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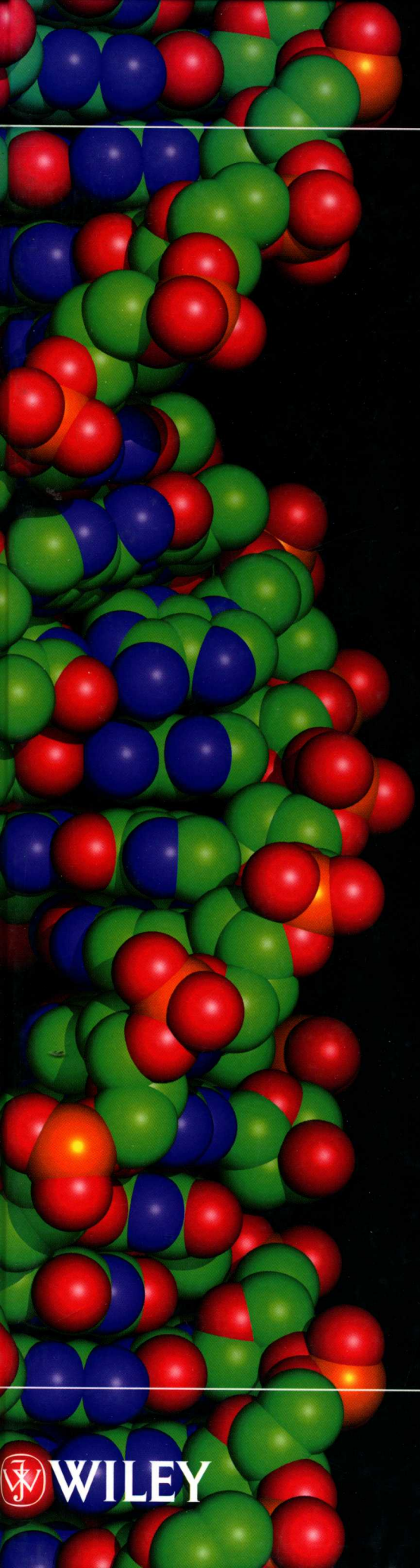

ENZYME KINETICS

Rapid-Equilibrium Applications of Mathematica®

VOLUME 53

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Enzyme Kinetics

Rapid-Equilibrium Applications of Mathematica

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METHODS OF BIOCHEMICAL ANALYSIS

Volume 53

Preface

Rapid-equilibrium enzyme kinetics has several advantages over steady-state enzyme kinetics. That is not to say that it is always applicable, but rapid-equilibrium enzyme kinetics yields simpler rate equations than steady-state enzyme kinetics. Since rapid-equilibrium rate equations are the simplest possible, they are the place to start. They are actually widely used. When rapid-equilibrium rate equations are applicable, Michaelis constants are equilibrium constants. For simple mechanisms, rapid-equilibrium rate equations are readily derived by hand, but, for more complicated mechanisms, they can be derived using a computer without having to write a computer program. This is especially useful when pH effects are involved, or there are many reactants, as for random A + B + C → products, or multiple forms of inhibition, activation, and modification. When the rapid-equilibrium assumption is applicable, all the reactions up to the rate-determining reaction are at equilibrium. The expression for the equilibrium concentration of the enzyme-substrate complex that yields products can be derived with a computer using Solve, which is an operation that solves a set of simultaneous polynomial equations. This operation is available in *Mathematica*^{R}, Maple^{R}, and MatLab^{R}. The input for Solve includes equilibrium expressions for an independent set of reactions and a conservation equation. The use of a computer has the additional advantage that the rapid-equilibrium rate equation is obtained in computer-readable form and does not have to be typed into the computer. Rapid-equilibrium rate equations can be derived with a computer for very complicated mechanisms.

Solve can also be used to calculate the values of the kinetic parameters in a rate equation from the minimum number of velocity measurements. This calculation can be made using steady-state rate equations as well as rapid-equilibrium rate equations. When rapid-equilibrium rate equations do not represent the experimental data, steady-state rate equations can be used, or empirical rate equations can be used.

There are two ways to organize a book on rapid-equilibrium kinetics. There could be chapters on deriving rate equations, estimating kinetic parameters, effects of pH, effects of inhibitors, etc. Or there could be chapters on various types of reactions with derivations of rate equations for different mechanisms, estimation of kinetic parameters, effects of pH, etc. This second option is followed here by treating different kinds of mechanisms separately so that a user can study various aspects of the kinetics of a certain type of enzyme-catalyzed reaction in a single chapter. The exceptions of this organizational plan are Chapter 1 on biochemical thermodynamics that is the foundation for the rapid-equilibrium assumption, the three chapters on inhibition, activation, and modification, and the last chapter on systems of enzyme-catalyzed reactions.

Biochemical thermodynamics is based on the Legendre transformed Gibbs energy G' that brings in the pH as an independent variable. This development has brought in new ideas about the use of thermodynamics in biochemistry. Enzyme kinetics necessarily contains biochemical thermodynamics because rate equations including the reverse reaction must yield the equilibrium composition at long times. When rate equations are derived on the assumption that there is a rate-determining reaction, the reactions before the rate-determining reaction are treated with biochemical thermodynamics. For example, the equilibrium composition of substrates, enzymatic sites, enzyme-substrate complexes, hydrogen ions, inhibitors, activators, and modifiers is calculated using an independent set of reactions. The relationship between biochemical thermodynamics and rapid-equilibrium enzyme kinetics is made evident by the Haldane relation that expresses the apparent equilibrium constant K' for the reaction that is catalyzed in terms of kinetic parameters in the rate equation. The existence of the Haldane relation means that the thermodynamic effects of temperature, pH, and ionic strength on equilibrium are all in the kinetic parameters. Of course biochemical thermodynamics is also built into transient kinetics, but that is not discussed in this book.

This book is concerned with six levels of understanding the kinetics of an enzyme-catalyzed reaction. (1) The first level is the understanding of the thermodynamics of the enzyme-catalyzed reaction. When an enzyme-catalyzed reaction occurs, the

composition changes until equilibrium is reached. The equilibrium concentrations of the substrates can be calculated using the apparent equilibrium constant, which is a function of temperature, pH and ionic strength. The apparent equilibrium constant may also be a function of $[Mg^{2+}]$, for example. This thermodynamic information does not tell us anything about the mechanism or rate constants because the apparent equilibrium constant depends entirely on the thermodynamic properties of the substrates. (2) The second level of understanding is to be sure that the velocity at specified temperature, pH, and ionic strength is directly proportional to the total enzyme concentration. This is true for all the mechanisms discussed here. (3) The third level of understanding involves the determination of the rate equation at a particular temperature, pH, and ionic strength. There are two types of rapid-equilibrium rate equations: rate equations for the forward reaction and complete rate equations that include the reverse rate equation as well. In this book, when the catalyzed reaction is represented by A + B → products, only the forward reaction is discussed, and when the catalyzed reaction is represented by A + B = P + Q, the rate equation includes the parameters for the reverse reaction, as well as the forward reaction. This book is concerned with rapid-equilibrium rate equations, but there is the possibility that a steady-state rate equation is required. Even an empirical rate equation may be required. A complete rate equation can be tested by using the Haldane relation to calculate the apparent equilibrium constant at the experimental temperature, pH, and ionic strength. (4) The fourth level of understanding involves the study of the effects of pH on the kinetics. Even when the substrates do not have p*K*s, velocities will in general depend on the pH. These effects are determined by p*K*s of the enzymatic site, the enzyme-substrate complexes, and the substrates. It is possibile that one or more hydrogen ions may be consumed in the mechanism. When a steady-state rate equation is required, these effects become much more complicated, but when the rapid-equilibium assumption is applicable, all of these effects can be represented by p*K*s and the number of hydrogen ions consumed in the mechanism. When these effects can be determined quantitatively for both the forward and reverse reactions, the Haldane relation must yield the correct dependence of the apparent equilbrium constant on the pH and ionic strength. It is important to distinguish between the number *n* of hydrogen ions consumed in the mechanism and the change in binding of hydrogen ions determined by thermodynamic measurements. The number *n* of hydrogen ions consumed in a reaction in a mechanism is an integer, but the change in binding of hydrogen ions in an enzyme-catalyzed reaction depends on the pH. (5) When the rapid-equilibrium assumption applies, Michaelis constants are equilibrium constants, and so the study of the effect of temperature can yield standard transformed Gibbs energies of reaction, standard transformed enthalpies of reaction, and standard transformed entropies of reactions in the mechanism. (6) The sixth level of understanding involves inhibition, activation, and modification by binding at the catalyic site or other sites. Modifiers can be inhibiting or activating, but they provide additional pathways to products. This book deals with all six levels of understanding.

Mathematica is a wonderful application for rapid-equilibrium enzyme kinetics because Solve can be used to derive rapid-equilibrium rate equations and also to estimate the kinetic parameters using the minimum number of velocity measurements. *Mathematica* has the advantage of including a word processor so that a book with text and live equations can be written. In *Mathematica* the symbols for variables and kinetic parameters have to start with lower case letters because operations like Solve, TableForm, and Round start with capital letters. The names of programs also have to start with lower case letters. Names of reactants and functions cannot involve spaces, hyphens, dots or other mathematical symbols.

Each chapter in this book is a *Mathematica* notebook that includes text as well as calculations. These chapters include descriptions of the background for calculations, the calculations, and discussion of their significance. The CD at the back of the book contains the entire book in *Mathematica*. This CD can be downloaded into a personal computer with *Mathematica* installed, but it can also be read in a computer with *MathReader*, which is freely available from Wolfram Research, Inc. (100 Trade Center Drive, Champaign, IL 61820-7237, and www.wolfram.com). All the calculations in a chapter can be run by use of Evaluate/Evaluate Notebook. Programs and calculations can be copied and pasted in a new notebook. Calculations can be rerun with different input. The input to programs can be changed to apply these programs to other reactions and conditions.

It is not necessary to be a programmer to use the programs in this book. More programs and calculations on rapid-equilibrium enzyme kinetics are available at *MathSource*. The references are at the end of the book, and this includes web URLs and notebooks in *MathSource*.

This book builds on my previous book "Biochemical Thermodynamics: Applications of *Mathematica*", Wiley, Hoboken, NJ (2006). That book provides a great deal of information on the thermodynamics of enzyme-catalyzed reactions that is relevant to enzyme kinetics. For example, it gives the apparent equilibrium constants for 229 enzyme-catalyzed reactions at 298.15 K, 0.25 M ionic strength, and pHs 5-9. More apparent equilibrium constants can be calculated for similar reactions.

This is the second book on enzyme kinetics written in *Mathematica*. The first is P. J. Mulquiney and P. W. Kuchel, Modelling Metabolism with *Mathematica*, CRC Press, Boka Raton, Florida (2003). Their book uses steady-state rate equations, and it gives a program in *Mathematica* to derive steady-state rate equations. This program is based on the method described by A. Cornish-Bowden (Biochem. J. 165, 55-59 (1977)). They give steady-state rate equations for about 30 enzyme-catalyzed reactions.

I am indebted to Professor William Martin McClain, Wayne State University, for catching some errors in my treatment of thermodynamic cycles, which have been corrected. He has written an excellent book "Symmetry Theory in Molecular Physics with *Mathematica*," Springer, 2009.

I am indebted to the National Institutes of Health for support of research on which this book is based (5-RO1-GM4834812). At Wiley I am indebted to my Editor Anita Lekhwani.

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Use of Mathematica

This book is written in *Mathematica* 7.0. Even if you are not familiar with *Mathematica* (Wolfram Research, Inc. 100 World Center Drive, Champaign, IL 61820-7237 and www.wolfram.com) you should be able to read this book. The concepts and calculations in biochemical kinetics are explained in words in the textual parts of the book. And the results of calculations are discussed in words. When *Mathematica* is used to make tables and figures, explanatory titles are given.

Since *Mathematica* is a high level language that uses commands like Solve and D (for differentiate), you can see what mathematical operations are involved in a program. Everything that is involved in making these calculations is shown. When a calculation is made, a semicolon is often put at the end of the input so that the result is not shown there. When calculations are performed in you computer, the semicolons can be omitted to see what the result is. Semicolons are used in the book to save space.

Each chapter is a *Mathematica* notebook. When a chapter has been downloaded, it can be run by using Evaluation/Evaluate Notebook. This will take a few seconds or a minute to evaluate the operations. Quit *Mathematica* and restart before opening another chapter. Calculations and programs can be copied and pasted into a notebook of your own. Then the input can be changed to solve your problem.

The page of Contents shows that this book contains 12 Chapters that are each a *Mathematica* notebook. These 12 notebooks are selfsufficient in the sense that they can each be run alone. As mentioned above, only one of these notebooks should be open at one time. Pages are numbered within each of the notebooks.

The rate equations in this book can be used to calculate velocities for different sets of kinetic parameters and substrate concentrations. This is a good way to learn about the behavoir of a mechanism or to test the kinetic parameters that have been determined.

A number of books have been written to introduce *Mathematica* to new users. A list of books is available at the Wolfram Store. Wolfram offers free online seminars for new users: http://www.wolfram.com/services/education/seminars/.

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1.1 Introduction

It is necessary to start a book on rapid-equilibrium enzyme kinetics with a chapter on biochemical thermodynamics for two reasons: (1) The complete rate equation for an enzyme-catalyzed reaction has to contain the biochemical thermodynamics of the reaction that is catalyzed. Setting the velocity in the complete rate equation equal to zero yields the Haldane relation for the apparent equilibrium constant K' in terms of the kinetic parameters at the specified temperature, pH and ionic strength. (2) The equilibrim concentration of the enzyme-substrate complex in the rate-determining reaction is calculated with a set of independent biochemical reactions. When an independent set of reactions is at equilibrium, other reactions that are the sums or differences of the reactions in the independent set are also at equilibrium.

In chemical thermodynamics, reactions are written in terms of species, and chemical reactions must balance the various atoms and electric charges. But in biochemical thermodynamics, reactions are written in terms of reactants (sums of species like ATP), and all atoms are balanced except for hydrogen atoms because the pH is held constant. Electric charges are also not balanced in a biochemical reaction. Conceptually, hydrogen ions are added or removed during the reaction so that the pH is held constant. In practice a buffer is used to hold the pH approximately constant.

This book is about rapid-equilibrium rate equations because they are simpler than steady-state rate equations. This is not to say that all enzyme-catalyzed reactions involve a rate-determining reaction, but simply to say that rapid-equilibrium rate equations are the place to start in the investigation of rate equations. When the kinetic data for an enzyme-catalyzed reaction cannot be represented by a rapid-equilibrium rate equation, steady-state rate equations or empirical rate equations have to be used.

The thermodynamics of enzyme-catalyzed reactions is based on chemical thermodynamics because it is the species of substrates, enzymatic sites, and enzyme-substrate complexes that react. Important new concepts are involved in biochemical thermodynamics because the pH is an independent variable like T and P; that is, the pH is chosen by the investigator. In chemical thermodynamics, on the other hand, the pH is calculated using the chemical equilibrium constants for an independent set of chemical reactions and the conservation of all atoms and electric charges.

To see how the specification of pH is handled in biochemical thermodynamics, it is necessary to consider the foundations of chemical thermodynamics. The most basic criterion for spontaneous change and equilibrium in chemical thermodynamics is provided by the entropy S because the second law specifies that the entropy of an isolated system can only increase and has its maximum value at equilibrium: thus $(\mathrm{d}S)_{U,V} \geq 0$, where U is the internal energy and V is the volume. This criterion is only applicable to isolated systems because this is the only way to hold the internal energy and volume constant. The internal energy U provides the criterion for spontaneous change and equilibrium at constant volume and entropy: $(\mathrm{d}U)_{V,S} \leq 0$. The internal energy decreases to a minimum when the system goes to equilibrium at constant V and S, but there is no way to hold the entropy constant. The enthalpy H is defined by the Legendre transform $H = U + PV$, and it provides the criterion for spontaneous change and equilibrium at specified P and S: $(\mathrm{d}H)_{P,S} \leq 0$. Again there is no way to hold the entropy constant. A Legendre transform is the defintion of a new thermodynamic property by subtracting the product of two conjugate variables from an existing thermodynamic property [12,17,19]. Gibbs defined what we now call the Gibbs energy G by use of the Legendre transform $G = H - TS$. The Gibbs energy is so useful in chemistry because it provides the criterion for spontaneous change at specified temperature and pressure: $(\mathrm{d}G)_{T,P} \leq 0$. Thus a chemical reaction at specified T and P can spontaneously go in the direction that decreases the Gibbs energy, and the Gibbs energy of a chemical reaction system is at its lowest value at equilibrium.

1.2 Chemical Thermodynamics

The fundamental equation for the Gibbs energy G of a chemical reaction system is given by [22]

$$dG = -SdT + VdP + \sum_{j=1}^{N} \mu_j \, d\, n_j \tag{1.2-1}$$

where μ_j is the chemical potential of species j, n_j is the amount of species j, and N is the number of different species. Equation 1.2-1 shows that T, P, and $\{n_j\}$ are the independent variables for the Gibbs energy of a chemical reaction system. For ideal solutions, the chemical potential of a species is given by

$$\mu_j = \mu_j^\circ + RT\ln[j] \tag{1.2-2}$$

where μ_j° is the standard chemical potential of species j and $[j]$ is the molar concentration of species j. When there is a single chemical reaction involving N species, the differential of the amount of species j is given by $dn_j = \nu_j \, d\xi$, where ν_j is the stoichiometric number for species j and ξ is the extent of the single reaction. In working with the fundamental equation, the chemical potential of a species is used, but, in working with experimental data, the symbol for the Gibbs energy of formation $\Delta_f G_j$ is used rather than μ_j. When there is a single chemical reaction in a system, equation 1.2-1 becomes

$$dG = -SdT + VdP + \sum_{j=1}^{N} \nu_j \, \Delta_f G_j \, d\xi \tag{1.2-3}$$

At constant temperature and pressure, the Gibbs energy of reaction is given by

$$\Delta_r G = dG/d\xi = \sum_{j=1}^{N} \nu_j \Delta_f G_j \tag{1.2-4}$$

Equation 1.2-2 for a chemical species can be written as

$$\Delta_f G_j = \Delta_f G_j^\circ + RT\ln[j] \tag{1.2-5}$$

where $\Delta_f G_j^\circ$ is the standard Gibbs energy of formation of species j. In chemical thermodynamics, this equation is used for ideal solutions and activity coefficients are introduced, but in biochemical thermodynamics this equation is used at the specified ionic

strength and the Debye-Huckel equation is used to account for the effects of ionic strength. Substituting equation 1.2-5 into equation 1.2-4 yields

$$\Delta_r G = \Delta_r G° + RT\ln Q \tag{1.2-6}$$

where $\Delta_r G°$ is the standard Gibbs energy of reaction $\sum \nu_j \Delta_f G_j °$, and Q is the reaction quotient. At equilibrium, $\Delta_r G = 0$ and Q becomes the chemical equilibrium constant K.

$$\Delta_r G° = - RT\ln K = \sum_{j=1}^{N} \nu_j \Delta_f G_j° \tag{1.2-7}$$

Chemists have developed tables of values of $\Delta_f G_j°$ for species with respect to the elements. In other words, the $\Delta_f G_j°$ for elements are taken to be zero in a defined reference state. These tables can be used to calculate a number of chemical equilibrium constants K that are of biochemical interest, but the Natonal Bureau of Standards Tables [10] are limited to C_2.

More information about chemical thermodynamics is given in text books on chemical thermodynamics like Beattie and Oppenheim [8], and in my two books on biochemical thermodynamics [20,23].

The first publication that applied chemical thermodynamics to biochemical reactions was by Burton and Krebs [2], and the first table of thermodynamic properties was published by Burton in Krebs and Kornberg, Energy Transformations in Living Matter [4]. Burton recognized that the equilibrium constants for enzyme-catalyzed reactions together with the standard Gibbs energies $\Delta_f G_j°$ of species determined with chemical methods can yield $\Delta_f G_j°$ biochemical species. He made a table that could be used to calculate equilibrium constants of biochemical reactions that have not been studied. But he ran into problems with reactants like ATP that are sums of species at pH 7. Wilhoit [5] extended these tables, but ATP remained a problem. I became involved with ATP through electrophoresis, and my group determined acid dissociation constants and magnesium complex dissociation constants of ATP [1,3]. I worked on the thermodynamics of petroleum processing in the 1980-1990 period and learned that when the concentration of a species (like H^+) is held constant, the criterion for equilibrium is provided by a transformed Gibbs energy [13,15].

There is one more aspect of chemical thermodynamics that needs to be mentioned. If the equilibrium constants of a set of chemical reactions are known, the equilibrium composition can be calculated, but an independent set of chemical reactions must be used in the calculation. A set of chemical reactions is independent if no reaction in the set can be obtained by adding and subtracting reactions in the set. Linear algebra is needed for a complete discussion, but it leads to a simple equation: $N = C + R$, where N is the number of different species, C is the number of components, and R is the number of independent reactions [20,23]. The number of components in chemical thermodynamics is the number of elements involved.

1.3 Transformed Thermodynamic Properties of Biochemical Reactants at a Specified pH

To obtain the criterion for spontaneous change when the pH is held constant in addition to the temperature and presssure, it is necessary to use another Legendre transform. Two examples of Legendre transforms are $H = U + PV$ and $G = H - TS$ that have been discussed in Section 1.1. The conjugate variables required to introduce the pH as an independent variable are the amount of hydrogen atoms in the system $n_c(H)$, which is an extensive property, and the chemical potential of hydrogen ions $\mu(H^+)$, which is the intensive property corrresponding to the specified pH. The amount of the hydrogen component $n_c(H)$ is expressed in moles, and $\mu(H^+)$ is expressed kJ mol^{-1}. The product of conjugate variables is always an energy, and so $n_c(H)\mu(H^+)$ has the units of kJ, as does G. The relation between the chemical potential of hydrogen ions and the pH is

$$\mu(H^+) = \Delta_f G°(H^+) - RT\ln(10)\,pH \tag{1.3-1}$$

where $\Delta_f G°(H^+)$ is the standard Gibbs energy of formation of hydrogen ions and $R = 8.31451$ J mol^{-1}. Thus the transformed Gibbs energy G' of a thermodynamic system at a specified pH is defined by the Legendre transform

$$G' = G - n_c(H)\,\mu(H^+) = G - n_c(H)\{\Delta_f G°(H^+) - RT\ln(10)\,pH\} \tag{1.3-2}$$

The transformed Gibbs energy is needed in biochemical thermodynamics because it provides the criterion for spontaneous change and equilibrium at specified temperature, pressure, and pH: $(dG')_{T,P,pH} \le 0$. Thus a biochemical reaction at specified T, P, and pH can react spontaneously in the direction that decreases G', and G' has its minimum value at equilibrium.

The fundamental equation for the transformed Gibbs energy G' for a biochemical reaction system when N' reactants are present is given by [13,20,23]

$$dG' = -S'dT + VdP + \sum_{i=1}^{N'} \Delta_f G_i'\, dn_i' + RT\ln(10) n_c(H)dpH \tag{1.3-3}$$

where $\Delta_f G_i'$ is the transformed Gibbs energy of formation of reactant i (sum of species), and n_i' is the amount of reactant i. A number of steps are involved in deriving this equation. Equation 1.3-3 shows that there is a new type of term in the fundamental equation for the transformed Gibbs energy that is proportional to dpH.

When the reactants in a biochemical reaction system are involved in a single biochemical reaction, $dn_i' = \nu_i' d\xi'$, where ν_i' is the stoichiometric number of reactant i in the biochemical reaction and $d\xi'$ is the differential of the extent of the biochemical reaction. The extent ξ' of a biochemical reaction is defined by $n_i' = (n_i')_0 + \nu_i'\xi'$, where $(n_i')_0$ the amount of reactant i when $\xi' = 0$. Replacing n_i' in equation 1.3-3 with this equation at constant T, P, and pH leads to the expression for the change in the transformed Gibbs energy in the biochemical reaction.

$$\Delta_r G' = \sum_{i=1}^{N'} \nu_i' \Delta_f G_i' \tag{1.3-4}$$

Thus equation 1.3-3 for a biochemical reaction system with a single biochemical reaction can be written as

$$dG' = -S'dT + VdP + \Delta_r G' d\xi' + RT\ln(10) n_c(H) dpH \tag{1.3-5}$$

Equation 1.3-5 can be used to derive the expression for the apparent equilibrium constant K' for a biochemical reaction at a specified pH. Substituting equation 1.3-4 in equation 1.3-5 yields

$$dG' = -S'dT + VdP + \sum_{i=1}^{N'} \nu_i' \Delta_f G_i' d\xi' + RT\ln(10) n_c(H) dpH \tag{1.3-6}$$

At specified T, P, and pH,

$$dG'/d\xi' = \sum_{i=1}^{N'} \nu_i' \Delta_f G_i' = \Delta_r G' \tag{1.3-7}$$

This is very much like equation 1.2-4 in chemical thermodynamics except that the i reactants are sums of species and ν_i' is the stoichiometric number of reactant i in the biochemical reaction at a specified pH. Substituting

$$\Delta_f G_i' = \Delta_f G_i'^{\circ} + RT\ln[i] \tag{1.3-8}$$

in equation 1.3-7 yields

$$\Delta_r G' = \sum_{i=1}^{N'} \nu_i' \Delta_f G'^{\circ} + RT\ln Q' = \Delta_r G'^{\circ} + RT\ln Q' \tag{1.3-9}$$

where $\Delta_r G'^{\circ}$ is the standard transformed Gibbs energy of reaction that is given by equation 1.3-8. Q' is the apparent reaction quotient. At equilibrium, $\Delta_r G' = 0$, and so

$$\Delta_r G'^{\circ} = -RT\ln K' \tag{1.3-10}$$

where K' is the apparent equilibrium constant for the biochemical reaction. The expression for K' is written in terms of reactant concentrations, except for H_2O.

Now we need to discuss the calculation of the standard transformed Gibbs energy of formation of a reactant $\Delta_f G_i'^\circ$ that was introduced in equation 1.3-8. The standard transformed Gibbs energies of formation $\Delta_f G_j'^\circ$ of the species that make up a reactant are given by

$$\Delta_f G_j'^\circ = \Delta_f G_j^\circ - N_H(j)\,\Delta_f G(H^+) \tag{1.3-11}$$

where $N_H(j)$ is the number of hydrogen atoms in species j. Since dilute solutions are assumed to be ideal, the Gibbs energy of species j is given by

$$\Delta_f G_j = \Delta_f G_j^\circ + RT\ln[j] \tag{1.3-12}$$

This equation can be applied to hydrogen ions at a specified pH.

$$\Delta_f G(H^+) = \Delta_f G^\circ(H^+) + RT\ln\left[10^{-pH}\right] = \Delta_f G^\circ(H^+) - RT\ln(10)\,pH \tag{1.3-13}$$

Substituting this equation into equation 1.3-11 yields

$$\Delta_f G_j'^\circ = \Delta_f G_j^\circ - N_H(j)\,(\Delta_f G^\circ(H^+) - RT\ln(10)pH) \tag{1.3-14}$$

When species have electric charges their standard thermodynamic properties need to be adjusted for the ionic strength I according to the extended Debye-Huckel theory.

$$\Delta_f G_j'^\circ(I) = \Delta_f G_j^\circ(I{=}0) - N_H(j)\,RT\ln(10)\,pH - RT\alpha\left(z_j^2 - N_H(j)\right) I^{1/2}\Big/\left(1 + 1.6\,I^{1/2}\right) \tag{1.3-15}$$

In the ionic strength term, z_j is the electric charge of species j, and $\alpha = 1.17582\ kg^{1/2}\ mol^{-1/2}$ at 298.15 K. This equation makes it possible to produce tables of standard transformed Gibbs energies of formation $\Delta_f G_j'^\circ$ of species at specified temperature, pH and ionic strength.

Now we can consider the relation between the standard transformed Gibbs energies of formation of the species and the standard transformed Gibbs energy of formation of the reactant at a specified temperature, pH, and ionic strength. Because of the entropy of mixing the species, the standard transformed Gibbs energy of formation is more negative than any of the species. The standard transformed Gibbs energy of formation of a reactant $\Delta_f G_i'^\circ$ is given in terms of the standard transformed Gibbs energies of formation $\Delta_f G_j'^\circ$ of the species (pseudoisomers) by [11]

$$\Delta_f G_i'^\circ = -RT\ln\sum_{j=1}^{N}\exp(-\Delta_f G_j'^\circ/RT) \tag{1.3-16}$$

where N is the number of different species in the pseudoisomer group. This summation is a partition function. The same result can be obtained by taking the mole-fraction-weighted average of the standard transformed Gibbs energies of formation of the species and adding a term for the transformed Gibbs energy of mixing. This equation made it possible to produce tables of standard transformed Gibbs energies of biochemical reactants at specified temperature, pH, and ionic strength. The equilibrium mole fractions r_j of the species in the reactant at a specified pH are given by

$$r_j = \exp[(\Delta_f G_i'^\circ - \Delta_f G_j'^\circ)/RT] \tag{1.3-17}$$

When the pH is specified, equation 1.2-7 is replaced with

$$\Delta_r G'^\circ = -RT\ln K' = \sum_{i=1}^{N'} \nu_i' \Delta_f G_i'^\circ \tag{1.3-18}$$

This makes it possible to produce tables of standard transformed Gibbs energies of reaction and tables of apparent equilibrium constants K' for biochemical reactions for which $\Delta_f G_i'^\circ$ (see equation 1.3-16) are known for all the reactants. It is important to notice that this does not require that K' has been measured for this reaction. More detailed derivations of these equations are given in references [20] and [23].

When the pH has been specified, the relation $N = C + R$ of chemical thermodynamics no longer applies; see the end of Section 1.2. It is replaced with $N' = C' + R'$, where N' is the number of reactants (sums of species), C' is the number of components, excluding hydrogen, and R' is the number of independent biochemical reactions [20,23]. The equilibrium compostion of a system of biochemical reactions can be calculated using an independent set of biochemical reactions. A set of reactions is independent if no reaction in the set can be obtained by adding or subtracting reactions in the set. This relation for determining the number R' of independent reactions will be used often in this book.

1.4 Calculation of the change in binding of hydrogen ions in a biochemical reaction at a specified pH by taking the derivative of logK' or $\Delta_r G'^\circ$ with respect to pH

The fourth term in equation 1.3-5 shows how the transformed Gibbs energy of a biochemical reaction system with one reaction changes with the pH, but there is no direct way to determine G' for a reaction system. However, the terms on the right hand side of equation 1.3-5 are related by Maxwell equations. The derivative of $\Delta_r G'$ with respect to pH is equal to the derivative of $RT\ln(10)n_c(\mathrm{H})$ with respect to the extent of reaction ξ'.

$$\partial \Delta_r G'/\partial \mathrm{pH} = \partial RT\ln(10)n_c(\mathrm{H})/\partial \xi' = RT\ln(10)\partial n_c(\mathrm{H})/\partial \xi' \tag{1.4-1}$$

Because of equation 1.3-9, the derivative $\partial \Delta_r G'/\partial \mathrm{pH}$ is the same as $\partial \Delta_r G'^\circ/\partial \mathrm{pH}$. Since $\Delta_r G'^\circ = -RT\ln K'$, equation 1.4-1 can be written as

$$\partial \ln K'/\partial \mathrm{pH} = -\ln(10)\partial n_c(\mathrm{H})/\partial \xi' \tag{1.4-2}$$

This shows that when the apparent equilibrium constant depends on the pH, the amount of hydrogen atoms in the system changes when the reaction occurs. $\partial n_c(\mathrm{H})/\partial \xi'$ is the increase in the binding of hydrogen ions, and so it is represented by $\Delta_r N_H$.

$$\Delta_r N_H = -\partial \log K'/\partial \mathrm{pH} \tag{1.4-3}$$