Effects of pH in Rapid-Equilibrium Enzyme Kinetics

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The effects of pH on the rates of enzyme-catalyzed reactions are very important because they yield information on the p*K*s of acidic groups in the enzymatic site and the various enzyme−substrate complexes. But many enzyme-catalyzed reactions produce or consume hydrogen ions in a way that cannot be explained with p*K*s. These pH effects extend over the whole pH range of interest. In investigating these effects, the rapid-equilibrium assumption is especially useful because a large number of chemical reactions have to be taken into account. In these calculations, all of the reactions up to the rate-determining reaction are treated with biochemical thermodynamics. Kinetic studies make it possible to determine the number of hydrogen ions consumed in the rate-determining reaction, a number that can be in the range of 0−8. It is shown that the experimental limiting velocity of the forward reaction V_{fexp} is equal to $10^{n\mathrm{pH}}V_{\mathrm{f}}$, where n is a negative integer and V_{f} varies with pH in the way determined by the p*K*s of the enzyme−substrate complex that reacts in the rate-determining reaction. A computer program for the initial reaction velocity makes it possible to investigate the rapid-equilibrium kinetics of enzymatic mechanisms that involve the consumption of hydrogen ions.

Introduction

The effects of pH on the rates of enzyme-catalyzed reactions are very important because they yield information on the p*K*s of acidic groups in the enzymatic site and the various enzyme−substrate complexes. Efforts to obtain these p*K*s are complicated by the p*K*s of the substrates and the fact that many enzyme-catalyzed reactions produce or consume hydrogen ions in a way that cannot be explained[1,2] with p*K*s. The consumption of hydrogen ions causes pH effects that extend over the whole pH range of interest, while p*K*s have their major effects over about two units of pH. Knowledge of the p*K*s of the reactants and the number of hydrogen atoms they contain at high pH make it possible to calculate the change in binding of hydrogen ions $\Delta_{\mathrm{r}}N_{\mathrm{H}}$ in the catalyzed reaction,[3,4] and this property can also be calculated[5] by taking the negative derivative of the logarithm of the apparent equilibrium constant with respect to pH. The change in the binding of hydrogen ions in an enzyme-catalyzed reaction is not due to the p*K*s of the substrates alone because, even reactions where the substrates do not have p*K*s, enzyme-catalyzed reactions may consume as much as 8 mol of hydrogen ions per mole of reaction.[3,4] The effects of pH on the initial velocity of an enzyme-catalyzed reaction are a consequence of the p*K*s of the substrates, enzymatic site, enzyme−substrate complexes, and the consumption of hydrogen ions. In investigating these effects in enzyme kinetics, the rapid-equilibrium method is especially useful because a large number of chemical reactions have to be taken into account. In this method, all of the reactions up to the rate-determining reaction are treated with biochemical thermodynamics. When the rapid-equilibrium assumption is used, the Michaelis constants are equilibrium constants.

When the kinetic parameters of both the forward and the reverse reactions can be determined as functions of pH, the Haldane equation makes it possible to calculate the apparent equilibrium constant K' as a function of pH. Thus, in principle, the determination of initial velocities of the forward and reverse reactions makes it possible to calculate $\Delta_{\mathrm{r}}N_{\mathrm{H}}$ as a function of pH. But kinetic studies can do more than this because they make it possible to determine the number of hydrogen ions consumed in the rate-determining reaction. In summary, measurements of apparent equilibrium constants as functions of pH provide information about the change of binding of hydrogen ions in the complete reaction, but measurements of initial reaction velocities as functions of pH provide information on the consumption of hydrogen ions in the rate-determing reaction.

The following five related types of equations are involved in the discussion of the effects of pH in biochemical thermodynamics and enzyme kinetics:

$$K' = K_{\mathrm{ref}}10^{n\mathrm{pH}}f(\mathrm{pH}) \tag{1}$$

$$\Delta_{\mathrm{r}}N_{\mathrm{H}} = -\mathrm{d}\log K'/\mathrm{dpH} = \sum \nu_i' \bar{N}_{\mathrm{H}}(i) \tag{2}$$

$$K' = \frac{V_{\mathrm{fexp}}K_{\mathrm{P}}K_{\mathrm{IQ}}}{V_{\mathrm{r}}K_{\mathrm{IA}}K_{\mathrm{B}}} \tag{3}$$

$$V_{\mathrm{fexp}} = 10^{n\mathrm{pH}}V_{\mathrm{f}} \tag{4}$$

$$V_{\mathrm{f}} = \frac{k_{\mathrm{f}}[\mathrm{E}]_{\mathrm{t}}}{1 + 10^{\mathrm{p}K_{2\mathrm{EAB}}-\mathrm{pH}} + 10^{\mathrm{pH}-\mathrm{p}K_{1\mathrm{EAB}}}} \tag{5}$$

The first equation has been used in discussing biochemical thermodynamics for some time[7−10]. K' is the apparent equilibrium constant for an enzyme-catalyzed reaction at a specified pH. The concentrations of reactants in the expression for the apparent equilibrium constant are concentrations of sums of species. K_{ref} is the equilibrium constant for a chemical reference reaction (that is, a reaction written in terms of species); n is the number of hydrogen ions in the reference reaction, and f(pH) brings in the p*K*s of the substrates. This equation shows that there are potentially two different types of effects of pH on the

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apparent equilibrium constant of an enzyme-catalyzed reaction; there are effects from the p*K*s of substrates, but the effect of 10^{npH} extends over the whole pH range of interest. In biochemical thermodynamics, this equation can take on many different forms because there are many different choices for chemical reference reactions, including reference reactions with $n = 0$. This is expected because the thermodynamics of biochemical reactions is independent of the enzymatic mechanism. Since the p*K*s of substrates can be determined by acid titrations, the effects of the p*K*s of substrates can be taken out of the expressions for the Michaelis constants. Thermodynamic measurements do not yield *n* in a mechanistic sense.

The second equation shows that the change in binding of hydrogen ions[5] in a biochemical reaction $\Delta_r N_H$ is given by the negative derivative of log*K*′ with respect to pH. This is not the only way to calculate $\Delta_r N_H$. When the p*K*s of the substrates and the number of hydrogen atoms they contain at high pH are known, the average number of hydrogen atoms in each substrate, $\bar{N}_H(i)$, can be calculated[3] as a function of pH. The change in binding of hydrogen ions can then be calculated using the second expression for $\Delta_r N_H$, where ν_i' is the stoichiometric number of substrate *i*. The prime on ν_i' is needed to differentiate it from the stoichiometric numbers of the species in the underlying chemical reactions. A new method for calculating $\Delta_r N_H$ is described in this paper.

The third equation is the Haldane equation for the A + B = P + Q reaction when hydrogen ions are consumed in the rate-determining reaction in the forward direction. This reaction is used as an example. All of the rate equations in this article are based on the assumption that reactions up to the rate-determining reaction are in equilibrium. The four Michaelis constants are equilibrium constants. There are corresponding Haldane equations for other reactions. V_{fexp} is the experimental limiting velocity in the forward direction. It can be obtained from the rapid-equilibrium velocity v in the forward direction at specified pH by use of Lineweaver−Burk plots.

The fourth equation shows that in general V_{fexp} is equal to the product of two factors[1] that depend on the pH. The first factor, 10^{npH}, is introduced by the consumption of *n* hydrogen ions in the rate-determining reaction. The second factor, V_f, brings in the p*K*s of the enzyme−substrate complex that yields products in the rate-determining reaction. The second factor also brings in the rate constant k_f for the rate-determining reaction and the total concentration of enzymatic sites. The first factor in V_{fexp} can be taken to be $10^{n(pH-x)}$, where *x* can be assigned an arbitrary value in calculating reaction rates, just like k_f can be assigned an arbitrary value in showing how kinetic properties change with pH.

The fifth equation gives the expression for the limiting velocity V_f in the forward

direction when $n = 0$ in the mechanism or is calculated by use of $V_f = 10^{-npH}V_{fexp}$. It is assumed here that the enzyme−substrate complex that reacts in the rate-determining reaction has two p*K*s and the intermediate ionized form reacts. The forward rate constant is k_f, and $[E]_t$ is the total concentration of enzymatic sites. Note that $pK_1 > pK_2$.

Kinetics can be used to determine *n* for a specific mechanism but thermodynamics cannot. Thermodynamics deals with the equilibria of complete reactions. Enzyme kinetics deals with half reactions; that is, the initial velocity of the forward reaction and the initial velocity of the reverse reaction. This makes it possible to determine the consumption of hydrogen ions in the rate-determining reaction. There are other ways kinetic parameters can be obtained, but this is the simplest to visualize. If

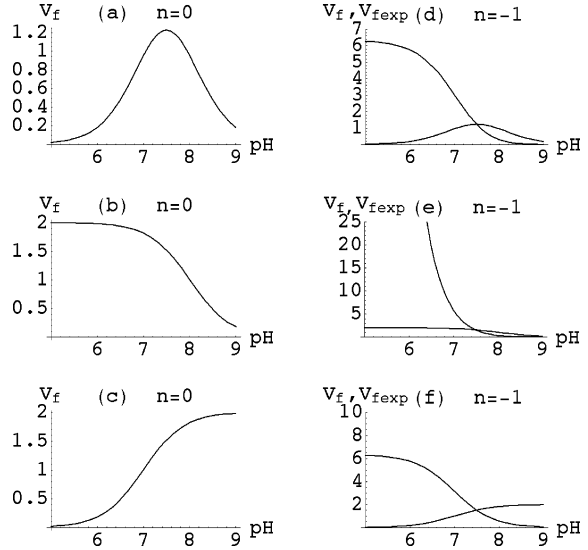

Figure 1. Plots of V_f and $V_{fexp} = 10^{n(pH-7.5)}V_f$ for three types of V_f plots with $n = 0$, and $n = -1$: (a) $pK_{1EAB} = 8$ and $pK_{2EAB} = 7$, (b) $pK_{1EAB} = 8$, (c) $pK_{2EAB} = 7$. Composite plots of V_f and V_{fexp} when $n = -1$ and (d) $pK_{1EAB} = 8$ and $pK_{2EAB} = 7$, (e) $pK_{1EAB} = 8$, (f) $pK_{2EAB} = 7$.

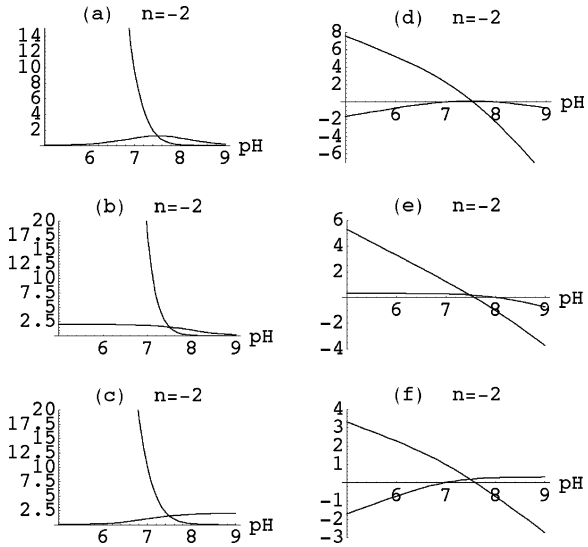

Figure 2. Left column: Composite plots of V_f and V_{fexp} when $n = -2$ and (a) $pK_{1EAB} = 8$ and $pK_{2EAB} = 7$, (b) $pK_{1EAB} = 8$, (c) $pK_{2EAB} = 7$ and $n = -2$ in V_{fexp}. Right column: Logarithmic plots when (d) $pK_{1EAB} = 8$ and $pK_{2EAB} = 7$, (e) $pK_{1EAB} = 8$, (f) $pK_{2EAB} = 7$ and $n = -2$ in V_{fexp}.

hydrogen ions are produced along with products, that does not affect the pH dependence of rate of the forward reaction. So *n* is always determined in the forward reaction. If the rate-determining reaction in the mechanism for an enzyme-catalyzed reaction produces hydrogen ions, the value of *n* has to be determined by studying the reverse reaction. When that is done, P and Q are reactants, and their reaction is the forward reaction.

Methods

1. Calculation of V_{fexp} and V_f as Functions of pH. The relation between V_{fexp} obtained from Lineweaver−Burk plots of experimental data and V_f obtained using $10^{-npH}V_{fexp}$ is illustrated by making numerical calculations. When $pK_{1EAB} = 8.0$, $pK_{2EAB} = 7.0$, $k_f[E]_t = 2$, and $n = 0$, the plot of V_f versus pH is given by Figure 1a. If pK_2 is very low or is nonexistent, V_f is given by Figure 1b. If pK_1 is very high or is nonexistent,

V_f is given by Figure 1c. When one H^+ is consumed in the rate-determining reaction, $n = -1$, and the pH dependencies of V_f and V_{fexp} are compared in Figure 1d−f. To make it convenient to compare the pH dependencies of V_f and V_{fexp}, $V_{fexp} = V_f 10^{-(pH-7.5)}$ was used in making plots d to f so that the superimposed plots cross at pH 7.5. Alternatively, the two plots can be compared by adjusting k_f in eq 5. The plots of V_{fexp} in Figure 1d and f look like V_f in Figure 1b, even though they are different mathematical functions. Thus, the failure to recognize that $n = -1$ can lead to the incorrect conclusion that $pK_{1EAB} = 7$ from Figure 1d or Figure 1f. The plots of V_{fexp} are displaced when the 7.5 in the exponential function is changed, but the conclusions are not changed.

Figure 2a−c shows the superimposed plots of V_{fexp} and V_f when two hydrogen ions are consumed in the rate-determining reaction ($n = -2$). The right column shows the corresponding log plots. Logarithmic plots of this type are often used to determine pKs by use of tangent lines.[11−13] Failure to recognize that $n = -2$ can lead to the incorrect conclusion that $pK_{1EAB} = 7$.

2. Ordered Mechanism for the Forward Reaction A + B = Products. Consider the enzymatic catalysis of the forward reaction A + B → products for the ordered mechanism.

$$E + A = EA \qquad K_{IA} = [E][A]/[EA] \tag{6}$$

$$EA + B = EAB \qquad K_B = [EA][B]/[EAB] \tag{7}$$

$$EAB \rightarrow \text{products} \tag{8}$$

Equal signs are used for reactions that are equilibrated rapidly, and → indicates the rate-determining reaction. The Michaelis constants K_{IA} and K_B are equilibrium constants. It is assumed that the enzymatic site and enzyme−substrate complexes each have two pKs and that A and B do not have pKs in the range of interest. This extension of the mechanism is represented by

$$\begin{array}{lll} E^- & EA^- & \\ \| & \| & \\ HE + A = HEA & K_{HEA} = [HE][A]/[HEA] & (9) \\ \| & \| & \\ H_2E^+ & H_2EA^+ & \end{array}$$

$$\begin{array}{lll} EA^- & EAB^- & \\ \| & \| & \\ HEA + B = HEAB & K_{HEAB} = [HEA][B]/[HEAB] & (10) \\ \| & \| & \\ H_2EA^+ & H_2EAB^+ & \end{array}$$

$$\begin{array}{l} \qquad EAB^- \\ \qquad \| \quad k_f \\ |n|H^+ + HEAB \text{ -> products} \qquad (11) \\ \qquad \| \\ \qquad H_2EAB^+ \end{array}$$

The absolute value of n is required in eq 11 because n is a signed quantity. The chemical equilibrium constant expressions for K_{HEA} and K_{HEAB} are always obeyed.

3. Calculation of Kinetic Parameters of the Forward Reaction A + B → Products when $n = 0$. A Mathematica program (see derordAB in Appendix) has been written to input 6 pKs, 2 chemical equilibrium constants, n, and $k_f[E]_t$ for the calculation the pH dependencies of V_{fexp}, V_f, K_{IA}, K_B, V_f/K_B, and $V_f/K_{IA}K_B$. This program also derives the equation for the initial velocity v of the forward reaction as a function of [A], [B], and pH.

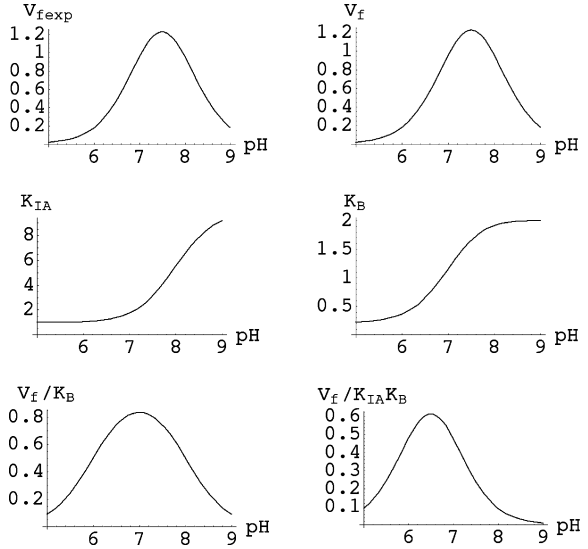

Figure 3. Plots of kinetic parameters for the ordered mechanism of the forward reaction A + B → products when $n = 0$. Note $V_{fexp} = V_f$.

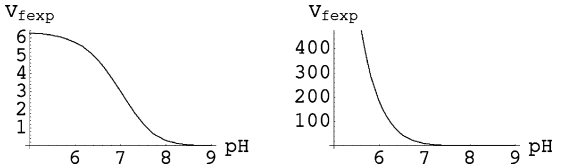

Figure 4. V_{fexp} as a function of pH when $n = -1$ (left) and $n = -2$ (right). V_f and the other kinetic parameters are the same as those shown in Figure 3 for $n = 0$.

To illustrate the use of derordAB, the following constants were used to calculate plots of kinetic parameters versus pH for the ordered mechanism for eqs 9−11 when $pK_{1E} = 7$, $pK_{2E} = 6$, $pK_{1EA} = 8$, $pK_{2EA} = 6$, $pK_{1EAB} = 8$, $pK_{2EAB} = 7$, $k_f[E]_t = 2$, $n = 0$, $K_{HEA} = 1$, and $K_{HEAB} = 2$. The plots for K_{IA} and K_B each involve five parameters, and so it is convenient to use the plots for V_f/K_B and $V_f/K_{IA}K_B$, which each yield three parameters. The plots of the kinetic parameters are given in Figure 3.

4. Calculation of Plots of V_{fexp} when $n = -1$ and -2. Figure 4 shows the pH dependencies of V_{fexp} when $n = -1$ and -2. The plot on the left makes it look like $pK_{1EAB} = 7$, but it is actually 8.

5. Three-Dimensional Plots of Initial Velocities as Functions of [A], [B], and pH. The initial reaction velocity v produced by computer program derordAB is a function of [A], [B], and pH for a specified set of input constants, which includes the value of n. This function can be treated like an experimental reaction system because v can be calculated for any set of [A], [B], and pH. Since v is a function of two variables at a specified pH, Plot3D in Mathematica can be used to plot v versus [A] and [B] at a specified pH. Figure 5 gives plots of initial velocities at pHs 5, 6, 7, and 8 when $n = 0$. Figure 6 gives plots of the initial velocities when $n = -1$.

6. Three-Dimensional Plots of Reciprocal Velocities versus Reciprocal Concentrations. Mathematica can be used to plot reciprocal velocities $1/v$ versus 1/[A] and 1/[B]; these plots can be called three-dimensional Lineweaver−Burk plots. These plots can be made for various pHs and for $n = 0, -1, -2, \ldots$ They are given in Figure 7 only for pHs 5, 6, 7, and 8 with $n = -1$.

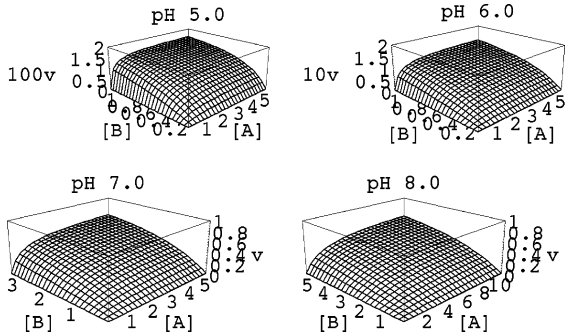

Figure 5. Plots of v at pHs 5, 6, 7, and 8 when $n = 0$. The input constants are given in connection with Figure 3. Notice that the plots are the same for pH 7 and 8. This shows that V_{fexp} is 1.8×10^{-2} at pH 5.

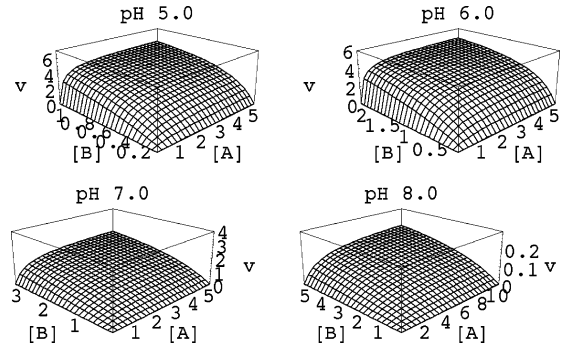

Figure 6. Plots of v at pHs 5, 6, 7, and 8 when $n = -1$. The other input constants are given in connection with Figure 3. This shows that V_{fexp} is 5 at pH 5. This is an illustration of the effect of $10^{-(pH-7.5)}$.

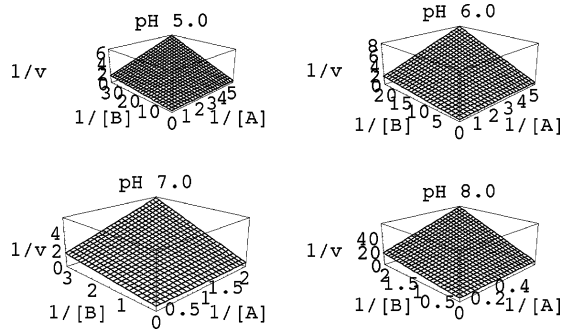

Figure 7. Reciprocal initial velocities for the ordered mechanism for A + B → products versus 1/[A] and 1/[B] at pHs 5, 6, 7, and 8 for $n = -1$.

The plots in Figure 7 are referred to in mathematics as ruled surfaces, and quite a bit has been written about ruled surfaces. Each of the surfaces in Figure 7 is characterized by three constants: the experimental limiting velocity V_{fexp} and Michaelis constants K_{IA} and K_B at the specified pH. These surfaces are not planes, but the lines are all straight. These surfaces emphasize the fact that the velocities at three points on one of these surfaces yields three simultaneous equations that can be solved for the values of V_{fexp}, K_{IA}, and K_B at that pH. It will be of interest to identify the most useful [A] and [B] for the three points that will give the most accurate values of V_{fexp}, K_{IA}, and K_B.

7. Relation between $\Delta_r N_H$ and n. In order to find this relation, it is necessary to concentrate on the rate-determining reaction in the mechanism because that is where n is defined. That cannot be accomplished by considering mechanism eqs 9−11 because that mechanism does not include the reverse reaction. The main line of reaction for the ordered mechanism for A + B = P + Q can be written as

TABLE 1: Arbitrary Parameters Used to Illustrate an Application of Eq 16

	pK_1	pK_2
A	8.0	7.0
B	7.7	6.5
P	8.5	7.0
Q	8.0	6.0

$$HE + H_2A = H_3EA \tag{12}$$

$$H_3EA + HB = H_4EAB \tag{13}$$

$$|n|H^+ + H_4EAB \leftrightarrow H_{n+4}EPQ^{n+} = H_{n+1}EQ^{n+} + H_3P \tag{14}$$

$$H_{n+1}EQ^{n+} = HE + H_nQ^{n+} \tag{15}$$

where ↔ indicates the rate-determining reaction. In this mechanism, each of the species (other than H^+) shown can lose H^+ or gain H^+; in other words, each species has two pKs. The expression for the apparent equilibrium constant is

$$K' = \frac{K_{ref}10^{n\mathrm{pH}}(1 + 10^{\mathrm{pH}-\mathrm{p}K_{1P}} + 10^{\mathrm{p}K_{2P}-\mathrm{pH}}) \times (1 + 10^{\mathrm{pH}-\mathrm{p}K_{1Q}} + 10^{\mathrm{p}K_{2Q}-\mathrm{pH}})}{(1 + 10^{\mathrm{pH}-\mathrm{p}K_{1A}} + 10^{\mathrm{p}K_{2A}-\mathrm{pH}})(1 + 10^{\mathrm{pH}-\mathrm{p}K_{1B}} + 10^{\mathrm{p}K_{2B}-\mathrm{pH}})} \tag{16}$$

where

$$K_{ref} = \frac{[H_3P][H_nQ^{n+}]}{[H^+]^n[H_2A][HB]} \tag{17}$$

An application of eq 16 is illustrated by arbitrarily using the values of the constants given in Table 1. The use of eq 2 ($\Delta_r N_H = -\mathrm{d}\log K'/\mathrm{dpH}$) yields

$$\Delta_r N_H = -n - \frac{10^{\mathrm{pH}-8.5} - 10^{7-\mathrm{pH}}}{1 + 10^{7-\mathrm{pH}} + 10^{\mathrm{pH}-8.5}} - \frac{10^{\mathrm{pH}-8} - 10^{6-\mathrm{pH}}}{1 + 10^{6-\mathrm{pH}} + 10^{\mathrm{pH}-8}} + \frac{10^{\mathrm{pH}-8} - 10^{7-\mathrm{pH}}}{1 + 10^{7-\mathrm{pH}} + 10^{\mathrm{pH}-8}} + \frac{10^{\mathrm{pH}-7.7} - 10^{6.5-\mathrm{pH}}}{1 + 10^{6.5-\mathrm{pH}} + 10^{\mathrm{pH}-7.7}} \tag{18}$$

This equation can be written

$$\Delta_r N_H = -n + n\mathrm{HP} + n\mathrm{HQ} - n\mathrm{HA} - n\mathrm{HB} \tag{19}$$

where nHP is the number of hydrogen atoms in P as a function of the pH, and so forth. These four terms can each be plotted versus pH to obtain the titration curve for that reactant where $n\mathrm{HP} = 0$ for the intermediate form, that is, HP.

Two ways to calculate $\Delta_r N_H$ are given in eq 2, and so this is an additional method. The values nHP and so forth are not equal to the average number of hydrogen atoms $\bar{N}_H(i)$ in a substrate because the latter are average numbers with respect to the number of hydrogen atoms at high pH that is known from the molecular formula. The values of $\bar{N}_H(i)$ can be used to calculate $\Delta_r N_H$, but the nHP, nHQ, nHA, and nHB values cannot be used to calculate it because they are with respect to an internal

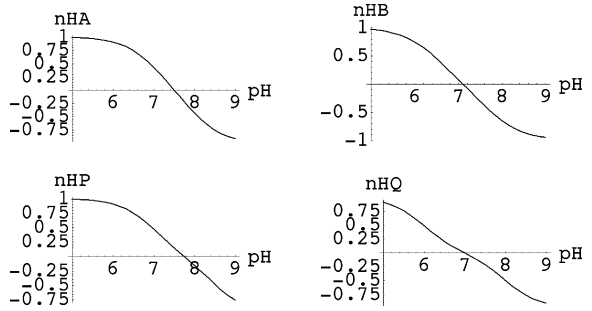

Figure 8. Titration curves for A, B, P, and Q with respect to the intermediate ionized form, where the p*K*s are given in Table 1.

reference, that is, the number of hydrogen atoms in the intermediate ionized form. The values of *n*HA, *n*HB, *n*HP, and *n*HQ are shown as functions of pH in Figure 8. When *n* can be determined from initial velocities of the forward reaction, eq 19 can be used to calculate $\Delta_r N_H$.

Discussion

In studying the mechanisms of enzyme-catalyzed reactions using the rapid-equilibrium assumption, it is important to recognize the consumption of hydrogen ions in the rate-determining reaction. When this happens, a 10^{npH} appears in the rate equation, where *n* is the number of hydrogen ions consumed. When this happens, the effect on the rate of reaction is large and extends over the whole range of pH of interest. Since the pH effect is large, it is not difficult to determine *n*. It is important not to confuse this 10^{npH} effect with the change in binding of hydrogen ions $\Delta_r N_H$ in an enzyme-catalyzed reaction. The 10^{npH} is associated with the half reaction for the forward reaction, but $\Delta_r N_H$ is a property of the whole reaction. These properties are related by eq 19.

When a mechanism involves a number of p*K*s and chemical equilibrium constants, rate equations become very complicated, and so mathematical applications like Mathematica are very useful. The rate equation in a computer is like an actual reaction system because it provides the reaction rate when the substrate concentrations are specified. In this way, it is possible to learn how a reaction system behaves. This program can be used to check kinetic parameters determined experimentally by calculating Lineweaver−Burk plots and other plots with these parameters.

A great deal is known about the changes in binding of hydrogen ions in enzyme-catalyzed reactions as functions of pH.[3−5] For some reactions, $\Delta_r N_H$ is zero at all pHs in the range pH 5−9. For others, $\Delta_r N_H$ varies with pH, and some are given by a positive or negative integer at all pHs in this range. When integer values are obtained, the substrates do not have p*K*s in the range of interest.

In contrast with biochemical thermodynamics that deals with complete reactions, rapid-equilibrium kinetics deals with forward half reactions and reverse half reactions. Initial rates of the forward reactions provide information about the consumption of hydrogen ions in the rate-determining reaction for the forward reaction. Initial rates of the reverse reaction provide information about the consumption of hydrogen ions in the rate-determining reaction for the reverse reaction.

Appendix

derordAB[pK1e_,pK2e_,pK1ea_,pK2ea_,pK1eab_,pK2eab_,-kfEt_,n_,kHEA_,kHEAB_]: = Module[{eafactor,efactor,-abfactor,vf,vfexp,kia,kb,v},(*Calculates kinetic parameters of the forward enzyme-catalyzed reaction ordered A + B = products as functions of pH when A and B do not have p*K*s in the pH range of interest. The output is a list of six functions of pH: vfexp,vf,kia,kb,vf/kb, and vf/kiakb. The initial velocity v is a function of [A] = a, [B] = b, and pH. The 7.5 in (pH−7.5) can be changed because it is equivalent to changing the value of $k_f E_t$.*)

efactor = $1 + 10^{pK_{2e}-pH}+10^{pH-pK_{1e}}$;

eafactor = $1 + 10^{pK_{2ea}-pH}+10^{pH-pK_{1ea}}$;

eabfactor = $1 + 10^{pK_{2eab}-pH}+10^{pH-pK_{1eab}}$;

vf = kfEt/eabfactor;

vfexp = (10^(*n**(pH−7.5)))*Vf;

kia = kHEA*efactor/eafactor;

kb = kHEAB*eafactor/eabfactor;

v = vfexp/(1+(kb/b)*(1+(kia/a)));

{vfexp,vf,kia,kb,vf/kb,vf/(kia*kb),v}]

References and Notes

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