Shapes of Curves of pH-Dependence of Reactions

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A simple case is considered in which the rate of a two-step reaction depends on pH because the intermediate formed in the first step has to gain (or lose) a proton before it can react in the second step, and in which the rate-determining step therefore changes with pH. The curves of reaction rate against pH are shown to be symmetrical, and the sharpest peak possible has a width at half its height of 1.53 pH units, i.e. of $2 \log(3 + 2\sqrt{2})$. Any particular curve for this situation proves to be identical with a curve that could be generated for the pH-dependence of a single-step reaction in which the rate is proportional to the concentration of a particular ionic form of a reactant. Curves for the latter situation, however, can have forms impossible for the former case in which the rate-determining step changes, but only if the protonations that activate and deactivate the reactant are co-operative. The peak can then become even sharper, and its width at half its height can fall to 1.14 pH units, i.e. to $2 \log(2 + \sqrt{3})$.

Of the several possible reasons for dependence of the rate of a reaction on H^+ concentration, two apply in cases where all the steps involved that consist of gain or loss of protons are fast enough never to be rate-determining. One is that the reactant must be in a particular ionic form to be reactive, and gain or loss of a proton makes it unreactive (Scheme 1*a*). This situation has been deeply explored by Michaelis (1914), Alberty & Massey (1954), Dixon & Webb (1958) and others. The other cause is that after an intermediate has been formed in one step of a reaction it has to lose or gain a proton before it can proceed further along the reaction pathway (Scheme 1*b*). This leads to a change in rate-determining step with pH (see Jencks, 1969). The present paper brings out certain similarities and one difference in the possible forms of pH-dependence of the two cases.

Change of Rate with pH because Protonation of the Intermediate is Required

Definition of the system

A simple system is considered in which the reactant A is converted, only when unprotonated, into an intermediate X. This is further converted, only when protonated, into the product. The rate and equilibrium (dissociation) constants are as shown in Scheme 1*b*). Since protonation reactions are assumed to be fast, only equilibrium constants need be shown for them.

Imine formation may be taken as an example since the starting amine $R-NH_2$ is reactive with $R'-CHO$ when unprotonated, but the intermediate carbinolamine, $R-NH-CHOH-R'$, is dehydrated only when protonated (see Jencks, 1969). The reverse situation

Scheme 1. Reaction schemes of the systems considered

(a) The pH-dependence is due to conversion of the reactant into inactive forms by protonation and deprotonation; (b) the pH-dependence is due to conversion of the reactant into an inactive form by protonation and of the intermediate into an inactive form by deprotonation.

in which AH^+ but not A, and X but not XH^+ , are reactive, would of course lead to similar equations.

Derivation of the rate equation

Following the standard procedure (Michaelis & Menten, 1913; Briggs & Haldane, 1925), v , the velocity of the reaction, is given by:

$$v = k_{+2}[\text{XH}^+]$$

To find $[\text{XH}^+]$, a steady state in intermediate is assumed, so that

$$k_{+2}[\text{XH}^+] + k_{-1} \frac{K'}{H} [\text{XH}^+] = k_{+1}[A]$$

where H is the activity of hydrogen ions.

$$\text{Hence } v = \frac{k_{+2}k_{+1}[A]}{k_{+2} + k_{-1}K'/H}$$

If the total concentration of reactant, i.e. $[A] + [\text{AH}^+]$, is called $[A]_t$, then:

$$[A] = \frac{[A]_t}{1 + H/K}$$

so that:

$$v = \frac{k_{+1}[A]_t}{\left(1 + \frac{k_{-1}K'}{k_{+2}H}\right) \left(1 + \frac{H}{K}\right)} \quad (1)$$

It is convenient to designate the expression $\left(\frac{k_{-1}K'}{k_{+2}K}\right)$ by the term μ^2 , so that eqn. (1) becomes:

$$v = \frac{k_{+1}[A]_t}{\left(1 + \frac{\mu^2 K}{H}\right) \left(1 + \frac{H}{K}\right)} \quad (2)$$

Limiting cases

When H is large, $[\text{XH}^+]$ is high and step 2 is fast compared with step 1, which becomes rate-determining. Then:

$$v = k_{+1}[A] = \frac{k_{+1}[A]_t}{1 + \frac{H}{K}}$$

i.e. v rises with pH as $[A]$ does, and this is the limiting form of eqn. 2 when $H \gg \mu^2 K$.

When H is small, step 2 can become rate-determining as step 1 increases in rate and XH^+ is converted into X. Since $v = k_{+2}[\text{XH}^+]$, and since under these conditions XH^+ will be in equilibrium with AH^+ , the following substitutions can be made:

$$[\text{XH}^+] = \frac{H}{K'}[X]; [X] = \frac{k_{+1}}{k_{-1}}[A]; [A] = \frac{K}{H}[\text{AH}^+]$$

so that:

$$v = \frac{k_{+2}k_{+1}K}{k_{-1}}[\text{AH}^+] = \frac{k_{+1}}{\mu^2}[\text{AH}^+] = \frac{k_{+1}[A]_t}{\mu^2(1 + K/H)}$$

Fig. 1. Dependence of reaction rate on pH when the rate-determining step changes with pH

The line marked v shows the rate, and is plotted from eqn. 2 for $\mu = 2$. At acid pH values it follows the curve of $k_{+1}[A]$ and at alkaline pH values that of $\frac{k_{+1}}{\mu^2}[\text{AH}^+]$.

The optimum pH ($\text{pH}_{\text{opt.}}$) is displaced by $\log \mu$ from the pK of AH^+ , in this case by 0.3.

i.e. v rises as $[\text{AH}^+]$ does, as the pH is diminished, and this is the limiting form of eqn. (2) when $\mu^2 K \gg H$.

Fig. 1 shows the plots of v against pH, together with the curves for the two limiting cases. In plotting the curve μ has been arbitrarily set at 2; other features of the curve shown in Fig. 1 are given below. Note that the use of the pH scale (i.e. $-\log H$) allows a convenient plot to be made.

Relation of the rate to the maximum rate

Let $H_{\text{opt.}}$ be the value of H when v reaches its maximum value $v_{\text{opt.}}$. This occurs when the denominator, f , in the expression for v in eqn. (2) is minimal (cf. Michaelis, 1914).

$$f = 1 + \frac{\mu^2 K}{H} + \frac{H}{K} + \mu^2$$

$$\frac{df}{dH} = -\frac{\mu^2 K}{H^2} + \frac{1}{K}$$

$$\text{Hence } \frac{\mu^2 K}{H_{\text{opt.}}^2} = \frac{1}{K}, \text{ or } H_{\text{opt.}} = \mu K$$

This allows us to give various forms of $\frac{v}{v_{\text{opt.}}}$, and those useful for bringing out features of the dependence include:

$$v/v_{\text{opt.}} = \frac{(1 + \mu)^2}{1 + \mu^2 + \frac{\mu^2 K}{H} + \frac{H}{K}} \quad (3)$$

and

$$v/v_{\text{opt.}} = \frac{\mu + 1/\mu + 2}{\mu + 1/\mu + \left(\frac{H_{\text{opt.}}}{H} + \frac{H}{H_{\text{opt.}}} \right)} \quad (4)$$

Overall features of the curve

(a) *Symmetry.* It follows from eqn. (4) that the curve of $v/v_{\text{opt.}}$ against pH is symmetrical about $\text{pH}_{\text{opt.}}$ (i.e. $-\log H_{\text{opt.}}$). This is because the value for $v/v_{\text{opt.}}$ at any value of $H = nH_{\text{opt.}}$ is the same when $H = H_{\text{opt.}}/n$, since $\left(\frac{H_{\text{opt.}}}{H} + \frac{H}{H_{\text{opt.}}} \right)$ is the same in the two cases, i.e. when the pH is $(\text{pH}_{\text{opt.}} + \log n)$ and $(\text{pH}_{\text{opt.}} - \log n)$.

(b) *Displacement of the centre of the curve from pK.* Since $H_{\text{opt.}} = \mu K$, $\text{pH}_{\text{opt.}} = \text{pK} - \log \mu$. The optimum pH is thus displaced from the pK by $\log \mu$, as shown in Fig. 1, where, since $\mu = 2$, the centre is 0.3 pH unit below pK, and the ratio of the heights of the limiting curves is 4 (i.e. μ^2).

(c) *Forms of the curve when μ is very large or small.* If $\mu \gg 1$, eqn. (3) approaches:

$$\frac{v}{v_{\text{opt.}}} = \frac{1}{1 + \frac{K}{H} + \frac{H}{\mu^2 K}}$$

At least one of the last two terms of the denominator must be negligible, so that any region of the curve is adequately represented either by:

$$\frac{1}{1 + \frac{K}{H}} \quad \text{or by} \quad \frac{1}{1 + \frac{H}{\mu^2 K}}$$

Hence the situation is that shown in Fig. 2, where two titration curves appear to be followed. Note that the upper of the two pK values (pK) is a true pK, but the

Fig. 2. Dependence of reaction rate on pH when μ is large

The limiting curves are dashed. The curves shown are for $\mu = 10^4$.

lower one, $\text{pK} - 2\log \mu$, is a mirage, partly determined by rate constants. This mirage, however, as Dr. J. R. Knowles has pointed out to me, contains pK' , the pK of the intermediate, although it is displaced by $\log k_{-1}/k_{+2}$ (cf. Schmidt & Westheimer, 1971), since $\mu^2 K = K' k_{-1}/k_{+2}$.

If $\mu \ll 1$, a similar form of curve is obtained, since $v/v_{\text{opt.}}$ approaches $1 / \left(1 + \frac{\mu^2 K}{H} + \frac{H}{K} \right)$. In this case, however, the apparent pK on the left-hand side of the curve is the pK of the substrate, and the apparent pK on the right-hand side is the mirage at $\text{pK} - 2\log \mu$, which is a value greater than the pK because $\log \mu$ is negative.

Change of Rate because Protonation or Deprotonation makes the Reactant Inactive

Equations

As pointed out by Michaelis (1914), Alberty & Massey (1954) and Dixon & Webb (1958) among others, if HAH, AH^- , HA^- and A^{2-} are the possible ionic forms of a compound that can lose two protons, then:

$$[\text{AH}^-] + [\text{HA}^-] = [\text{A}]_t / \left(1 + \frac{H}{K_1} + \frac{K_2}{H} \right)$$

where $[\text{A}]_t = [\text{HAH}] + [\text{AH}^-] + [\text{HA}^-] + [\text{A}^{2-}]$, and K_1 and K_2 are the two molecular (titration, macroscopic) dissociation constants. Since the ratio of $[\text{AH}^-]$ to $[\text{HA}^-]$ is independent of H , each of them varies with H in the same way as their sum. Hence the rate, v , of a reaction that is first order with respect to one of them is given by:

$$v \propto \frac{[\text{A}]_t}{1 + \frac{H}{K_1} + \frac{K_2}{H}}$$

Treatment to find $H_{\text{opt.}}$, the value of H when v attains its maximum value $v_{\text{opt.}}$, is similar to that given above in deriving eqns. (3) and (4), and shows that $H_{\text{opt.}} = \sqrt{K_1 K_2}$ and that:

$$\frac{v}{v_{\text{opt.}}} = \frac{1 + 2\sqrt{K_2/K_1}}{1 + \frac{H}{K_1} + \frac{K_2}{H}} \quad (5)$$

or, putting $K_1 = m^2 K_2$ (i.e. defining m as $\sqrt{K_1/K_2}$):

$$\frac{v}{v_{\text{opt.}}} = \frac{1 + 2/m}{1 + \frac{H}{m^2 K_2} + \frac{K_2}{H}} \quad (6)$$

or, putting $K_2 = H_{\text{opt.}}/m$ (since $H_{\text{opt.}}^2 = K_1 K_2$ and $K_1 = m^2 K_2$):

$$v/v_{\text{opt.}} = \frac{m + 2}{m + \left(\frac{H}{H_{\text{opt.}}} + \frac{H_{\text{opt.}}}{H} \right)} \quad (7)$$

Features of the curve

As is well known, the curve of $v/v_{\text{opt.}}$ against pH is symmetrical about $\text{pH}_{\text{opt.}}$, as is clear from eqn. (7) by the same reasoning as from eqn. (4), since both equations contain the term $\left(\frac{H}{H_{\text{opt.}}} + \frac{H_{\text{opt.}}}{H}\right)$ as their only variable. When m is very large the curve takes the broad form shown in Fig. 2, in a way similar to eqn. (4) when μ was very large or very small. In this case $\text{p}K_1$ will be on the left and $\text{p}K_2$ on the right.

Width of the curve

Alberty & Massey (1954) pointed out that useful information could be obtained from the values of H , H_1 and H_2 , at which $v/v_{\text{opt.}}$ attained half its maximum value. From eqn. 7, the condition for this is that:

$$m + \frac{H}{H_{\text{opt.}}} + \frac{H_{\text{opt.}}}{H} = 2 \left(m + \frac{H_{\text{opt.}}}{H_{\text{opt.}}} + \frac{H_{\text{opt.}}}{H_{\text{opt.}}} \right)$$

and this simplifies to:

$$H^2 - (m+4)H_{\text{opt.}}H + H_{\text{opt.}}^2 = 0 \quad (8)$$

The sum of the roots H_1 and H_2 of eqn. (8) is given by:

$$H_1 + H_2 = mH_{\text{opt.}} + 4H_{\text{opt.}} = K_1 + 4H_{\text{opt.}} \quad (9)$$

Since H_1 , H_2 and $H_{\text{opt.}}$ can all be determined directly from the observed plot of activity (e.g. Fig. 3), K_1 can be found, and hence K_2 .

The function needed to find the width of the peak of the graph of $v/v_{\text{opt.}}$ against pH at half its maximum height is $H_1/H_{\text{opt.}}$, where H_1 is the larger root of eqn. (8).

$$\text{Since } H_1/H_{\text{opt.}} = \frac{m+4 + \sqrt{m^2+8m+12}}{2} \quad (10)$$

values for the width ($2 \log H_1/H_{\text{opt.}}$) can be tabulated (Table 1) as done by Alberty & Massey (1954). This function has no true minimum; it gets smaller as m

Fig. 3. *Narrow peaks of pH-dependence*

— represents the sharpest the peak can get ($m=0$);
 ---- represents the sharpest it can get without cooperative protonation ($m=2$), and this is its form when the proton-binding sites are identical and independent; this is also the narrowest the peak can be when the pH-dependence is due to a change of rate-determining step with pH ($\mu=1$).

Table 1. *Peak widths of various pH-dependence curves*

The curves are those of activity against pH when the activity is proportional to one ionic form whose protonation ($\text{p}K_1$) or deprotonation ($\text{p}K_2$) gives an inactive form. The $\text{p}K$ values given are molecular (macroscopic). The widths are calculated from eqn. (10). The same curves can also represent activity when the pH-dependence is due to a change of rate-determining step with pH, provided that $\text{p}K_2 \geq \text{p}K_1 + 0.6$.

$\text{p}K_2 - \text{p}K_1$ (i.e. $2 \log m$)	Width of curve at half maximum height	$m = \sqrt{K_1/K_2}$	$H_1/H_{\text{opt.}}$
5	5.01		
4	4.03	100	
3	3.10		
2	2.28	10	$7+4\sqrt{3}$
1	1.69	2	$3+2\sqrt{2}$
0.6*	1.53	1	$\frac{5+\sqrt{21}}{2}$
0	1.36	0	$2+\sqrt{3}$
-1	1.22		
-2	1.17	0.1	
$-\infty$	1.14	0	

* This is the case of identical and independent proton-binding sites.

diminishes, so that its lowest value occurs when $m = 0$, and the peak is then 1.14 pH units wide (at half its maximum height). At this limit the concentrations of AH^- and HA^- become infinitesimal in comparison with $[A]$.

Comparison of the Two Situations

Eqns. 4 and 7 (similarly eqns. 3 and 6) are very similar, and they become identical if:

$$m = \mu + 1/\mu \quad (11)$$

By its definition as $k_{-1}^{\frac{1}{2}} k_{+2}^{\frac{1}{2}} K'^{\frac{1}{2}} K^{-\frac{1}{2}}$, μ can have any positive value from 0 to ∞ . Differentiation of eqn. (11) shows that m is minimal when $\mu = 1$, and the value of m is then 2. By definition, however, m is $\sqrt{K_1/K_2}$ and can have any value from 0 to ∞ .

Hence eqn. (4) can be represented by eqn. (7) for any value of μ . Eqn. (7), however, can exist when $0 < m < 2$ but then there is no identical form of eqn. (4), since m cannot then obey eqn. (11). Eqn. (7) shows some interesting features when $m < 2$, although, as shown below, this represents an exceptional situation.

Just as eqn. (8) was derived from eqn. (7), eqn. (4) similarly gives an equation for the values of H at which $v/v_{opt.}$ is half-maximal:

$$\mu H^2 - (1 + 4\mu + \mu^2)H_{opt.} H + \mu H_{opt.}^2 = 0 \quad (12)$$

This equation is equally well derived by combining eqns. (8) and (11).

The sum of the roots of this equation is given by:

$$H_1 + H_2 = (1 + 4\mu + \mu^2)H_{opt.}/\mu$$

Unlike eqn. (9) (i.e. $H_1 + H_2 = K_1 + 4H_{opt.}$; Alberty & Massey, 1954), this does not give a useful way of finding K . Hence in cases where K is close to $\mu^2 K$ in eqn. (4), it may be better to find K_1 and K_2 by eqn. (9), i.e. to make use of the fact that some value of m allows eqn. (7) to be used instead of eqn. (4) for any value of μ . But the values thereby found are not K and $\mu^2 K$, although they approach them when μ is large, since $m = \mu + 1/\mu$ (eqn. 11), so that $m \rightarrow \mu$ when $\mu \gg 1$. Use of eqn. (11) would then allow calculation of $\mu^2 K$ and K from K_1 and K_2 , since $\mu K = H_{opt.} = mK_2$.

The minimum width of the curve of $\frac{v}{v_{opt.}}$ against pH for eqn. (4) can be found either from the solutions of eqn. (12)

$$[H_1/H_{opt.} =$$

$$(1 + 4\mu + \mu^2 + \sqrt{1 + 8\mu + 14\mu^2 + 8\mu^3 + \mu^4})/2\mu$$

and this shows a true minimum when $\mu = 1$, or, more simply, from eqn. 8 when $m = 2$. Then, from eqn.

$$(10), 2 \log \frac{H_1}{H_{opt.}} \text{ is } 2 \log (3 + 2\sqrt{3}) \text{ or } 1.53 \text{ pH units, as}$$

entered in Table 1. This minimum width occurs when $\mu = 1$ so that the limiting curves (Fig. 1) are the same height.

The meaning of m

So far the dissociations of HAH have been specified only by the molecular constants K_1 and K_2 . But as shown in Scheme 2 (Edsall & Wyman, 1958) this is not a complete description. The circumstances in which K_1 can fall below $4K_2$, i.e. when m can fall below 2, and so when Scheme 1(a) can give curves that cannot be given by Scheme 1(b), are noteworthy. They can be investigated by expressing K_b (Scheme 2) as $a \cdot K_a$, i.e. by supposing that group B is a stronger acid than group A by a factor a , and that the degree to which each group is strengthened as an acid by protonation of the other is a factor q , i.e. $K'_a = q \cdot K_a$ and $K'_b = q \cdot K_b$. From these definitions:

$$\frac{K_1}{K_2} = m^2 = q \cdot \frac{(a+1)^2}{a}$$

Hence m^2 is minimal when $a = 1$, and then $m^2 = 4q$. Hence m cannot be less than 2 when $q \geq 1$, i.e. when the loss of the two protons is not co-operative.

When the proton-binding groups are identical (in acidity; $a = 1$) and independent ($q = 1$), then K_1/K_2 has its statistical value of 4. Since, however, protonation of a nearby group usually raises the dissociation constant of a particular group, q usually exceeds unity, sometimes very greatly. Thus for the thiol and amino groups of cysteine $q = 10^{1.50}$, i.e. $pK_a - pK'_a = 1.50$ (see Edsall & Wyman, 1958), and for 3-hydroxypyridine $q = 10^{2.89}$ (Metzler & Snell, 1955). Co-operative binding of protons can, of course, occur, especially

Scheme 2. Relationship between molecular and group dissociation constants

HAH is a substance that can lose a proton from each of two groups. Group A has dissociation constant K_a when group B is dissociated and K'_a when group B is protonated. Other constants are as shown. The molecular constants K_1 and K_2 are given by $K_1 = K'_a + K'_b$ and $\frac{1}{K_2} = \frac{1}{K_a} + \frac{1}{K_b}$.

if two protons exchange for one other ligand (chelation), but it is relatively rare. Only if it occurs can the curve of rate against pH exhibit a sharper maximum under eqn. (7) than under eqn. (4).

It is important to note that K_2 can exceed K_1 only because of co-operative effects, since K_1 is the dissociation constant of the first proton to dissociate from HAH. If activity required, for example, that a group of pK_5 should be protonated, and one of pK_9 should be unprotonated, this would not represent a cross-over of pK_1 and pK_2 , since pK_1 and pK_2 are molecular, not group, constants. Such a case can be represented in Scheme 2 if pK'_b is 5 and pK_a is 9, and HA^- , not AH^- , is the active form. Provided that q is not very far from unity, i.e. the groups are largely independent, then the large difference between pK'_b and pK_a means that a is large. Hence pK_1 is almost pK'_b (i.e. 5) and pK_2 is almost pK_a (i.e. 9), even though the group pK values for protonating and deprotonating the active form HA^- are 9 and 5, not 5 and 9. This is an application of the well-known principle (see Jencks, 1969) that pH-dependence cannot indicate the position of a proton, in this case whether it is required on the group of pK_5 or on that of pK_9 .

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