Factorization of the Michaelis Functions

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(Received 22 May 1975)

Each Michaelis function that expresses the concentration of one of the species AL_2 , AL and A in terms of the concentration of free ligand (or its logarithm) is the product of two functions each of which represents the degree of ligation or de-ligation of a single site. These hypothetical sites have pK values of $pK^* \pm \log(\alpha + \sqrt{\alpha^2 - 1})$, where pK^* and α are defined by writing the two molecular pK values as $pK^* \pm \log 2\alpha$. The factors are thus real if $\alpha \geq 1$, i.e. if the binding of L by A is not positively co-operative. The dependence of $[AL]$ on $\ln[L]$ is compared with relations that represent other ligand-dependent equilibria.

Michaelis (1914) gave expressions for the concentrations of the three forms AL_2 , AL and A of a molecule that possesses two sites for a ligand L ; these expressions were functions of the concentration of free ligand. Various related expressions have since been used. The present paper shows that the expression for any one of these species is the product of two factors, each of which has the same dependence on the concentration of free ligand as has the degree of ligation or de-ligation of a molecule with a single site.

Theoretical

(a) Presentation

In order to present the expressions of Michaelis (1914) for $[AL_2]$, $[AL]$ and $[A]$ explicitly on a logarithmic scale, such as is frequently used for the concentration of free ligand, $[L]$, Dixon (1974), following Haldane (1930) and Irving & Williams (1949), used hyperbolic functions. The equations could be expressed in terms of a parameter $\alpha = \sqrt{K_1/4K_2}$ (cf. Bjerrum, 1941; α is his 'spreading factor'), where K_1 and K_2 are the molecular dissociation constants for the first and second ligand molecules to dissociate, and in terms of a variable $x = \ln[L] - \frac{1}{2} \ln K_1 K_2$. Thus $x = \pm \ln 2\alpha$ when $[L] = K_1$ and when $[L] = K_2$. It is also convenient to define $K^* = \sqrt{K_1 K_2}$, so that $x = \ln[L] - \ln K^*$. This notation is used below.

(b) Extreme forms AL_2 and A

If AL_2 is the bi-ligated form of a molecule A that can bind two molecules of ligand L , and if $[AL_2]/[A]$, i.e. the relative concentration of AL_2 , is called y , then in the terms used by Michaelis (1914; see also Dixon & Webb, 1958; Edsall & Wyman, 1958)

$$y = 1/(1 + K_1/[L] + K_1 K_2/[L]^2)$$

Since quadratic expressions have factors, this is the product of two terms $1/(1 + A/[L])$ and $1/(1 + B/[L])$, where A and B are given by $\frac{1}{2}K_1(1 \pm \sqrt{1 - 4K_2/K_1})$. In alternative expressions for y , such as $\frac{1}{2} - \frac{1}{2}(\alpha - \sinh x)/(\alpha + \cosh x)$ (Dixon, 1974, eqn. 5), or $e^x/(2\alpha + e^x + e^{-x})$, or $\frac{1}{2}(\sinh x + \cosh x)/(\alpha + \cosh x)$, factorization is simplified by substituting $\cosh d$ for α . In these terms the two factors are $1/(1 + e^{-(x+d)})$ and $1/(1 + e^{-(x-d)})$, which may also be expressed as $\frac{1}{2} + \frac{1}{2} \tanh \frac{1}{2}(x \pm d)$. These forms show that each factor is the expression that represents the degree of ligation of a single site.

The conclusion that the concentration of the doubly ligated form is given by the product of two one-site saturation functions is obvious if the two sites are independent, because the fraction of molecules doubly ligated is simply the product of the degree of ligation at one site and of the degree of ligation at the other. The above factorization generalizes the conclusion to interacting sites, where the filling of each of them follows not a one-site saturation curve, but one that may be doubly sigmoid (Dixon & Tipton, 1973; Dixon, 1974). It is restricted, however, to bindings of negative or zero co-operativity, because if the co-operativity is positive, $4K_2$ can exceed K_1 , so that α can fall below unity and no real value of d can exist; the factors then cease to be real.

If the sites are identical and independent, $\alpha = 1$ and the co-operativity is zero. Since $\alpha^2 = (2 + K_A/K_B + K_B/K_A) \cdot K_A/4K'_A$ (Dixon & Tipton, 1973) where K_A and K_B are the dissociation constants of groups A and B when the other group is ligated, and K'_A is the dissociation constant of group A when group B is not ligated (Scheme 1), sites with different dissociation constants can also give a value of α of unity, i.e. zero co-operativity at the molecular level, but only if $K'_A > K_A$, i.e. when there is positively co-operative binding at the group level. When $\alpha = 1$, $d = 0$, so that the factors are identical and y becomes the square of

Scheme 1. Ligation of a molecule with two binding sites

The molecular dissociation constants K_1 and K_2 are related to the groups constants shown by the equations $K_1 = K_A + K_B$ and $1/K_2 = 1/K_A' + 1/K_B'$ (see, for example, Edsall & Wyman, 1958).

the degree of ligation at a single site. Under these conditions $y = 1/4$ when $x = 0$, and the curve shows a point of inflexion when $y = 4/9$.

When α is large, i.e. when $K_1 \gg K_2$, y approaches the one factor of $\frac{1}{2} + \frac{1}{2} \tanh \frac{1}{2}(x-d)$ and it thus exhibits a one-site ligation curve. This factor then approaches $[L]/([L] + K_1)$. The other factor, i.e. $\frac{1}{2} + \frac{1}{2} \tanh \frac{1}{2}(x+d)$, which under these conditions approaches $[L]/([L] + K_2)$, can be ignored because it will be almost unity except at values of x (or $[L]$) that make the factor $\frac{1}{2} + \frac{1}{2} \tanh \frac{1}{2}(x-d)$ close to zero.

The concentration of the non-ligated species A can be similarly treated.

(c) The intermediate form AL

The relative concentration of the intermediate form AL is given (Haldane, 1930; Dixon, 1974) by the expression $z = [AL]/[A]_t = \alpha/(\alpha + \cosh x)$. The fractional concentrations of the three species AL_2 , AL and A are thus given by $e^x/(2\alpha + e^x + e^{-x})$, $2\alpha/(2\alpha + e^x + e^{-x})$ and $e^{-x}/(2\alpha + e^x + e^{-x})$. When, as above, α is replaced by $\cosh d$, z can be expressed as

$$\frac{e^d + e^{-d}}{e^d} \cdot \frac{1}{1 + e^{-(x+d)}} \cdot \frac{1}{1 + e^{(x-d)}}$$

that is, as $(1 + e^{-2d})$ multiplied by the product of the degree of saturation of a single site that exhibits a dissociation constant of $K^* \cdot e^d$ [i.e. the function $1/(1 + e^{-(x+d)})$, or $\frac{1}{2} + \frac{1}{2} \tanh \frac{1}{2}(x+d)$] and the degree of desaturation of a single site of dissociation constant $K^* \cdot e^{-d}$ [i.e. the function $1/(1 + e^{(x-d)})$, or $\frac{1}{2} - \frac{1}{2} \tanh \frac{1}{2}(x-d)$].

The quantity z is equally well expressed as

$$\frac{e^d + e^{-d}}{e^{-d}} \cdot \frac{1}{1 + e^{-(x-d)}} \cdot \frac{1}{1 + e^{(x+d)}}$$

that is, as $(1 + e^{2d})$ multiplied by the products of the same two saturation and desaturation functions with their dissociation constants interchanged (Fig. 1). The constants $1 + e^{\pm 2d}$ by which the products of the functions are multiplied are $K_1(1 \pm \sqrt{1 - 4K_2/K_1})/2K_2$.

A third expression for the relative concentration of AL is given by

$$z = \frac{1}{1 + e^{-(x+d)}} \cdot \frac{1}{1 + e^{(x-d)}} + \frac{1}{1 + e^{-(x-d)}} \cdot \frac{1}{1 + e^{(x+d)}}$$

It is thus obtained by adding the product of the saturation function characterized by one dissociation constant ($K^* \cdot e^{-d}$) and the desaturation function characterized by the other constant ($K^* \cdot e^d$) to the product of the saturation and desaturation functions with the constants interchanged.

Again this conclusion is obvious if the sites are independent, because one form of AL is obtained if one site (A) is ligated and the other (B) is de-ligated, and these ligations will follow one-site curves. The other form of AL is obtained by ligation at site B but not at site A. Indeed if the sites are independent, then α^2 simplifies to $(2 + K_A/K_B + K_B/K_A)/4$, so that the dissociation constants characterizing the factor functions (i.e. $K^* \cdot e^{\pm d}$) reduce to the group dissociation constants K_A and K_B . But the above treatments show that the same applies to the filling of negatively interacting sites (which do not individually follow one-site saturation curves).

(d) The nature of the factors

The functions of saturation and desaturation that are factors of the Michaelis functions are plotted in Fig. 1. They have dissociation constants of $\frac{1}{2}K_1(1 \pm \sqrt{1 - 4K_2/K_1})$, or more simply, $K^* \cdot e^{\pm d}$. Since $d = \ln(\alpha + \sqrt{\alpha^2 - 1})$, the pK values of the curves are $pK^* \pm \log(\alpha + \sqrt{\alpha^2 - 1})$ [i.e. $pK^* + \log(\alpha \pm \sqrt{\alpha^2 - 1})$], whereas pK_2 and pK_1 are $pK^* \pm \log 2\alpha$. Thus the pK values of the functions that form the factors do not differ significantly from pK_1 and pK_2 when $\alpha \gg 1$.

Dixon (1974) pointed out that provided that $\alpha \geq 1$, i.e. that the binding is not positively co-operative, the actual degree of ligation of a molecule with two binding sites was the sum of two functions each of the form of the degree of ligation of a single site (and that measurements of the degree of saturation could therefore not distinguish between independent and interacting sites). It is exactly these two functions (i.e. those whose sum gives the degree of saturation) that now prove to be the factors of the Michaelis functions for the species present, and which are shown in Fig. 1.

It should be emphasized that there are no actual sites or molecular species present with these dissociation constants. The constants K_1 and K_2 are the molecular constants for the first and second molecules of ligand to dissociate, in whatever way this

Fig. 1. Factorization of Michaelis functions into one-site binding functions

The bell-shaped curve shows the concentration of AL in the AL_2 -AL-A system as a fraction of all forms of A. It is plotted for a value of $\sqrt{K_1/4K_2} = \alpha = \cosh d$ of 1.25 from the equation $[AL]/[A]_t = \alpha/(\alpha + \cosh x)$. It is given first by $(1 + e^{-2d})$ times (here 1.25 times) the product of functions a and b , the one-site desaturation and saturation functions with mid-points at $x = \pm d$ (here ± 0.69), secondly by $(1 + e^{2d})$ times (here 5 times) the product of functions c and d , the same as functions a and b but with mid-points interchanged, and thirdly by the sum of the product of functions a and b and the product of functions c and d . Functions a , b , c and d are likewise factors for the other Michaelis functions, since the product of functions a and c gives the concentration of AL_2 and the product of b and d gives the concentration of A. No such simple relations exist between $[A]$, $[AL]$ and $[AL_2]$ and one-site functions of dissociation constants K_1 and K_2 , i.e. those with mid-points at $x = \pm \ln 2\alpha$ (here ± 0.92).

dissociation may be partitioned between the two actual sites.

Discussion

The factorization of the Michaelis functions helps to correlate the several ways in which the well-known bell-shaped curve of general formula (Haldane, 1930; Dixon, 1974) $y = \alpha/(\alpha + \cosh x)$, alternatively $y = 1/(1 + a \cosh x)$ where $a = 1/\alpha$, can arise. This curve, expressed as a fraction of its maximum, has the form $(\alpha + 1)/(\alpha + \cosh x)$. When y represents the concentration of uni-ligated form, AL, considered above, α can be less than unity, but only if the ligand binding is positively co-operative, and the curve can then be as sharp as $1/\cosh x$ (Dixon, 1973, 1974).

Provided that $\alpha \geq 1$, then, as shown above, the expression has factors, and these are simple one-site ligation and de-ligation expressions. Thus the product of the concentrations of the ligated form of any molecule present and the unligated form of any other gives

the same expression; hence the frequent occurrence of this dependence in chemical kinetics. The sharpest peak in this commoner case is $2/(1 + \cosh x)$, which gives a width at half the maximal height of $1.53 \log$ units (Dixon, 1973). Wood (1974) noted that the product of the concentration of an acid and its conjugate base had this form. Dixon (1973) noted that this was also the sharpest possible dependence on ligand concentration for the rate of a reaction whose rate-determining step changed because of the binding or liberation between two steps of one ligand molecule.

Another rather different phenomenon gives the same type of curve; John & Jones (1974), in considering electrophoretic mobilities of acids, gave the expression for the difference in degree of ligation of two substances that have different dissociation constants. If the saturation curves of the two substances are represented as $\frac{1}{2} + \frac{1}{2} \tanh \frac{1}{2}(x \pm d)$, the difference between these is $(\sinh d)/(\cosh d + \cosh x)$, and thus differs only in amplitude and not in form from the expression $(\cosh d)/(\cosh d + \cosh x)$ used above for the concentration of uni-ligated form. This difference in ligation between two binding molecules depends most sharply on ligand concentration as their two dissociation constants approach each other; its limit is clearly the slope of the one-site binding curve, which has already been noted to be of this form (Irving *et al.*, 1949; Dixon, 1974).

Thus the expression for the bell-shaped curve arises in (1) the concentration of a uni-ligated form, (2) the product of concentrations of a ligated species and an unligated species, (3) the rate dependence of a reaction with a change of rate-determining step, and (4) the difference between two one-site ligations. The present factorization relates the first two of these. It applies only when binding is not positively co-operative, and therefore excludes the one condition when the first of these cases gives a sharper dependence on $\log [L]$ than any of the others can.

I thank Dr. J. L. Wood, Professor M. Dixon, Dr. M. A. H. MacCallum and Dr. K. F. Tipton for valuable advice, and I thank Dr. Wood for showing me his paper before it was published.

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