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Calorimetric determination of the standard transformed enthalpy of a biochemical reaction at specified pH and pMg

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Abstract

In a biochemical reaction there is generally a change in the binding of hydrogen ions and metal ions. Therefore, calorimetric measurements of enthalpies of reaction have to be adjusted for the enthalpies of reaction of the hydrogen ions and metal ions produced or consumed with the buffer. It can be shown that this yields the standard transformed enthalpy of reaction that determines the change in the apparent equilibrium constant K' (written in terms of sums of concentrations of species of a reactant) with temperature at the chosen pH and concentration of free metal ion. The derivations are based on the assumption that the changes in pH and free metal ion concentrations in the calorimetric experiment are small. This assumption is experimentally realized if a solution is well buffered for hydrogen and metal ions. The derived equations are discussed in terms of the implications they have for the performance and interpretation of calorimetric measurements.

1. Introduction

The thermodynamic description of biochemical reactions frequently requires one to deal with mixtures of species that differ only in the number of hydrogen or metal ions attached to a central biochemical species. These related species are referred to as pseudoisomers [1] at a specified pH and concentrations of free metal ions. The thermodynamic description of a biochemical reaction is done in terms of the sums of the amounts of these pseudoisomers, unlike an ordinary chemical reaction where one deals with only single species as reactants. The thermodynamic formalism is necessarily different, and one uses transformed thermodynamic quantities and an apparent equilibrium constant K' written in terms of sums of concentrations of pseudoisomers. In a biochemical reaction there is also generally a change in the binding of hydrogen ions and metal ions. Therefore, calorimetric results of enthalpies of reaction have to be adjusted for the enthalpies of reaction of the hydrogen ions and metal ions produced or consumed by the buffer. A principal aim of this paper is to establish formally the relationship between the calorimetric enthalpy of reaction and the standard transformed enthalpy of reaction that determines the change in the apparent equi-

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librium constant K' with temperature at a given pH and concentration of free metal ion. The treatment is general and is applicable to all types of reactions and degrees of complexity. Since the ATP hydrolysis reaction contains many of the generic features found in biochemical reactions and since the necessary thermodynamic information is available for detailed calculations, the derived principles are applied both to this reaction and to the reactants that participate in it.

The apparent equilibrium constant is a function of T, P, pH, pMg, and ionic strength I. In this paper we will consider cases where Mg^{2+} is bound, but the equations which will be developed are similar for other metal ions. When T, P, pH, and pMg are independent variables, the criterion of equilibrium is the transformed Gibbs energy G' [1]. This quantity is defined by the Legendre transform

$$G' = G - n'(H^+)\mu(H^+) - n'(Mg^{2+})\mu(Mg^{2+}). \tag{1}$$

Here G is the Gibbs energy, $n'(H^+)$ is the total amount of hydrogen in the system, and $\mu(H^+)$ is the specified chemical potential of H^+. A transformed entropy S' can be obtained by use of the fundamental equation of thermodynamics since

$$S' = -(\partial G'/\partial T)_{T,P,\mathrm{pH},\mathrm{pMg}}. \tag{2}$$

A transformed enthalpy can be defined with

$$H' = G' + TS'. \tag{3}$$

The apparent equilibrium constant K' leads to the standard transformed Gibbs energy of reaction

$$\Delta_r G'^{\circ} = -RT \ln K', \tag{4}$$

and it can be shown [2] that the standard transformed enthalpy of reaction $\Delta_r H'^{\circ}$ at a specified T, P, pH, pMg, and I is obtained from the temperature derivative of the apparent equilibrium constant at that pH, pMg, and I. If $\Delta_r H'^{\circ}$ is independent of temperature,

$$\Delta_r H'^{\circ} = \{RT_1T_2/(T_2 - T_1)\} \ln (K_2'/K_1'). \tag{5}$$

If the standard transformed heat-capacity change $\Delta_r C_P'^{\circ}$ is not equal to zero and is dependent of temperature, the standard transformed enthalpy of reaction varies with temperature according to:

$$\Delta_r H'^{\circ}(T) = \Delta_r H'^{\circ}(T') + \Delta_r C_P'^{\circ}(T - T'). \tag{6}$$

The reference temperature T' is usually taken as 298.15 K. In this case, $\Delta_r G'^{\circ}$ and K' vary with temperature according to [3]:

$$\begin{aligned}\Delta_r G'^{\circ}(T) &= -RT \ln K'(T)\\ &= \Delta_r H'^{\circ}(T') + \Delta_r C_P'^{\circ}(T - T')\\ &\quad + T\{\Delta_r G'^{\circ}(T') - \Delta_r H'^{\circ}(T')\}/T'\\ &\quad - T\Delta_r C_P'^{\circ} \ln(T/T')\end{aligned} \tag{7}$$

Additional terms containing $(\partial \Delta_r C_P'^{\circ}/\partial T)_P$ and higher order derivatives may be needed for extremely accurate data or for a very wide temperature range. The standard transformed enthalpy of reaction for a number of enzyme-catalyzed reactions has been determined with the above equations. The standard transformed enthalpy of reaction can also be determined from calorimetric measurements at a single temperature. The standard transformed enthalpy of reaction $\Delta_r H'^{\circ}$ at 298.15 K, 1 bar, pH 7, pMg 3, and an ionic strength I of 0.25 M has been calculated from equilibrium constants K for reactions written in terms of species and the corresponding standard enthalpy of reaction for the reactions catalyzed by glucose-6-phosphatase (EC 3.1.3.9), glucokinase (EC 2.7.1.2), and several reactions in the adenosine 5'-triphosphate series [4].

It is important to distinguish between the standard reaction enthalpy $\Delta_r H^{\circ}$ of a chemical reaction at T, P, and I and the standard transformed enthalpy $\Delta_r H'^{\circ}$ of a biochemical reaction at T, P, pH, pMg, and I. If $\Delta_r H^{\circ}$ is known for a reference reaction and the acid dissociation constants and magnesium complex dissociation constants are known for all of the reactants, $\Delta_r H'^{\circ}$ can be calculated. The standard transformed enthalpy of reaction is important because eq. (5) determines the temperature dependence of K' if $\Delta_r H'^{\circ}$ is independent of temperature. If $\Delta_r C_P'^{\circ}$ is not zero, but is constant, eq. (7) has to be used.

This paper is primarily concerned with the calorimetric determination of $\Delta_r H'^{\circ}$ at specified T, P, pH, pMg, and I.

2. Calculation of the standard transformed enthalpy of reaction from standard formation properties

The standard transformed enthalpy of a reaction can be expressed in terms of the standard transformed enthalpies of formation $\Delta_f H_i'^{\circ}$ of the reactants, including water if it is a reactant, at the specified T, P, pH, pMg, and I by the equation

$$\Delta_r H'^{o} = \sum_{i=1}^{N'} \nu_i \Delta_f H_i'^{o} \tag{8}$$

where N' is the number of reactants (sums of species) in the reaction and ν_i is the stoichiometric number of reactant i in the biochemical equation. The standard transformed enthalpy of formation of a reactant at a specified pH and pMg can be calculated if the standard Gibbs energies and enthalpies of formation are known for all of the species of the reactant that have a significant concentration at the specified pH and pMg. This calculation [2] requires the adjustment of the standard enthalpy of formation of each species j of a reactant at the desired ionic strength to the specified pH and pMg with the equation

$$\Delta_f H_j'^{\circ} = \Delta_f H_j^{\circ} - N_H(j)\Delta_f H^{\circ}(H^+) - N_{Mg}(j)\Delta_f H^{\circ}(Mg^{2+}) \tag{9}$$

where $N_H(j)$ is the number of hydrogens (ions or atoms) in species j and $\Delta_f H^{\circ}(H^+)$ is the standard molar enthalpy of formation of H^+ at the desired T, P and I; similar terms $\{N_{Mg}$ and $\Delta_f H^{\circ}(Mg^{2+})\}$ are also given for magnesium in eq. (9). The standard transformed enthalpy of formation $\Delta_f H_i'^{\circ}$ of a reactant (sum of species) is the mole fraction-weighted average of the standard transformed enthalpies of formation $\Delta_f H_j'^{\circ}$ of the species at the specified pH and pMg, which is given by

$$\begin{aligned}\Delta_f H_i'^{\circ} &= \sum_{j=1}^{N(\mathrm{iso})} r_j \Delta_f H_j'^{\circ} \\ &= \sum_{j=1}^{N(\mathrm{iso})} r_j \Delta_f H_j^{\circ} - \Delta_f H^{\circ}(H^+) \sum_{j=1}^{N(\mathrm{iso})} r_j N_H(j) \\ &\quad - \Delta_f H^{\circ}(Mg^{2+}) \sum_{j=1}^{N(\mathrm{iso})} r_j N_{Mg}(j)\end{aligned} \tag{10}$$

where r_j is the equilibrium mole fraction of species j in a reactant under the specified conditions and the summations are over the N(iso) pseudoisomer species [1] that comprise reactant i. Since $\Sigma r_j \Delta_f H_j^{\circ}$ is equal to the average standard enthalpy of formation of the reactant i, it is convenient to replace it with $\langle \Delta_f H_i^{\circ} \rangle$. Since $\Sigma r_j N_H(j)$ is equal to the average number of hydrogens in reactant i, it is convenient to replace it with $\bar{N}_H(i)$. Thus eq. (10) can be written as

$$\Delta_f H_i'^{\circ} = \langle \Delta_f H_i^{\circ} \rangle - \bar{N}_H(i)\Delta_f H^{\circ}(H^+) - \bar{N}_{Mg}(i)\Delta_f H^{\circ}(Mg^{2+}) \tag{11}$$

Substituting eq. (10) for a reactant into eq. (8) for a reaction yields

$$\begin{aligned}\Delta_r H'^{\circ} &= \sum_{i=1}^{N'} \nu_i \langle \Delta_f H_i^{\circ} \rangle - \Delta_f H^{\circ}(H^+) \sum_{i=1}^{N'} \nu_i \bar{N}_H(i) \\ &\quad - \Delta_f H^{\circ}(Mg^{2+}) \sum_{i=1}^{N'} \nu_i \bar{N}_{Mg}(i) \\ &= \Sigma \nu_i \langle \Delta_f H_i^{\circ} \rangle - \Delta_r N_H \Delta_f H^{\circ}(H^+) \\ &\quad - \Delta_r N_{Mg} \Delta_f H^{\circ}(Mg^{2+})\end{aligned} \tag{12}$$

where N' is the number of reactants (sums of species) involved in the reaction. The changes in binding of H^+ and Mg^{2+} in the reaction are given by

$$\Delta_r N_H = \sum_{i=1}^{N'} \nu_i \bar{N}_H(i) \tag{13}$$

$$\Delta_r N_{Mg} = \sum_{i=1}^{N'} \nu_i \bar{N}_{Mg}(i). \tag{14}$$

Table 1

Mole fraction-weighted average thermodynamic properties of the reactants in biochemical eq. (17) and reaction quantities at 298.15 K, 1 bar, pH 7, pMg 3, and $I = 0.25$ *M*

Reactant	$\langle \Delta_f H_i^o \rangle$ (kJ mol^{-1})	$\bar{N}_H(i)$	$\bar{N}_{Mg}(i)$
ATP(aq)	−3394.46	12.0325	0.897398
ADP(aq)	−2192.32	12.1038	0.423528
Orthophosphate (aq)	−1310.23	1.30201	0.0252822
$H_2O(l)$	−285.83	2	0
Reaction quantities	177.74	−0.62669	−0.448588

Various conventions can be used in counting hydrogens, but the recommended convention is to count them all [2,4]. The *sign convention* used here for $\Delta_r N_H$ and $\Delta_r N_{Mg}$ is the *opposite* of that used earlier [5,6]. The quantities $N_x(i)$, $\bar{N}_x(i)$, and $\Delta_r N_x$ are dimensionless.

The form of eq. (12) can be understood in terms of a thought experiment in which the enzyme-catalyzed reaction is carried out in a reaction chamber in contact with a heat reservoir, a pressure reservoir (the atmosphere), and pH and pMg reservoirs, which are connected with the reaction chamber through semipermeable membranes which allow only H^+ and Mg^{2+} (with counter ions) to pass between the reaction chamber and the reservoirs. When the reaction goes from an initial state of reactants (sums of species) at 1 *M* to products at 1 *M*, the change in binding of H^+ is $\Delta_r N_H$ and the change in binding of Mg^{2+} is $\Delta_r = N_{Mg}$.

The change in binding can be calculated from the acid and magnesium complex dissociation constants of the reactants or it can be determined by measuring K' at a series of pH and pMg values [5,6]:

$$\Delta_r N_H = -(\partial \log_{10} K'/pH)_{T,P,pMg,I} \quad (15)$$

$$\Delta_r N_{Mg} = -(\partial \log_{10} K'/pMg)_{T,P,pH,I}. \quad (16)$$

Thus the change in binding of protons can be determined without knowing the acid dissociation constants for the two reactants. These methods apply to calorimetric measurements on proteins and polynucleotides as well as to simpler weak acids. The change in binding of H^+ can also be determined with a pHstat.

The magnitudes of the terms in eq. (12) for the standard transformed enthalpy of reaction can be illustrated by the hydrolysis of ATP [1]

$$\mathrm{ATP(aq)} + H_2O(l) = \mathrm{ADP(aq)} + \mathrm{orthophosphate(aq)} \quad (17)$$

because the mole fractions of the species of these reactants have been calculated at 298.15 K, 1 bar, pH 7, pMg 3, and $I = 0.25$ *M* [4]. The mole fraction-weighted average standard enthalpies of formation, H binding, and Mg binding of the reactants under these conditions are given in Table 1. At 298.15 K, 1 bar, and $I = 0.25$ *M*, $\Delta_f H^o(H^+) = 0.41$ kJ mol^{-1} and $\Delta_f H^o(Mg^{2+}) = -465.36$ kJ mol^{-1}, and so eq. (12) yields

$$\begin{aligned}\Delta_r H'^o &= 177.74 - 0.41(-0.62669) \\ &\quad + 465.36(-0.448588) \\ &= -30.76 \text{ kJ mol}^{-1}. \end{aligned} \quad (18)$$

It is important to note the tremendous impact of the outflow of Mg^{2+} from the reaction chamber to the pMg reservoir in the thought experiment represented by eq. (17). This term is larger than $\Sigma \nu_i \langle \Delta_f H_i^o \rangle$ for the reaction and has the opposite sign. Also, note that this insight was obtained by considering the mole fraction-weighted average standard enthalpies of formation. These quantities which are given in Table 1 and defined by $\Sigma r_j \Delta_f H_j^o$ are physically different quantities than the standard transformed enthalpies of formation (i.e. the mole fraction-weighted average of the standard transformed enthalpies of formation of the pseudoisomers comprising that reactant at a specified pH, pMg, and *I*) defined by eq. (9) and given in Table V in ref. [4].

3. Calorimetric determination of the standard transformed enthalpy of reaction

The standard transformed enthalpy of reaction is in general not obtained directly from a calori-

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[1] Abbreviations used in this paper are: AMP, adenosine 5′-monophosphate; ADP, adenosine 5′-diphosphate; ATP, adenosine 5′-triphosphate; NAD, nicotinamide adenine-dinucleotide; NADH, nicotinamide adenine-dinucleotide (reduced form).

metric experiment because when H^+ and Mg^{2+} are produced or consumed, their reactions with the buffer produce an energy effect that depends on the buffer and is not a characteristic of the biochemical reaction being studied. The quantities $\Delta_r N_H$ and $\Delta_r N_{Mg}$ can be calculated if all of the acid dissociation and magnesium dissociation constants for the system are known, but they are sometimes unknown. When reactants have many ionizing groups, it may be almost impossible to determine all of the individual dissociation constants. In either case, $\Delta_r N_H$ and $\Delta_r N_{Mg}$ can be determined by measuring K' as a function of pH and pMg and with eqs. (15) and (16). When the individual dissociation constants are not known, there is a problem in determining pMg, but if the concentrations of the reactants are low compared with the concentration of the buffer, pMg can be calculated if the buffer binds Mg^{2+} and the acid and magnesium complex dissociation constants of the buffer are known. A pHstat can also be used to determine $\Delta_r N_H$ directly.

Now we wish to consider the correction for the enthalpy of reaction of H^+ and Mg^{2+} with the buffer. In order to do that we will consider a very simple enzyme-catalyzed reaction, the isomerization of a weak acid in the absence of metal ion binding. As a first step, we need the equations for the system in the absence of a buffer. The isomerization is represented by

$$\begin{tikzcd}
A^- + H^+ \arrow[r, shift left] \arrow[d, shift left] & B^- + H^+ \arrow[l, shift left] \arrow[d, shift left] \\
HA \arrow[u, shift left, "K_{HA}"] & HB \arrow[u, shift left, "K_{HB}"']
\end{tikzcd} \qquad (19)$$

where the acid dissociation constants are for the experimental ionic strength.[2] This reaction can also be represented by

$$A_{tot} = B_{tot}, \qquad (20)$$

where A_{tot} and B_{tot} represent, respectively, the total amounts of the A and B pseudoisomers.

The change in standard transformed enthalpy for reaction (20) can be written as

$$\Delta_r H'^{\circ} = \Delta_f H'^{\circ}(B_{tot}) - \Delta_f H'^{\circ}(A_{tot}) \qquad (21)$$

where these properties are for a specified T, P, pH, and I. The standard transformed enthalpies of formation of the reactants (sums of species) can be calculated with eq. (10). Thus for the reactants A_{tot} and B_{tot}, the standard transformed enthalpies of formation are given by

$$\begin{aligned}\Delta_f H'^{\circ}(A_{tot}) &= r(A^-)\Delta_r H^\circ(A^-) + r(HA)\Delta_f H^\circ(HA) \\ &\quad - r(HA)\Delta_f H^\circ(H^+) \qquad (22)\end{aligned}$$

$$\begin{aligned}\Delta_f H'^{\circ}(B_{tot}) &= r(B^-)\Delta_f H^\circ(B^-) + r(HB)\Delta_f H^\circ(HB) \\ &\quad - r(HB)\Delta_f H^\circ(H^+) \qquad (23)\end{aligned}$$

where $r(A^-)$ is the equilibrium mole fraction of A^- in the (A^-, HA) pseudoisomer group. Here it is assumed that HA and HB each contains one hydrogen, but, in making thermodynamic tables, adjustments would be made for all H in the species [2,4].

Substituting eqs. (22) and (23) in eq. (21) yields

$$\begin{aligned}\Delta_r H'^{\circ} &= r(B^-)\Delta_f H^\circ(B^-) + r(HB)\Delta_f H^\circ(HB) \\ &\quad - r(A^-)\Delta_f H^\circ(A^-) \\ &\quad - r(HA)\Delta_f H^\circ(HA) \\ &\quad - \{r(HB) - r(HA)\}\Delta_f H^\circ(H^+) \\ &= r(B^-)\Delta_f H^\circ(B^-) + r(HB)\Delta_f H^\circ(HB) \\ &\quad - r(A^-)\Delta_f H^\circ(A^-) \\ &\quad - r(HA)\Delta_f H^\circ(HA) \\ &\quad - \Delta_r N(H^+)\Delta_f H^\circ(H^+) \qquad (24)\end{aligned}$$

where $\Delta_r N_H = \{r(HB) - r(HA)\}$ is the change in binding of H^+ in the reaction. If more H^+ is bound per mole of B_{tot} than per mole of A_{tot}, 2;$\Delta_r N_H$ is positive. During the reaction of A_{tot} to

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[2] The effects of ionic strength can be rigorously treated if the relative apparent molar enthalpies of the reactants and products are known. In the absence of this information, the extended Debye–Hückel theory has sometimes been used to estimate these effects [4,7].

B_{tot}, $\Delta_r N_H$ moles of H^+ per mole of reaction must flow into the reaction chamber in the thought experiment. This reduces the transformed enthalpy change as described above.

Calorimetric measurements are usually made with buffered solutions so that the pH is nearly constant during the calorimetric experiment. Nevertheless, if H^+ is produced or consumed, there is a displacement of the buffer equilibrium with an additional enthalpy effect not discussed above. The standard enthalpy of reaction of the buffer at the T, P, I of the calorimetric experiment is therefore involved. For the buffer ionization reaction

$$\mathrm{HBuff} = \mathrm{H}^+ + \mathrm{Buff}^- \tag{25}$$

the standard enthalpy of ionization is given by

$$\Delta_r H^\circ(\mathrm{Buff}) = \Delta_f H^\circ(\mathrm{H}^+) + \Delta_f H^\circ(\mathrm{Buff}^-) - \Delta_f H^\circ(\mathrm{HBuff}). \tag{26}$$

If we consider the volume of solution that contains one mole of the A, B mixture and b moles of buffer at $\mathrm{p}H = \mathrm{p}K(\mathrm{HBuff})$ at the T, P, I of the experiment, the molar enthalpy of the initial solution is given by

$$\begin{aligned}\mathrm{H(initial)} &= r(\mathrm{A}^-)\Delta_f H^\circ(\mathrm{A}^-) + r(\mathrm{HA})\Delta_f H^\circ(\mathrm{HA}) \\ &\quad + (b/2)\Delta_f H^\circ(\mathrm{HBuff}) \\ &\quad + (b/2)\Delta_f H^\circ(\mathrm{Buff}^-) + h\Delta_f H^\circ(\mathrm{H}^+)\end{aligned} \tag{27}$$

where h is the amount of free H^+ in the volume of solution considered. The enthalpy of the final solution is given by

$$\begin{aligned}\mathrm{H(final)} &= r(\mathrm{B}^-)\Delta_f H^\circ(\mathrm{B}^-) + r(\mathrm{HB})\Delta_f H^\circ(\mathrm{HB}) \\ &\quad + \{(b/2) - \Delta_r N_H\}\Delta_f H^\circ(\mathrm{HBuff}) \\ &\quad + \{(b/2) + \Delta_r N_H\}\Delta_f H^\circ(\mathrm{Buff}^-) \\ &\quad + h\Delta_f H^\circ(\mathrm{H}^+)\end{aligned} \tag{28}$$

where it is assumed that the pH has not changed significantly. Thus the enthalpy change $\Delta_r H(\mathrm{cal})$ in the calorimetric experiment is given by

$$\begin{aligned}\Delta_r H(\mathrm{cal}) &= \mathrm{H(final)} - \mathrm{H(initial)} \\ &= r(\mathrm{B}^-)\Delta_f H^\circ(\mathrm{B}^-) + r(\mathrm{HB})\Delta_f H^\circ(\mathrm{HB}) \\ &\quad - \mathrm{r}(\mathrm{A}^-)\Delta_f H^\circ(\mathrm{A}^-) \\ &\quad - r(\mathrm{HA})\Delta_f H^\circ(\mathrm{HA}) \\ &\quad - \Delta_r N_H\{\Delta_f H^\circ(\mathrm{HBuff}) - \Delta_f H^\circ(\mathrm{Buff}^-)\}.\end{aligned} \tag{29}$$

Substituting eqs. (24) and (26) yields

$$\begin{aligned}\Delta_r H(\mathrm{cal}) &= \Delta_r H'^\circ + \Delta_r N(\mathrm{H}^+)\Delta_f H^\circ(\mathrm{H}^+) \\ &\quad - \Delta_r N_H\{\Delta_f H^\circ(\mathrm{H}^+) - \Delta_r \mathrm{H}^\circ(\mathrm{Buff})\} \\ &= \Delta_r H'^\circ + \Delta_r N_H \Delta_r H^\circ(\mathrm{Buff}).\end{aligned} \tag{30}$$

Thus, the calorimetric enthalpy change is made up of two components and the standard transformed enthalpy of the enzyme-catalyzed reaction $\Delta_r H'^\circ$ is equal to the calorimetric enthalpy per mole of reaction minus $\Delta_r N_H \Delta_r H^\circ(\mathrm{Buff})$. The buffer correction will be smaller if the buffer has a small enthalpy of acid dissociation. If $\Delta_r H(\mathrm{cal})$ is determined in two different buffers that have significantly different values of $\Delta_r H^\circ(\mathrm{Buff})$, one would have two equations in the two unknowns $\Delta_r H'^\circ$ and $\Delta_r N_H$. These two equations could then be solved to obtain these two quantities.

If the reactant anions and the buffer anion bind Mg^{2+}, terms have to be added to the above equations. The enthalpy change for the reaction

$$\mathrm{MgBuff}^+ \rightleftharpoons \mathrm{Mg}^{2+} + \mathrm{Buff}^- \tag{31}$$

is given by

$$\Delta_r H^\circ(\mathrm{MgBuff}) = \Delta_f H^\circ(\mathrm{Mg}^{2+}) + \Delta_f H^\circ(\mathrm{Buff}^-) - \Delta_f H^\circ(\mathrm{MgBuff}^+). \tag{32}$$

Thus, eq. (24) is replaced by

$$\begin{aligned}\Delta_r H'^{\circ} = {} & r(\mathrm{B}^-)\Delta_f H^{\circ}(\mathrm{B}^-) + \mathrm{r}(\mathrm{HB})\Delta_f H^{\circ}(\mathrm{HB}) \\ & + r(\mathrm{MgB}^+)\Delta_f H^{\circ}(\mathrm{MgB}^+) \\ & - r(\mathrm{A}^-)\Delta_f H^{\circ}(\mathrm{A}^-) \\ & - r(\mathrm{HA})\Delta_f H^{\circ}(\mathrm{HA}) \\ & + r(\mathrm{MgA}^+)\Delta_f H^{\circ}(\mathrm{MgA}^+) \\ & - \Delta_r N_{\mathrm{H}}\Delta_f H^{\circ}(\mathrm{H}^+) \\ & - \Delta_r N_{\mathrm{Mg}}\Delta_f H^{\circ}(\mathrm{Mg}^{2+}). \qquad (33)\end{aligned}$$

The equations for H(initial) and H(final) have additional terms, and eq. (30) becomes

$$\begin{aligned}\Delta_r H(\mathrm{cal}) = {} & \Delta_r H'^{\circ} + \Delta_r N_{\mathrm{H}}\Delta_r H^{\circ}(\mathrm{Buff}) \\ & + \Delta_r N_{\mathrm{Mg}}\Delta H^{\circ}(\mathrm{MgBuff}). \qquad (34)\end{aligned}$$

If other metal ions ligands were involved, additional terms would have to be added to eq. (34).

4. Discussion

This paper has concentrated on the hydrolysis of ATP to ADP and orthophosphate because of the availability of detailed information on the acid dissociation constants and the metal ion complex dissociation constants, but we note that eq. (30) is also applicable to reactions involving proteins and nucleic acids. With larger molecules it usually is not possible to determine the individual dissociation constants, but the amount of acid produced or consumed by the reaction can also be determined with a pHstat. The amount of acid produced or consumed can also be determined by measuring the apparent equilibrium constant K' as a function of pH. This latter method cannot be applied when it is impractical to measure an apparent equilibrium constant or when the reactants are heterogeneous. In this case the concept of a single biochemical reaction cannot be applied.

If $\Delta_r H'^{\circ}$ is measured calorimetrically for a biochemical reaction at specified T, P, pH, pMg, and I, the value of $\Delta_r H^{\circ}$ (ref) for any chosen chemical reference reaction can be calculated if the acid dissociation and magnesium complex dissociation constants and corresponding enthalpies of dissociation are known for all of the reactants. For example, the hydrolysis of ATP was written as a biochemical reaction (see eq. 17). A chemical reference reaction for this biochemical reaction is

$$\begin{aligned}& \mathrm{ATP}^{4-}(\mathrm{aq}) + \mathrm{H_2O(l)} \\ & \quad \rightleftharpoons \mathrm{ADP}^{3-}(\mathrm{aq}) + \mathrm{HPO_4^{2-}}(\mathrm{aq}) + \mathrm{H}^+(\mathrm{aq}). \qquad (35)\end{aligned}$$

The biochemical reaction is written in terms of sums of species and unlike the chemical reaction it does not balance charge and atoms of H and Mg. The choice of the chemical reference reaction is arbitrary and there are a number that could have been selected. Equations that relate thermodynamic quantities that pertain to a reference reaction and those that pertain to a biochemical reaction have been given by Alberty [5] for the hydrolysis of ATP and by Goldberg and Tewari [7] for the disproportionation reaction of ADP to AMP and ATP. Both $\Delta_r H'^{\circ}$ for a biochemical reaction and $\Delta_r H^{\circ}$(ref) for a chemical reaction can be obtained either from equilibrium constants determined as a function of temperature or from calorimetric measurements.

The above equations also have implications for the performance and interpretation of all calorimetric and equilibrium measurements. The most important is the assumption that the measurements are performed at constant pH, pMg, and I (the temperature and pressure are easily kept constant in a properly constructed calorimeter or thermostat). In an equilibrium measurement the pH, pMg, and I are those determined for the system at equilibrium. Note that while the pH can be measured with an electrode, pMg and I are quantities which must be calculated and are therefore dependent upon both a knowledge of the equilibrium constants of the various reactions occurring in solution and on some knowledge or assumptions about the activity coefficients of the substances in the solution. However, in a calorimetric experiment there may be changes in pH, pMg, and I as a consequence of the chemical reactions occurring in the solution. For this reason, it is important that the system under investigation be studied in a solution that is well buffered with respect to pH and pMg; the ionic strength cannot be buffered. If this buffering is not done, the chemical reactants and products may themselves be reacted with the hydrogen ions or magnesium ions that are produced or absorbed as a consequence of the biochemical reaction. Also not that the calculation of $\Delta_r H'^{\circ}$ from apparent equilibrium constants which have been determined as a function of temperature requires that pH, pMg, and I be the same at each temperature (see eqs. 5 and 7). Thus, values of $\Delta_r H'^{\circ}$ determined from either calorimetric or equilibrium measurements with the above equations may be complicated by changes in pH, pMg, or I. However, it is likely that these errors are small if these changes are also kept small by careful experimentation or if they are adjusted to a common set of conditions.

The most general treatment of the results of calorimetric measurements requires the solution of the chemical equilibrium equations (see ref. [8]) to obtain the concentrations of the various species in solution for both the initial state and the final state of the reacting system. The enthalpy of the initial state H(initial) and the enthalpy of the final state H(final) can then be calculated with

$$H = \Sigma n_j \Delta_f H_j^{\circ} \tag{36}$$

where n_j is the amount of species j in solution, $\Delta_f H_j^{\circ}$ is the enthalpy of formation of species j, and the summation is over all chemical species present in the solution. The measured enthalpy of reaction is

$$q = H(\text{final}) - H(\text{initial}). \tag{37}$$

The molar enthalpy of reaction is given by $\{H(\text{final}) - H(\text{initial})\}/\xi$, where ξ is the extent of reaction which must be measured. [3] The solution of the chemical equilibrium equations requires a knowledge of the equilibrium constants for the various reactions occurring in solution. The use of eq. (36) requires a knowledge of the enthalpies of formation are not known for all the species in a given chemical reaction, it will be necessary to assign arbitrary values (i.e. zeros) to all but one of the enthalpies of formation in that reaction. It is also necessary to assume a value of the standard molar enthalpy of reaction $\Delta_r H^{\circ}$(ref) for the chemical reference reaction occurring in solution and that is the object of the study. The final step is to perform the above calculation and adjust the value of $\Delta_r H^{\circ}$ (ref) to obtain a least-squares fit of the model to the measured molar enthalpies of reaction. This very general calculation would have to couple to solution of the chemical equilibrium equations (a set of simultaneous non-linear equations) with the regression of $\Delta_r H^{\circ}$ (ref). Because of the difficulties of this approach, simplifying as to the constancy of pH, pMg, and I are generally made and simpler calculations are performed. As pointed out above, these calculations should be adequate if the solutions are well buffered. While the above equations and treatment were geared towards biochemical reactions which generally involve the use of enzyme catalysis, all that has been discussed above will apply to non-enzymatic reactions as well.

In the above discussion we have assumed that there was no interaction between the enzyme and the reactants and products of the reaction which the enzyme catalyzes. One way of testing this assumption (and that is appropriate to the interpretation of calorimetric experiments) is to measure the enthalpy of mixing of a solution containing the enzyme that catalyzes a given reaction with another solution containing the equilibrium amounts of reactants and products for that reaction. This second solution must be synthetically prepared from the pure reactants and products and requires a knowledge of the apparent equilibrium constant at the appropriate T, pH, pMg, and I. If the enthalpy of binding of the enzyme to the reactants and the products is zero or if there is no binding, the measured enthalpy of mixing will be zero.

Some comparisons are now made (see Table 2) between values of standard transformed enthalpies of reaction obtained from calorimetric

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[3] Under favorable conditions the extent of reaction can be determined by measuring the enthalpy of reaction from both directions of the reaction. In such cases it is also possible to calculate the apparent equilibrium constant for the reaction [9].

Table 2

Calorimetric enthalpies (in kJ mol^{-1}) of reaction $\Delta_r H$(cal) and standard transformed enthalpies of reaction $\Delta_r H'^{o}$ obtained from these calorimetric measurements and calculated from apparent equilibrium constants measured as a function of temperature. In some cases, the enthalpies of reaction were obtained from the direction of reaction *opposite* to that given below or from both directions. The results given below pertain to 298.15 K and the direction of the reaction as written

Reaction	$\Delta_r H$(cal) (kJ mol^{-1})	Reference	Buff. Corr. [a] (kJ mol^{-1})	$\Delta_r H'^{o}$ [b] (kJ mol^{-1})	$\Delta_r H'^{o}$ [c] (kJ mol^{-1})	Reference
glucose(aq) → fructose(aq)	2.76	Tewari and Goldberg [10]	0.00	2.76	2.6 [d]	Van Tilburg [11]
					2.7	Lloyd and Chan [12]
					2.6	Tewari and Goldberg [10]
					3.7	Olivier and du Toit [13]
fumarate(aq) + $H_2O(l) \rightleftharpoons$ malate(aq)	−16.0	Ohlmeyer [14]	0.00	−16.0	−14.1	Krebs and Smyth [17]
	−15.5	Kitzinger and Hems [15]	0.00	−15.5	−14.9	Scott and Powell [18]
	−15.7	Gajewski [16]	0.00	−15.7	−16.6	Bock and Alberty [19]
(S)-lactate(aq) + NAD(aq) ⇌ pyruvate(aq) + NADH(aq)	41.0	Katz [a] [20]	3.5	44.5	46.7	Hakala et al. [23]
	57.8	Donovan et al. [21]	4.1	61.9	–	
	50.4	Schmid and Hinz [22]	4.2	54.6	–	
ATP(aq) + glucose(aq) ⇌ ADP(aq) + glucose 6-phosphate(aq)	−74.9	McGlothlin and Jordan [24]	47.3	−27.6	– [f]	
	−71.6	McGlothlin and Jordan [24]	44.4	−27.2	–	
	−66.1	McGlothlin and Jordan [24]	37.7	−28.4	–	
	−54.0	McGlothlin and Jordan [24]	26.4	−27.6	–	
ADP(aq) + phosphocreatine(aq) ⇌ ATP(aq) + creatine(aq)	– [g]	Woledge and Reilly [25], and Alberty [6]	– [g]	−10.0 [g]	−11.9	Teague and Dobson [26]

[a] The buffer correction is equal to $-\Delta_r N_H \Delta_r H^{o}$ (Buff) where $\Delta_r H^{o}$ (Buff) is the standard enthalpy of ionization of the buffer.

[b] Calculated with $\Delta_r H'^{o} = \Delta_r H\ (\mathrm{cal}) - \Delta_r N_H \Delta_r H^{o}\ (\mathrm{Buff})$.

[c] Calculated from apparent equilibrium constants measured as a function of temperature.

[d] Results from several earlier equilibrium measurements on this reaction are summarized in ref. [10].

[e] Katz used (R)-lactate in his study.

[f] The apparent equilibrium constant for this reaction has not been measured as a function of temperature.

[g] The result of $\Delta_r H'^{0} = -10.0$ kJ mol^{-1} was obtained from a thermochemical cycle calculation [26] involving two separate reactions and independent buffer protonation corrections.

measurements and values obtained from apparent equilibrium constants determined as a function of temperature. The first two examples involve relatively simple reactions where $\Delta_r N_H = 0$, and it is seen that there is reasonable agreement between the results for $\Delta_r H'^{\circ}$ obtained from both calorimetry and from the apparent equilibrium constants which were measured as a function of temperature. For the first reaction, $I K' = K$ (ref) and $\Delta_r H'^{\circ} = \Delta_r H^{\circ}$ (ref). For the second reaction, these relations also apply for $\text{pH} > 6$ providing the reference reaction is taken to be

$$\text{fumarate}^{2-}(\text{aq}) + H_2O(\text{l}) \rightleftharpoons \text{malate}^{2-}(\text{aq}). \tag{38}$$

For the oxidation of (S)-lactate to pyruvate with NAD, there are the results of three calorimetric studies and only the result of Hakala et al. [23] for $\Delta_r H'^{\circ}$ from a second law treatment. For this reaction $\Delta_r N(H^+) = -1.0$ over a very wide pH range and both $\Delta_r H(\text{cal})$ and $\Delta_r H'^{\circ}$ are predicted to be independent of pH. The result from Hakala et al. [23] has a statistical uncertainty of approximately 6 kJ mol^{-1} and is judged to be in agreement with the result obtained from the calorimetric measurements of Katz [20] and of Schmid and Hinz [22]. The result of Donovan et al. [21] was obtained at $I = 0.5$ M, while the results of the other studies were obtained at $I = 0.10$ M to $I = 0.20$ M. McGlothlin and Jordan [24] measured enthalpy changes ($T = 298.15$ K, pH 8.0, pMg 1.6 to 3.7, and $I \approx 0.5$ M) for the hexokinase catalyzed phosphorylation of glucose in four different buffers (tris(hydroxymethyl) aminomethane, glycylglycine, glycylglycylglycine, and *N,N*-bis(2-hydroxyethyl)glycine). After the buffer protonation corrections were applied, the results obtained for $\Delta_r H'^{\circ}$ from these four different buffers were consistent. The apparent equilibrium constant I' has not been determined as a function of temperature for this latter reaction. The values for $\Delta_r H'^{\circ}$ ($T = 298.15$ K, pH 7.0, pMg 3.0, and $I = 0.25$ M) obtained for the last reaction in Table 2 from calorimetry and from a second law treatment are also judged to be in agreement.

The importance of the state of ionization of reactants and products and of the buffer protonation correction appears to have been first recognized for biochemical calorimetry by Dobry and Sturtevant [27], by Dobry et al. [28], and by Meyerhoff and Shatas [29]. The equations derived above confirm the necessity of the corrections used by these investigators and make clear the relationship between the calorimetric enthalpy of reaction and the standard transformed enthalpy of reaction.

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