Evolution of Enzyme Catalytic Power

CHARACTERISTICS OF OPTIMAL CATALYSIS EVALUATED FOR THE SIMPLEST PLAUSIBLE KINETIC MODEL

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1. Evolutionary changes in the structure of an enzyme that provide an increase in its K_m value are considered. Provided that K_m increases as a result of increases in the forward rate constants of the catalysis relative to the reverse rate constants, the enzyme catalyses the conversion of a fixed concentration of its substrate more rapidly when its structure provides that $K_m > [S]$ than when $K_m < [S]$. 2. Catalytic efficiency of enzymes is discussed in terms of the simplest plausible model, the Haldane [(1930) *Enzymes*, Longmans, London] reversible three-step model:

The rate equation for the forward reaction of this model (formation of P) may be written in the simple form:

$$v = \frac{k_{cat.}[E]_T[S]}{\left[\frac{k_{cat.}}{k_{+1}} \left(1 + \frac{k_{+1}}{k_{-3}} \cdot \frac{1}{K_{eq.}} + \frac{k_{-1}}{k_{+2}} \right) \right] + [S]}$$

$K_{eq.}$ is the equilibrium constant ($= [P]_{eq.}/[S]_{eq.}$), and $k_{cat.} = V/[E]_T$, where $[E]_T$ is the total enzyme concentration. 3. To assess the effectiveness of an enzyme, it is necessary only to determine the extent to which the constraints of a particular kinetic mechanism permit v_2 (v when $K_m \gg [S]$) to approach v_d (the diffusion-limited rate). 4. The value of the optimal rate of catalysis ($v_{opt.}$, the maximal value of v_2) is dictated by the equilibrium constant for the reaction, $K_{eq.}$; $v_2 = v_d/a$, where

$$a = \left(1 + \frac{1}{K_{eq.}} + \frac{k_{-1}}{k_{+2}} \right)$$

when k_{+1} is assumed equal to k_{-3} , and $v_{opt.} = v_d/a_{min.}$. When $K_{eq.} \gg 1$, it is necessary that $k_{+2} \gg k_{-1}$ for a to take its minimum value, $a_{min.}$; when $K_{eq.} \ll 1$, it is necessary only that $k_{+2} \gg K_{eq.} \cdot k_{-1}$, i.e. a can equal $a_{min.}$ even if $k_{+2} < k_{-1}$. When $K_{eq.} \gg 1$, $v_{opt.} = v_d$; when $K_{eq.} = 1$, $v_{opt.} = v_d/2$, and when $K_{eq.} \ll 1$, $v_{opt.} = K_{eq.} \cdot v_d$. 5. The analysis, together with predicted effects of evolutionary pressure, suggests that in practice the rates of the fastest enzyme-catalysed freely reversible reactions might be expected to be lower than the value of $k_{+1}[E]_T[S]$ by about an order of magnitude, particularly if $K_{eq.} < 1$. 6. The existing literature suggests that, in general, appropriate values of K_m have evolved for the provision of high rates of catalysis but that many values of $k_{cat.}$ are not large enough to provide optimal rates of catalysis unless the value of k_{+1} *in vivo* is lower than its value in free solution.

Although analysis of evolution has concentrated on evolution of structure, e.g. primary sequences of proteins (see Margoliash, 1972), interest is growing in exploring the ways in which enzyme function might respond to evolutionary pressure (see, e.g., Fersht, 1974; Cornish-Bowden, 1976a; Koshland, 1976; Margoliash *et al.*, 1976; Knowles, 1976).

One aspect of the effect of evolutionary pressure on enzyme function concerns optimization of catalytic power (see Cleland, 1976), and this has been discussed by Fersht (1974), Cornish-Bowden (1976a) and Brocklehurst & Cornish-Bowden (1976) in terms of the simple irreversible two-step mechanism of Briggs & Haldane (1925) shown in Scheme 1. It has

Scheme 1. *Irreversible two-step kinetic model of enzymic catalysis (Briggs & Haldane, 1925)*

Scheme 2. *Reversible three-step kinetic model of enzymic catalysis (Haldane, 1930)*

been conventional to discuss simple aspects of enzyme catalysis in terms of this model, but this practice is not without its dangers. There is a growing awareness of the necessity to ascertain whether conclusions thus reached apply also to other, more plausible, models for enzyme catalysis, in which binding and catalytic steps are clearly distinguished and reversibility of all steps is acknowledged (see, e.g., Cornish-Bowden, 1976b). Haldane (1930, pp. 80–83) pointed out that the simplest plausible model for enzyme catalysis is not that given in Scheme 1, but rather the fully reversible three-step model given in Scheme 2.

In the present paper it is demonstrated that substrate at a fixed concentration is transformed into product by a given amount of a particular enzyme more rapidly when $K_m > [S]$ than when $K_m < [S]$. This conclusion applies to both optimal and suboptimal rates of catalysis, provided that the larger K_m value derives from larger values of the forward rate constants of the kinetic model. The requirements for optimal rates of catalysis are delineated mathematically in terms of the diffusion-limited rate of collision of enzyme and substrate for the Haldane (1930) kinetic model of Scheme 2. The optimal rate of a freely reversible catalysis is generally less than the diffusion-limited value and depends on the equilibrium constant, $K_{eq.}$, for the overall reaction $S \rightleftharpoons P$.

This thermodynamic constraint is strengthened by predicted effects of evolutionary pressure on the relative values of the rate constants for all unimolecular steps in the catalysis, and on the relative values of K_m and the substrate concentrations *in vivo*. The combination of these effects suggests that optimal rates of catalysis should in practice be about an order of magnitude less than the rates predicted by the value of the diffusion-limited rate constant, k_{+1} , and the concentrations of enzyme and substrate. A number of enzymes appear to exhibit optimal catalytic power if the value of k_{+1} is taken to be the traditional value of $1 \times 10^9 \text{ M}^{-1} \cdot \text{s}^{-1}$. Many more enzymes appear to have K_m values appropriate to their substrate concentrations *in vivo*

for rapid rates of catalysis, but the relatively low values of their catalytic rate constants provide for rates that are suboptimal unless environmental factors *in vivo* provide values of k_{+1} substantially lower than $1 \times 10^9 \text{ M}^{-1} \cdot \text{s}^{-1}$. Increases in $k_{cat.}$ occasioned by increases in forward rate constants are reflected in parallel increases in K_m as optimal rates are closely approached. For suboptimal catalyses, increases in K_m that are greater than those provided by increases in $k_{cat.}$ must result from increases in reverse rate constants.

Argument

(1) *Optimal rate of catalysis, $v_{opt.}$, and the maximum velocity, V*

An enzymic catalysis whose rate, v (strictly the rate of increase of concentration of product; see *IUPAC Manual of Symbols and Terminology for Physicochemical Quantities and Units*, 1973 edn., Butterworths, London, 1975, pp. 31–32), obeys the Michaelis–Menten equation (eqn. 1) is considered.

$$v = \frac{V[S]}{K_m + [S]} = \frac{k_{cat.}[E]_T[S]}{K_m + [S]} \quad (1)$$

The characterizing parameters V , $k_{cat.}$ and K_m are defined empirically: $K_m = [S]$ when $v = V/2$, V is the value that v approaches when $[S] \gg K_m$, and $k_{cat.}$ is V divided by the total enzyme active-site concentration, $[E]_T$.

Eqn. (1) is commonly applied in studies *in vitro* involving one particular enzyme, where K_m is a constant and $[S]$ is a variable. In attempting to evaluate optimal catalysis, however, it is necessary to reverse this situation and to consider $[S]$ to be constant at a metabolically appropriate value. The effect on the rate of catalysis of variations in K_m occasioned by evolutionary pressure is then considered.

When $K_m \gg [S]$, eqn. (1) approximates closely to a second-order rate equation and the rate, v_2 (eqn. 2), cannot exceed v_d , the diffusion-limited rate of collision of E and S (eqn. 3). It is assumed that in a particular cellular environment k_{+1} , the second-order rate constant for the collision of a particular enzyme, E, and a particular substrate, S, is a constant and is not significantly changed by structural changes in E occasioned by evolutionary pressure. In addition, the particular value of k_{+1} should be insensitive to small structural changes in S such as those that occur when many substrates are transformed into their products. Thus in such cases it should be legitimate to assume that for a reversible model (Scheme 2) k_{+1} is closely approximated by k_{-3} , the rate constant for the diffusion-limited collision of E and P.

$$v_2 = \frac{k_{\text{cat.}}}{K_m} [E]_T [S] \quad (2)$$

$$v_d = k_{+1} [E] [S] \quad (3)$$

Since under the conditions considered $[E]$ approximates closely to $[E]_T$, $k_{\text{cat.}}/K_m$ can be written as k_{+1}/a (eqn. 4), where $a \geq 1$.

$$a = k_{+1} \cdot \frac{K_m}{k_{\text{cat.}}} \quad (4)$$

By using the expression for K_m provided by eqn. (4), the Michaelis-Menten equation (eqn. 1) can be written as eqn. (5).

$$v = \frac{k_{\text{cat.}} [E]_T [S]}{\frac{a \cdot k_{\text{cat.}}}{k_{+1}} + [S]} \quad (5)$$

Eqn. (5) shows that v_2 (v when $K_m \gg [S]$) and V (v when $K_m \ll [S]$) are given by eqns. (6) and (7) respectively. When $a = 1$, $v_2 = v_d$ [see eqns. (3) and (4)] and thus $v_2 \gg V$ which is the opposite situation to that commonly encountered in studies *in vitro* on one enzyme when K_m is constant and $[S]$ is allowed to vary. (The relationship between v_2 and V when $a > 1$ is discussed below.)

$$v_2 = \frac{k_{+1}}{a} [E]_T [S] \quad (6)$$

$$V = \frac{k_{+1}}{a} [E]_T K_m \quad (7)$$

Thus to assess the effectiveness of an enzyme as a catalyst for the conversion of S into P, it is necessary only to determine the extent to which the constraints of a particular kinetic mechanism will permit v_2 to approach the diffusion-limited rate, v_d . It is demonstrated below that the theoretical limit on v_2 (in terms of v_d) is dictated by the value of the equilibrium constant for the conversion of S into P ($K_{\text{eq.}}$). Cleland (1976) has pointed out that the upper limit on the rate of an enzyme catalysis is a thermodynamic one. It is this theoretical limiting value of v_2 (which may take values between v_d , when $K_{\text{eq.}} \gg 1$, and $K_{\text{eq.}} \cdot v_d$, when $K_{\text{eq.}} \ll 1$) that is referred to as the optimal rate of catalysis, $v_{\text{opt.}}$. The relationship between v_d and $v_{\text{opt.}}$ is given by eqn. (8), in which $a_{\text{min.}}$ is the minimum value of the factor a defined in eqn. (4).

$$v_{\text{opt.}} = \frac{v_d}{a_{\text{min.}}} \quad (8)$$

It is clearly important to distinguish $v_{\text{opt.}}$ from the 'maximum velocity', V (commonly given the

symbol $V_{\text{max.}}$), which is the term used conventionally to describe the saturation rate.

When a [see eqn. (4)] is greater than unity it is important to identify the circumstances in which v_2 is still greater than V , which is their relationship when $a = 1$.

Consider a structural change in an enzyme, E, that raises the K_m from a 'low' value, K_{m1} , much less than the ambient substrate concentration $[S]$ (which provides that v approximates closely to V) to a 'high' value, K_{mh} , much greater than $[S]$ (which provides that v approximates closely to v_2). This structural change in E could result also in a change in $k_{\text{cat.}}$ (from $k_{\text{cat.1}}$ to $k_{\text{cat.h}}$). An increase in $k_{\text{cat.}}$ would result from increases in the rate constants of forward steps of the catalysis (or from decreases in certain reverse rate constants such as k_{-2} of Scheme 2 [see eqn. (11) below], and it seems reasonable to assume that such changes would be required to increase the catalytic power of the enzyme.

The relationship between v_2 and V may be expressed as eqn. (9) [making use of eqns. (4), (6) and (7)].

$$\frac{v_2}{V} = \frac{k_{\text{cat.h}}}{k_{\text{cat.1}}} \cdot \frac{[S]}{K_{mh}} \quad (9)$$

Eqn. (9) shows that v_2 will be greater than V unless the increase in $k_{\text{cat.}}$ from $k_{\text{cat.1}}$ to $k_{\text{cat.h}}$ is outweighed by K_{mh} , the increased value of K_m . Since $K_{m1} \ll [S]$, $K_{m1}/K_{mh} \ll [S]/K_{mh}$ and thus $K_{mh}/K_{m1} \gg K_{mh}/[S]$. Eqn. (9) shows that $v_2 \gg V$ if $k_{\text{cat.h}}/k_{\text{cat.1}} \gg K_{mh}/[S]$, and thus $v_2 \gg V$ if $k_{\text{cat.h}}/k_{\text{cat.1}} \geq K_{mh}/K_{m1}$, although this condition may be excessive. Only if $k_{\text{cat.h}}/k_{\text{cat.1}} \leq K_{mh}/K_{m1}$ does the possibility arise that v_2 may not be greater than V . This may be simply illustrated by using the Briggs & Haldane (1925) model (Scheme 1). For this model, if k_{+1} is constant, $k_{\text{cat.}}$ is the only forward rate constant susceptible to evolutionary pressure, and $K_m = (k_{-1} + k_{\text{cat.}})/k_{+1}$. It is possible to envisage that an increase in K_m might result solely or in part from an increase in k_{-1} , and this could provide a value of $v_2 \leq V$. Evolution of catalytic power would, however, require an increase in $k_{\text{cat.}}$ relative to k_{-1} , as the catalysis approaches the extreme mechanism envisaged by Van Slyke & Cullen (1914) (see Brocklehurst & Cornish-Bowden, 1976). This relative increase ensures that $k_{\text{cat.h}}/k_{\text{cat.1}} \geq K_{mh}/K_{m1}$ and thus that $v_2 > V$. It is possible to demonstrate an analogous conclusion by using the Haldane (1930) model, although in this case the parameters $k_{\text{cat.}}$ and K_m are more complex assemblies of rate constants. Once again, if the increase in K_m results from increases in the rate constants of the forward unimolecular steps of the catalysis relative to the values of the rate constants of the reverse unimolecular steps, $v_2 > V$. As discussed below, the extent to which the forward rate constants can increase relative to the reverse rate constants is constrained thermodynamically by the well-known

Haldane relationship, which for the model of Scheme 2 is given by:

$$K_{eq.} = k_{+1}k_{+2}k_{+3}/k_{-1}k_{-2}k_{-3}$$

(2) *Characteristics of optimal catalysis delineated for the reversible three-step model of Haldane (1930) (Scheme 2)*

Enzymic catalyses proceeding according to Scheme 2 are considered. The concentrations of substrate and product are assumed to be maintained essentially constant either by the usual laboratory techniques, e.g. maintaining $[S] \gg [E]_T$ and measuring initial rates, or in a metabolic context by other reactions which provide substrate and consume product. Even in the latter context, complications arising from high enzyme concentrations (see Cornish-Bowden, 1976a; Laidler, 1955) are not considered.

The forward reaction ($S \rightarrow P$) of Scheme 2 is considered, and $[P]$ (but not $[EP]$!) is set to zero to reveal the maximum potential of this reaction. With this constraint applied, the steady-state rate equation for Scheme 2 may be written as eqn. (5) in which factor a (eqn. 10) is simply derived by using eqn. (4) and the expressions for K_m and $k_{cat.}$ given by Haldane (1930, p. 81; see also, e.g., Cornish-Bowden, 1976c, p. 30), i.e. eqns. (11) and (12). $K_{eq.}$ is given by the well-known Haldane relationship, $K_{eq.} = k_{+1}k_{+2}k_{+3}/k_{-1}k_{-2}k_{-3}$.

$$a = 1 + \frac{1}{K_{eq.}} \cdot \frac{k_{+1}}{k_{-3}} + \frac{k_{-1}}{k_{+2}} \quad (10)$$

$$k_{cat.} = \frac{k_{+2}k_{+3}}{k_{-2} + k_{+2} + k_{+3}} \quad (11)$$

$$K_m = \frac{k_{-1}k_{-2} + k_{-1}k_{+3} + k_{+2}k_{+3}}{k_{+1}(k_{-2} + k_{+2} + k_{+3})} \quad (12)$$

For optimal catalysis, k_{+1} and k_{-3} are taken to be equal to the diffusion-limited value (say $1 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$; see below and Brocklehurst & Cornish-Bowden (1976), and eqn. (10) then shows that the required condition for a to take its minimum value is that

$$\left(1 + \frac{1}{K_{eq.}}\right) \gg \frac{k_{-1}}{k_{+2}}$$

Thus optimal catalysis requires that $k_{+2} \gg k_{-1}$, unless $K_{eq.} \ll 1$. If $K_{eq.} \ll 1$, it is necessary only that $k_{+2} \gg K_{eq.} \cdot k_{-1}$, which means that there can then be optimal catalysis even if $k_{+2} < k_{-1}$.

For catalyses with $K_{eq.} = 1$ and $k_{+2} = k_{-2}$, $v_2 = v_{opt.}$ if $k_{+2} \gg k_{-1}$ (and necessarily, therefore, $k_{+3} \ll k_{-2}$). The value of $v_{opt.}$, however, is not v_d (the value for $v_{opt.}$ predicted by consideration of Scheme 1; see Brocklehurst & Cornish-Bowden,

Scheme 3. *Restricted version of Scheme 2: the 'acyl-enzyme intermediate' mechanism (Hartley & Kilby, 1954) commonly used to describe many hydrolytic enzymes*

1976) but only 50% of this value. The energy barrier for $ES \rightarrow EP$ is below those for the other two steps, which are the only two that contribute significantly to the rate limitation, and hence $v_{opt.} = v_d/2$. The same relationship obtains even if $k_{+2} \gg k_{-2}$. This corresponds to the mechanism discussed by Jencks (1975) for so-called 'one-way' enzymes operating under non-equilibrium conditions; a particularly large V in one direction is accompanied obligatorily by much tighter binding of P than of S , as appears to be the case for the reaction catalysed by ATP-L-methionine *S*-adenosyl-transferase (EC 2.5.1.6).

One much-studied class of reactions for which $K_{eq.} \gg 1$ is that catalysed by the extracellular hydrolases. These catalyses are often discussed in terms of the 'irreversible' three-step 'acyl-enzyme intermediate' mechanism of Scheme 3 (Hartley & Kilby, 1952, 1954). For such reactions $v_{opt.} = v_d$, just as when optimal catalysis is considered in terms of Scheme 1. The fact that $K_{eq.} \gg 1$ means that the energy barriers for $ES \rightarrow EP$ and for $EP \rightarrow E + P$ can be below that for $E + S \rightarrow ES$, which can therefore become rate-limiting. If substrate binding and product binding are equally strong, the necessity that $k_{+2} \gg k_{-1}$ for optimal catalysis implies that $k_{+2} \gg k_{+3}$. In other cases, $v_2 = v_d = v_{opt.}$ whatever the relative values of k_{+2} and k_{+3} , provided that $k_{+2} \gg k_{-1}$.

For reactions with $K_{eq.} \ll 1$ ['uphill' reactions; see Knowles (1976) and Newsholme & Start (1973)], v_2 is always $\ll v_d$ and $v_{opt.} = K_{eq.} \cdot v_d$.

(3) *Conclusions, optimal enzymic catalysis, enzyme action and control in vivo, and possible manifestations of evolutionary pressure*

One reason for attempting to evaluate the characteristics of 'optimal enzymic catalysis' is to assist in the identification of enzymes that fall a long way short of providing optimal catalysis. This may stimulate a search for the reasons for these shortcomings and possibly contribute to our understanding of the control of metabolism.

In the present paper, optimal catalysis has been evaluated for the simplest plausible model of enzyme action. This has revealed features of optimal catalysis not revealed by previous analyses that used the two-step irreversible model. Thus, although consideration

of both models leads to the conclusion that optimal catalysis demands K_m to be appreciably larger than the ambient substrate concentration, consideration of the plausible model reveals other features of optimal catalysis. Thus $v_{opt.}$ may be significantly lower than v_d , and, when $K_{eq.} \ll 1$, not only is $v_{opt.} \ll v_d$, but optimal catalysis does not require k_{+2} to be larger than k_{-1} , which is required when $K_{eq.} \geq 1$ and when optimal catalysis is evaluated by using the simple model of Scheme 1. Other features of enzymes as hypothetical 'perfect' catalysts may be revealed when more realistic models are considered. These should take account of multiple substrates, the effect of activators and inhibitors, including co-operative effects, high enzyme concentrations and the organization of enzymes into sequences. The smallest catalytic 'unit' that is realistic metabolically might be considered to be three enzyme catalyses in sequence.

One qualification of the results provided in the present paper should be noted, and presumably applies equally to consideration of more complex models. It is becoming increasingly apparent that intermediates in enzyme-catalysed reactions partition much more evenly between product formation and retrogression to substrate than has often been supposed. Knowles (1976), pointing out that this feature of even partitioning may well be an inevitable consequence of the evolution of catalytic function, wrote: 'Consider a catalysed reaction for which one free energy barrier is much higher than all the others. Assuming a selective pressure exists to improve the flux between substrate(s) and product(s), then mutational events that result in the lowering of this rate-limiting transition state will be selected for. Every 1.4 kcal/mol that the barrier is lowered will provide an enzyme ten times more effective. At the point where the barrier has been lowered to the level of some *other* transition state in the multistep reaction, then the most that can be gained in rate terms by lowering the energies of either of the two transition states is two-fold. One may expect then, that for enzymes whose function is just catalysis (e.g. those for which no catalytic advantage is being sacrificed to the possibly higher good of control) two or more transition states will be rather close in free energy.' Thus it might be anticipated that the demand that $k_{+2} \gg k_{-1}$ for the hypothetical optimal enzyme (assuming $K_{eq.} \geq 1$) might be modulated to $k_{+2} \simeq k_{-1}$ by the considerations given above. The condition $k_{+2} = k_{-1}$ alone represents quite a small sacrifice in effectiveness of catalysis: when $K_{eq.} > 1$, v_2 is decreased from $v_{opt.} = v_d$ to $v_d/2$, and, when $K_{eq.} = 1$, v_2 is decreased from $v_{opt.} = v_d/2$ to $v_d/3$. When, however, this is combined with the possibility that the concentrations of the substrates for many enzymes *in vivo* may be close to their K_m values (see below and

Cornish-Bowden, 1976a), the highest rates of catalysis actually achieved might be predicted to be appreciably below the value of $k_{+1}[E]_T[S]$. Eqn. (5) shows that, when $K_m = [S]$, v is $k_{+1}[E]_T[S]/2a$, i.e. 50% of v_2 . Thus, with $K_m = [S]$ and $k_{+2} = k_{-1}$, v_2 would be one-sixth of the value of $k_{+1}[E]_T[S]$ for the catalysis of a reaction with $K_{eq.} = 1$ and even less for a reaction with $K_{eq.} < 1$.

Care must be exercised in describing the rate provided by a particular mechanism in terms of v_d at substrate concentrations other than $[S] \ll K_m$. This is because v_d is defined in terms of $[E]$ (i.e. not $[E]_T$) (eqn. 3), and thus the optimal value of v at given total concentrations of E and S is v_d/a at all substrate concentrations. This value, however, becomes less than the value of $k_{+1}[E]_T[S]/a$ as K_m decreases with respect to $[S]$ [see eqn. (13)].

$$v = \frac{k_{+1}}{a} [E][S] = \frac{k_{+1}}{a} \cdot \frac{K_m}{(K_m + [S])} \cdot [E]_T[S] \quad (13)$$

The fact that v is as much as 50% of v_2 when $K_m = [S]$ (eqn. 5) means that the evolutionary pressure that is postulated to drive K_m to values greater than $[S]$ will be much decreased when K_m has become approximately equal to $[S]$. This argument is analogous to that used by Knowles (1976) (see above) in connexion with the even partitioning of intermediates. Thus, whereas the suggestion by Fersht (1974) that K_m should be $\gg [S]$ for optimal rates is analytically correct, the suggestion by Cornish-Bowden (1976a) that K_m should approximate to $[S]$ (perhaps be a little greater) might be more likely to describe the situation in practice.

The literature (e.g. Achs *et al.*, 1971; Barman, 1969) suggests that many enzyme catalyses are characterized by K_m values approximately equal to, or up to an order of magnitude higher than, their probable substrate concentrations *in vivo*. To this extent, evolutionary pressure could be held to have succeeded in providing appropriate K_m values for the achievement of high rates of catalysis. Unless environmental factors *in vivo* provide values of k_{+1} significantly lower than $1 \times 10^9 \text{ M}^{-1} \cdot \text{s}^{-1}$, the traditional value for reactions of E and S in free solution [see Peller & Alberty (1959), who quote a likely range for k_{+1} as 1×10^8 – $10^{10} \text{ M}^{-1} \cdot \text{s}^{-1}$], however, most of these rates are probably significantly sub-optimal in the sense discussed above.

The possibility that values of k_{+1} might be substantially lower than $1 \times 10^9 \text{ M}^{-1} \cdot \text{s}^{-1}$ in some cellular environments is an intriguing one. Attention has previously been focused rather on the ways in which diffusion limitation may be obviated, e.g. in multienzyme systems (see, e.g., Saks *et al.*, 1976), and on the possibility that, *in vitro* at least, $1 \times 10^9 \text{ M}^{-1} \cdot \text{s}^{-1}$ may be a low estimate for k_{+1} (see, e.g., Chou & Jiang, 1974; Li & Chou, 1976). However, Jonsson *et al.* (1976) have provided evidence that the

rate constant for the reaction of carbonic anhydrase with buffer molecules *in vitro* is about $1 \times 10^8 \text{ M}^{-1} \cdot \text{s}^{-1}$, i.e. an order of magnitude below the traditional diffusion limit. Many enzyme catalyses are characterized by values of K_m in the range $100 \mu\text{M}$ – 1 mM (see Barman, 1969). The present analysis suggests that optimal rates should correspond to values of $k_{\text{cat.}}/K_m$ about one-tenth of the value of k_{+1} . If, therefore, k_{+1} is taken to be $1 \times 10^9 \text{ M}^{-1} \cdot \text{s}^{-1}$, $k_{\text{cat.}}/K_m$ should be about $1 \times 10^8 \text{ M}^{-1} \cdot \text{s}^{-1}$.

A number of enzyme catalyses do have values of $k_{\text{cat.}}/K_m$ in this region (Peller & Alberty, 1959; see also Knowles, 1976), but many others have values significantly lower. Catalyses with K_m values of $100 \mu\text{M}$ would require $k_{\text{cat.}}$ to be 10^4 s^{-1} (to achieve 'practical' optimal rates of catalysis), whereas more typically $k_{\text{cat.}}$ is in the range 10^2 – 10^3 s^{-1} .

Although the uncertainty about the value of k_{+1} *in vivo* adds to the difficulty of assessment of catalytic efficiency, the apparent success in providing appropriate values of K_m and the apparent failure generally to provide equally appropriate values of $k_{\text{cat.}}$ raise interesting questions about the extent to which $k_{\text{cat.}}$ and K_m evolve independently (see also Cornish-Bowden, 1976a) and about possible relationships of this with the demands of control of metabolism.

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