Relations between the dissociation constants of dibasic acids

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The relationships between the molecular pK values of a dibasic acid, its titration pK values and the pH values at which the concentration of monohydrated species is half-maximal are presented. These enable any pair of the values to be found from any other pair.

INTRODUCTION

Enzymes often show bell-shaped curves of activity against pH , most simply explained by supposing that addition of H^+ to one group in the enzyme activates, and addition to another deactivates, the enzyme. Hence the pH -dependence of the concentration of the singly ligated form of a dibasic acid provides a model for the pH -dependence of enzyme activity (see, e.g., Tipton & Dixon, 1979).

Dibasic acids are easy to consider when their molecular pK values are well separated, because each dissociation of a hydron, H^+ , can then be treated as a one-site dissociation. Complexities arise if the second hydron starts to dissociate appreciably as the pH is raised while the first is still appreciably associated. Since dissociation at one group changes the dissociation constant at the other, **group** constants change during titrations, so are not useful for considering the dissociation, and indeed they cannot be determined (Dixon, 1976), so **molecular** constants are used. These are the constants K_1 and K_2 , defined (Adams, 1916) for a dibasic acid, H_2A , such that $K_1 = [H^+][HA^-]/[H_2A]$, and $K_2 = [H^+][A^{2-}]/[HA^-]$, where $[HA^-]$ represents the sum of the concentrations of the two species formed by losing H^+ from H_2A , one species formed by losing it from one acidic group, and the other species by losing it from the other group. Hence K_1 is the dissociation constant for the loss of the first hydron, irrespective of how this loss is divided between the two acidic groups, and K_2 that for the loss of the second.

Molecular pK values (i.e. $-\log K$, where K is a molecular dissociation constant) are useful in considering pH -dependences. This is because the concentration of each of the two species whose sum is $[HA^-]$ is a fixed fraction of $[HA^-]$. Hence the pH -dependence of each concentration is the pH -dependence of $[HA^-]$, so it is this dependence that is of interest.

Michaelis (1914) pointed out that the fractional concentration of HA^- , i.e. $[HA^-]/([H_2A] + [HA^-] + [A^{2-}])$, or more simply $[HA^-]/[A]_t$, is equal to $1/(1 + [H^+]/K_1 + K_2/[H^+])$, and he gave similar functions for the concentrations $[H_2A]$ and $[A^{2-}]$.

Molecular constants differ from one-site constants. Thus a molecule with two identical and independent groups of dissociation constant K will have molecular constants of $2K$ and $K/2$. So for much physical chemistry 'statistical correction' of the molecular constants has to be applied. The simplest treatment in terms of one-site constants is that of the **titration** constants given by Simms (1926). He pointed out that the titration of a

dibasic acid was identical with that of an equimolar mixture of two monobasic acids whose dissociation constants are the titration constants of the dibasic acid. He defined the values of the titration constants, G_1 and G_2 , such that $K_1 = G_1 + G_2$ and $1/K_2 = 1/G_1 + 1/G_2$.

The concentration $[HA^-]$ can be expressed as the product of the concentrations of the hydronated form of one monobasic acid and of the dehydronated form of another. The dissociation constants of these two (hypothetical) monobasic acids are again the titration values given by Simms (1926), and they are unreal if $K_1 < 4K_2$ (Dixon, 1975).

Another easily measured pair of constants are the values of $[H^+]$, H_1 and H_2 , at which $[HA^-]$ is half its maximum value (Alberty & Massey, 1954).

All three of the pairs pK_1 and pK_2 , pG_1 and pG_2 and pH_1 and pH_2 have the same mean. Further, pK_1 , pG_1 and pH_1 will all approach one value, and pK_2 , pG_2 and pH_2 another, if the values in any pair differ greatly. But if the two constants of a pair are close together, then the three pairs all differ.

RESULTS AND DISCUSSION

Dixon (1979) gave functions for calculating each pair of pK or pH values from one or both of the other pairs. The main use of this is to obtain molecular and titration values from the easily observed width of a bell-shaped curve. Titration values are useful because they provide factors of simple form for analysing more complex pH -dependence (see, e.g., Dixon *et al.*, 1987).

Table 1 in Dixon (1979) was somewhat complex in form, which may have limited its usefulness. Table 1 of the present paper gives the relationships more completely, and more simply, as it is in terms of three numbers, m , p and q , defined so that $2 \log m$ is the difference in molecular pK values, $2 \log p$ is the difference in titration pK values, and $2 \log q$ is the difference in half-height pH values. Thus, for example, the width at half height of a bell of pH -dependence, $2 \log q$, can be used to give q ; then, since $m = q - 4 + 1/q$, this enables the difference in molecular pK values, $pK_2 - pK_1$, to be given as $2 \log(q - 4 + 1/q)$ (which may be negative).

Table 1 brings out several regularities. Line 1 and column 1 show that q is the same function of $m + 4$ as p is of m . A change of sign in any of the $\pm$ signs in the Table changes the sign of the logarithm that contains it, since this is always of the form $\log[x \pm \sqrt{(x^2 - 1)}]$. These $\pm$ signs occur only in the expressions for p and q , since there is no physical significance in interchanging the titration constants or the half-height pH values; this is

Table 1. Relationships between various kinds of dissociation constants of dibasic acid

The molecular pK values, pK_1 and pK_2 , the titration pK values, pG_1 and pG_2 , and the pH values at which the monohydrated form is at half its maximal value, pH_1 and pH_2 , all have the same mean, pK^* . The constants m , p and q are defined by letting $2\log m = pK_2 - pK_1$, $2\log p = pG_2 - pG_1$ and $2\log q = pH_2 - pH_1$; thus $K_1/K_2 = m^2$, $K_1/K^* = m$, $G_1/K^* = p$, $H_1/K^* = q$ etc. Then the expressions for each of these in terms of the others are:

$$m = p + 1/p = q - 4 + 1/q$$

$$p = [m \pm \sqrt{(m^2 - 4)}]/2 = [q^2 - 4q + 1 \pm (q - 1)\sqrt{(q^2 - 6q + 1)}]/2q$$

$$q = [m + 4 \pm \sqrt{(m^2 + 8m + 12)}]/2 = [p^2 + 4p + 1 \pm (p + 1)\sqrt{(p^2 + 6p + 1)}]/2p$$

Most of the derivations are given by Dixon (1979); the others follow simply. These relations give the following entries.

Difference between molecular pK values ($pK_2 - pK_1$)	Difference between titration pK values ($pG_2 - pG_1$)	Difference between pH values at which the concentration of the monohydrated form is half maximal ($pH_2 - pH_1$)
$2\log m$ $2\log(p + 1/p)$ $2\log(q - 4 + 1/q)$	$2\log\{[m \pm \sqrt{(m^2 - 4)}]/2\}$ $2\log p$ $2\log\{[q^2 - 4q + 1 \pm (q - 1)\sqrt{(q^2 - 6q + 1)}]/2q\}$	$2\log\{[m + 4 \pm \sqrt{(m^2 + 8m + 12)}]/2\}$ $2\log\{[p^2 + 4p + 1 \pm (p + 1)\sqrt{(p^2 + 6p + 1)}]/2p\}$ $2\log q$

Fig. 1. Dependences on the difference in molecular pK values of a dibasic acid of (curve *A*) the difference in titration pK values and of (curve *B*) the width at half height of the pH-dependence of the concentration of its monohydrated form, HA^- .

Positive co-operativity of hydronation can allow the difference in molecular pK values to be negative, i.e. the dissociation constant for the second hydron to be greater than that for the first. If this occurs, the titration constants cease to be real.

reflected in the fact that $pK_2 - pK_1$ is the same function of p as of $1/p$, and of q as of $1/q$ (column 1). By contrast, a change in sign of $pK_2 - pK_1$ is a change in the co-operativity of hydronation. A further point to notice is the similarity of the expressions for p in terms of q , and for q in terms of p , the new entries in Table 1. This results from the fact that $p + 1/p = q + 1/q - 4$, since both these quantities are equal to m .

Six plots are needed to show how the difference between the two constants of one type depends on the difference between the two constants of any other, but only one of these (Fig. 1, curve *B*), giving the width of the bell-shaped curve at half its height as a function of

Fig. 2. Dependences on the difference in titration pK values of a dibasic acid of (curve *A*) the difference in molecular pK values and of (curve *B*) the width at half height of the pH-dependence of the concentration of its monohydrated form, HA^- .

difference in molecular values, has been published (Tipton & Dixon, 1979). All six graphs are shown in Figs. 1-3.

The main utility of graphs is to make relationships plain. The titration pK values are unreal if $pK_2 - pK_1 < 0.6$ (a condition implying positively co-operative hydronation). From Fig. 1 it is clear that these titration pK values are identical when $pK_2 - pK_1 = 0.6$ (the situation for identical and independent groups). As the difference in molecular pK values, $pK_2 - pK_1$, rises, the difference in titration pK values rapidly approaches it, with the two types of pK differing negligibly when $pK_2 - pK_1$ exceeds about 1.2.

Fig. 1 also shows the way the width at half height of the bell representing the pH-dependence of $[AH^-]$ approaches a limiting value of 1.14 [i.e. $2\log(2 + \sqrt{3})$;

Fig. 3. Dependences of the differences in (curve A) molecular pK values and (curve B) titration pK values on the width at half height of the pH-dependence of the concentration of the monohydrated form, HA^- , of a dibasic acid

Dixon (1973)] as the binding becomes progressively more positively co-operative, so that the bell falls towards zero in height. This width is 1.53 [i.e. $2\log(3+2\sqrt{2})$] when $pK_2 - pK_1 = 0.6$, the lowest it can get without positive co-operativity. The difference in molecular pK values has to reach much larger values for it to differ negligibly from

the width at half height than for it to differ negligibly from the difference in titration values.

Fig. 2 shows these points in another way: the difference in molecular values rapidly approaches the difference in titration values as this rises above zero, whereas the width at half height approaches more slowly.

Fig. 3 brings out the difficulty of measuring the molecular pK values when the hydrations are positively co-operative; the slope of the line of difference in molecular pK values against width at half height is so steep, being infinite at first and remaining high up to a width of about 2. Similarly the difference in titration pK values is hard to measure until it has become about unity, when the titration pK values approach the molecular values.

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