2O is omitted in equilibrium expressions for dilute aqueous solutions. Calculations of the standard transformed Gibbs energies of formation of total carbon dioxide and total ammonia at specified pH are discussed, and the average bindings of hydrogen ions by these reactants are calculated by differentiation. Since both of these reactants are involved in the urease reaction, the apparent equilibrium constants and changes in the numbers of hydrogen ions bound are calculated for this reaction as functions of pH.

Keywords: Water; Thermodynamics; Standard transformed Gibbs energy of formation; Binding of hydrogen ions; Legendre transforms; Carbon dioxide; Ammonia; Urease

1. Introduction

I first met John T. Edsall in 1945 at Harvard Medical School because E.J. Cohn, J.T. Edsall, and others were responsible for a wartime project on plasma proteins, and I was employed by the part of the project at the University of Wisconsin under the direction of J.W. Williams. When I met John at that time, I remember hearing from him about Henderson's 1913 book '*The Fitness of the Environment*' [1]. I later learned that John Edsall and Jeffries Wyman were both students of Henderson. This remarkable book describes many aspects that make the earth fit for life and includes a chapter on water, which I want to discuss here from the viewpoint of thermodynamics of systems of biochemical reactions in dilute aqueous solutions. As a young faculty member, I worked on electrophoresis, protein fractionation, and enzyme kinetics, which led to many contacts with John Edsall. Later I served as an Associate Editor of the *Journal of Biological Chemistry* when he was Editor. After I moved to Cambridge, MA, in 1967 I saw more of John and in particular had many

---

*Tel.: +1-617-253-2456; fax: 1-617-253-7030.
E-mail address: alberty@mit.edu (R.A. Alberty).

0301-4622/03/$ - see front matter © 2002 Elsevier Science B.V. All rights reserved.
PII: S0301-4622(02)00280-6

discussions with him about the thermodynamics of biochemical reactions. The work of our group and that of others in the 1960s and 1970s lead IUPAC, IUB, and IUPAB to form a committee involving John to make recommendations on biochemical thermodynamics [2]. I was invited to join the committee, but I felt that I was too busy as a Dean. However, I did follow the discussions of the committee and made suggestions. Later, I became chairman of a successor committee sponsored by IUPAC and IUBMB that wrote a report entitled ‘Recommendations for Nomenclature and Tables in Biochemical Thermodynamics’ [3].

The origin of this second report was unusual because it really grew out of my research on the thermodynamics of petroleum processing in the period 1980–1990. In working on the thermodynamics of chemical reactions in ideal gases with Oppenheim [4], I had learned about the use of Legendre transforms to define new thermodynamic potentials that provide the criterion for spontaneous change and equilibrium when the partial pressure or concentration of a species is held constant in addition to T and P. In 1991 I recognized for the first time that when the pH is specified, the Gibbs energy G does not provide the criterion for spontaneous change and equilibrium in biochemical reactions described in terms of reactants like ATP, which is a sum of species. The transformed Gibbs energy G' that does is defined by [5,6]

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

where $n_c(\mathrm{H})$ is the amount of the hydrogen component in the system (i.e. the total amount of hydrogen atoms) and $\mu(\mathrm{H}^+)$ is the specified chemical potential of hydrogen ions. These are conjugate variables. This Legendre transform can be used to show that the standard transformed Gibbs energy of formation $\Delta_f G'^0_i$ of a species at 298.15 K can be calculated using

$$\begin{aligned}\Delta_f G'^0_i = {} & \Delta_f G^0_i + N_H(i)RT\ln(10)\mathrm{pH}\\ & -2.91482(z_i^2 - N_H(i))I^{1/2}/(1+1.6I^{1/2})\end{aligned} \tag{2}$$

where $\Delta_r G^0_i$ is the standard Gibbs energy of formation of the species, z_i is its electric charge, $N_H(i)$ is the number of hydrogen ions in the species, I is the ionic strength and $\mathrm{pH} = -\log[\mathrm{H}^+]$. The extended Debye–Hückel equation is used to adjust the standard transformed Gibbs energy of formation of a species to the desired ionic strength. Note that when $N_H(i) = 0$, there is no dependence on pH, and when $z_i^2 - N_H(i) = 0$, there is no dependence on ionic strength. When the pH is held constant, species that differ only with respect to the number of hydrogen ions bound become pseudoisomers. When the pseudoisomers in a group are at equilibrium with each other, the standard transformed Gibbs energy of formation of the pseudoisomer group (i.e. a reactant like ATP) is given by

$$\Delta_f G'^0 = -RT\ln\sum_{i=1}^{N'_{\mathrm{iso}}}\exp\left(-\Delta_f G'^0_i/RT\right) \tag{3}$$

where N'_{iso} is the number of species in the pseudoisomer group. Since $\Delta_f G'^0_i$ contains information on the numbers of hydrogen atoms in the species, the average number of hydrogen atoms $\bar{N}_H$ in a reactant can be calculated using

$$\bar{N}_H = \frac{1}{RT\ln(10)}\left(\frac{\partial\Delta_f G'^0_i}{\partial\mathrm{pH}}\right)_{T,P} \tag{4}$$

Calculating the average number of hydrogen atoms bound by using a derivative with respect to pH traces back to Wyman [7], but Eq. (4) can also be derived from the fundamental equation for the transformed Gibbs energy.

The apparent equilibrium constant K' for a biochemical reaction can be calculated using

$$\Delta_r G'^0 = \sum \nu'_i \Delta_f G'^0_i = -RT\ln K' \tag{5}$$

where the ν'_i are the stoichiometric numbers of the reactants and the $\Delta_f G'^0_i$ are calculated using Eq. (3) if the reactant consists of more than one species. The change in binding of hydrogen ions in a biochemical reaction can be calculated using

$$\begin{aligned}\Delta_r N_H &= \frac{1}{RT\ln(10)}\left(\frac{\partial\Delta_r G'^0_i}{\partial\mathrm{pH}}\right)_{T,P}\\ &= \frac{-1}{\ln(10)}\\ &\quad\times\left(\frac{\partial\ln K'}{\partial\mathrm{pH}}\right)_{T,P} = -\left(\frac{\partial\log K'}{\partial\mathrm{pH}}\right)_{T,P}\end{aligned} \tag{6}$$

My initial interests in the thermodynamics of biochemical reactions were on the hydrolysis of ATP and related reactions [8], but when I got to reactions involving carbon dioxide, I ran into problems that caused me to seek out John Edsall. Enzyme-catalyzed reactions involving carbon dioxide are often written in terms of CO_2 (g), but it is more interesting to calculate the total concentration of carbon dioxide in the aqueous phase. In thinking about carbon dioxide in dilute aqueous solutions, I remembered John's research on the thermodynamics and kinetics of CO_2, H_2CO_3, HCO_3^-, and CO_3^{2-} that are so interesting from the standpoint of our exhalation of gaseous carbon dioxide, and so I went to see him. He found a copy of the NASA report [9] that he had written in 1969 and gave it to me. This report contains the value of K_h for the hydration reaction

$$CO_2(sp)+H_2O(l)=H_2CO_3(sp) \tag{7}$$

at 298.15 K: $K_h=2.584\times10^{-3}$. The sp refers to species. It should be noted that this equilibrium constant was obtained from kinetic measurements, not thermodynamic measurements. I had first learned about this slow reaction in titrating carbon dioxide solutions with dilute sodium hydroxide as an undergraduate because of the slow fading of the endpoint using methyl orange as an indicator. At pH 7 the relaxation time is approximately 15 s. Although the NBS tables [10] have entries that might appear to be CO_2 (sp), H_2CO_3 (sp), HCO_3^- (sp), and CO_3^{2-} (sp), its Introduction contains a warning that $\Delta_f G^0(H_2CO_3,ao)=\Delta_f G^0(CO_2,ao)+\Delta_f G^0(H_2O,ao)$, where ao indicates the property of an undissociated molecule in water. This convention is accompanied by the explanation that the equilibrium constant K_h for Eq. (7) is taken arbitrarily to be unity. This difference in equilibrium constants by a factor of 400 was the first problem I had with water in thermodynamic calculations. The calculation of $\Delta_f G'^0$ and $\Delta_f H'^0$ for the sum of species, referred to as CO_2tot, led me to consider this problem in three papers [11–13].

The second problem that I ran into with water was caused by the convention used in writing expressions for equilibrium constants of reactions involving water; i.e., the activity of water is taken as unity in dilute aqueous solutions. This creates a problem because conservation matrices and stoichiometric number matrices for systems of biochemical reactions are inconsistent when water is a reactant [14].

When the activity of water is constant and water is a reactant at a specified pH, the criterion for spontaneity and equilibrium is provided by the further transformed Gibbs energy G'' that is defined by [14]

$$G''=G'-n_c(O)\mu'^0(H_2O) \tag{8}$$

where $n_c(O)$ is the amount of the oxygen component in the system (i.e. the total amount of oxygen atoms) and $\mu'^0(H_2O)$ is the standard transformed chemical potential of water at the specified pH. These are conjugate properties. The further transformed Gibbs energy G'' is used when it is necessary to recognize that when H_2O is produced or consumed in an enzyme-catalyzed reaction, it is not conserved in the reaction system because it comes or goes from an essentially infinite reservoir in dilute aqueous solutions. The Legendre transform in Eq. (8) indicates that the standard further transformed Gibbs energy of formation of a species is given by

$$\Delta_f G''^0_i=\Delta_f G'^0_i-N_O(i)\Delta_f G'^0(H_2O) \tag{9}$$

where $N_O(i)$ is the number of oxygen atoms in species i and $\Delta_f G'^0(H_2O)$ is the standard transformed Gibbs energy of formation of H_2O at the specified pH and ionic strength. Note that Eq. (9) shows that the further transformed Gibbs energy of formation of H_2O is 0. In dilute aqueous solutions at specified pH, reactants that differ from other reactants only with respect to the number of oxygen atoms and hydrogen atoms they contain become pseudoisomers. The standard further transformed Gibbs energy of formation of a pseudoisomer group is given by

$$\Delta_f G''^0=-RT\ln\sum_{i=1}^{N''_{iso}}\exp\left(-\Delta_f G''^0_i/RT\right) \tag{10}$$

where N''_{iso} the number of pseudoisomer groups when the activity of water is constant.

The apparent equilibrium constant K'' for a biochemical reaction involving water can be calculated using

$$\Delta_r G''^0 = \sum \nu''_i \Delta_f G''^0_i = -RT \ln K'' \tag{11}$$

where the ν''_i are the stoichiometric numbers of the reactants and the $\Delta_f G''^0_i$ are calculated using Eq. (10) if the reactant consists of more than one species. Since $\Delta_f G''^0$ contains information about the number of hydrogen atoms in a pseudoisomer group, the change in the number of hydrogen ions bound in a biochemical reaction involving H_2O can be calculated using

$$\begin{aligned}\Delta_r N_H &= \frac{1}{RT \ln(10)}\left(\frac{\partial \Delta_r G''^0}{\partial \mathrm{pH}}\right)_{T,P,a(H_2O)}\\ &= \frac{-1}{\ln(10)}\left(\frac{\partial \ln K''}{\partial \mathrm{pH}}\right)_{T,P,a(H_2O)}\\ &= -\left(\frac{\partial \log K'}{\partial \mathrm{pH}}\right)_{T,P,a(H_2O)}\end{aligned} \tag{12}$$

where $a(H_2O)$ is the activity of H_2O.

The solutions to these two problems are illustrated here by calculation of the thermodynamic properties of carbon dioxide and ammonia in dilute aqueous solutions and the application of these properties to the urease reaction. The calculations have been carried out using MATHEMATICA® [15] and thermodynamic data on the web [16] for 119 biochemical reactants.

2. Standard thermodynamic properties of carbon dioxide in dilute aqueous solutions as a function of pH

In the NBS tables [10] some species in aqueous solution are listed with two or more formulas that differ only in the number of molecules of water they contain. Examples of interest to biochemists are CO_2–H_2CO_3 and NH_3–NH_4OH, but there are a dozen more examples in the NBS tables. The introduction to the NBS tables explains that the thermodynamic properties of each pair are connected by the formal chemical relationship

$$A + nH_2O(l) = A\cdot nH_2O \tag{13}$$

The standard reaction properties are taken to be

$$\Delta_r G^0 = \Delta_r H^0 = \Delta_r S^0 = 0 \tag{14}$$

Table 1
Standard Gibbs energies of formation in aqueous solution at 298.15 K, 1 bar, and $I = 0$ from the NBS tables [10] and for species [11]

	$\Delta_f G^0$(ao) ($\mathrm{kJ\ mol^{-1}}$)	$\Delta_f G^0$(sp) ($\mathrm{kJ\ mol^{-1}}$)
CO_2	−385.98	−385.97
H_2CO_3	−623.08	−608.33
HCO_3^-	−586.77	−586.77
CO_3^{2-}	−527.81	−527.81

as a convention of the table. At first sight it appears strange to take the equilibrium constants for all these hydration reactions to be equal to unity. In my first calculations [11] of $\Delta_f G'^0(CO_2\mathrm{tot})$ and $\Delta_f H'^0(CO_2\mathrm{tot})$ I used Edsall's recommended $K_h = 2.584\times10^{-3}$ to calculate the standard formation properties of species (indicated by sp) that are given the second column of Table 1.

Table 1 indicates that the pK for H_2CO_3(sp) is 3.78 at 298.15 K and 0 ionic strength. This is quite different from the value calculated from K_1, which corresponds with p$K_1 = 6.37$ at 0 ionic strength.

$$K_1 = \frac{K_{H_2CO_3}}{1+1/K_h} = \frac{1.668\times10^{-4}}{1+1/2.584\times10^{-3}} = 4.299\times10^{-7} \tag{15}$$

Note that $\Delta_f G^0(H_2CO_3,\mathrm{ao}) = -385.98 - 237.13 = -623.11\ \mathrm{kJ\ mol^{-1}}$, where $\Delta_f G^0(H_2O,\mathrm{ao}) = -237.13\ \mathrm{kJ\ mol^{-1}}$. (There is an error of 0.03 $\mathrm{kJ\ mol^{-1}}$ in the NBS tables for H_2CO_3(ao).)

In 1995 [11] I calculated the standard transformed properties of CO_2tot as a function of pH and ionic strength by using the species values in Table 1. In 1997 [12] I calculated the standard transformed properties of CO_2tot by an entirely different route using equilibrium constants for reactions of species in Table 1. In 1998 [13] I found that the same values could be obtained directly from the NBS tables by using values for H_2CO_3(ao), HCO_3^-(ao), and CO_3^{2-}(ao). This shows that K_h is not needed to calculate $\Delta_f G'^0(CO_2\mathrm{tot})$. Table 2 gives values of $\Delta_f G'^0(CO_2\mathrm{tot})$ calculated using data and programs

Table 2
Standard transformed Gibbs energies of formation (in kJ mol^{-1}) and average numbers of hydrogen ions bound by CO_2tot and ammonia at 298.15 K and 0 ionic strength

	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9	pH 10
$\Delta_f G'^0(CO_2tot)$	−577.46	−566.13	−555.50	−547.33	−541.17	−535.52	−530.64
$\bar{N}_H(CO_2tot)$	2.00	1.96	1.70	1.19	1.02	0.96	0.68
$\Delta_f G'^0$(ammonia)	12.02	34.86	57.68	80.50	103.21	125.08	144.33
$\bar{N}_H$(ammonia)	4.00	4.00	4.00	3.99	3.95	3.64	3.15

in Ref. [16] at 298.15 K and 0 ionic strength from pH 4 to 10. Values of $\bar{N}_H(CO_2tot)$ were calculated using Eq. (4).

Values of $\Delta_f G'^0(CO_2tot)$ and $\bar{N}_H(CO_2tot)$ at 298.15 K and 0 ionic strength from pH 4 to 10 are plotted in Fig. 1. It is of interest to note that the inflection in the binding plot is centered at pH 6.5, rather than at pH 3.78 that corresponds to the pK for $H_2CO_3(sp)$.

A general way to summarize the complication of water of hydration in thermodynamics is to say that if only dilute aqueous solutions are considered, and this is the case here, it is impossible to determine n in Eq. (13) or K_h by thermodynamic measurements because the activity of water is not variable. When more concentrated aqueous solutions are considered, the thermodynamic treatment becomes much more complicated and is not discussed here. The NBS tables provide a service in giving $\Delta_f G^0(H_2CO_3,ao)$, even though it is equal to $\Delta_f G^0(CO_2,ao)+\Delta_f G^0(H_2O,l)$, because it is convenient to consider that $H_2CO_3(ao)=H^+(ao)+HCO_3^-(ao)$, after all it gives the correct value of K_1. In other words, the same value of $\Delta_f G'^0(CO_2tot)$ is obtained by assuming that K_h is 2.584×10^{-3} or unity.

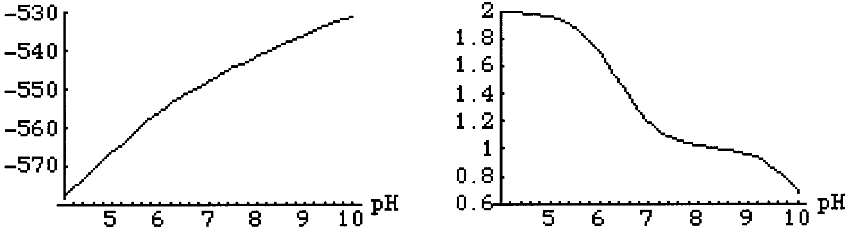
3. Calculation of the standard transformed thermodynamic properties of ammonia as a function of pH

The thermodynamic treatment of ammonia in dilute aqueous solutions involves consideration of the species NH_3, NH_4^+, and NH_4OH, but in contrast with CO_2tot, the hydration constant for

$$NH_3(sp)+H_2O(l)=NH_4OH(sp) \qquad (16)$$

is not known. The NBS tables give values for $NH_3(ao)$, $NH_4^+(ao)$, and $NH_4OH(ao)$, where $\Delta_f G^0(NH_4OH,ao)=\Delta_f G^0(NH_3,ao)+\Delta_f G^0(H_2O,l)$ based on the convention that $K_h=1$ for Eq. (16).

The standard transformed Gibbs energy of formation of ammonia can be calculated from $\Delta_f G^0(NH_3,ao)$ and $\Delta_f G^0(NH_4^+,ao)$ because they are pseudoisomers at a specified pH. The values of $\Delta_f G'^0$(ammonia) and $\bar{N}_H$(ammonia) can be



(a) (b)

Fig. 1. (a) $\Delta_f G'^0(CO_2tot)$ in kJ mol^{-1} at 298.15 K and 0 ionic strength. (b) $\bar{N}_H(CO_2tot)$ obtained by taking the derivative of $\Delta_f G'^0(CO_2tot)$ with respect to pH as indicated by Eq. (6).

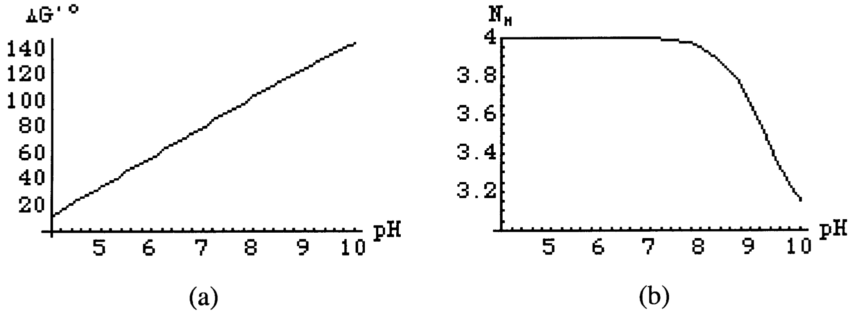


Fig. 2. (a) $\Delta_f G'^0$(ammonia) in kJ mol^{-1} at 298.15 K and 0 ionic strength. (b) $\bar{N}_H$(ammonia) obtained by taking the derivative of $\Delta_f G'^0$(ammonia) with respect to pH, as indicated by Eq. (5).

calculated using data and programs in Ref. [16] as functions of pH at 298.15 K and the desired ionic strength. Values are given in Table 2 and are plotted in Fig. 2.

4. Use of standard further transformed Gibbs energies of formation for systems of reactions involving water as a reactant

The second problem referred to above occurs when water is a reactant in a series or cycle of biochemical reactions [14]. For a single reaction it is easy enough for an investigator to remember to include H_2O in the calculation of $\Delta_r G'^0$ and leave H_2O out of the expression for the apparent equilibrium constant. But when series or cycles of reactions are considered and a computer is used to calculate the equilibrium composition, it is necessary to use conservation matrices $\boldsymbol{A}'$ and stoichiometric number matrices $\boldsymbol{\nu}'$ where

$$\boldsymbol{A}'\boldsymbol{\nu}' = 0 \tag{17}$$

and

$$(\boldsymbol{\nu}')^T(\boldsymbol{A}')^T = 0 \tag{18}$$

The apparent conservation matrix $\boldsymbol{A}'$ is $C' \times N'$, where C' is the number of apparent components and N' is the number of reactants. The apparent stoichiometric number matrix is $N' \times R'$, where R' is the number of independent reactions. Eq. (17) shows that $\boldsymbol{\nu}'$ is the null space of $\boldsymbol{A}'$, and it can be calculated by hand for small matrices or by using a computer for large matrices. Note that neither matrix is unique. Eq. (18) shows that $(\boldsymbol{A}')^T$ is the null space of $(\boldsymbol{\nu}')^T$, and so these matrices are interconvertible, and that is useful when computers are used to calculate equilibrium compositions.

Matrices provide a convenient way to input and output data in using computers, and Krambeck [17] has written convenient programs for calculating equilibrium compositions by use of the Newton–Raphson method. The program EQUCALCC requires a matrix of the coefficients in the conservation equations, a vector of the standard Gibbs energies of formation (or standard transformed Gibbs energies of formation) of the reactants, and a vector of the initial amounts of the reactants. The program EQUCALCRX requires the stoichiometric number matrix, a vector of the equilibrium constants (or apparent equilibrium constants) of a set of independent reactions, and a vector of the initial amounts of reactants. These programs are available in digital form in BasicBiochemData2 [16].

When H_2O is a reactant, it is necessary to include it in the stoichiometric number matrix so that apparent equilibrium constants for the reaction system can be calculated using

$$\Delta_f \boldsymbol{G}'^0 \boldsymbol{\nu}' = \Delta_f \boldsymbol{G}'^0 = -RT \ln \boldsymbol{K}' \tag{19}$$

where matrices are indicated by the use of bold face type. The matrix of standard transformed Gibbs energies of formation is $1 \times N'$. The matrix for the standard transformed Gibbs energies of reaction is $1 \times R'$, and the matrix of apparent equilibrium constants is also $1 \times R'$. But when H_2O

is a reactant, oxygen atoms are not conserved in a system of reactions because they can be obtained from an essentially infinite reservoir of oxygen atoms in dilute aqueous solutions. This means that Eqs. (17) and (18) do not apply. This problem can be solved by using the further transformed Gibbs energy G'' defined in Eq. (6). When this is done, the column and row for H_2O are deleted in the conservation matrix $\boldsymbol{A}'$ to obtain $\boldsymbol{A}''$. When the constancy of the activity of water is taken into account by the definition of G'', the conservation and stoichiometric matrices may be changed by more than the deletion of the row and columns for H_2O. When the pH and $a(H_2O)$ are specified some of the reactants become pseudoisomers. For example, citrate, isocitrate, and *cis*-aconitate become pseudoisomers under these conditions, and so the number of columns in the conservation matrix is also reduced. Then program EQUCALCC yields the equilibrium composition when a vector of further transformed Gibbs energies of formation and a vector of amounts of pseudoisomer groups excluding water are used.

There is another way to calculate the equilibrium composition when water is a reactant that is more convenient. That method involves the use of EQUCALCRX with a stoichiometric number matrix omitting water and a vector of apparent equilibrium constants K'' for the set of independent reactions. When EQUCALCRX is used, the stoichiometric number matrix is utilized by the program to construct a corresponding conservation matrix. This avoids using standard transformed Gibbs energies of formation directly. In both cases the program uses a conservation matrix, but in EQUCALCRX this matrix is calculated by the computer program from the stoichiometric number matrix where water is not included as a reactant. This has the advantage that standard further transformed Gibbs energies of formation do not have to be calculated.

When oxygen atoms are not conserved, their conservation equation is omitted in the new conservation matrix $\boldsymbol{A}''$ and stoichiometric numbers for H_2O are omitted in the corresponding $\boldsymbol{\nu}''$, where

$$\boldsymbol{A}''\boldsymbol{\nu}''=\boldsymbol{0} \tag{20}$$

and

$$(\boldsymbol{\nu}'')^{\mathrm{T}}(\boldsymbol{A}'')^{\mathrm{T}}=\boldsymbol{0} \tag{21}$$

5. Calculation of the standard transformed Gibbs energy of the urease reaction

The urease reaction (EC 3.5.1.5) involves both carbon dioxide and ammonia, and, since the thermodynamic properties of urea are known [10], its apparent equilibrium constant can be calculated at 298.15 K for a range of pH values. In Enzyme Nomenclature [18] the urease reaction is written as

$$\mathrm{Urea}+H_2O=CO_2+2NH_3 \tag{22}$$

However, to calculate the apparent equilibrium constant K' in the aqueous phase over a range of pH, it is necessary to write the urease reaction as

$$\mathrm{Urea}+2H_2O=CO_2\mathrm{tot}+2\mathrm{ammonia} \tag{23}$$

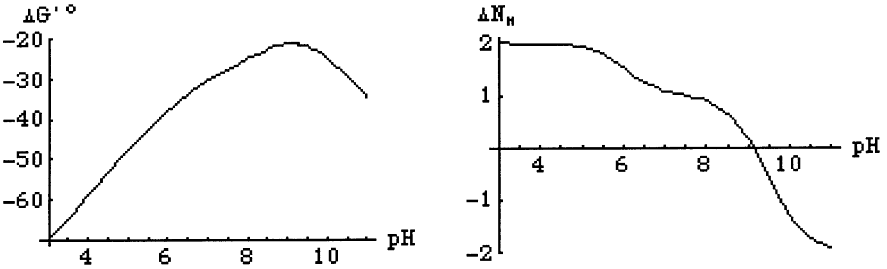
Note that when a reaction involving CO_2 gas is rewritten in terms of the total concentration of carbon dioxide in the aqueous phase, it is necessary to add an H_2O on the other side of the biochemical equation. There are two ways to calculate the apparent equilibrium constant for the urease reaction as a function of pH. The first is the usual method using the standard transformed Gibbs energies of formation of the four reactants. Using the database and programs in MATHSOURCE [16], the standard transformed Gibbs energy at 298.15 K for Eq. (23) can be calculated by simply typing in the reaction. Fig. 3 gives a plot of the standard transformed Gibbs energy of reaction at 298.15 K and 0.25 M ionic strength and the change in the binding of hydrogen ions in the reaction.

The extreme values of this plot are readily understood. At very low pH the predominant chemical reaction is

$$CON_2H_4+2H_2O+2H^+=H_2CO_3+2NH_4^+ \tag{24}$$

At very high pH the predominant chemical reaction is

$$CON_2H_4+2H_2O=CO_3^{2-}+2NH_3+2H^+ \tag{25}$$



(a) (b)

Fig. 3. (a) Plot of the standard transformed Gibbs energy of the urease reaction (Eq. (23)) at 298.15 K and ionic strength 0.25 M. (b) Change in the binding of hydrogen ions in the reaction.

The second way to calculate the standard transformed Gibbs energy of reaction is needed when a computer program using the conservation matrix is used to calculate the equilibrium composition of a system containing this reaction alone or as part of a system of reactions. This calculation requires the further transformed Gibbs energies of formation $\Delta_f G''^0$ of urea, CO_2tot, and ammonia because the activity of water does not change in the reaction. When this method is used, the reaction is written as

$$\text{Urea} = CO_2\text{tot} + 2\text{ammonia} \tag{26}$$

The stoichiometric number for H_2O is taken as 0 because oxygen atoms are not conserved, and so the standard further transformed Gibbs energy $\Delta_r G''^0$ is used.

The difference between the uses of Eqs. (23) and (26) is clarified by the use of matrices. The conservation matrix for Eq. (23) is

$$\boldsymbol{A}' = \begin{array}{ccccc} & \text{urea} & H_2O & CO_2\text{tot} & \text{ammonia} \\ C & 1 & 0 & 1 & 0 \\ O & 1 & 1 & 3 & 0 \\ N & 2 & 0 & 0 & 1 \end{array} \tag{27}$$

The stoichiometric matrix for Eq. (23) is

$$(\boldsymbol{\nu}')^T = \begin{array}{cccc} \text{urea} & H_2O & CO_2\text{tot} & \text{ammonia} \\ -1 & -2 & 1 & 2 \end{array} \tag{28}$$

The dot product $\boldsymbol{A}'\boldsymbol{\nu}'$ gives a zero matrix, as expected. These matrices treat the system as if the concentration of H_2O changes during the reaction.

The conservation matrix for Eq. (26) is obtained by deleting the row and column for oxygen in matrix Eq. (27):

$$\boldsymbol{A}'' = \begin{array}{cccc} & \text{urea} & CO_2\text{tot} & \text{ammonia} \\ C & 1 & 1 & 0 \\ N & 2 & 0 & 1 \end{array} \tag{29}$$

The stoichiometric number matrix for Eq. (26) is

$$(\boldsymbol{\nu}'')^T = \begin{array}{ccc} \text{urea} & CO_2\text{tot} & \text{ammonia} \\ -1 & 1 & 2 \end{array} \tag{30}$$

The dot product $\boldsymbol{A}'\boldsymbol{\nu}'$ gives a zero matrix, as expected. These matrices treat the system as if the concentration of H_2O does not change during the reaction.

The standard further transformed Gibbs energies of the reactants are calculated using the following equations:

$$\Delta_f G''^0(\text{urea}) = \Delta_f G'^0(\text{urea}) - \Delta_f G'^0(H_2O) \tag{31}$$

$$\Delta_f G''^0(CO_2\text{tot}) = \Delta_f G'^0(CO_2\text{tot}) - 3\Delta_f G'^0(H_2O) \tag{32}$$

$$\Delta_f G''^0(\text{ammonia}) = \Delta_f G'^0(\text{ammonia}) \tag{33}$$

where Eq. (9) has been used. These properties incorporate 2 mol of H_2O into $\Delta_r G''^0$ when Eq. (26) is used, so that the standard further transformed Gibbs energy of reaction is equal to the standard transformed Gibbs energy of reaction at each pH. Eq. (26) leads to

$$\begin{aligned}\Delta_r G''^0(\text{Eq. 26}) &= \Delta_f G''^0(\text{CO}_2\text{tot}) + 2\Delta_f G''^0(\text{ammonia}) - \Delta_f G''^0(\text{urea})\\ &= \Delta_r G'^0(\text{Eq. 23})\end{aligned} \quad (34)$$

Thus $K''=K'$. Eq. (34) leads to the same plots of $\Delta_f G'^0$ and $\Delta_r N_H$ as functions of pH as in Fig. 3.

6. Discussion

The two problems with H_2O in the thermodynamics of biochemical reactions in dilute aqueous solutions are (1) the fact that the hydration equilibrium constant K_h does not have to be known when hydrated forms exist and (2) there is an inconsistency between the conservation matrix and the stoichiometric number matrix when H_2O is a reactant. The examples of carbon dioxide and ammonia show that it is not necessary to have values of hydration constants K_h to make thermodynamic calculations on dilute aqueous solutions on species that are in equilibrium with their hydrates.

Other species in enzyme-catalyzed reactions may be hydrated, but this hydration can also be ignored. A prime example of this is the hydrogen ion. Although the species is represented as H^+, it is certainly not a bare proton in aqueous solutions. Hydrogen ions in water are often indicated by H_3O^+, but that under represents the complexity of aqueous solutions. Other forms like $H_5O_2^+$ may exist, but the point is that thermodynamic calculations can be made without knowledge of these hydration constants or others. The use of H_3O^+ in balancing chemical reactions is not satisfactory because it does not adequately represent the actual situation and is unnecessary in thermodynamic calculations on dilute aqueous solutions.

In fact thermodynamics works even though it is not possible to measure $\Delta_f G^0(H^+)$ directly. This value is taken as 0 at each temperature as a convention in making thermodynamic tables. The same type of convention has been useful more recently in calculations on biochemical reactions. When Alberty and Goldberg [8] made calculations on the ATP series, it was not possible to connect any of the reactants containing adenosine with the elements, and so the convention was adopted that $\Delta_f G^0(\text{adenosine}) = \Delta_f H^0(\text{adenosine}) = 0$. More recently, Boerio-Goates et al. [19] have obtained these properties of adenosine calorimetrically. This makes it possible to drop the convention used earlier for the whole ATP series and changes the standard thermodynamic properties, but this does not change the values of apparent equilibrium constants and heats of reaction calculated using the earlier table.

The thermodynamics of liquid water itself is an extreme example of not having to deal with hydration because the structure of water does not have to be analyzed in terms of H_2O, $(H_2O)_2$, $(H_2O)_3$,… As Edsall and Wyman [20] wrote ‘the structure of water is truly unique.’ The remarkable thing about thermodynamics is that measurement of the heat of combustion of molecular hydrogen and heat capacity measurements of H_2, O_2, and H_2O down to nearly absolute zero yields a value of $\Delta_f G^0(H_2O,l)$ that can be used to calculate the contribution of water to the apparent equilibrium constant at a specified pH for any reaction in dilute aqueous solutions. This neglect of hydrated forms may not be appropriate in kinetics because alternate pathways can contribute, as in the hydration of CO_2(sp) to form H_2CO_3(sp) that is catalyzed by carbonic anhydrase.

The second problem encountered when H_2O is a reactant has been illustrated by the urease reaction. This problem is not serious when a single enzyme-catalyzed reaction is considered, but it is serious when conservation matrices are used in calculating equilibrium compositions for series or cycles of enzyme-catalyzed reactions. When H_2O is a reactant in dilute aqueous solutions, oxygen atoms are not conserved in the reaction system because they can be withdrawn from or moved to an essentially infinite reservoir of water molecules. The calculations with urease show how H_2O can be omitted in the biochemical reaction used for quantitative thermodynamic calculations.

There are many more important aspects of water in connection with thermodynamic calculations that are not discussed here, but this article has been concerned with two aspects of quantitative calculations on systems of biochemical reactions.

Acknowledgments

I am indebted to Robert N. Goldberg and Irwin Oppenheim for many helpful discussions. This research was supported by NIH Grant 5-R01-GM48358-05.

References

[1] J.L. Henderson, The Fitness of the Environment, Macmillan, New York, 1913.

[2] (a) I. Wadsö, H. Gutfreund, P. Privalov, et al., Recommendations for measurement and presentation of biochemical equilibrium data, J. Biol. Chem. 251 (1976) 6879–6885
(b) I. Wadsö, H. Gutfreund, P. Privalov, et al., Recommendations for measurement and presentation of biochemical equilibrium data, Q. Rev. Biophys. 9 (1976) 439–456.

[3] R.A. Alberty, A. Cornish-Bowden, Q.H. Gibson, et al., Recommendations for nomenclature and tables in biochemical thermodynamics, Pure Appl. Chem. 66 (1994) 1641–1666, Reprinted in Eur. J. Biochem. 240 (1996) 1–14. Available from http://www.chem.gmw.as.uk/iabmb/thermod/.

[4] R. Alberty, I. Oppenheim, Fundamental equation for systems in chemical equilibrium, J. Chem. Phys. 89 (1988) 3689.

[5] R.A. Alberty, Equilibrium calculations on systems of biochemical reactions, Biophys. Chem. 42 (1992) 117–131.

[6] R.A. Alberty, Calculation of transformed thermodynamic properties of biochemical reactants at specified pH and pMg, Biophys. Chem. 43 (1992) 239–254.

[7] J. Wyman, Heme proteins, Adv. Protein Chem. 4 (1948) 407–531.

[8] R.A. Alberty, R.N. Goldberg, Calculation of thermodynamic formation properties for the ATP series at specified pH and pMg, Biochemistry 31 (1992) 10610–10615.

[9] J.T. Edsall, CO_2: chemical, biochemical, and physiological aspects, NASA SP-188 (1969).

[10] D.D. Wagman, W.H. Evans, V.B. Parker, et al., The NBS tables of chemical thermodynamic properties, J. Phys. Chem. Ref. Data 11 (Suppl. 2) (1982).

[11] R.A. Alberty, Standard transformed Gibbs energy of formation of carbon dioxide in aqueous solution at specified pH, J. Phys. Chem. 99 (1995) 11028–11034.

[12] R.A. Alberty, Apparent equilibrium constants and standard transformed Gibbs energies of biochemical reactions involving carbon dioxide, Arch. Biochem. Biophys. 348 (1997) 116–124.

[13] R.A. Alberty, Calculation of standard transformed Gibbs energies and standard, transformed enthalpies of biochemical reactants, Arch. Biochem. Biophys. 353 (1998) 116–130.

[14] R.A. Alberty, Calculation of equilibrium compositions of biochemical reaction systems involving water as a reactant, J. Phys. Chem. B 105 (2001) 1109–1114.

[15] Wolfram Research, Inc., 100 World Trade Center, Champaign, IL 61820-7237.

[16] R.A. Alberty, BasicBiochemData2. Available from http://www.mathsource.com/cgi-bin/msitem?0211-622.

[17] F.J. Krambeck, in: F.J. Krambeck, A.M. Sapre (Eds.), Chemical Reactions in Complex Systems, Van Nostrand Reinhold, New York, 1991.

[18] E.V. Webb, Enzyme Nomenclature, Academic Press, New York, 1992.

[19] J.A. Boerio-Goates, M.R. Francis, R.N. Goldberg, M.A.V. Ribeiro da Silva, M.D.M.C. Ribeiro da Silva, Y.B. Tewari, Thermochemistry of adenosine, J. Chem. Thermo. 33 (2001) 929–947.

[20] J.T. Edsall, J. Wyman, Biophysical Chemistry, vol. I, Academic Press, New York, 1958.