ELSEVIER

Biophysical Chemistry 124 (2006) 11 – 17

Biophysical Chemistry

http://www.elsevier.com/locate/biophyschem

Relations between biochemical thermodynamics and biochemical kinetics

Robert A. Alberty *

Department of Chemistry, Massachusetts Institute of Technology, Room 6-215, MIT, 77 Mass. Ave., Cambridge, MA 02139, USA

Received 15 May 2006; received in revised form 18 May 2006; accepted 18 May 2006
Available online 12 June 2006

Abstract

The parameters in steady-state or rapid-equilibrium rate equations for enzyme-catalyzed reactions depend on the temperature, pH, and ionic strength, and may depend on the concentrations of specific species in the buffer. When the complete rate equation (i.e. the equation with parameters for the reverse reaction as well as the forward reaction) is determined, there are one or more Haldane relations between some of the kinetic parameters and the apparent equilibrium constant for the reaction that is catalyzed. When the apparent equilibrium constant can be calculated from the kinetic parameters, the equilibrium composition can be calculated. This is remarkable because the kinetic parameters all depend on the properties of the enzymatic site, but the apparent equilibrium constant and the equilibrium composition do not. The effects of ionic strength and pH on the unoccupied enzymatic site and the occupied enzymatic site have to cancel in the Haldane relation or in the calculation of the apparent equilibrium constant using the rate constants for the steps in the mechanism. Several simple enzymatic mechanisms and their complete rate equations are discussed.

Keywords: Kinetics of enzyme-catalyzed reactions; Rate equations; Haldane relations; Apparent equilibrium constants; Rate constants; Kinetic parameters

1. Introduction

This article describes the close connection between the biochemical thermodynamics and biochemical kinetics of an enzyme-catalyzed reaction. The distinction between chemical thermodynamics and biochemical thermodynamics is the specification of the pH as an independent variable in biochemical thermodynamics. In chemical thermodynamics, the Gibbs energy G provides the criterion for spontaneous change and equilibrium when the temperature, pressure and amounts of species n_j are independent variables; in chemical thermodynamics these are usually referred to as the natural variables for G. Thus the amount of hydrogen ions $n(H^+)$ is a natural variable in chemical thermodynamics like T and P, but the conjugate thermodynamic property, the chemical potential of hydrogen ions $\mu(H^+)$, which is related to the pH, is not. To understand the significance of the specification of the pH as an independent variable in biochemical thermodynamics, it is necessary to go back to Gibbs in 1873. Before Gibbs famous paper in the Transactions of the Connecticut Academy, the internal energy U, the enthalpy H, and the entropy S were known. These properties of a homogeneous system are related by $H=U+PV$, which is referred to as a Legendre transform. In a spontaneous change in an isolated system, the entropy increases, but this is not a very practical criterion for spontaneity in a chemical reaction system. Gibbs recognized that to provide a criterion for spontaneous change and equilibrium at constant temperature and pressure, it was necessary to use another Legendre transform that we now write as $G=H-TS$. Note that to introduce the temperature as an independent variable he subtracted the product of two conjugate variables. One conjugate variable is T, which is an intensive variable, and the other conjugate variable is S, which is an extensive variable. The product of conjugate variables is always an energy. In a spontaneous change at specified T and P, the Gibbs energy G decreases, and at equilibrium it has its minimum value. This shows that to obtain a criterion for spontaneous change and equilibrium at specified T, P, and pH, it is necessary to use the Legendre transform $G'=G-n_c(H)\mu(H^+)$ to define a transformed Gibbs energy G'. The conjugate properties in this

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

0301-4622/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.bpc.2006.05.024

equation are the amount of the hydrogen component $n_c(H)$, that is the total amount of hydrogen atoms in the system, and the chemical potential of hydrogen ions μ, which is related to the pH. In a process at specified T, P, and pH, the transformed Gibbs energy G' decreases and at equilibrium it has its minimum value. There are similar Legendre transforms that define the transformed enthalpy H' and the transformed entropy S' of a system at specified T, P, and pH. The specification of pH as an independent variable in biochemical thermodynamics has another advantage, and that is that adenosine triphosphate can be treated as ATP, rather than the species ATP^{4-}, $HATP^{3-}$, and H_2ATP^{2-}, which is required in chemical thermodynamics. Sums of species like ATP are referred to as reactants in biochemical thermodynamics, rather than species. Properties of reactants like the standard transformed Gibbs energy of formation $\Delta_f G'^\circ$ are represented by mathematical functions of T, pH, and ionic strength, and properties of enzyme-catalyzed reactions like the apparent equilibrium constant K' are also represented by mathematical functions of T, pH, and ionic strength. Biochemical kinetics is closely related to biochemical thermodynamics, and so rate constants in enzyme-catalyzed reactions can also be represented by mathematical functions of T, pH and ionic strength. Recent books [1,2] provide more information on biochemical thermodynamics.

The term biochemical kinetics is used here to refer to complete steady-state or rapid-equilibrium rate equations or the direct determination of forward and reverse rate constants for steps in a mechanism. A complete steady-state or rapid-equilibrium rate equation includes both the forward and reverse reactions and every mechanism has at least one Haldane relation [3] that expresses the apparent equilibrium constant K' for the catalyzed reaction in terms of kinetic parameters. This means that, in a sense, biochemical kinetics includes biochemical thermodynamics; for example, the determination of the kinetic parameters in a complete rate equation makes it possible to calculate the equilibrium composition for the enzyme-catalyzed reaction at the temperature, pH, and ionic strength of the kinetics measurements. This is quite remarkable because the kinetic parameters all depend on the properties of the enzyme, but the apparent equilibrium constant for the catalyzed reaction and the equilibrium composition do not depend in any way on the properties of the enzyme.

Choosing the pH as an independent variable involves the assumption that during an enzyme-catalyzed reaction the pH is held constant by adding hydrogen ions or neutralizing hydrogen ions. Most enzyme-catalyzed reactions produce or consume hydrogen ions when they occur at a specified pH. Biochemists do not routinely use pHstats to hold the pH constant, but they interpret their experiments as if they had. In determining the apparent equilibrium constant for an enzyme-catalyzed reaction, a buffer is used to keep the pH near the desired value, and the pH is measured at equilibrium. This is the pH at which the reaction system is at equilibrium. Since it is understood that hydrogen ions are added or neutralized during the reaction, biochemical equations do not balance hydrogen ions or electric charge, but they must balance numbers of atoms of all other elements [4]. Hydrogen ions are not ignored in biochemical thermodynamics because the change in binding of hydrogen ions in a reaction $\Delta_r N_H$ can be obtained by taking the partial derivative of $\log K'$ with respect to pH [5]. $\Delta_r N_H$ can also be calculated using the pK values of the reactants [2]. It is this change in binding of hydrogen ions in an enzyme-catalyzed reaction that causes the apparent equilibrium constant to change with pH.

In chemical thermodynamics, activity coefficients γ are used so that chemical equilibrium constants K are independent of ionic strength, but in biochemical thermodynamics it is more convenient to take the apparent equilibrium constant and transformed thermodynamic properties of reactants like ATP to be functions of ionic strength in addition to temperature and pH. This is really required because biochemists have used various ionic strengths in their studies of thermodynamics and kinetics. Since biochemical reactions do not balance hydrogen atoms or electric charges, other ions should not appear in biochemical equations because they suggest that electric charges are to be balanced. For example, Fe^{3+} and Fe^{2+} in a biochemical reaction can be replaced with ferric and ferrous; this is also better for another reason, and that is that these ions are likely to be complexed with anions in the buffer. If H_2O is required to balance oxygen atoms, it should be included in the biochemical equation, but its concentration is not included in the expression for the apparent equilibrium constant; this is a convention for dilute aqueous solutions inherited from chemical thermodynamics.

Now we turn to the kinetics of enzyme-catalyzed reactions. Again the assumption is that the pH is held constant during the reaction, and so the reaction equation used in biochemical thermodynamics is used in biochemical kinetics. Rate equations are written in terms of concentrations of reactants like ATP, just as in biochemical thermodynamics. Biochemical equations for some enzyme-catalyzed reactions can be written in different ways. For example, the gases CO_2, NH_3, O_2, H_2, NO, etc., can be used to balance biochemical equations, but this is not very suitable for representing biochemical reactants in kinetic experiments or in living cells. When CO_2 is dissolved in a buffer, there is an equilibrium between four species (CO_2(aq), H_2CO_3, HCO_3^-, and CO_3^{2-}) that depends on the temperature, pH, and ionic strength. This equilibrium mixture is represented by CO_2tot in BasicBiochemData3 [6]. When NH_3 is dissolved in a buffer, there is equilibrium between NH_3 and NH_4^+, and that reactant can be referred to as ammonia.

A significant difference between biochemical thermodynamics and chemical kinetics is that there is no known way to adjust rate constants for reactions involving enzymatic sites for effects of ionic strength. In biochemical thermodynamics the extended Debye–Hückel equation is used to calculate activity coefficients of ions [1,2]. This involves the assumption of a spherically symmetrical ion atmosphere around an ion, and so it is not applicable to sites in or on an enzyme molecule.

Biochemical thermodynamics and biochemical kinetics are quite different fields, but they have to come together at equilibrium. The Haldane relations provide this connection. The way that works is described by starting with the simplest possible mechanism for the interconversion of S and P.

2. Consideration of the effect of ionic strength on a simple mechanism

For the mechanism

$$E + \underset{k_{-1}}{\overset{k_1}{S}} \quad \underset{k_{-2}}{\overset{k_2}{EX}} \quad E + P \tag{1}$$

the complete steady-state rate equation is

$$v = \frac{(V_f/K_S)[S] - (V_r/K_P)[P]}{1 + [S]/K_S + [P]/K_P} \tag{2}$$

where $V_f = k_2[E]_t$, $V_r = k_{-1}[E]_t$, $K_S = (k_{-1}+k_2)/k_1$, and $K_P = (k_{-1}+k_2)/k_{-2}$. Here S, P, E, and EX are treated as single species. The Haldane relation yields the following expression for the apparent equilibrium constant K' [7–9]

$$K' = \frac{[P]_{eq}}{[S]_{eq}} = \frac{V_f K_P}{V_r K_S} = \frac{k_1 k_2}{k_{-1} k_{-2}} \tag{3}$$

There are two reactions in this mechanism, and so the thermodynamic properties of these two reactions are considered first. In chemical thermodynamics it is often convenient to use equilibrium constants written in terms of activities. The equilibrium constants K_{Schem} and K_{Pchem} for the two reactions in Eq. (1) can be written in terms of activities so that they are not functions of ionic strength.

$$K_{Schem} = \frac{[E]_{eq}\gamma_E[S]_{eq}\gamma_S}{[EX]_{eq}\gamma_{EX}} \tag{4}$$

$$K_{Pchem} = \frac{[E]_{eq}\gamma_E[P]_{eq}\gamma_P}{[EX]_{eq}\gamma_{EX}} \tag{5}$$

The activity coefficients of the species are represented by γ_S, γ_P, γ_E, and γ_{EX}. The activity coefficients of S and P can be estimated using the extended Debye–Hückel equation in the ionic strength range zero to about 0.35 M. But this equation cannot be used for γ_E and γ_{EX}. The chemical equilibrium constant K for the reaction S=P is equal to the ratio of K_{Pchem} to K_{Schem}.

$$K(S = P) = \frac{K_{Pchem}}{K_{Schem}} = \frac{[P]_{eq}\gamma_P}{[S]_{eq}\gamma_S} \tag{6}$$

This equilibrium constant is independent of ionic strength.

However, in the thermodynamics of biochemical reactions, it is useful to consider equilibrium constants to be functions of temperature, pH, and ionic strength. The two biochemical equilibrium constants are calculated from the rate constants in Eq. (1) and are functions of the ionic strength:

$$K_{Sbioch} = K_{Schem}\frac{\gamma_{EX}}{\gamma_E\gamma_S} = \frac{[E]_{eq}[S]_{eq}}{[EX]_{eq}} = \frac{k_{-1}}{k_1} \tag{7}$$

$$K_{Pbioch} = K_{Pchem}\frac{\gamma_{EX}}{\gamma_E\gamma_P} = \frac{[E]_{eq}[P]_{eq}}{[EX]_{eq}} = \frac{k_2}{k_{-2}} \tag{8}$$

The apparent equilibrium constant K' for the biochemical reaction S=P at a specified ionic strength is given by the ratio of K_{Pbioch} to K_{Sbioch}.

$$K'(S = P) = \frac{K_{Pbioch}}{K_{Sbioch}} = \frac{K_{Pchem}\gamma_S}{K_{Schem}\gamma_P} = \frac{[P]_{eq}}{[S]_{eq}} = \frac{k_1 k_2}{k_{-1} k_{-2}} \tag{9}$$

This shows that the apparent equilibrium constant K' for the biochemical reaction S=P is equal to $k_1k_2/k_{-1}k_{-2}$. Note that in calculating K_{Pbioch}/K_{Sbioch} (see Eqs. (4) and (5)), γ_E and γ_{EX} have cancelled so that K' (S=P) depends only on two ratios: the first is K_{Pchem}/K_{Schem} that does not depend on ionic strength, and the second is γ_S/γ_P that can be calculated using the extended Debye–Hückel equation. The V_f, V_r, K_S, and K_P in the Haldane relation (Eq. (3)) each involve the properties of E and EX, for which activity coefficients cannot be calculated, but V_fK_P/V_rK_S varies with ionic strength as γ_S/γ_P that can be calculated using the extended Debye–Hückel equation.

Mechanism (1) has the disadvantage that when S, P, E, and EX are each made up of several species with different numbers of hydrogen atoms, the pH dependencies of V_f and V_r are the same. But studies of the effects of pH on a number of enzyme-catalyzed reactions have shown that in general V_f and V_r have different pH dependencies, and so the following mechanism has to be used:

$$E + \underset{k_{-1}}{\overset{k_1}{S}} \quad \underset{k_{-2}}{\overset{k_2}{ES}} \quad \underset{k_{-3}}{\overset{k_3}{EP}} \quad E + P \tag{10}$$

The complete steady-state rate equation for this mechanism is given by Eq. (2), and the Haldane equation in terms of kinetic parameters is the same as for mechanism (1). The expressions for the kinetic parameters in terms of rate constants in Eq. (10) are given a number of places in the literature [7–9], and substituting these expressions into the Haldane equation yields

$$K' = \frac{k_1 k_2 k_3}{k_{-1} k_{-2} k_{-3}} = K_1 K_2 K_3 = \frac{[P]_{eq}}{[S]_{eq}} \tag{11}$$

where the equilibrium constants for the three reactions in mechanism (10) are given by K_1=[ES]/[E][S], K_2=[EP]/[ES], and K_3=[E][P]/[EP]. The product of these three equilibrium constants is independent of the thermodynamic properties of the unoccupied enzymatic site or the occupied enzymatic sites.

3. Consideration of the effect of pH on a simple mechanism

Now consider that there are multiple forms of each reactant in mechanism (10) with different numbers of hydrogen atoms. In this section the complications of activity coefficients are avoided by specifying the ionic strength and using equilibrium constants like K_{Sbioch} that are functions of the ionic strength. As further simplifications, the rapid-equilibrium approximation is used and only the forward reaction is considered at first. The

mechanism for the forward reaction that brings in the pH is as follows:

$$\begin{array}{ccccc} E^- & & S^- & & HES^- \\ \| & & \| & & \| \\ HE & + & HS & = & H_2ES \end{array} \text{ -> products} \qquad (12)$$
$$\begin{array}{ccccc} \| & & \| & & \| \\ H_2E^+ & & H_2S^+ & & H_3ES^+ \end{array}$$

The equal signs represent equilibria that are assumed to be adjusted very rapidly. The electric charges are arbitrary, but indicate differences between various protonated forms. The smallest acid dissociation constant in each case is labeled K_1, and so $K_{1S}=[S^-][H^+]/[HS]$ and $K_{1H2S}=[HES^-][H^+]/[H_2ES]$ [1,2]. Acid dissociation constants are functions of temperature and ionic strength in dilute aqueous solutions. Additional species of substrate, enzymatic site, and enzyme–substrate complex can be included, but that can be done by the reader later when the form of the rate equation is clear. The chemical reaction $HS+HE=H_2ES$ is assumed to be fast enough that it remains in equilibrium; therefore,

$$K_{H_2ES} = [HS][HE]/[H_2ES] \qquad (13)$$

The initial rate of formation of products is given by $v=k_f[H_2ES]$. There are six equations for acid dissociation constants, the expression for the equilibrium constant K_{H2ES}, and the following conservation equations for enzyme and substrate that have to be obeyed:

$$[E]_t = [E^-] + [HE] + [H_2E^+] + [HES^-] + [H_2ES] + [H_3ES^+] \qquad (14)$$

$$[S] = [S^-] + [HS] + [H_2S^+] \qquad (15)$$

The concentrations of HES^-, H_2ES, and H_3ES^+ are assumed to be negligible compared to the concentrations of S^-, HS, and H_2S^+. Substituting the expressions for the acid dissociation constants in Eqs. (14) and (15) yields the following conservation equations.

$$[E]_t = [HE](1 + K_{1E}/[H^+] + [H^+]/K_{2E}) + [H_2ES](1 + K_{1ES}/[H^+] + [H^+]/K_{2ES}) \qquad (16)$$

$$[S] = [HS](1 + K_{1S}/[H^+] + [H^+]/K_{2S}) \qquad (17)$$

The [HE] in Eq. (16) can be eliminated by use of Eq. (13), and [HS] can be eliminated by use of Eq. (17). Thus the total concentration of enzyme is given by

$$[E]_t = [H_2ES]\{(K_{H2ES}/[S](1 + K_{IE})/[H^+] + [H^+]/K_{2E}) \times (1 + K_{1S}/[H^+] + [H^+]/K_{2S}) + (1 + K_{1ES}/[H^+] + [H^+]/K_{2ES})\} \qquad (18)$$

The initial rate of the enzyme-catalyzed reaction is given by

$$v = k_f[H_2ES] = \frac{k_f[E]_t/(1 + K_{1ES})/[H^+] + [H^+]/K_{2ES}}{1 + \frac{K_{H2ES}}{[S]}\frac{(1+K_{1E}/[H^+]+[H^+]+[H^+]/K_{2E})(1+K_{1S}/[H^+]+[H^+]/K_{2S})}{(1+K_{1ES}/[H^+]+[H^+]/K_{2ES})}} \qquad (19)$$

This equation can be written as

$$v = V_f/(1 + K_S/[S]) \qquad (20)$$

where

$$V_f = k_f[E]_t/(1 + K_{1ES}/[H^+] + [H^+]/K_{2ES}) \qquad (21)$$

$$K_S = \frac{K_{H2ES}(1 + K_{1E}/[H^+] + [H^+]/K_{2E})(1 + K_{1S}/[H^+] + [H^+]/K_{2S})}{1 + K_{1ES}/[H^+] + [H^+]/K_{2ES}} \qquad (22)$$

The limiting rate V_f yields a symmetrical curve when plotted versus pH, but K_S has a very complicated pH dependence [7–9].

4. Inclusion of the pH-dependent reverse reaction

Consider the following mechanism:

$$\begin{array}{ccccccc} S^- & & E^- & & HES^- & & HEP^- \quad E^- \quad P^- \\ \| & & \| & & \| & & \| \quad \| \quad \| \\ HS & + & HE & = & H_2ES & \underset{k_r}{\overset{k_f}{\rightleftharpoons}} & H_2EP = HE + HP \\ \| & & \| & & \| & & \| \quad \| \quad \| \\ H_2S^+ & & H_2E^+ & & H_3ES^+ & & H_3EP^+ \quad H_2E^+ \quad H_2P^+ \end{array} \qquad (23)$$

The steady-state rate equation for mechanism (10) has been derived by a number of investigators. It can be derived by the King–Altman method [10], and Roberts [9] has derived it using determinants. The steady-state rate equation for mechanism (10) is given by Eq. (2). When the values of k_2 and k_{-}sub $_2$ are very small (i.e., when the rapid-equilibrium assumption is used), the form of Eq. (2) is not changed.

The pH dependence of the initial velocity v when the reverse reaction is included is obtained by substituting Eqs. (21) and (22) and the following two equations for the reverse reaction in Eq. (10).

$$V_r = k_r[E]_0/(1 + K_{1EP}/[H^+] + [H^+]/K_{2EP}) \qquad (24)$$

$$K_P = \frac{K_{H2EP}(1 + K_{1E}/[H^+] + [H^+]/K_{2E})(1 + K_{1P}/[H^+] + [H^+]/K_{2P})}{(1 + K_{1EP}/[H^+] + [H^+]/K_{2EP})} \qquad (25)$$

Note that $K_{H2EP}=[HP][HE]/[H_2EP]$ is the equilibrium constant for a chemical reaction.

Setting $v=0$ in the expanded version of Eq. (2) yields the Haldane relation for the apparent equilibrium constant K' for the reaction S=P.

$$K' = \frac{[P]_{eq}}{[S]_{eq}} = \frac{V_fK_P}{V_rK_S} = \frac{k_fK_{H2EP}(1 + K_{1P}/[H^+] + [H^+]/K_{2P})}{k_rK_{H2ES}(1 + K_{1S}/[H^+] + [H^+]/K_{2S})} \qquad (26)$$

where $K_{ref}=k_fK_{H2EP}/k_rK_{H2ES}=[HP]_{eq}/[HS]_{eq}$ is the chemical equilibrium constant for the reference reaction HS=HP, which is a reaction between species. The dependencies of the four acid dissociation constants in Eq. (26) and K_{ref} on the ionic strength can be calculated using the extended Debye–Hückel equation.

Thus the ionic strength and pH effects of the enzyme cancel in using the Haldane relation.

5. Consideration of the effects of pH on the rapid-equilibrium rate equation for the reaction A + B = P + Q

The rate equation for this reaction is considered here only for a simple mechanism and only for the rapid-equilibrium case. The reactions and their equilibrium constants (except for the rate-determining step) are as follows:

$$E + A = EA \quad K_A = [E][A]/[EA] \tag{27}$$

$$EA + B = EAB \quad K_{IB} = [EA][B]/[EAB] \tag{28}$$

$$EAB \underset{k_{-r}}{\overset{k_f}{}} EPQ \tag{29}$$

$$EPQ = EQ + P \quad K_{IP} = [EQ][P]/[EPQ] \tag{30}$$

$$EQ = E + Q \quad K_Q = [E][Q]/[EQ] \tag{31}$$

It is assumed that the equilibria of the reactions represented by equal signs in Eqs. (27)–(31) are rapid.

First, consider the reaction rate in the forward direction when P and Q are not present. The total enzyme concentration is given by

$$\begin{aligned}[E]_t &= [E] + [EA] + [EAB] \\ &= K_A[EA]/[A] + [EA] + [EA][B]/K_{IB}\end{aligned}$$

$$= [EA]\{K_A/[A] + 1 + [B]/K_{IB}\} \tag{32}$$

The initial velocity in the forward direction is given by $k_f[EAB]$. Several steps are required to obtain the following rate equation from Eq. (32):

$$v_f = \frac{k_f[E]_t[A][B]/K_AK_{IB}}{1 + \frac{[A]}{K_A}\left(1 + \frac{[B]}{K_{IB}}\right)} \tag{33}$$

Second, consider the reaction rate in the reverse direction when A and B are not present. The total enzyme concentration is given by

$$\begin{aligned}[E]_t &= [E] + [EQ] + [EPQ] \\ &= K_Q[EQ]/[Q] + [EQ] + [EQ][P]/K_{IP}\end{aligned}$$

$$= [EQ]\{K_Q/[Q] + 1 + [P]/K_{IB}\} \tag{34}$$

The initial velocity in the reverse direction is given by $k_r[EPQ]$.

$$v_r = \frac{k_r[E]_t[P][Q]/K_{IP}K_Q}{1 + \frac{[Q]}{K_Q}\left(1 + \frac{[P]}{K_{IP}}\right)} \tag{35}$$

The complete rate equation is given by

$$v = \frac{k_f[E]_t[A][B]/K_AK_{IB} - k_r[E]_t[P][Q]/K_{IP}K_Q}{1 + \frac{[A]}{K_A}\left(1 + \frac{[B]}{K_{IB}}\right) + \frac{[Q]}{K_Q}\left(1 + \frac{[P]}{K_{IP}}\right)} \tag{36}$$

The Haldane relation is

$$K'(A + B = P + Q) = \frac{k_fK_{IP}K_Q}{k_rK_AK_{IB}} = \frac{[P]_{eq}[Q_{eq}]}{[A]_{eq}[B]_{eq}} \tag{37}$$

When the rate-determining step is EAB ⇄ EPQ, the pH-dependent rate equation for the forward reaction is derived from the following two-step mechanism:

$$\begin{array}{lll} E^- & A^- & HEA^- \\ \| & \| & \| \\ HE + & HA = & H_2EA \\ \| & \| & \| \\ H_2E^+ & H_2A^+ & H_3EA^+ \end{array} \tag{38}$$

$$\begin{array}{lll} HEA^- & B^- & H_2EAB^- \\ \| & \| & \| \\ H_2EA + & HB = & H_3EAB \\ \| & \| & \| \\ H_3EA^+ & H_2B^+ & H_4EAB^+ \end{array} \xrightarrow{k_f} \text{products} \tag{39}$$

The reverse reaction is accomplished by the following two steps:

$$\begin{array}{lll} E^- & Q^- & HEQ^- \\ \| & \| & \| \\ HE + & HQ = & H_2EQ \\ \| & \| & \| \\ H_2E^+ & H_2Q^+ & H_3EQ^+ \end{array} \tag{40}$$

$$\begin{array}{lll} HEQ^- & P^- & H_2EPQ^- \\ \| & \| & \| \\ H_2EQ + & HP = & H_3EPQ \\ \| & \| & \| \\ H_3EQ^+ & H_2P^+ & H_4EPQ^+ \end{array} \xrightarrow{k_r} \text{products} \tag{41}$$

pH dependencies for the equilibrium constants and limiting velocities defined in Eqs. (27)–(31) are as follows:

$$K_A = \frac{K_{H2EA}(1 + K_{1E}/[H^+] + [H^+]/K_{2E})(1 + K_{1A}/[H^+] + [H^+]/K_{2A})}{(1 + K_{1EA}/[H^+] + [H^+]/K_{2EA})} \tag{42}$$

$$K_{IB} = \frac{K_{H3EAB}(1 + K_{1EA}/[H^+] + [H^+]/K_{2EA})(1 + K_{1B}/[H^+] + [H^+]/K_{2B})}{(1 + K_{1EAB}/[H^+] + [H^+]/K_{2EAB})} \tag{43}$$

$$V_f = k_f[E]_t/(1 + K_{1EAB}/[H^+] + [H^+]/K_{2EAB}) \tag{44}$$

$$K_Q = \frac{K_{H2EQ}(1 + K_{1E}/[H^+]/K_{2E})(1 + K_{1Q}/[H^+] + [H^+]/K_{2Q})}{(1 + K_{1EQ}/[H^+] + [H^+]/K_{2EQ})} \tag{45}$$

$$K_{IP} = \frac{K_{H3EPQ}(1 + K_{1EP}/[H^+] + [H^+]/K_{2EP})(1 + K_{1P}/[H^+] + [H^+]/K_{2P})}{(1 + K_{1EPQ}/[H^+] + [H^+]/K_{2EPQ})} \tag{46}$$

$$V_r = k_r[E]_t/(1 + K_{1EPQ}/[H^+] + [H^+]/K_{2EPQ}) \tag{47}$$

These six functions can be substituted into Eq. (36), but that yields a very large function for the rate equation. However, that is not necessary, and it is more interesting to substitute Eqs. (42)–(47)

into the Haldane relation (Eq. (37)).

$$K' = \frac{k_f K_{H3EPQ} K_{H2EQ}(1 + K_{1P}/[H^+] + [H^+]/K_{2P})(1 + K_{1Q}/[H^+] + [H^+]/K_{2Q})}{k_r K_{H3EAB} K_{H2EA}(1 + K_{1A}/[H^+] + [H^+]/K_{2A})(1 + K_{1B}/[H^+] + [H^+]/K_{2B})}$$

$$= K_{ref} \frac{(1 + K_{1P}/[H^+] + [H^+]/K_{2P})(1 + K_{1Q}/[H^+] + [H^+]/K_{2Q})}{(+K_{1A}/[H^+] + [H^+]/K_{2A})(1 + K_{1B}/[H^+] + [H^+]/K_{2B})} \quad (48)$$

where K_{ref} is the equilibrium constant for the chemical reference reaction HA+HB=HP+HQ, which is independent of pH. As in the cases of simpler mechanisms this shows that in the use of Haldane relations, the pH effects introduced by the unoccupied enzymatic site and the occupied sites cancel so that the correct expression is obtained for the catalyzed reaction.

Segel [7] has given a rapid equilibrium rate equation for v_f for the reaction A+B=P based on the assumption that if the binding of one substrate changes the dissociation constant of the other substrate by the factor α, the reverse is true. This makes it possible to derive a rate equation with the same general form as Eq. (33), but it raises the question as to how α varies with pH and whether α has the same value for the reverse reaction.

6. Expression of the apparent equilibrium constant for A + B = P + Q in terms of the rate constants of the steps in the forward and reverse direction

Penner and Frieden [11] determined individual rate constants in the mechanism of the dihydrofolate reductase reaction (EC 1.5.1.3) that involves the random addition of substrates. The determination of individual rate constants utilized several experimental methods and the use of KINSIM, a kinetics simulation program [12]. They obtained forward and reverse rate constants for thirteen steps in the mechanism for the following reaction:

$$7,8 - \text{dihydrofolate} + NADP_{red} = 5,6,7,8 - \text{tetrahydrofolate} + NADP_{ox} \quad (49)$$

The apparent equilibrium constant for this reaction can be calculated by two five-step paths. For each step, an equilibrium constant K can be calculated by taking the ratio of the rate constant in the forward direction to the rate constant in the reverse direction. The apparent equilibrium constant for reaction (49) can be calculated for the two paths using

$$K' = K_1 K_3 K_5 K_6 K_8 \quad (50)$$

$$K' = K_2 K_4 K_5 K_7 K_9 \quad (51)$$

where the numbering system is as published. This is the form of the Haldane relation when rate constants of individual steps are determined. For 20 °C, pH 7.2, and 0.05 M ionic strength, Eqs. (50) and (51) yield $K'=4400$ and $K'=4700$. This is as good agreement as can be expected because some of the rate constants are known to a single significant figure.

These rate constants and equilibrium constants in the mechanism for reaction (49) all depend on the properties of the unoccupied enzymatic site and the various occupied enzymatic sites. It is therefore of interest to see how the ionic strength effects on the five rate constants in the forward direction and the five rate constants in the reverse direction cancel so that K' depends only on the ionic strength effects arising from the four reactants in reaction (49).

Rather than writing out an expression for the ionic strength effects for Eqs. (50) and (51), consider only the expressions for K_1 and K_8. Eq. (7) shows that

$$K_1 = K_{1chem} \frac{\gamma_{EH2folate}}{\gamma_E \gamma_{H2folate}} \quad (52)$$

$$K_8 = K_{8chem} \frac{\gamma_E \gamma_{H4folate}}{\gamma_{EH4folate}} \quad (53)$$

where K_{1chem} and K_{8chem} are chemical equilibrium constants that are independent of ionic strength. The product of K_1 and K_8 is given by the following equation:

$$K_1 K_8 = K_{1chem} K_{8chem} \frac{\gamma_{EH2folate} \gamma_{H4folate}}{\gamma_{H2folate} \gamma_{EH4folate}} \quad (54)$$

Notice that γ_E has cancelled, but $\gamma_{EH2folate}$ and $\gamma_{EH4folate}$ remain to be cancelled in carrying out the multiplications in Eqs. (50) and (51). Therefore, the apparent equilibrium constants in Eqs. (50) and (51) depend only on the properties of the reactants in Eq. (49).

7. Discussion

The significance of the fact that K' for an enzyme-catalyzed reaction can be calculated using parameters from the complete rate equation or rate constants for the steps in the mechanism can be emphasized in three ways. First, knowledge of K' makes it possible to calculate the equilibrium composition of reactants for given initial concentrations of the reactants. It also makes it possible to calculate whether an enzyme-catalyzed reaction will go to the right or the left for specified concentrations of the reactants. Second, values of K' can be used to calculate $\Delta_r G'^\circ$ using $-RT\ln K'$. This is very important because partial derivatives of $\Delta_r G'^\circ$ can be used to calculate $\Delta_r H'^\circ$, $\Delta_r S'^\circ$, and $\Delta_r N_H$. Third, values of K' or $\Delta_r G'^\circ$ can be used to calculate values of $\Delta_f G^\circ$ of species of a new reactant if the $\Delta_f G^\circ$ are known for species of all the other reactants. Goldberg has pointed out that in the Goldberg–Tewari series of reviews [13] that evaluate the thermodynamic information on enzyme-catalyzed reactions, fifty-four entries are for K' calculated from kinetic data. More work is involved in obtaining a complete steady-state or rapid equilibrium rate equation or determining forward and reverse rate constants for each step than to measure equilibrium concentrations, but fifty-four is an impressive number of entries in the table of evaluated thermodynamic data.

The kinetic parameters for an enzyme-catalyzed reaction depend on the electric charges and the pK values of the unoccupied enzymatic site and the occupied enzymatic sites, but the apparent equilibrium constant for the catalyzed reaction is independent of these properties. The way this works out has

been demonstrated here for rapid equilibrium treatments of mechanisms for the catalysis of S=P and A+B=P+Q. In using the King–Altman method [10] steps like ES=EP and EAB=EPQ are usually omitted because they do not introduce new terms in to the steady-state rate equation [14]. In the present discussion the steps ES=EP and EAB=EPQ have been included so that the limiting velocities of the forward and reverse reactions can have different pH dependencies.

Equilibrium constants for chemical reactions in aqueous solutions are expressed in terms of activities of species, as shown in Eqs. (3) and (4), so that the equilibrium constants K_{chem} are independent of ionic strength, but apparent equilibrium constants K' in biochemistry are expressed in terms of concentrations of reactants (sums of species) and are functions of pH and ionic strength, as well as temperature. Thus biochemical thermodynamics is compatible with biochemical kinetics because concentrations of reactants (sums of species) are used and parameters in rate equations are considered to be functions of pH and ionic strength, as well as temperature. Both thermodynamics and kinetics of enzyme-catalyzed reactions can be discussed in terms of chemical thermodynamics and chemical kinetics (that is in terms of species); in fact, the most efficient way to store information on the thermodynamics of enzyme-catalyzed reactions is to store standard Gibbs energies of formation and standard enthalpies of formation of species [6]. This article has followed the prevailing nomenclature of biochemical kinetics by representing equilibrium constants for steps in the mechanism at specified pH with K, but it would be better to use K' when the pH is specified. Similarly, it would be better to indicate rate constants at specified pH by k' so that they will not be confused with rate constants k in the underlying chemical reactions. These changes would make it clearer that when the pH is specified, concentrations of reactants (sums of species) are used in biochemical kinetics rather than concentrations of species and $[H^+]$ is omitted in both rate equations and expressions for apparent equilibrium constants.

Since kinetic parameters involve properties of the unoccupied enzymatic site and the occupied enzymatic sites, there is a problem in determining the pH dependencies of kinetic parameters that I did not recognize when I was doing it [15,16]. That is, the enzyme may bind species in the buffer that affect the kinetic properties of the enzymatic site. For example, when the pH of a phosphate buffer is changed, the ratio $[H_2PO_4^-]/[HPO_4^{2-}]$ changes, and the binding of these ions by the enzyme at or near the enzymatic site may change in addition to the pH. This is usually not a problem in studying the thermodynamics of enzyme-catalyzed reactions because presumably $H_2PO_4^-$ and HPO_4^{2-} are not bound by substrates. However, in biochemical thermodynamics the binding of buffer ions by substrates may affect the thermodynamics of a reaction like $2\text{ferrous}+\text{NAD}_{ox}=2\text{ferric}+\text{NAD}_{red}$. One way to reduce buffer effects in both kinetics and thermodynamics, is to use buffers for which the ionic strength is primarily supplied by NaCl or KCl.

When magnesium ions or other divalent cations are present, their effects on the thermodynamics of enzyme-catalyzed reactions can be handled in the same way as effects of hydrogen ions; that is by using a Legendre transform to introduce pMg as an independent variable of the transformed Gibbs energy in addition to pH. Unfortunately, pK_{Mg} values are not known for many magnesium complex ions. Standard transformed thermodynamic properties of $ATP+H_2O=ADP+P_i$ and $ATP+2H_2O=AMP+2P_i$ have been calculated as functions of temperature, pH, pMg, and ionic strength [2].

Strictly speaking, the conclusions reached here apply to the mechanisms and conditions specified, but I think the conclusion that the ionic strength and pH effects contributed by the unoccupied and occupied enzymatic sites cancel in the Haldane relation is a general one.

Acknowledgements

I am indebted to Carl Frieden (Washington University) and Robert N. Goldberg (NIST) for helpful discussions of these issues. Grateful thanks to NIH for support of this research by award number 5-RO1-GM48348-10.

References

[1] R.A. Alberty, Thermodynamics of Biochemical Reactions, Wiley, Hoboken, NJ, 2003.
[2] R.A. Alberty, Biochemical Thermodynamics: Applications of Mathematica, Wiley, Hoboken, NJ, 2006.
[3] J.B.S. Haldane, Enzymes, Longmans Green, London, 1930.
[4] R.A. Alberty, A. Cornish-Bowden, Q.H. Gibson, R.N. Goldberg, G.G. Hammes, W. Jencks, K.F. Tipton, R. Veech, H.V. Westerhoff, E.C. Webb, Recommendations for nomenclature and tables in biochemical thermodynamics, Pure Appl. Chem. 66 (1994) 1641–1666 (Reprinted in Europ. J. Biochem. 240 (1996), pp. 1–14. http://www.chem.qmw.ac.uk/iubmb/thermod/).
[5] R.A. Alberty, Degrees of freedom in biochemical reaction systems at specified pH and pMg, J. Phys. Chem. 96 (1992) 9614–9621.
[6] R.A. Alberty, BasicBiochemData3.nb http://library.wolfram.com/infocenter/MathSource/5704.
[7] I.H. Segel, Enzyme Kinetics, Wiley, Hoboken, NJ, 1975.
[8] A. Cornish-Bowden, Fundamentals of Enzyme Kinetics, 3rd ed.Portland Press, London, 2004.
[9] D.V. Roberts, Enzyme Kinetics, Cambridge University Press, 1977.
[10] E.L. King, C. Altman, A systematic method of deriving rate laws for enzyme-catalyzed reactions, J. Phys. Chem. 60 (1956) 1375–1378.
[11] M.H. Penner, C. Frieden, Kinetic analysis of the mechanism of *Escherichia coli* dihydrofolate reductase, J. Biol. Chem. 262 (1987) 15908–15914.
[12] B.A. Barshop, R.F. Wrenn, C. Frieden, Analysis of numerical methods for computer simulation of kinetic processes: development of KINSIM – a flexible, portable system, Anal. Biochem. 130 (1983) 134–145.
[13] R.N. Goldberg, Thermodynamics of enzyme-catalyzed reactions: Part 6. 1999 update, J. Phys. Chem. Ref. Data 28 (1999) 931–965.
[14] W.W. Cleland, The kinetics of enzyme-catalyzed reactions with two or more substrates or products: I. Nomenclature and rate equations, Biochim. Biophys. Acta 67 (1963) 104–137.
[15] R.A. Alberty, V. Massey, On the interpretation of the pH variation of the maximum initial velocity of an enzyme-catalyzed reaction, Biochim. Biophys. Acta 13 (1954) 347–353.
[16] V. Massey, R.A. Alberty, Ionization constants of fumarase, Biochim. Biophys. Acta 13 (1954) 354–359.