The Steady-State Kinetics of Isotope Exchange at Equilibrium: One Substrate–One Product Enzymic Mechanisms where Two Molecules of Substrate or Product are Bound to an Enzyme Molecule

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The conclusion that the steady-state kinetics of isotope exchange at equilibrium do not show first-order behaviour for some one substrate–one product enzymic mechanisms in which two molecules of substrate or product can be combined with an enzyme molecule at the one time was shown to be erroneous.

Fleck (1972) has shown that the frequent assertion of first-order behaviour for isotope-exchange kinetics at equilibrium, regardless of mechanism, is not always valid. He pointed out that if two labelled molecules appear on the same side of one or more of the elementary reaction steps in a mechanism, then the kinetics of isotope-exchange need not be first-order. Since many enzyme mechanisms (especially ‘allosteric’ mechanisms) contain species with more than one molecule of substrate or product bound to an enzyme molecule, one could therefore expect that isotope-exchange kinetics of some such mechanisms might yield data showing that the radioactive material is not distributed among the substrate and product components according to a first-order law.

Darvey (1973) has derived steady-state equations for isotope-exchange kinetics at equilibrium for some

particular one substrate–one product enzymic mechanisms. These mechanisms can be obtained from a general mechanism (Mechanism I), which is shown in the lower right-hand portion of Scheme 1 by the steps connecting species E, EX, XE and XEX. In Mechanism I, two molecules of substrate or product can be combined with an enzyme molecule at the one time. From an analysis of the equations describing the steady-state kinetics of isotope exchange at equilibrium, Darvey (1973) concluded that the usual assumption of first-order behaviour was not valid for the mechanisms contained in Mechanism I. He also suggested a procedure for recognizing whether isotope-exchange data follow first-order kinetics.

In the presence of radioactively labelled substrate and product one has to consider all the species shown

Scheme 1. Species and steps required to describe Mechanism I in the presence of radioactively labelled substrate and product

$k_{+1}, k_{-1}, k_{+2}, k_{-2}, \dots$ denote the velocity constants for the various steps; S, P and E denote substrate, product and enzyme respectively; EX, XE and XEX denote enzyme intermediates; radioactively labelled species are denoted by placing a * on the appropriate symbol; each arrow in the Scheme is associated with a sum of two terms with each term representing an elementary step whereby one enzyme species is converted into another enzyme species: e.g. the step $\text{E} \xrightarrow{k_{+1}\text{S}+k_{-2}\text{P}} \text{EX}$ represents the two elementary steps $\text{E}+\text{S} \xrightarrow{k_{+1}} \text{EX}$ and $\text{E}+\text{P} \xrightarrow{k_{-2}} \text{EX}$.

Table 1. Expressions for the coefficients appearing in eqn. (2)

Coefficient	Expression in terms of velocity constants
a_0	$(k_{-3} + k_{+4} + k_{-7} + k_{+8})^2 [k_{-1}k_{-2}(k_{-5} + k_{+6}) + k_{-5}k_{-6}(k_{-1} + k_{+2})]$
a_1	$(k_{-3} + k_{+4} + k_{-7} + k_{+8})[k_{-1}k_{-2}(k_{+7} + \bar{K}k_{-8})(k_{-3} + k_{+4}) + k_{-3}k_{-4}(k_{+1} + \bar{K}k_{-2})(k_{-5} + k_{+6})$ $+ k_{-5}k_{-6}(k_{+3} + \bar{K}k_{-4})(k_{-7} + k_{+8}) + k_{-7}k_{-8}(k_{+5} + \bar{K}k_{-6})(k_{-1} + k_{+2}) + (k_{-1}k_{+5}k_{-8} + k_{+2}k_{-6}k_{+7})(k_{-3} + k_{+4})$ $+ (k_{+1}k_{-4}k_{-5} + k_{-2}k_{+3}k_{+6})(k_{-7} + k_{+8}) + (k_{+1}k_{+6}k_{+8}/\bar{K} + k_{-2}k_{-5}k_{-7})(k_{+3} + \bar{K}k_{-4})$ $+ (k_{+2}k_{+4}k_{+5}/\bar{K} + k_{-1}k_{-3}k_{-6})(k_{+7} + \bar{K}k_{-8})]$
a_2	$(k_{-3} + k_{+4} + k_{-7} + k_{+8})(k_{+1} + \bar{K}k_{-2} + k_{+5} + \bar{K}k_{-6})[k_{+3}k_{-8}(k_{+4} + k_{-7}) + k_{-4}k_{+7}(k_{-3} + k_{+8})]$ $+ (k_{+1}k_{+8}k_{-8} + k_{-2}k_{+7}k_{-7})(k_{+3} + \bar{K}k_{-4})(k_{-3} + k_{+4}) + (k_{+3}k_{-3}k_{-6} + k_{+4}k_{-4}k_{+5})(k_{+7} + \bar{K}k_{-8})(k_{-7} + k_{+8})]$
a_3	$\frac{k_{+1}k_{+3}}{k_{-1}k_{-3}}(k_{-3} + k_{+4})(k_{-7} + k_{+8})[k_{+3}k_{-8}(k_{+4} + k_{-7}) + k_{-4}k_{+7}(k_{-3} + k_{+8})]$
b_0	$(k_{-3} + k_{+4} + k_{-7} + k_{+8})^2(k_{-1} + k_{+2})(k_{-5} + k_{+6})$
b_1	$(k_{-3} + k_{+4} + k_{-7} + k_{+8})[(k_{+7} + \bar{K}k_{-8})(k_{-3} + k_{+4})(k_{-1} + k_{+2}) + (k_{+3} + \bar{K}k_{-4})(k_{-7} + k_{+8})(k_{-5} + k_{+6})]$
b_2	$(k_{+3} + \bar{K}k_{-4})(k_{+7} + \bar{K}k_{-8})(k_{-3} + k_{+4})(k_{-7} + k_{+8})$

in Scheme 1. Darvey (1973) erroneously omitted the steps leading to the formation of species X^*EX and XEX^* (see Scheme 1) when he derived his rate equations. Hence the conclusions reached by Darvey (1973) are not valid.

The correct steady-state rate equation describing isotope-exchange kinetics at equilibrium for Mechanism I may be derived by the procedure by Alberty *et al.* (1962) and written as:

$$v^* = -\frac{d[S^*]}{dt} = \frac{d[P^*]}{dt} = R \left(\frac{[S^*]}{[S]} - \frac{[P^*]}{[P]} \right) \quad (1)$$

with

$$R = \frac{(a_3[S]^3 + a_2[S]^2 + a_1[S] + a_0)\bar{K}[S][E]}{b_2[S]^2 + b_1[S] + b_0} \quad (2)$$

where v^* is the steady-state rate of isotope exchange at equilibrium, $[S^*]$ and $[P^*]$ are the steady-state concentrations of S^* and P^* at time t respectively, $[S]$, $[P]$ and $[E]$ are the equilibrium concentrations of

S , P and E respectively, and $\bar{K}$ is the equilibrium constant for the reaction $S \rightleftharpoons P$, i.e.:

$$\bar{K} = \frac{k_{+1}k_{+2}}{k_{-1}k_{-2}} = \frac{k_{+3}k_{+4}}{k_{-3}k_{-4}} = \frac{k_{+5}k_{+6}}{k_{-5}k_{-6}} = \frac{k_{+7}k_{+8}}{k_{-7}k_{-8}} = \frac{[P]}{[S]} \quad (3)$$

a_0 , a_1 , a_2 , a_3 , b_0 , b_1 and b_2 are constants which only involve various combinations of sums of products of velocity constants and are given in Table 1.

Contrary to the equation for v^* derived by Darvey (1973), the expression for R in eqn. (1) [see eqn. (2)] does not involve $[S^*]$ or $[P^*]$. Thus the steady-state kinetics of isotope exchange at equilibrium for the mechanisms contained in Mechanism I show first-order behaviour and the conclusions reached by Darvey (1973) are erroneous.

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