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Two different ways that hydrogen ions are involved in the thermodynamics and rapid-equilibrium kinetics of the enzymatic catalysis of S═P and S + H$_2$O═P

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Abstract

Hydrogen ions are involved in two different ways in the thermodynamics and rapid-equilibrium kinetics of enzyme-catalyzed reactions. The two ways are through p*K*s and through the production or consumption of hydrogen ions in the mechanism. These ways are examined for the catalyzed reactions S═P and S + H$_2$O═P. Since the apparent equilibrium constant K' can be calculated from the kinetic parameters by use of the Haldane equation, the treatment of the effects of pH must be consistent in thermodynamics and kinetics. This leads to a new kind of Haldane equation that involves 10^{pH} or $10^{-\mathrm{pH}}$ in addition to the kinetic parameters when hydrogen ions are produced or consumed. These concepts are applicable to more complicated reactions and rate equations. Derivations of equations for calculating these two types of pH effects are discussed in thermodynamics and rapid-equilibrium kinetics. A computer program is used to make four plots of apparent equilibrium constants and changes in the binding of hydrogen ions in the catalyzed reaction.

Keywords: Apparent equilibrium constants; Enzyme kinetics; Rate equations; pH effects in kinetics; Haldane equations

1. Introduction

Hydrogen ions are involved in two different ways in the thermodynamics and kinetics of enzyme-catalyzed reactions. The two ways are indicated by the following equation for the pH dependence of the apparent equilibrium constant K' of the catalyzed reaction [1–3]:

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

K_{ref} is the equilibrium constant for a chemical reference reaction, n is the number of hydrogen ions produced in the chemical reference reaction, and f(pH) is a function of pH that brings in the p*K*s of the substrates in the catalyzed reaction. When hydrogen ions are consumed, n is a negative number. Since the complete rate equation for any enzyme-catalyzed reaction yields a Haldane equation that expresses K' of the catalyzed reaction in terms of kinetic parameters, the rapid-equilibrium rate equation must also involve the two pH effects in Eq. (1).

If K' is determined as a function of pH by equilibrium measurements, the change in the binding of hydrogen ions in the catalyzed reaction $\Delta_r N_H$ can be calculated by use of the following equation [4]:

$$\Delta_r N_H = -\frac{\mathrm{dlog}K'}{\mathrm{dpH}} \tag{2}$$

This article is concerned with the catalysis of the reaction S═P, but it also applies to S + H$_2$O═P. The thermodynamic and kinetic equations are rather complicated even for these simple reactions, but the same ideas apply to reactions with more substrates. Since kinetics is discussed in terms of mechanisms, it is of interest to apply thermodynamics to the mechanisms of enzyme-catalyzed reactions. The rapid-equilibrium assumption is used here in deriving rate equations. When this is done, the Michaelis constants are equilibrium constants [5]. Rapid-equilibrium derivations are especially appropriate in treating pH effects because acid dissociations are equilibrated rapidly.

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2. Thermodynamics of three possible mechanisms for the uncatalyzed reaction S=P

The thermodynamics of this reaction in which S and P each exist in three forms can be discussed in terms of three mechanisms:

Mechanism 1:

$$\begin{array}{ccc} S^- & & P^- \\ \| & k_{f1} & \| \\ HS & \Longleftrightarrow & HP \\ \| & k_{r1} & \| \\ H_2S^+ & & H_2P^+ \end{array} \tag{3}$$

Mechanism 2:

$$\begin{array}{ccc} & k_{f2} & \\ S^- & \Longleftrightarrow & P^- \\ \| & k_{r2} & \| \\ HS & & HP \\ \| & & \| \\ H_2S^+ & & H_2P^+ \end{array} \tag{4}$$

Mechanism 3:

$$\begin{array}{ccc} S^- & & P^- \\ \| & & \| \\ HS & & HP \\ \| & k_{f3} & \| \\ H_2S^+ & \Longleftrightarrow & H_2P^+ \\ & k_{r3} & \end{array} \tag{5}$$

The equilibria represented by equal signs are assumed to be adjusted rapidly and the double arrows indicate the rate-determining step. The electric charges are arbitrary, but they indicate differences between different protonated species. In each case the apparent equilibrium constant K' is given by the following equation:

$$K' = \frac{[P]}{[S]} = \frac{[HP]+[P^-]+[H_2P^+]}{[HS]+[S^-]+[H_2S^+]} \tag{6}$$

The thermodynamic treatments of these three mechanisms involve the following three chemical equilibrium expressions:

$$\frac{k_{f1}}{k_{r1}} = \frac{[HP]}{[HS]} \tag{7}$$

$$\frac{k_{f2}}{k_{r2}} = \frac{[P^-]}{[S^-]} \tag{8}$$

$$\frac{k_{f3}}{k_{r3}} = \frac{[H_2P^+]}{[H_2S^+]} \tag{9}$$

Eq. (6) can be expressed in terms of pH by use of

$$10^{-pK_{1S}} = \frac{10^{-pH}[S^-]}{[HS]} \tag{10}$$

$$10^{-pK_{2S}} = \frac{10^{-pH}[HS]}{[H_2S^+]} \tag{11}$$

and the similar equations that apply to P. The use of these chemical equilibrium equations in Eq. (6) yields the following expressions for the apparent equilibrium constant:

$$K' = \frac{k_{f1}(1+10^{pH-pK_{1P}}+10^{-pH+pK_{2P}})}{k_{r1}(1+10^{pH-pK_{1S}}+10^{-pH+pK_{2S}})} \tag{12}$$

$$K' = \frac{k_{f2}(1+10^{-pH+pK_{1P}}+10^{-2pH+pK_{1P}+pK_{2P}})}{k_{r2}(1+10^{-pH+pK_{1S}}+10^{-2pH+pK_{1S}+pK_{2S}})} \tag{13}$$

$$K' = \frac{k_{f3}(1+10^{pH-pK_{2P}}+10^{2pH-pK_{1P}-pK_{2P}})}{k_{r3}(1+10^{pH-pK_{2S}}+10^{2pH-pK_{1S}-pK_{2S}})} \tag{14}$$

These three equations, which are of the form $K_{ref}f(pH)$, involve different functions of pH, but they must yield the same values of K' because the apparent equilibrium constant of the catalyzed reaction is independent of mechanism. They must also yield the same change in the binding of hydrogen ions $\Delta_r N_H$ when Eq. (2) is used. The ratios k_f/k_r have different values. It is readily shown that

$$\frac{k_{f2}}{k_{r2}} = \frac{k_{f1}}{k_{r1}} 10^{pH1S-pH1P} \tag{15}$$

$$\frac{k_{f3}}{k_{r3}} = \frac{k_{f1}}{k_{r1}} 10^{pH2P-pH2S} \tag{16}$$

When K' has been determined experimentally for S=P over a range of pH by direct measurement or from kinetic data by use of the Haldane equation, the four pKs can be calculated.

The apparent equilibrium constant calculated with Eq. (12) is shown in Fig. 1 as a function of pH when $pK_{1S}=8$, $pK_{2S}=6$, $pK_{1P}=7$, $pK_{2P}=5$, and $k_f/k_r=1$.

The use of Eq. (2) yields $\Delta_r N_H$ as a function of pH, as shown in Fig. 2.

These plots were made by using the Mathematica® program calcthpropsfpH given in the Appendix. The plots of K' for mechanisms (1)–(3) have the same shape, but different ordinates. The plots of $\Delta_r N_H$ are the same for the three mechanisms because their K' plots have the same shape, even though they have different ordinates.

Measurements of K' at a series of pHs make it possible to determine the four pKs. However, these four pKs can be determined more directly by acid titrations of the two substrates. In

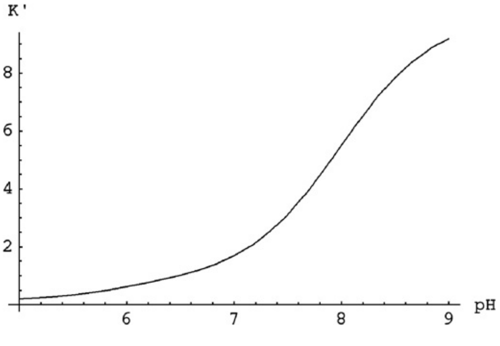

Fig. 1. Apparent equilibrium constant for S=P as a function of pH for mechanism (1) when $pK_{1S}=8$, $pK_{2S}=6$, $pK_{1P}=7$, $pK_{2P}=5$, and $k_f/k_r=1$.

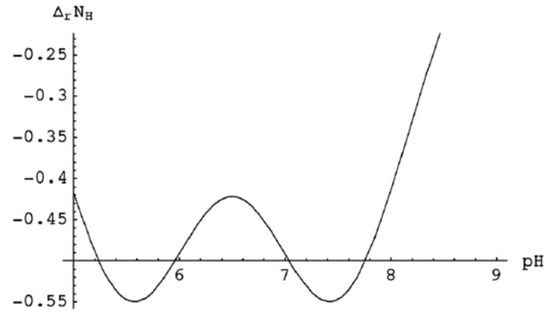

Fig. 2. Change in the binding of hydrogen ions for S═P as a function of pH for mechanism (1) when $pK_{1S}=8$, $pK_{2S}=6$, $pK_{1P}=7$, $pK_{2P}=5$, and $k_f/k_r=1$.

Figs. 1 and 2, S has the higher pKs, and so at any pH, S binds more hydrogen ions than P. Therefore, the binding of hydrogen ions $\Delta_r N_H$ decreases when S is converted to P, and hydrogen ions are produced.

3. Thermodynamics of mechanism (1) in which hydrogen ions are produced in the rate-determining step

The second way that hydrogen ions can be produced or consumed is in the rate-determining step: $H_2S \Leftrightarrow HP + H^+$. When this occurs the mechanism is represented by

$$\begin{array}{ccccc} HS^- & & P^{2-} & & \\ \| & k_{f4} & \| & & \\ H_2S & \Longleftrightarrow & HP^- & + & H^+ \\ \| & k_{r4} & \| & & \\ H_3S^+ & & H_2P & & \end{array} \tag{17}$$

The apparent equilibrium constant for this mechanism is given by

$$K' = \frac{[P]}{[S]} = \frac{[P^{2-}] + [HP^-] + [H_2P]}{[HS^-] + [H_2S] + [H_3S^+]} \tag{18}$$

Substituting equations like Eqs. (10) and (11) for the pKs in this equation yields

$$K' = \frac{[HP^-](1 + 10^{-pK_{1P}+pH} + 10^{-pH+pK_{2P}})}{[H_2S](1 + 10^{-pK_{1S}+pH} + 10^{-pH+pK_{2S}})} \tag{19}$$

Mechanism (17) shows that

$$\frac{k_{f4}}{k_{r4}} = \frac{[HP^-]10^{-pH}}{[H_2S]} = K_{ref} \tag{20}$$

where K_{ref} is the chemical equilibrium constant for $H_2S=HP^-+H^+$. Eliminating $[HP^-]/[H_2S]$ between Eqs. (19) and (20) yields

$$K' = \frac{K_{ref}10^{pH}(1 + 10^{-pK_{1P}+pH} + 10^{-pH+pK_{2P}})}{(1 + 10^{-pK_{1S}+pH} + 10^{-pH+pK_{2S}})} \tag{21}$$

This equation introduces the second way that hydrogen ions are involved in the thermodynamics of the reaction S═P, and has the form of Eq. (1).

It may be desirable to write the reference reaction as $HS^-=P^{2-}+H^+$, instead of $H_2S=HP^-+H^+$. The reason is that there is no pH where $H_2S=HP^-+H^+$ fully represents the reaction, but in the limit of high pH the reaction is represented by $HS^-=P^{2-}+H^+$. According to mechanism (17) the chemical reaction $HS^-=P^{2-}+H^+$ is the sum of three chemical reactions that have the indicated equilibrium constants:

$$HS^- + H^+ = H_2S \quad 10^{pK_{1S}} \tag{22}$$

$$H_2S = H^+ + HP^- \quad k_f/k_r \tag{23}$$

$$HP^- = H^+ + P^- \quad 10^{-pK_{1P}} \tag{24}$$

The equilibrium constant of $HS^-=P^{2-}+H^+$ is the product of the equilibrium constants of Eqs. (22)–(24).

$$\begin{aligned} HS^- &= P^{2-} + H^+ \quad K_{refhighpH} = (k_f/k_r)10^{pK_{1S}-pK_{1P}} \\ &= K_{ref}10^{pK_{1S}-pK_{1P}} \end{aligned} \tag{25}$$

Replacing K_{ref} in Eq. (21) with $K_{refhighpH}10^{pK_{1P}-pK_{1S}}$ yields

$$K' = \frac{K_{refhighpH}10^{pK_{1P}-pK_{1S}}10^{pH}(1 + 10^{-pK_{1P}+pH} + 10^{-pH+pK_{2P}})}{(1 + 10^{-pK_{1S}+pH} + 10^{-pH+pK_{2S}})} \tag{26}$$

In the calculations given above, $pK_{1S}=8$ and $pK_{1P}=7$, and so $K_{refhighpH}=10K_{ref}$. In principle, any integer number of hydrogen ions can be produced or consumed in mechanism (17). When S and P do not have pKs in the pH range of interest, $\Delta^{r}N_H=-1$, independent of pH.

Fig. 3 gives the base 10 logarithm of the apparent equilibrium constant for S═P when the mechanism is given by Eq. (17) and $pK_{1S}=8$, $pK_{2S}=6$, $pK_{1P}=7$, $pK_{2P}=5$, and $k_f/k_r=1$.

Fig. 4 gives $\Delta_r N_H$ calculated using Eq. (2) for mechanism (17).

Notice that these values of $\Delta_r N_H$ are one unit more negative than in Fig. 2 because of the production of a hydrogen ion every time H_2S is converted to HP^-. When these ideas are applied to

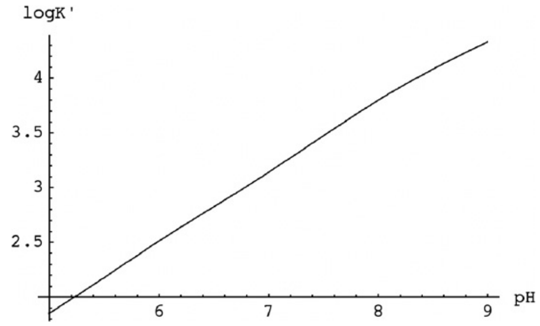

Fig. 3. Base 10 logarithm of the apparent equilibrium constant for S═P as a function of pH for mechanism (17) when $pK_{1S}=8$, $pK_{2S}=6$, $pK_{1P}=7$, $pK_{2P}=5$, and $k_f/k_r=1$.

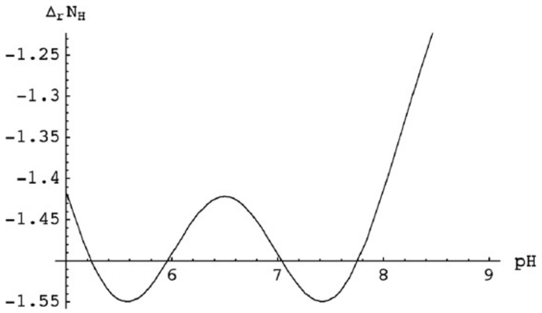

Fig. 4. Change in the binding of hydrogen ions for S═P as a function of pH for mechanism (17) when $pK_{1S}=8$, $pK_{2S}=6$, $pK_{1P}=7$, $pK_{2P}=5$, and $k_f/k_r=1$.

S+H_2O═P no new p*K*s are involved, but the hydrogen atoms in H_2O have to be included in the calculation of $\Delta_r N_H$.

4. Rapid-equilibrium rate equation for the enzymatic catalysis of S═P

The derivation of the rapid-equilibrium rate equation for the following mechanism has been given earlier [6], but the nomenclature has been changed here.

$$\begin{array}{ccccccccccc} S^- & & E^- & & HES^- & & HEP^- & & E^- & & P^- \\ \| & & \| & & \| & k_f & \| & & \| & & \| \\ HS & + & HE & = & H_2ES & \Longleftrightarrow & H_2EP & = & HE & + & HP \\ \| & & \| & & \| & k_r & \| & & \| & & \| \\ H_2S^+ & & H_2E^+ & & H_2ES^+ & & H_2EP^+ & & H_2E^+ & & H_2P^+ \end{array} \tag{27}$$

The equilibria represented by equal signs are assumed to be adjusted rapidly. The rate-determining step in the forward direction has the rate constant k_f and the rate-determining step in the reverse reaction has the rate constant k_r. Roberts [7] has provided an insightful discussion of the effects of pH in enzyme kinetics.

The concentration of substrate is always given by

$$\begin{aligned}[S] &= [HS] + [H_2S^+] + [S^-] \\ &= [HS](1 + 10^{pK_{2S}-pH} + 10^{pH-pK_{1S}}) = [HS]f_{HS}\end{aligned} \tag{28}$$

where the HS factor is given by

$$f_{HS} = 1 + 10^{pK_{2S}-pH} + 10^{pH-pK_{1S}} \tag{29}$$

Equations like this can be written for [HE], [H_2ES], [H_2EP], and [HP]. In addition, the following two expressions for chemical equilibrium constants are always satisfied.

$$K_{H_2ES} = [HS][HE]/[H_2ES] \tag{30}$$

$$K_{H_2EP} = [HP][HE]/[H_2EP] \tag{31}$$

It is well known that mechanism (27) yields the following rapid-equilibrium rate equation at a specified pH:

$$v = \frac{\frac{V_f[S]}{K_S} - \frac{V_r[P]}{K_P}}{1 + \frac{[S]}{K_S} + \frac{[P]}{K_P}} \tag{32}$$

The limiting velocity in the forward direction is given by

$$V_f = \frac{k_f[E]_t}{1 + 10^{pK_{2ES}-pH} + 10^{pH-pK_{1ES}}} \tag{33}$$

where $[E]_t$ is the total concentration of free and bound enzymatic sites. The limiting velocity in the reverse direction is given by

$$V_r = \frac{k_r[E]_t}{1 + 10^{pK_{2EP}-pH} + 10^{pH-pK_{1EP}}} \tag{34}$$

The Michaelis constant for substrate S is an equilibrium constant of the form

$$K_S = \frac{K_{H_2ES}(1 + 10^{pK_{2E}-pH} + 10^{pH-pK_{1E}})(1 + 10^{pK_{2S}-pH} + 10^{pH-pK_{1S}})}{(1 + 10^{pK_{2ES}-pH} + 10^{pH-pK_{1ES}})} \tag{35}$$

The Michaelis constant for product P is an equilibrium constant of the form

$$K_P = \frac{K_{H_2EP}(1 + 10^{pK_{2E}-pH} + 10^{pH-pK_{1E}})(1 + 10^{pK_{2P}-pH} + 10^{pH-pK_{1P}})}{(1 + 10^{pK_{2EP}-pH} + 10^{pH-pK_{1EP}})} \tag{36}$$

In biochemical thermodynamics, apparent equilibrium constants like K_S have primes to indicate that they are written in terms of sums of species and are therefore pH dependent. However, the primes are omitted here because they have not been used in enzyme kinetics.

The ratio V_f/K_S is of special interest because when pK_{1S} and pK_{2S} are known, the p*K*s of the enzymatic site can be obtained.

$$\frac{V_f}{K_S} = \frac{k_f[E]_t}{K_{H_2ES}(1 + 10^{pK_{2E}-pH} + 10^{pH-pK_{1E}})(1 + 10^{pK_{2S}-pH} + 10^{pH-pK_{1S}})} \tag{37}$$

The same pK_{1E} and pK_{2E} should be obtained from V_r/K_P. The Haldane equation is given by

$$\begin{aligned}K' &= \frac{V_f K_P}{V_r K_S} = \frac{k_f K_{H_2EP}(1 + 10^{pK_{2P}-pH} + 10^{pH-pK_{1P}})}{k_r K_{H_2ES}(1 + 10^{pK_{2S}-pH} + 10^{pH-pK_{1ES}})} \\ &= K_{ref}\frac{(1 + 10^{pK_{2P}-pH} + 10^{pH-pK_{1P}})}{(1 + 10^{pK_{2S}-pH} + 10^{pH-pK_{1S}})}\end{aligned} \tag{38}$$

where $K_{ref}=k_f K_{H_2EP}/k_r K_{H_2ES}$ is the equilibrium constant for the chemical reaction HS═HP. This is in agreement with Eq. (12) for the uncatalyzed reaction.

When the pK_1's are very high, and the pK_2's are very low, the net reaction for the mechanism is HS═HP, which does not produce or consume hydrogen ions, but when there are p*K*s in the range of experimental interest, the reaction S═P may produce or consume hydrogen ions as shown by Fig. 2.

5. Kinetics of the enzymatic catalysis of S═P when a hydrogen ion is produced in the rate-determining step

When a single hydrogen ion is produced in the rate-determining step, mechanism (27) is extended to

$$\begin{array}{ccccccccc} HS^- & & E^- & & H_2ES^- & & HEP^{2-} & & \\ \| & & \| & & \| & k_f & \| & & \\ H_2S & + & HE & = & H_3ES & \Longleftrightarrow & H_2EP^- & + & H^+ \\ \| & & \| & & \| & k_{rH} & \| & & \\ H_3S^+ & & H_2E^+ & & H_4ES^+ & & H_3EP & & \end{array} \tag{39}$$

$$\begin{array}{ccccc} \mathrm{HEP}^{2-} & & \mathrm{E}^{-} & & \mathrm{P}^{2-} \\ \| & & \| & & \| \\ \mathrm{H_2EP}^{-} & = & \mathrm{HE} & + & \mathrm{HP}^{-} \\ \| & & \| & & \| \\ \mathrm{H_3EP} & & \mathrm{H_2E}^{+} & & \mathrm{H_2P} \end{array} \tag{40}$$

The following two chemical equilibrium constants are always satisfied:

$$K_{\mathrm{H_3ES}} = [\mathrm{H_2S}][\mathrm{HE}]/[\mathrm{H_3ES}] \tag{41}$$

$$K_{\mathrm{H_2EP^-}} = [\mathrm{HP^-}][\mathrm{HE}]/[\mathrm{H_2EP^-}] \tag{42}$$

There are some changes in symbols for species, but the significant difference from the treatment of mechanism (27) is that k_r is replaced with $k_{rH}[H^+]$. This effect of the production of a hydrogen ion in the rate-determining step is quite different from the effects of p*K*s. Section 3 shows that the limiting velocity for the reverse reaction for S═P when a hydrogen ion is not produced in the rate-determining step is given by Eq. (34). When k_r is replaced with $k_{Hr}10^{-\mathrm{pH}}$ in rate Eq. (32), it can be done in two ways. If $k_{Hr}10^{-\mathrm{pH}}$ is substituted for k_r in Eq. (34), the following equation is obtained:

$$V_r = \frac{k_{rH}10^{-\mathrm{pH}}[\mathrm{E}]_t}{1 + 10^{\mathrm{p}K_{2\mathrm{EP}}-\mathrm{pH}} + 10^{\mathrm{pH}-\mathrm{p}K_{1\mathrm{EP}}}} \tag{43}$$

However, this is not a useful equation because the plot of V_r versus pH is no longer the bell-shaped curve that is obtained experimentally. The other way is to put the $10^{-\mathrm{pH}}$ outside of V_r, and that yields

$$v = \frac{\frac{V_f[\mathrm{S}]}{K_S} - \frac{V_r[\mathrm{P}]10^{-\mathrm{pH}}}{K_P}}{1 + \frac{[\mathrm{S}]}{K_S} + \frac{[\mathrm{P}]}{K_P}} \tag{44}$$

This is the correct place for the important factor of $10^{-\mathrm{pH}}$. Thus when a single hydrogen ion is produced in the rate-determining step, a $10^{-\mathrm{pH}}$ term is introduced into the rapid-equilibrium rate equation. Setting the numerator equal to zero yields a new type of Haldane equation:

$$\begin{aligned} K' &= \frac{10^{\mathrm{pH}}V_f K_P}{V_r K_S} \\ &= \frac{K_{\mathrm{ref}}10^{\mathrm{pH}}(1 + 10^{\mathrm{p}K_{2P}-\mathrm{pH}} + 10^{\mathrm{pH}-\mathrm{p}K_{1P}})}{(1 + 10^{\mathrm{p}K_{2S}-\mathrm{pH}} + 10^{\mathrm{pH}-\mathrm{p}K_{1S}})} \end{aligned} \tag{45}$$

This is a big change from the usual Haldane equation, which is $K' = V_f K_P / V_r K_S$. Eq. (45) agrees with Eq. (21), as it must, because kinetics and thermodynamics must yield the same apparent equilibrium constant. The equilibrium constant for the chemical reference reaction H_2S═$HP^- + H^+$ is $K_{\mathrm{ref}} = k_f K_{\mathrm{H_2EP}^-} / k_{rH} K_{\mathrm{H_3ES}}$. The pH dependencies of V_f, V_r, K_S, and K_P are not affected by the production of hydrogen ions in the rate-determining step. Thus Figs. 3 and 4 apply to the mechanisms (39)–(40) as well as to mechanism (17).

When a hydrogen ion is consumed in the rate-determining step of the forward reaction, the rate equation is

$$v = \frac{\frac{V_f[\mathrm{S}]10^{-\mathrm{pH}}}{K_S} - \frac{V_r[\mathrm{P}]}{K_P}}{1 + \frac{[\mathrm{S}]}{K_S} + \frac{[\mathrm{P}]}{K_P}} \tag{46}$$

The expression for the apparent equilibrium constant is

$$\begin{aligned} K' &= \frac{10^{-pH}V_f K_P}{V_r K_S} \\ &= \frac{K_{ref}10^{-pH}(1 + 10^{pK2P-pH} + 10^{pH-pK1P})}{(1 + 10^{pK2S-pH} + 10^{pH-pK1S})} \end{aligned} \tag{47}$$

Eqs. (45) and (47) are special cases of Eq. (1). The limiting velocities and Michaelis constants can be determined in kinetics experiments, but the calculation of the pH dependence of K' requires the inclusion of $10^{n\mathrm{pH}}$. When the molecular formulas are known for the reactants, n can be obtained from the balanced chemical equation used as a reference reaction.

Eqs. (45) and (47) both show that

$$\frac{V_f K_P}{V_r K_S} = \frac{K_{\mathrm{ref}}(1 + 10^{\mathrm{p}K_{2P}-\mathrm{pH}} + 10^{\mathrm{pH}-\mathrm{p}K_{1P}})}{(1 + 10^{\mathrm{p}K_{2S}-\mathrm{pH}} + 10^{\mathrm{pH}-\mathrm{p}K_{1S}})} = K_{\mathrm{ref}} f(\mathrm{pH}) \tag{48}$$

where *f*(pH) is the function that brings in the p*K*s of the reactants. Thus measurements of V_f, V_r, K_S, and K_P can yield the p*K*s of the reactants, but these measurements alone do not yield K' as a function of pH when hydrogen ions are produced or consumed in the rate-determining step, if the reactants do not have p*K*s in the pH range of interest, *f*(pH) = 1; in other words, the value of K_{ref} for a chemical reaction is obtained from Eq. (48).

6. Discussion

This article has clarified how $K' = K_{\mathrm{ref}}10^{n\mathrm{pH}}f(\mathrm{pH})$ arises in both the thermodynamics and rapid-equilibrium enzyme kinetics of S═P. These concepts apply to the enzymatic catalysis of reactions with more substrates, but they are more clearly introduced by considering the simplest enzyme mechanism. The pH effects on various thermodynamic properties of an enzyme-catalyzed reaction are all represented by Eq. (1) in the sense that the standard transformed Gibbs energy $\Delta_r G'^\circ$ is given by $-RT\ln K'$, and the other standard transformed thermodynamic properties of the enzyme-catalyzed reaction can be obtained by taking partial derivatives of $\Delta_r G'^\circ$ [4]. This includes the change in binding of hydrogen ions in the reaction that is catalyzed, as shown in Eq. (2).

The changes in binding of hydrogen ions $\Delta_r N_H$ have been calculated for 229 enzyme-catalyzed reactions [8,9] at 298.15 K, 0.25 M ionic strength, and pHs 5, 6, 7, 8, and 9 using data in BasicBiochemData3 [10]. Some enzyme-catalyzed reactions produce or consume as many as 8 hydrogen ions. A previous article [8] has verified $\Delta_r N_H$ at high pH for the following five reactions by counting hydrogen atoms in the reference reaction:

$$\begin{aligned} &\text{EC 1.2.1.3 Acetaldehyde} + \mathrm{NAD_{red}} + \mathrm{H_2O} \\ &\quad = \text{acetate} + \mathrm{NAD_{red}} \end{aligned} \tag{49}$$

$$\begin{aligned} &\text{EC 1.2.2.4 Carbonmonoxide} + 2\mathrm{H_2O} \\ &\quad + 2\ \text{ferricytochromeb} - 561 \\ &\qquad = \mathrm{CO_2tot} + 2\ \text{ferrocytochromeb} - 561 \end{aligned} \tag{50}$$

$$\text{EC 1.7.1.4 Nitrite} + 3\text{NADP}_{\text{red}} = \text{ammonia} + 3\text{NADP}_{\text{ox}} + 2\text{H}_2\text{O} \quad (51)$$

$$\text{EC 1.7.2.2 Nitrite} + 6\ \text{ferrocytochromec} = \text{ammonia} + 2\text{H}_2\text{O} + 6\ \text{ferricytochromec} \quad (52)$$

$$\text{EC 1.8.7.1 Sulfite} + 3\text{ferredoxin}_{\text{red}} = \text{hydrogensulfide} + 3\text{ferredoxin}_{\text{ox}} + 3\text{H}_2\text{O} \quad (53)$$

The values of $\Delta_r N_H$ for these reactions at pH 9 are −2.0, −3.2, 4.6, 7.6, and 7.0, respectively. These values are important because they show how much changing the pH affects the apparent equilibrium constant of the reaction that is catalyzed. Note that the most striking effects of pH on the apparent equilibrium constant occur in reaction (52) because the reactants do not contain any hydrogen atoms, but the products contain eight hydrogen atoms. Therefore, at high pH, $K' = 10^{8\text{pH}} K_{\text{ref}}$.

If magnesium ions are involved in an enzyme-catalyzed reaction, their role in the thermodynamics and kinetics of enzyme-catalyzed reactions is like that of hydrogen ions. There are two problems in carrying out the calculations described here when magnesium ions are involved: First, not many dissociation constants for magnesium complex ions are known. Second, pMg has to be calculated from the total concentration of magnesium ions in the solution.

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Appendix A

```
calcthpropsfpH[pK1s_, pK2s_, pK1p_, pK2p_] := Module
[{sfactor, pfactor, kprime1, dNH1, kprime2, logkprime2,
dNH2}, (*Calculates four functions of pH : (1) K′(S==P)for
three species of S and three species of P, (2) ΔrNH (S==P)for
three species of S and three species of P, (3) logK′(S==P)for
three species of S and three species of P that produces one
hydrogen ion in the rate-determining step, (4) ΔrNH (S==P)for
three species of S and three species of P that produces one
hydrogen ion in the rate-determining step. These four functions
can be used to make tables and plots.*)

  sfactor = 1 + 10^(pK2s − pH) + 10^(pH − pK1s);
  pfactor = 1 + 10^(pK2p − pH) + 10^(pH − pK1p);
  kprime1 = pfactor/sfactor;
  dNH1 = (−1/Log[10])*D[Log[(pfactor/sfactor)], pH];
  kprime2 = (10^pH)*pfactor/sfactor;
  logkprime2 = (1/Log[10])*Log[kprime2];
  dNH2 = (− 1/Log[10])*D[Log[kprime2], pH];
  {kprime1, dNH1, logkprime2, dNH2}]
```

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