never visited America, and his two periods of sabbatical leave from Cambridge University were spent at the Molteno Institute of Parasitology and at King's College, respectively 100 and 400 metres from the Department of Biochemistry.

In the years leading up to his official retirement in 1966, and in the years following, Malcolm Dixon continued to pursue research, usually, as had been his custom, carrying out all the stages himself. He had only had a personal laboratory assistant for five of his years of research, and had never had a secretary—all his writing being typed personally on an ancient but immaculate typewriter.

His active Christianity and his interest in music (he is a most accomplished pianist) have provided him with a lifelong circle of friends. He is held in great esteem by several generations of biochemical students who have passed through Cambridge, by all those who have been his personal students or colleagues and by enzymologists throughout the world who may know him only through his published work. It is very pleasant to note that his eightieth year has been marked, not only by appropriate ceremonies, but also by the publication of the third edition of 'Enzymes', the work for which he had occupied Malcolm Dixon for much of his, and the century's, seventies.

C. J. R. THORNE

Kinetic mechanism and enzyme function*

KEITH F. TITPON
Department of Biochemistry, Trinity College, Dublin 2, Ireland

There have been many attempts to analyse the behaviour of metabolic pathways based on a knowledge of the properties of the individual enzymes involved, ranging from approaches using computer simulation [see, e.g., Garfinkel et al. (1970) and McMinn & Ottaway (1976)] to considerations of the responsiveness of metabolic systems to changes in their biochemical environments [see, e.g., Savageau (1972), Atkinson (1969), Kaeser & Burns (1973, 1979) and Crabtree & Newsholme (1978)]. In this review I shall take a much more simple-minded approach by considering the applicability of the results from simple steady-state kinetic studies to such systems and by speculating on whether the kinetic mechanism obeyed by an individual enzyme can be advantageous in terms of its specific metabolic functions. A great deal of attention has been devoted to enzymes that show co-operative or allosteric kinetics, since these clearly may be important in regulating the response of pathways to changing cellular conditions, but here I shall restrict myself to considering those enzymes whose behaviour approximates to that predicted by the Michaelis–Menten equation:

$$v = \frac{V}{1 + \frac{K_m}{s}}$$

where $v$ and $V$ represent the initial and maximum velocities respectively, $K_m$ is the substrate concentration that will give half of this latter value and $s$ is the substrate concentration.

The validity of the Michaelis–Menten equation

The Michaelis–Menten equation only provides a valid description of the behaviour of an enzyme-catalysed reaction if the total substrate concentration is much greater than that of the enzyme-substrate complex. If this is not the case, depletion of free substrate occurs, and the kinetic equation necessary to define the system becomes more complicated [see, e.g., Laidler (1955) and Griffiths (1979)]. Under such conditions the substrate concentration necessary to give half-maximal velocity is increased by $e/2$, where $e$ represents the total enzyme concentration, and the Michaelis curve will not be a simple rectangular hyperbola [see, e.g., Dixon (1972) and Griffiths (1978)]. Since the concentrations of many enzymes within the cell are similar to, or even greater than, the concentrations of their substrates (Sera, 1968; Sole & Marco, 1970) and in many cases the latter are similar to the $K_m$ values of the enzymes that act upon them (see below), it has often been argued that the Michaelis–Menten equation will not provide an adequate description of the behaviour of intracellular enzymes.

This view has arisen because it is normal to assume that the total substrate concentration is much greater than that of the enzyme (so that no significant depletion of free substrate concentration $s$) occurs in complex-formation ($s_f = s - es = s$) when deriving the Michaelis–Menten equation, whether assuming that rapid-equilibrium or steady-state conditions apply. However, this is not a requirement for the equation to be valid; and it will be obeyed, whatever the relative concentrations of enzyme and substrate, if the free substrate concentration is substituted for the total value in eqn. (1).

Within the cell most substrates will bind to more than a single enzyme and also, perhaps, to other cellular components [see, e.g., Passonneau et al. (1979) and Ottaway (1979)], and thus it is essential to know the concentration of free, unbound, substrate if any useful conclusions are to be reached about the behaviour of the enzyme within the cell, whatever rate equations are used. This is precisely the condition necessary to ensure the validity of the Michaelis–Menten equation. The requirement for the satisfactory use of the Michaelis–Menten equation to predict the behaviour of enzymes within the cell is thus independent of the relative magnitudes of the enzyme and substrate concentrations; rather it is simply dependent on our ability to determine or estimate the free substrate concentration and that this does not fluctuate so rapidly that a steady-state is not achieved.

The possible significance of kinetic mechanism

The full kinetic mechanism and steady-state rate equation for an enzyme represent a convenient way of summarizing the information which, taken together with the maximum velocity and the Michaelis and inhibitor constants for the substrates, products and any other effectors, will allow the activity to be calculated at any defined concentrations of these reactants. If such information is to be applied to predicting the behaviour of enzymes within the cell, it is, of course, necessary that the determinations in vitro are carried out under conditions approximating to those believed to exist intracellularly.

When attempts are made to simulate the behaviour of a metabolic pathway by combining the kinetic data of each of the enzymes involved, it must be remembered that the errors in the determination of individual kinetic parameters are often quite large [see, e.g., Baker & Mahler (1962), Henderson (1979) and Cornish-Bowden & Eisenhal (1974)] and that the effects of

* Dedicated to Professor Malcolm Dixon on his 80th Birthday.
compounding kinetic parameters in this way can severely restrict the accuracy of this approach in providing quantitatively accurate descriptions of metabolic flux, and its usefulness as an aid to revealing the presence of previously undiscovered complexities.

Several workers have discussed the significance of the relative values of $K_m$ and the free substrate concentration in terms of enzyme catalysis (Fersht, 1974; Crowley, 1975; Cornish-Bowden, 1976). Although the conclusions reached by these analyses are by no means identical, they are similar in that they propose that for an intracellular enzyme whose function is to maximize catalytic efficiency the ratio $k_{cat}/K_m$ (where $k_{cat} = V/\text{enzyme concentration}$) must be as large as possible and that the ratio $V/K_m$ will be close to, or less than, unity. A high value of $k_{cat}/K_m$ is also necessary to ensure the rapid response of an enzyme to changes in the supply of its substrates, as can be seen, to a first approximation, by substituting the equations describing the behaviour of coupled assay systems [see, e.g., McClure (1969) and Storer & Cornish-Bowden (1974)] to describe the situation where the first enzyme in a sequence undergoes a rapid change from one steady-state rate to another.

In many cases where data are available it appears that these predictions are generally, but not invariably, true for intra-cellular enzymes and their substrates [see, e.g., Lowry & Passonneau (1964) and Purich & Fromm (1972)], although in many cases comparisons are difficult because the effects of product inhibition on the effective $K_m$ values have not been taken into account (see Cornish-Bowden, 1976). There are sufficient exceptions to indicate that evolutionary perfection has not yet been achieved in this respect. The possibility that the simple maximization of catalytic rates may not be the only important factor in enzyme function should also be borne in mind. Cornish-Bowden (1976) has discussed exceptions in the case of enzymes whose function is to maintain a steady flow of metabolites under conditions of fluctuating substrate supply, and Atkinson (1969) has shown that the rate-limiting step in this respect is a pathway to respond to changes in flux, which will depend on the ratio $s/K_m$ will also be an important factor. Such a consideration may be particularly important in cases where different enzymes compete for the same substrate, in which case the relative importance of the two pathways will be dependent on the $K_m$ and $V$ values of the enzymes involved [see, e.g., Dixon & Webb (1964) and Turner et al. (1974)].

The evolution of relatively high $K_m$ values means that the value of the maximum velocity determined with all substrates saturating will be an overestimate of the effective activity of an enzyme within the cell. For example, an enzyme obeying a double-displacement mechanism [see eqn. (3)] will have a velocity of $V/3$ under conditions where both substrates are present at their true $K_m$ concentrations, and in the case of enzymes that give rise to more complicated rate equations the decrease may be considerably larger. In the case of carbamoyl phosphate synthetase (EC 6.3.4.16) from ox liver there are, for example, 24 terms in the denominator of the initial rate equation (Elliott & Tipton, 1974), suggesting that it may never function at more than a small fraction of its maximum catalytic activity under conditions in vivo.

In some cases the kinetic mechanism obeyed by an enzyme may be advantageous in terms of its function in affecting its response to fluctuations in the concentrations of its substrates (see Tipton, 1972). The general kinetic equation obeyed by bi-substrate enzymes that give rectangularly hyperbolic kinetics can be written in the form [see, e.g., Cleland (1967) and Tipton (1974)]:

$$ V = \frac{K_A K_B}{a K_m^A + b} \frac{K_A^B K_B^B}{a + b} \frac{K_m^A K_m^B}{K_m^{AB}} \frac{K_m^K}{a b} (2) $$

where $K_A^A$ and $K_B^B$ are the Michaelis constants for substrates A and B, whose concentrations are represented by $a$ and $b$, and $K_m^K$ is the apparent dissociation constant of the complex between the enzyme and A that can be determined in the absence of B. The equation obeyed by a double-displacement (Ping Pong) mechanism differs in that $K_m^K$ is zero (see Tipton, 1974) and thus the $K_m^K$ term is absent.

The behaviour of enzymes obeying these two types of kinetic equation towards fluctuations in their substrate concentrations can be very different in terms of their abilities to buffer the activity towards one substrate against changes in the concentrations of the other. Fig. 1 compares the behaviour of two enzymes, monoamine oxidase (EC 1.4.3.4) and yeast alcohol dehydrogenase (EC 1.1.1.1). The former enzyme exhibits a $K_m$ value for oxygen which is equal to or greater than the concentration of oxygen in air-saturated water at a similar temperature [see Tipton (1968) and Houslay & Tipton (1973)]; thus it might be expected that the activity of this enzyme in catalysing the oxidative deamination of the biogenic amines might be sensitive to fluctuations in the local oxygen concentration that could perhaps arise from competition with other mitochondrial oxygen-requiring enzymes. Such an effect could impair the activity of the enzyme to remove biogenic amines at times of high cellular catabolic activity. The double-displacement mechanism obeyed by this enzyme (Tipton, 1968; Houslay & Tipton, 1973), however, results in its activity being relatively insensitive to fluctuation in the oxygen concentration around the $K_m$ value if the concentrations of the amine substrates are relatively low (Fig. 1a), thus minimizing the effects of any transitory falls in the local concentration of oxygen.

In the case of an enzyme, such as yeast alcohol dehydrogenase, which obeys eqn. (2) (Sund & Theorell, 1962), the activity towards either of its substrates will be dependent upon that of the other at all levels provided that its concentration is not sufficient completely to saturate the enzyme (Fig. 1b). Such behaviour might be more advantageous in terms of the role of this enzyme within the cell, in allowing its activity to be sensitive to changes in the concentrations of either NADH or acet-aldehyde, perhaps reflecting the need to remove toxic acet-aldehyde as well as to maintain glycolytic flux.

The mathematical basis of this behaviour can easily be seen by comparing the behaviour of the two systems under limiting conditions. The initial-rate equation for a double-displacement mechanism will be:

$$ V = \frac{V}{1 + \frac{K_m^A + K_m^B}{a}} \frac{K_m^A K_m^B}{a + b} (3) $$

which may be written in the form:

$$ V = \frac{V}{1 + \frac{a}{K_m^A + b}} \frac{K_m^A}{K_A^B} \frac{K_m^B}{K_B^B} \frac{a}{a + b} \frac{a}{a + b} (4) $$

which describes the hyperbolic response of initial velocity upon the concentration of substrate A at a fixed concentration of B. The initial slope of this curve at a very low concentration of A $a = K_m^A/(K_m^A + b)$ will be given by $v = \frac{V}{K_A^B} a$, which is independent of the concentration of B. Thus the effect of changing the concentration of B will be on the effective maximum velocity [which will be $V' = v/K_m^B(K_m^A + b)$], not on the initial slope (see Fig. 1a). The conditions necessary for the activity of the enzyme to be insensitive to fluctuations in the concentration of substrate B depend upon the relative relationships between the substrates and their Michaelis constants (Fig. 2); for example this will apply if $a < 4.5 K_A^B/6$ when $b = 5 K_B^B$ if
Fig. 1. Michaelis curves for enzyme reactions involving two substrates

(a) Curves for monoamine oxidase with tyramine as the substrate plotted by using the following kinetic constants (Tipton, 1968): $K_m^{\text{tyramine}} = 234 \mu M$ and $K_m^{\text{tyramine}} = 240 \mu M$. The oxygen concentrations were: A, 5$K_m$; B, 2$K_m$; C, $K_m$; D, $K_m/2$.

(b) Curves for yeast alcohol dehydrogenase plotted by using the following kinetic constants (Wratten & Cleland, 1963): $K_m^{\text{NADH}} = 11 \mu M$, $K_m^{\text{acetaldehyde}} = 780 \mu M$, and assuming that $K_m^{\text{NADH}} = K_m^{\text{NADH}}$. The NADH concentrations were as indicated in Fig. 1(a).

Fig. 2. Effect of the concentration of one substrate on the sensitivity of the initial velocity to variations in the concentration of the other for an enzyme obeying eqn. (3)

$$v = \frac{V}{1 + \frac{K_m^a}{V} + \frac{K_m^b}{a}}$$

$$V = 100; K_m^a = K_m^b = 10.$$

where $a < K_m^a/2$ when $b = K_m^a$ and if $a < K_m^a/6$ when $b = K_m^a/5$. When the concentration of B is sufficient to saturate the enzyme completely, its activity towards A will, of course, be unaffected by any further increase.

In the case of enzymes that obey double-displacement mechanisms the presence of one or both of the products will lead to the appearance of a $K_m^a K_m^b/ab$ term in eqn. (3), which would result in a departure from the behaviour predicted above. In the case of monoamine oxidase, however, the nature of the products of the reaction are such that this is unlikely to have a significant effect (Turner et al., 1974).

Eqn. (2) may be written in the form

$$v = \frac{V \cdot b}{(K_m^a + b) a + (K_m^a \cdot b + K_m^b \cdot K_m^a)}$$

which will give an initial slope, when $a < (K_m^a \cdot b + K_m^a K_m^b)/(K_m^a + b)$, given by $v = V \cdot b \cdot a/(K_m^a \cdot b + K_m^b \cdot K_m^a)$, which will thus be dependent on the concentration of B (see Fig. 1b).

The sensitivities of the two enzyme mechanisms to variations in the concentration of substrate B at a fixed concentration 0.1$K_m$ for substrate A is shown in Fig. 3, under conditions where $K_m^a = K_m^b$ and $K_m^a = 0$ (eqn. (3)) or $K_m^a$ (eqn. (2)).

Eqn. (5) shows that the sensitivity of the rate at low concentrations of one substrate to fluctuations in the concentration of the other will depend on the relative value of the term $K_m^a K_m^b$ tending, of course, to the invariance described by eqn. (4) as this term becomes negligible. In the case of phosphofructokinase (EC 2.7.1.11), initial-rate studies in the presence of high concentrations of AMP, when the enzyme shows no co-operative effects towards its substrates, give patterns of apparently parallel lines in double-reciprocal plots, which have been interpreted in terms of a double-displacement mechanism. Isotope-exchange studies (Hulme & Tipton, 1971)
and more detailed initial-rate kinetic studies (Bar-Tana & Cleland, 1974) have, however, shown that the kinetic mechanism is in fact sequential, but that $K_A^*$ must have a very low value indeed. This behaviour, like that of the true double-displacement mechanism, indicates that at relatively low values of fructose 6-phosphate the activity of the enzyme will be relatively insensitive to fluctuations of the concentration of ATP around its $K_a$ value, and vice versa. Since the allosteric nature of this enzyme ensures that under cellular conditions it will only function hyperbolically when the concentrations of AMP are relatively high and those of ATP are thus low, such behaviour may be advantageous in allowing the enzyme to function as efficiently as possible under conditions where the concentration of its substrate is falling (cf. Garfinkel et al., 1979). If this were to be the case, the low value of $K_A^*$ might be of selective advantage to the enzyme despite the catalytic advantage of having a high value of such a constant.

Clearly it would be naive to suppose that the considerations discussed above are the only, or even the most important, ones affecting the kinetic mechanism obeyed by an enzyme. This must depend on a number of factors, such as the nature of the substrates involved and the chemical reaction catalysed, but, since the mechanism followed depends simply on the relative values of individual kinetic constants, it would not be surprising if random mutations affected these, and thus the kinetic mechanism. If this were to occur, there might be some selection pressure to retain a mechanism that was more suited to the specific role of an enzyme within the cell.

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Fig. 3. Comparison of the sensitivities of enzymes obeying eqns. (2) and (3) to fluctuations of the concentrations of one substrate (b) at a low concentration of the other

Curve (a), $v = \frac{\frac{K_m^a}{a + \frac{K_m^b}{b}}}{K_m^a + \frac{K_m^b}{b}}$

Curve (b), $v = \frac{\frac{K_m^a}{a + \frac{K_m^b}{b}}}{K_m^a + \frac{K_m^b}{b}}$

$V = 100; K_m^a = K_m^b = 10. \ln (b) K_m^b = 10. a = 1.$