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The Pre-eminence of $k_{cat.}$ in the Manifestation of Optimal Enzymic Activity Delineated by using the Briggs–Haldane Two-Step Irreversible Kinetic Model

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1. The suggestion by Fersht [(1974) *Proc. R. Soc. London Ser. B* **187**, 397–407] that enzymes that provide maximal rates of catalysis should be characterized by values of K_s, the dissociation constant of the enzyme–substrate complex, greater than 10 times the value of the ambient substrate concentration has been examined. 2. For such enzymes, K_s is not relevant, and attention is best focused on the relative numerical values of $k_{cat.}$ (in units of s^{-1}) and the substrate molarity. It is necessary only that the former be about 10^{10}–10^{11} times the latter to ensure that the rate of product formation be diffusion-limited and thus maximal.

Simple aspects of enzyme catalysis are sometimes discussed in terms of the kinetic model given in Scheme 1 and its characterizing parameters K_m and $k_{cat.}$, with $K_m = (k_{-1}+k_{cat.})/k_{+1}$ if the usual Briggs & Haldane (1925) conditions apply. For this model, it is only when substrate binding is maintained at equilibrium (Michaelis & Menten, 1913), i.e. $k_{cat.} \ll k_{-1}$, that K_m is equal to K_s, the dissociation constant of the enzyme–substrate complex (i.e. k_{-1}/k_{+1}).

Fersht (1974) discussed enzyme catalysis in terms of Scheme 1 and suggested that an enzyme that has evolved to achieve maximum rates of catalysis should be characterized by a value of K_s more than 10 times greater than the usual substrate concentration *in vivo*. The implication that K_s is provided by experimentally determined values of K_m (Fersht, 1974) is not an essential part of the argument provided that it is expressed as statements about K_m, not about K_s. The advantage of identifying K_m with K_s is that it permits Scheme 1 to be discussed in simple thermodynamic terms, and both Fersht (1974) and Cornish-Bowden (1976) found this to be useful; but this advantage is clearly outweighed by the doubtful validity of the assumption.

In this present paper we show that for an enzyme that is close to catalytic perfection K_s is most unlikely to be approximated by K_m, and it is therefore inappropriate to use K_s as a parameter to characterize such an enzyme. Instead, attention should be focused on $k_{cat.}$: it is necessary only to compare $k_{cat.}$ with the likely substrate concentration, [S] (allowing for the difference in dimensions), to determine whether such an enzyme is operating under conditions of substrate saturation.

For many real enzymes, and certainly for a hypothetical enzyme close to catalytic perfection, the rate constant for the formation of ES approaches the diffusion-controlled limit. Thus k_{+1} would be expected to be about $10^9 M^{-1} \cdot s^{-1}$ (Peller & Alberty, 1959; see also Knowles, 1976), but might be as high as $10^{10} M^{-1} \cdot s^{-1}$ (Chou & Jiang, 1974; Li & Chou, 1976). We use the latter value to illustrate the discussion in the remainder of this present paper, but the main character of the conclusions would be unaffected by assuming a lower value for k_{+1}.

The question of the effectiveness with which the enzyme catalyses the conversion of S into P is concerned with the relative values of k_{-1} and $k_{cat.}$. For an enzyme far removed from catalytic perfection, $k_{cat.}$ might be much less than k_{-1}, so that $K_m = K_s$. [This may apply rarely *in vivo* even though several examples are known for enzymes studied extensively *in vitro*, because many of the most detailed studies have been made with an atypical group of enzymes, the extracellular hydrolases, with unnatural substrates under unnatural conditions. For example, a recent book,

$$E+S \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} ES \xrightarrow{k_{cat.}} E+P$$

Scheme 1. *Enzyme catalysis depicted in terms of the Briggs & Haldane* (1925) *kinetic model*

Enzyme Structure and Function (Blackburn, 1976), is entirely devoted to these enzymes and their close analogues, and it is common practice, though a dangerous one, to regard them as archetypes of enzymes in general; see Blackburn (1976), pp. 8–9.] Improvement from this state would best be achieved by an increase in $k_{cat.}$, not merely as such, but also in relation to k_{-1}. Only when $k_{cat.}$ has become appreciably larger than k_{-1} can $k_{cat.}/K_m$, the second-order rate constant for the whole reaction, approach the diffusion limit. Thus one would expect K_s for a highly efficient enzyme to be appreciably less than K_m.

If $k_{cat.} \gg k_{-1}$, Scheme 1 approaches the restricted mechanism envisaged by Van Slyke & Cullen (1914), in which both steps of the reaction are irreversible, and K_m approaches $k_{cat.}/k_{+1}$. Philipp & Bender (1973) have suggested that this situation may apply to the α-chymotrypsin-catalysed hydrolysis of its best synthetic substrates, though in this case $k_{cat.}/K_m$ is only $1.5 \times 10^7 \text{M}^{-1} \cdot \text{s}^{-1}$, far below the limit of $10^{10} \text{M}^{-1} \cdot \text{s}^{-1}$ proposed by Chou & Jiang (1974) and indeed well below the more traditional limit of $10^9 \text{M}^{-1} \cdot \text{s}^{-1}$. If k_{+1} is assumed to have its maximum value of $10^{10} \text{M}^{-1} \cdot \text{s}^{-1}$, and $k_{cat.}$ is assumed to be large compared with k_{-1}, K_m has the value of $10^{-10} \times k_{cat.}$ and the Michaelis–Menten equation takes the following form:

$$v = \frac{k_{cat.}[E]_T[S]}{10^{-10} \times k_{cat.} + [S]} \quad (1)$$

in which the factor 10^{-10} is not a pure number, but has the units M·s. This equation reveals the conditions under which such an enzyme approaches saturation with substrate: the substrate molarity must be at least $10^{-9} \times k_{cat.}$ with $k_{cat.}$ expressed in s^{-1} (i.e. $[S] \gg 10^{-10} \times k_{cat.}$). If this condition is satisfied any increase in rate would have to be brought about by an increase in $[E]_T$ or an increase in $k_{cat.}$, because eqn. (1) approximates to $v = k_{cat.}[E]_T$.

Eqn. (1) also reveals the condition for a maximum rate of catalysis: if $k_{cat.}$ (in s^{-1}) reaches a value of about 10^{11} times the substrate molarity, eqn. (1) simplifies to the following:

$$v \simeq 10^{10} \times [E]_T[S] \quad (2)$$

which is an expression of the diffusion limit to the rate of product formation. Even if k_{+1} is somewhat less than $10^{10} \text{M}^{-1} \cdot \text{s}^{-1}$ and subject to some variation, the comparison must be made between [S] and $k_{cat.}/k_{+1}$, not between [S] and K_s as suggested by Fersht (1974).

Thus, if catalytic efficiency is considered solely a matter of maximizing the rate of product formation (which is without doubt an oversimplification), the numerical value of $k_{cat.}$ in s^{-1} should be about 10^{10}–10^{11} times the numerical value of the substrate molarity. This is equivalent to the view that, for an enzyme close to catalytic perfection, i.e. one for which $K_m \simeq k_{cat.}/k_{+1}$, K_m should be about 10 times the substrate concentration (Fersht, 1974; Cornish-Bowden, 1976). For enzymes that are required to work under conditions of saturation, such as some digestive enzymes (Cornish-Bowden, 1976), it is sufficient for K_m to be about $0.1 \times [S]$, i.e. for the numerical value of $k_{cat.}$ in s^{-1} to be about 10^9 times the substrate molarity.

It is important to emphasize that, in the present paper, enzyme catalysis is discussed in terms of the very simple, two-step, irreversible kinetic model of Scheme 1. Before the nature of enzyme catalytic efficiency can be fully understood, it will be necessary to take account of various complications. Some of these may arise from the necessity to consider kinetic models more complex (and more realistic) than that given in Scheme 1 and others from factors such as those discussed by Cornish-Bowden (1976), i.e. the presence of products and other inhibitors, the high enzyme concentrations that sometimes exist *in vivo* and the need to control the activities of some enzymes.

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