

# THERMODYNAMIC PROPERTY VALUES FOR ENZYME-CATALYZED REACTIONS

**ROBERT N. GOLDBERG**

\*Biochemical Science Division, National Institute of Standards and Technology,  
Gaithersburg, Maryland 20899, U.S.A.

and

Department of Chemistry and Biochemistry, University of Maryland, Baltimore  
County, Baltimore MD 21250, U.S.A.

**E-Mail:** [robert.goldberg@nist.gov](mailto:robert.goldberg@nist.gov)

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## ABSTRACT

This chapter deals with how one can obtain values of thermodynamic properties – specifically the apparent equilibrium constant  $K'$ , the standard molar transformed Gibbs energy change  $\Delta_r G'^\circ$ , and the standard molar transformed enthalpy change  $\Delta_r H'^\circ$  for biochemical reactions – and, in particular, for enzyme-catalyzed reactions. In addition to direct measurement, these property values can be obtained in a variety of ways: from thermochemical cycle calculations; from tables of standard molar formation properties; by estimation from property values for a chemically similar reaction or substance; by means of estimation by using a group-contribution method; by combining a known value of the standard molar enthalpy change  $\Delta_r H'^\circ$  and an estimated value for the standard molar entropy change  $\Delta_r S'^\circ$  in order to obtain the standard molar Gibbs energy change  $\Delta_r G'^\circ$  for a given reaction; and by use of computational chemistry.

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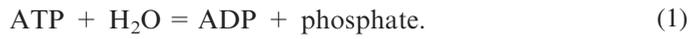
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## INTRODUCTION AND GENERAL PRINCIPLES

This chapter deals with how one can obtain values of thermodynamic properties – specifically the apparent equilibrium constant  $K'$ , the standard molar transformed Gibbs energy change  $\Delta_r G'^\circ$ , and the standard molar transformed enthalpy change  $\Delta_r H'^\circ$  for biochemical reactions – and, in particular, for enzyme-catalyzed reactions. Much of the interest in these property values arises from applications in bioprocess engineering, where the aim is to optimize product yield and energy utilization [1]. Another interest arises from the use of thermodynamics to model metabolic processes [2]. This approach has been expanded in recent years to also include kinetic considerations in the modeling calculations [3, 4].

Firstly, it is important to appreciate that, for biochemical reactions, thermodynamic quantities are, in general, functions of temperature  $T$ , pH, pX, and ionic strength  $I$ . Here,  $\text{pX} = -\log_{10}[X]$ , where  $[X]$  is the concentration of a species  $X$ , typically an ion, that binds to one or more of the reactants. This dependency on pH and pX arises because of the multiple states of ionization and metal ion binding in which the reactant molecules can exist. This point is illustrated by means of a generic reaction – the hydrolysis of adenosine 5'-triphosphate (ATP) to adenosine 5'-diphosphate (ADP) and phosphate (all reactions discussed in this chapter pertain to aqueous media unless indicated otherwise)



The apparent equilibrium constant  $K'$  for this reaction is

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

By convention the concentration of water has been omitted in the expression for  $K'$ . The concentrations used in eqn. (2) are *total* concentrations of the various ionic and metal bound forms of the reactants and products. For example

$$[\text{ATP}] = [\text{ATP}^{4-}] + [\text{HATP}^{3-}] + [\text{H}_2\text{ATP}^{2-}] + [\text{H}_3\text{ATP}^-] + [\text{MgATP}^{2-}] \\ + [\text{MgHATP}^-] + [\text{MgH}_2\text{ATP}^-] + [\text{Mg}_2\text{ATP}], \quad (3)$$

$$[\text{ADP}] = [\text{ADP}^{3-}] + [\text{HADP}^{2-}] + [\text{H}_2\text{ADP}^-] + [\text{MgADP}^-] + \\ [\text{MgHADP}], \quad (4)$$

$$[\text{phosphate}] = [\text{PO}_4^{3-}] + [\text{HPO}_4^{2-}] + [\text{H}_2\text{PO}_4^-] + [\text{H}_3\text{PO}_4] \quad (5)$$

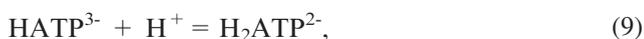
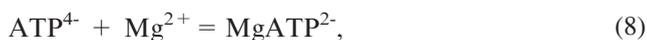
If calcium or other divalent metal ions are present, one must also consider additional, analogous species such as  $\text{CaATP}^{2-}$ . The essential point is that, because biochemical reactants such as ATP, ADP, and phosphate exist in several different ionic and metal bound

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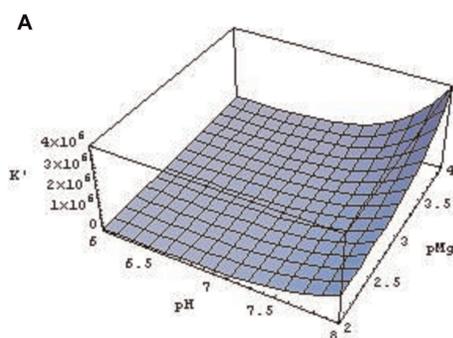
forms, there is a multiplicity of species that make up each of these reactants. This, in turn, leads to the aforementioned dependencies of thermodynamic quantities on pH and pX. Illustrations of these dependencies are shown in Fig. 1. These surface plots were calculated by using the equilibrium constant for the chemical reference reaction

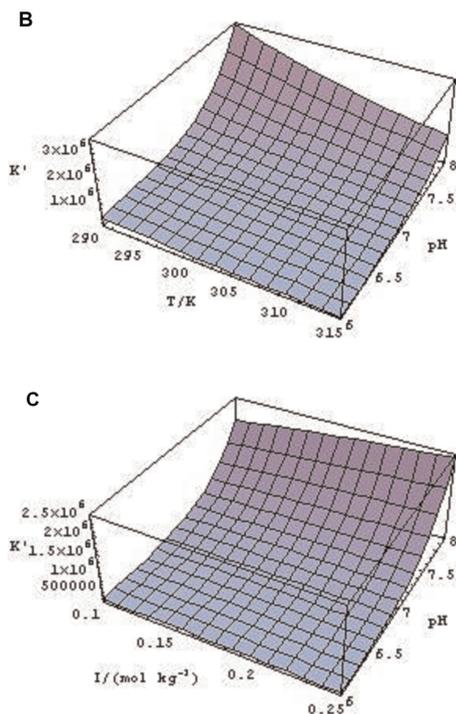


and equilibrium constants for the pertinent  $\text{H}^+$  and  $\text{Mg}^{2+}$  binding constants:



It is important to recognize that the equilibrium constants  $K$  for reactions (6) to (10) pertain to specific chemical species. Clearly, these chemical reactions must balance both the number of atoms and the charges. While equilibrium constants  $K$  depend on temperature and ionic strength they do not depend on pH or pX as do apparent equilibrium constants  $K'$ . Thus, it is important to maintain a clear distinction between  $K$  and  $K'$  [5]. The book *Thermodynamics of Biochemical Reactions* [6] contains a definitive treatment of transformed thermodynamic properties and many examples involving biochemical reactions.





**Figure 1.** The apparent equilibrium constant  $K'$  for the hydrolysis reaction ( $\text{ATP} + \text{H}_2\text{O} = \text{ADP} + \text{phosphate}$ ) as a function of temperature  $T$ , pH, pMg, and ionic strength  $I$ . Since it is not possible to represent a five dimensional surface in two dimensions, three 3-D projections with appropriate constraints are shown. These constraints are:  $T=298.15$  K and  $I=0.25$  mol·dm<sup>-3</sup> (A);  $I=0.25$  mol·dm<sup>-3</sup> and pMg=3.0 (B); and  $T=298.15$  K and pMg=3.0 (C).

Values of standard molar enthalpies of reaction  $\Delta_r H^\circ$  can be used to adjust values of  $K$  from one temperature to another over a relatively narrow temperature range by means of the van't Hoff equation. If one is operating over a wider temperature range, one also needs values of the standard molar heat capacity changes  $\Delta_r C_p^\circ$  for the reactions of interest. For this purpose, some useful formulas are [7]

$$\Delta_r G^\circ(T) = (T/T_{\text{ref}}) \cdot \Delta_r G^\circ(T_{\text{ref}}) + \Delta_r H^\circ(T_{\text{ref}}) \cdot (T_{\text{ref}} - T)/T_{\text{ref}} + \Delta_r C_p^\circ \cdot \{(T - T_{\text{ref}}) - T \ln(T/T_{\text{ref}})\} \quad (11)$$

$$K = \exp\{-\Delta_r G^\circ(T)/(RT)\} \quad (12)$$

Here,  $R$  is the gas constant,  $T$  is the temperature of interest, and  $T_{\text{ref}}$  is a reference temperature, typically 298.15 K. Also, since reactions are carried out at ionic strengths where non-ideality has to be accounted for, one also needs values of the activity coefficients  $\gamma$  of the species for use in the equilibrium equations. In the absence of values of activity coefficients  $\gamma$  for the vast majority of biochemical species, a common practice has been to estimate values of the activity coefficient  $\gamma_i$  of an ion  $i$  by using the extended Debye-Hückel equation

$$\log_e \gamma_j = -A_m z_i^2 I^{1/2} / (1 + BI^{1/2}). \quad (13)$$

Here  $A_m$  is the Debye-Hückel constant ( $A_m = 1.1758 \text{ kg}^{1/2} \cdot \text{mol}^{-1/2}$  at 298.15 K),  $z_i$  is the charge of species  $i$ , and  $B$  is an empirical constant, which has often been set at  $1.6 \text{ kg}^{1/2} \cdot \text{mol}^{-1/2}$ . Values of  $A_m$  have been tabulated by Clarke and Glew [8] for the temperature range 273 K to 423 K.

The principles discussed above provide a basis for performing calculations of the type that led to the results shown in Fig. 1. These calculations depend on having the necessary thermodynamic data available for both the chemical reference reaction {e. g., reaction (6)} and the pertinent  $\text{H}^+$  and metal-ion binding constants {e. g., reactions (7) to (10)}. One must also be able to solve the simultaneous non-linear equations that describe these complex reaction systems. Fortunately, this numerical problem can be handled routinely by using computer algorithms to calculate the extent of reaction for each chemical reaction, the concentrations of all chemical species and of the biochemical reactants, and finally the value of  $K'$ . These algorithms can also be used to calculate how calorimetrically determined molar enthalpy changes  $\Delta_r H(\text{cal})$  and changes in binding  $\Delta_r N$  of  $\text{H}^+$  and X also vary with pH, pX,  $T$ , and  $I$ .

As illustrated in Fig. 1, these calculations allow one to obtain all of this information as a function of  $T$ , pH, pX, and  $I$ . *Thus, the formalism outlined above makes it possible to obtain an essentially complete thermodynamic picture of these important reactions.*

Having a sound thermodynamic framework for dealing with complex reactions is essential. However, reliable property values are required for performing practical calculations. A substantial body of experimental results for enzyme-catalyzed reactions has accumulated over many years and has been systematized in several review articles [9–15] and on the web [16]. The equilibrium data has been obtained by using a variety of analytical methods, with chromatography, spectrophotometry, and enzymatic assays being the most commonly used. Molar enthalpies of reaction have been obtained either from calorimetric results or from equilibrium constants which have been measured as a function of temperature.

The essential point is that if one knows  $K'$  at a given  $T$ , pH, pMg, and  $I$ , it is possible to calculate a value of  $K$  for a chemical reference reaction. This value of  $K$  can then be used with the pKs of the chemical reactants and products in the reference reaction to calculate

values of  $K'$  as a function of  $T$ , pH, and  $I$ . A similar approach applies to calorimetrically determined molar enthalpy changes  $\Delta_r H(\text{cal})$ . In this case one needs, in addition to the p*K*s, values of standard molar enthalpies of reaction  $\Delta_r H^\circ$  for the relevant proton and metal ion binding reactions. Many of these p*K* and  $\Delta_r H^\circ$  values can be found in existing databases [17, 18]. However, if a p*K* or  $\Delta_r H^\circ$  value has not been measured, it may be possible to estimate a value to a sufficient degree of accuracy by using property values for structurally similar substances [19]. To summarize, one has a substantial body of experimental data,  $K'$  and  $\Delta_r H(\text{cal})$ , that can be used to calculate values of  $K$  and  $\Delta_r H^\circ$  for chemical reference reactions that correspond to overall biochemical reactions. These calculated values of  $K$  and  $\Delta_r H^\circ$  can then be used together with values of  $K$  and  $\Delta_r H^\circ$  for the relevant proton and metal ion binding reaction to calculate  $K'$  as a function of  $T$ , pH, pMg, and  $I$ . These calculations can be performed relatively conveniently by using published algorithms [20–22].

## COMPUTATIONAL AND ESTIMATION METHODS

However, if  $K'$  has not been measured for a reaction of interest, it may still be possible to obtain a value of  $K'$  by a variety of means. Possible approaches are:

- Calculate  $K$  and/or  $K'$  by means of thermochemical cycle calculations or by using tables of standard molar formation properties.
- Estimate the desired property value by using property values for a chemically similar reaction.
- Estimate the desired property value by using a group-contribution or Benson approach.
- If a value of  $\Delta_r H(\text{cal})$  has been measured, one can calculate  $\Delta_r H^\circ$  for an appropriate chemical reference reaction. A value of  $\Delta_r S^\circ$  can then be estimated and combined with  $\Delta_r H^\circ$  to give a value of  $\Delta_r G^\circ$  for the reference reaction. This value of  $\Delta_r G^\circ$  can then be used in conjunction with values of  $K$  and  $\Delta_r H^\circ$  for the relevant proton and metal ion binding reactions to obtain  $K'$  at the desired  $T$ , pH, pMg, and  $I$ .
- Use computational chemistry to calculate the desired property values.

A brief discussion of each of these approaches follows.

## TABLES OF STANDARD MOLAR FORMATION PROPERTIES

The method by which one adds or subtracts chemical reactions and combines the thermodynamic properties of these reactions to obtain the thermodynamic property for the summed reaction is well-known and will not be described herein. The generalization of this method leads to tables of formation properties. Thus, while extensive tables of formation properties exist [23] for inorganic substances and for organic substances that have one or two carbons,

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tabulations of the formation properties of biochemical substances is relatively limited. The earliest thermodynamic tables for biochemical substances appear to have been prepared by Krebs, Kornberg, and Burton [2]. These pioneering tables contain standard molar Gibbs energies of formation  $\Delta_f G^\circ$  for 88 species – and the property values for 21 of these species came from the NBS thermochemical tables [24]. The 1969 tables of Wilhoit [25] cover a much larger number of substances than Krebs *et al.* [2] and include values of the standard molar enthalpy of formation  $\Delta_f H^\circ$ , the standard molar entropy  $S^\circ$ , and the standard molar heat capacity  $C_p^\circ$  in addition to the standard molar Gibbs energy of formation  $\Delta_f G^\circ$ .

The 1989 tables of Goldberg and Tewari [26] are limited to the thermodynamic properties ( $\Delta_f G^\circ$ ,  $\Delta_f H^\circ$ ,  $S^\circ$ , and  $C_p^\circ$ ) of carbohydrates, specifically the pentoses and the hexoses, and their monophosphates. Goldberg and Tewari [26] constructed their tables by using a “reaction catalog” which consists of a table of experimental property values for the reactions and substances that are the basis for the calculation of the formation properties. The experimental property values were weighted according to their estimated accuracies. The advantage to this approach is that, as new experimental results become available, the reaction catalog can be updated relatively easily and new thermodynamic tables can be calculated promptly from the new reaction catalog.

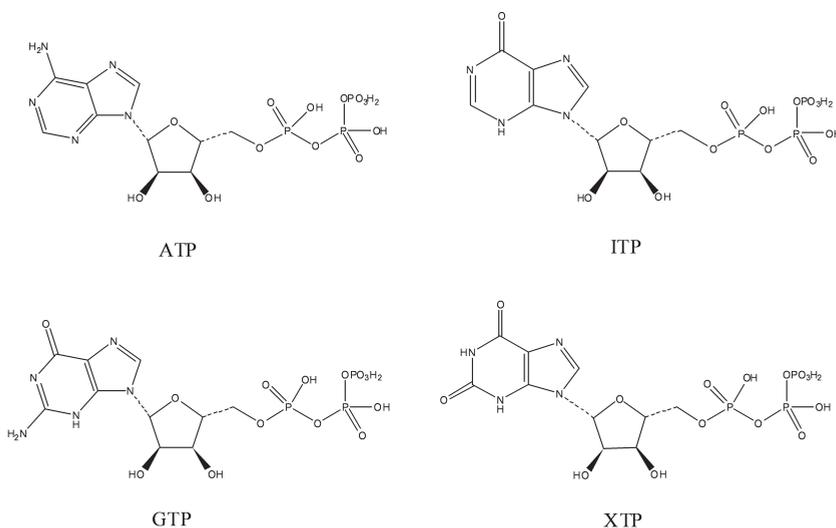
The 1990 tables of Miller and Smith-Magowan [27] are limited in their coverage to the substances found in the Krebs cycle. It should be noted that their [27] tabulated values of  $\Delta_f G^\circ$  pertain to an ionic strength of  $0.1 \text{ mol}\cdot\text{dm}^{-3}$ , while the other thermochemical tables use the conventional thermochemical standard state based upon a hypothetical ideal solution of unit molality. The thermochemical tables of Alberty [21, 22] are the most extensive of those available. They contain values of  $\Delta_f G^\circ$  for all of the species of 199 biochemical reactants (sums of species). Values of  $\Delta_f H^\circ$  are also given for the species that comprise 94 of these biochemical reactants. Alberty [21, 22] also gives computer programs that allow one to use the property values in his tables to calculate standard transformed Gibbs energy changes  $\Delta_r G^\circ$ , standard transformed enthalpy changes  $\Delta_r H^\circ$ , and apparent equilibrium constants  $K'$  for biochemical reactions as a function of  $T$ , pH, and  $I$ . The user of any of these tables is cautioned about the risks in using data from two or more different tables to calculate property values for a given reaction. Specifically, while each of the aforementioned thermochemical tables is internally consistent, the values may not be consistent with the values given in another table. Thus, serious errors can result if values from two or more tables are combined to calculate property values for a given reaction. While substantial progress has been made in thermodynamic tables for biochemical substances since the early work of Krebs, Kornberg, and Burton [2], an inspection of the total amount of thermodynamic data available for enzyme-catalyzed reactions [9–16] shows that there are many substances for which values of standard molar formation properties can be calculated from existing data. In addition to property values for enzyme-catalyzed reactions, there are also substantial amounts of data for standard molar enthalpies of combustion, standard molar entropies, saturation molalities (solubilities), standard molar enthalpies of solution, and  $pK$ s and stan-

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standard molar enthalpies of reaction for proton and metal-ion binding reactions that tie into the calculation of standard molar formation properties for biochemical substances. It will be a major challenge to pull together all of these thermodynamic property values together and to produce the equivalent of the tables that currently exist for inorganic substances and for small organic molecules in aqueous media.

## ESTIMATION OF A PROPERTY VALUE BY USING PROPERTY VALUES FOR A CHEMICALLY SIMILAR REACTION

The correlation of property values with structure can be usefully exploited to obtain property values for reactions and substances that have not been the subjects of direct measurements. This approach will be illustrated by a few examples. Boerio-Goates *et al.* [28] used the structural similarity (see Fig. 2) between the inosine 5'-triphosphate series (ITP, IDP, and IMP) and the adenosine 5'-triphosphate series (ATP, ADP, and AMP) to estimate the  $pK_s$  and  $\Delta_r H^\circ$  values for the  $H^+$  and  $Mg^{2+}$  binding reactions of the ITP series from the known values for the ATP series of similar reactions.



**Figure 2.** The structures of adenosine 5'-triphosphate (ATP), inosine 5'-triphosphate (ITP), guanosine 5'-triphosphate (GTP), and xanthosine 5'-triphosphate (XTP)

Alberty [29] later used this same structural similarity and assumed that  $\Delta_r G^\circ$  for the hydrolysis reactions in the GTP series and the XTP series were the same as in the ITP series. Also, Goldberg *et al.* [30] measured standard molar enthalpy changes for the hydrolysis reactions of maltose, maltotriose, maltotetraose, maltohexaose, and maltoheptaose to form glucose at 298.15 K. It was found that the values of  $\Delta_r H^\circ/N$ , where  $N$  is the number of  $\alpha$ -1,4 linkages in the aforementioned substances, had a constant value equal to  $-(4.53 \pm 0.04) \text{ kJ}\cdot\text{mol}^{-1}$ . Consideration of additional calorimetric and equilibrium measurements [30] showed that,

for reactions involving the making/breaking of  $N$  saccharide linkages, the assignment of characteristic values of  $\Delta_r H^\circ/N$  or  $\Delta_r G^\circ/N$  or  $\Delta_r S^\circ/N$  for a specified linkage, was accurate in predicting the values of  $\Delta_r H^\circ$ ,  $\Delta_r G^\circ$ , and  $\Delta_r S^\circ$  for reactions involving saccharides containing multiples or combinations of such linkages.

Finally, Tewari and Goldberg [31], in their summary of the results of the hydrolysis reactions of several six-carbon disaccharides, found that the values of  $\Delta_r S^\circ$  for these reactions were in the range  $32 \text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$  to  $48 \text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$ . This relatively narrow range of values has a practical use in that a value of  $\Delta_r G^\circ$  for a disaccharide hydrolysis reaction can be estimated from its measured value of  $\Delta_r H^\circ$  by using a typical value of  $\Delta_r S^\circ \approx 40 \text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$  based on the aforementioned range of values.

Clearly, there are many other biochemical reactions where knowledge of the property values for one reaction can lead to reliable property values for a structurally similar reaction.

### ESTIMATION OF THE DESIRED PROPERTY VALUE BY USING A GROUP-CONTRIBUTION METHOD

Closely related to the aforementioned method based on structural similarity is its generalization which assigns characteristic values to distinct chemical groups. In this approach, a chemical substance is broken down into its characteristic groups, values are assigned to each group, and a property value for the substance of interest is obtained by summation of these values.

This approach is commonly referred to as the group-contribution or Benson method, named after Sidney W. Benson, the principal developer of this approach [32–34]. This method is made possible by the availability of a large array of data for the standard molar enthalpies of formation  $\Delta_f H^\circ$ , standard molar heat capacities  $C_p^\circ$ , and standard molar entropies  $S^\circ$  of organic compounds. Thus, in their 1993 review, Domalski and Hearing [35] were able to provide  $\approx 3700$  comparisons between measured and calculated values for  $\Delta_f H^\circ$ ,  $S^\circ$ , and  $C_p^\circ$  for organic compounds in the gas, liquid, and solid phases.

However, if one wishes to utilize the group-contribution values tabulated by Domalski and Hearing [35] to estimate a value of  $\Delta_f H^\circ$ ,  $\Delta_r G^\circ$ ,  $S^\circ$ , and  $C_p^\circ$  for a substance in the aqueous phase, one must first use their [35] tabulated group-contribution values to calculate these property values for the substance (to take an example) in the solid phase. The values of  $\Delta_f H^\circ$  and  $S^\circ$  can then be used to calculate a value of  $\Delta_r G^\circ$  for this substance. In order to calculate  $\Delta_f H^\circ$  and  $\Delta_r G^\circ$  for this substance in aqueous solution, one then needs values for the standard molar enthalpy of solution  $\Delta_{\text{sol}} H^\circ$  and for the standard molar Gibbs energy of solution  $\Delta_{\text{sol}} G^\circ$ . A value of  $\Delta_{\text{sol}} G^\circ$  can be obtained from measurements of the saturation molality (or solubility) along with a value of the activity coefficient of the substance in solution at the saturation molality.

If the condensed phase is hydrated, one also needs the number of waters of hydration associated with the condensed phase and a value of the activity of the water. A value of  $\Delta_{\text{sol}}H^\circ$  can be obtained either from a direct calorimetric measurement or by use of the van't Hoff equation with values of  $\Delta_{\text{sol}}G^\circ$  at several temperatures.

Domalski [36] has discussed an approach to develop an estimation method for molar enthalpies of formation of organic compounds in water. This same and similar approaches can also be applied to the estimation of molar entropies and Gibbs energies of formation of organic compounds in water.

A limited set of such group-contribution values was developed by Cabani *et al.* [37] for 58 chemical groups. Mavrovouniotis [38, 39] has also developed a table of group-contribution values for biochemical substances in water. It should be noted that the table of Mavrovouniotis [38] appears to be based on values of apparent equilibrium constants obtained at a variety of conditions ( $T$ , pH, pMg, and  $I$ ).

Thus, the tabulated group-contribution values may have inconsistencies caused by variations in the conditions of the measurements on which the group-contribution values are based. Clearly, the construction of extensive tables of group-contribution values such as the tables of Domalski and Hearing [35] were enabled by the existence of a large array of property data, most of which came from measurements of standard molar enthalpies of combustion and third law molar entropies. Thus, the existence of an extensive table of  $\Delta_f H^\circ$ ,  $\Delta_f G^\circ$ , and  $S^\circ$  values for aqueous biochemical species is a prerequisite for the development of accurate group-contribution values for biochemical substances.

### ESTIMATION OF $K'$ AND $\Delta_r G'^\circ$ FROM CALORIMETRIC RESULTS

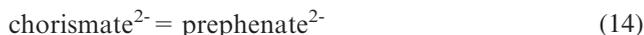
For some reactions, the position of equilibrium lies too far in a given direction to permit the practical measurement of an apparent equilibrium for that reaction. In such cases, and in the absence of either a thermochemical pathway or tabulated values of  $\Delta_r G^\circ$  for the reactants and products, it may still be possible to obtain a reasonable estimate of  $K'$  and  $\Delta_r G'^\circ$  for the reaction of interest if one has a value of the calorimetrically determined molar enthalpy change  $\Delta_r H(\text{cal})$  for the overall biochemical reaction. Specifically, one can use  $\Delta_r H(\text{cal})$  to calculate  $\Delta_r H^\circ$  for a suitable chemical reference reaction. This calculation will most likely involve a correction for the enthalpy of protonation of the buffer [40] and will also require values of  $K$  and  $\Delta_r H^\circ$  for the proton and metal-ion binding for the biochemical reactants. Once  $\Delta_r H^\circ$  has been calculated, one must then estimate the standard molar entropies  $S^\circ$  of the chemical reactants and products in the chemical reference reaction. These estimates are best done on the basis of structural similarity and are greatly aided if there are reasonably extensive tables of standard molar entropies available. In the absence of reliable property

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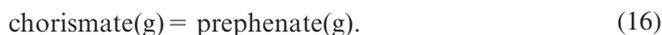
values for similar substances, one must rely on a Benson or group-contribution method. This approach is useful if an apparent equilibrium constant is too large (or too small) to measure and if there are no thermochemical pathways to  $\Delta_r G^\circ$  for the reaction of interest.

## COMPUTATIONAL CHEMICAL RESULTS

Computational chemistry continues to make considerable progress [41] and is approaching a state where some practically useful results can be obtained for thermodynamic properties for biochemical reactions in aqueous media in which the reactant molecules are not too large. Clearly, the principal bottleneck is treating the hydration of the molecules, which, in turn, impacts conformational equilibria and energies. What has been done in some cases [41, 42], as an alternative to the explicit inclusion of waters in the calculations, is to use a polarizable continuum model (PCM). In the cited studies [42, 43], comparisons were made between measured and calculated values of  $\Delta_r H^\circ$  for two biochemical reactions:



For reaction (14), the experimental value for the standard molar enthalpy change was  $\Delta_r H^\circ = -(55.4 \pm 2.3) \text{ kJ}\cdot\text{mol}^{-1}$  and the computed (PCM) value was  $\Delta_r H^\circ = -46.4 \text{ kJ}\cdot\text{mol}^{-1}$ . For reaction (15), the experimental value was  $\Delta_r H^\circ = -(144 \pm 7) \text{ kJ}\cdot\text{mol}^{-1}$  and the computed (PCM) value was  $\Delta_r H^\circ = -154 \text{ kJ}\cdot\text{mol}^{-1}$ . While it was not possible to assign uncertainties to the computed  $\Delta_r H^\circ$  values, it is clear that any reasonable assignment of possible error to the computed values would demonstrate that they were in agreement with the experimental values. Clearly, the calculation of  $\Delta_r S^\circ$  is more difficult than the calculation of  $\Delta_r H^\circ$  for a given reaction. Nevertheless, the value  $\Delta_r S^\circ = 3 \text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$  was obtained for reaction (14) by computation in which PCM was again used. In the absence of any experimental value for either  $\Delta_r G^\circ$  or for  $\Delta_r S^\circ$  for reaction (14), a comparison of a computationally derived and a Benson estimate for  $\Delta_r S^\circ$  was made for the reaction



The Benson estimate led to  $\Delta_r S^\circ = 9 \text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$  for the above reaction and the computationally derived value was  $\Delta_r S^\circ = 20 \text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$ . It is hoped that advances in computational chemistry will eventually make it possible to have values of  $\Delta_r H^\circ$ , and, eventually,  $\Delta_r G^\circ$  and  $\Delta_r S^\circ$  for biochemical reactions that are comparable in accuracy to experimentally derived values. Clearly, at the present time, accurately measured property values along with property values calculated by means of thermochemical pathways remain at the top of the hierarchy for the reliability of property values obtained by the various methods discussed herein.

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## ACKNOWLEDGMENTS

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## GLOSSARY OF SYMBOLS AND TERMINOLOGY

$A_m$	Debye-Hückel constant (1.1758 kg <sup>1/2</sup> mol <sup>-1/2</sup> at 298.15 K)	kg <sup>1/2</sup> ·mol <sup>-1/2</sup>
$B$	empirical constant in the extended Debye-Hückel equation	kg <sup>1/2</sup> ·mol <sup>-1/2</sup>
$C_p^\circ$	molar heat capacity	J·K <sup>-1</sup> ·mol <sup>-1</sup>
$\Delta_r C_p^\circ$	standard molar heat capacity change <sup>a</sup>	J·K <sup>-1</sup> ·mol <sup>-1</sup>
$\Delta_r G^\circ$	standard molar Gibbs energy change <sup>a</sup>	kJ·mol <sup>-1</sup>
$\Delta_r G'^\circ$	standard molar transformed Gibbs energy change	kJ·mol <sup>-1</sup>
$\Delta_r H^\circ$	molar enthalpy change for a reaction <sup>a</sup>	kJ·mol <sup>-1</sup>
$\Delta_r H'^\circ$	standard molar transformed enthalpy change	kJ·mol <sup>-1</sup>
$\Delta_r H(\text{cal})$	calorimetrically determined molar enthalpy change	kJ·mol <sup>-1</sup>
$I_c$	ionic strength, concentration basis	mol·dm <sup>-3</sup>
$I_m$	ionic strength, molality basis	mol·kg <sup>-1</sup>
$K$	equilibrium constant	dimensionless
$K'$	apparent equilibrium constant	dimensionless
$N$	number of entities	dimensionless
$\Delta_r N_X$	change in binding of species X	dimensionless
$P$	pressure	Pa
pH	$-\log_{10}[\text{H}^+]$	dimensionless
pK	$-\log_{10}K$	dimensionless
pMg	$-\log_{10}[\text{Mg}^{2+}]$	dimensionless
$R$	gas constant (8.314 472 J·K <sup>-1</sup> ·mol <sup>-1</sup> )	J·K <sup>-1</sup> ·mol <sup>-1</sup>
$T$	temperature	K
$z_i$	charge number of species i	dimensionless
$\gamma$	activity coefficient	dimensionless

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<sup>a</sup> The subscript “r” denotes a reaction. Subscripts “f” and “sol” are used, respectively, to denote formation and solution reactions.

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