ON THE KINETIC DISTINCTION OF ORDERED AND RANDOM BIREACTANT ENZYME SYSTEMS

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Summary: It is shown that under certain conditions it could be difficult to distinguish kinetically between an ordered or random addition of two substrates to an enzyme. An ordered mechanism in which substrate binding steps are in rapid equilibrium and which includes the formation of an inactive enzyme-second substrate complex from both directions is indistinguishable by initial velocity, product inhibition, or alternate substrate kinetic studies from a mechanism involving random addition of substrates with ternary complex interconversion as the rate limiting step.

One of the questions of concern to enzymologists is that the order of addition of substrate to an enzyme which catalyzes a reaction involving more than one substrate. For enzymes which utilize two substrates and are not of the ping-pong variety, several kinetic procedures have been devised to differentiate between the various mechanisms. As was shown in 1953, the derivation of an ordered substrate addition using the steady state assumption and random substrate addition using rapid equilibrium assumptions results in the same expression for the initial velocity dependence (1), namely,

$$v_o = \frac{V_{AB}}{1 + \frac{K_A}{A} + \frac{K_B}{B} + \frac{K_{AB}}{(A)(B)}} \qquad (1)$$

where A and B are the substrates and K_A, K_B and K_{AB} are the Michaelis constants.

Although the initial velocity equations are identical, it has been shown that it is possible to differentiate these cases on the basis of,

for example, product inhibition (2,3), use of alternate substrates or competitive inhibitors (4,5) and isotope exchange experiments (6).

The purpose of this note is to point out that it is possible to obtain kinetic data which are characteristic of a random rapid equilibrium substrate addition mechanism, but which reflect an ordered binding of substrates in the kinetic process. The system which gives rise to this situation is one in which the binding of substrates is ordered and in rapid equilibrium but with the additional formation of non-productive enzyme-second substrate complexes. It has been noted previously that the rapid equilibrium derivation of an ordered system without such complexes is clearly distinguishable from other cases on the basis of initial velocity kinetics (1). This mechanism is shown in I

$$E + A \overset{K_1}{\rightleftharpoons} EA \qquad E + Q \overset{K_4}{\rightleftharpoons} EQ$$

$$EA + B \overset{K_2}{\rightleftharpoons} EAB \qquad EQ + P \overset{K_5}{\rightleftharpoons} EPQ \qquad \text{(I)}$$

$$EAB \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} EPQ$$

and gives the initial velocity expression

$$V_o = \frac{V_{AB}}{1 + \frac{K_2}{B} + \frac{K_1K_2}{AB}} \qquad (2)$$

where K_1, K_2, K_4 and K_5 represent dissociation constants and the rate limiting step is either k_3 or k_{-3}.

With the additional assumption of the formation of an inactive enzyme-second substrate complex

$$E + B \overset{K_B}{\rightleftharpoons} EB \qquad \text{(Ia)}$$

The initial velocity equation becomes

$$V_o = \frac{V_{AB}}{1 + \frac{K_B}{B} + \frac{K_1K_2}{K_BA} + \frac{K_1K_2}{AB}} \qquad (3)$$

a form identical with equation (1). Thus on the basis of initial velocity experiments this mechanism is kinetically indistinguishable from a rapid equilibrium random addition or steady state ordered addition of substrates.

Product inhibition studies would distinguish these mechanisms since for the various mechanisms the product P could either be competitive for B (rapid equilibrium random) non-competitive for B (steady state ordered) or show no inhibition (rapid equilibrium ordered). However if the non-productive complex EP were also to form:

$$E + P \overset{K_P}{\rightleftharpoons} EP \qquad \text{(Ib)}$$

product inhibition studies would give the same results expected for the rapid equilibrium random addition case (B and P are competitive). The Haldane relationships (7) are also the same for these two mechanisms. The additional formation of inactive EIaB or EIqP complexes (where Ia and Iq are competitive inhibitors or alternative substrates for A) could make this mechanism indistinguishable from a rapid equilibrium random equilibrium one on the basis of use of substrate analogs as inhibitors (5) or alternative substrates (4). In addition direct binding studies, or any method of this sort, will not distinguish the mechanisms. On the other hand, isotope exchange at equilibrium (6) would distinguish an ordered mechanism from a rapid equlibrium random addition case since it would be expected that high concentrations of B would decrease an A to Q exchange in the ordered case with non-productive complexes. No decrease of this exchange would be expected in the rapid equilibrium random case. The similarity in resulting kinetic equations between these two mechanisms (rapid equilibrium random addition and rapid equilibrium ordered addition with non-productive EB and EP complexes) appears to have been overlooked in most discussions related to the kinetic distinction of ordered and random substrate addition.

Although somewhat more complex, similar arguments may be made for enzymes involving three substrates. Thus, on the basis of initial

velocity or product inhibition studies, a rapid equilibrium random addition case may be kinetically indistinguishable from a rapid equilibrium ordered case if non-productive complexes can form.

One important question when considering the likelihood of this mechanism is whether non-productive EB (and EP) complexes may indeed form. It could be noted that excess substrate inhibition is sometimes evidence either for this type of complex or an abortive complex of the form EAP or EQB (both of which could also form in the proposed mechanism).

Intuitively, it is not difficult to visualize the possibility of such binding in terms of a ligand exclusion type model (8). Thus the substrates A and B may have different subsites for binding but are arranged in such a way that binding of B prevents the approach of A to its site. That is, A binds beneath B to a different set of subsites.

In conclusion, the equation for an ordered addition of substrates with the additional formation of non-productive enzyme-second substrate complexes when derived assuming that the ternary complex conversion is rate limiting is kinetically indistinguishable from a rapid equilibrium random addition of substrates to form productive complexes. The existence of this mechanism indicates that kinetic (or binding) studies may not be as useful as previously indicated in distinguishing different enzyme mechanisms although these cases would be mechanistically quite distinct.

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