The Steady-State Kinetics of Isotope Exchange for One Substrate–One Product Enzymic Reactions

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Steady-state kinetic equations for isotope exchange are derived for a number of 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 (e.g. allosteric mechanisms). The usual assumption, that the radioactive material is distributed among the substrate and product components according to a first-order law, is not valid. One can recognize whether isotope-exchange kinetics of an enzyme reaction follows first-order behaviour by using various initial concentrations of the labelled substance added to a mixture.

It is frequently asserted that, regardless of mechanism, 'the rate at which a small amount of radioactive material, introduced into an equilibrium mixture, will distribute itself among the various species always follows a first-order law' (Benson, 1960). Fleck (1972) has shown, for chemical systems, that this generalization is not valid. It is valid for mechanisms where only one labelled molecule appears on either side of the elementary steps in a reaction mechanism. If two labelled molecules appear on the same side of an elementary step, then the reaction need not follow a first-order law.

Most studies on the kinetics of isotope exchange for enzyme reactions consider only mechanisms where one labelled molecule appears on either side of the elementary steps in a reaction mechanism; i.e., only one labelled molecule of substrate or product is combined with an enzyme molecule at the one time (Boyer, 1959; Alberty *et al.*, 1962; Boyer & Silverstein, 1963; Cleland, 1967, 1970; Plowman, 1972). Given this assumption, it is valid to assert that a first-order equation describes the distribution of radioactive material among the substrate and product components, as many have done (Boyer, 1959; Boyer *et al.*, 1959; Alberty *et al.*, 1962; Graves & Boyer, 1962; Fromm *et al.*, 1964; Silverstein & Boyer, 1964*a,b*; Cleland, 1967, 1970; Hansen *et al.*, 1969; Yagil & Hoberman, 1969; Cole & Schimmel, 1970; Dinovo & Boyer, 1971; Plowman, 1972; Purich & Fromm, 1972). However, many mechanisms for enzyme reactions (especially 'allosteric' mechanisms) invoke steps where more than one molecule of substrate or product can be combined with an enzyme molecule at the one time. For these mechanisms, the assumption that the radioactive material is distributed among the substrate and

product components according to a first-order law need not be valid.

This paper examines procedures for recognizing whether isotope-exchange data follow first-order kinetics. Steady-state kinetic equations for isotope exchange are derived for several one substrate–one product enzyme mechanisms in which two molecules of substrate or product can be combined with an enzyme molecule at the one time. An analysis of these equations shows that one can recognize whether isotope-exchange kinetics of an enzyme reaction follows first-order behaviour by adding various initial concentrations of the labelled substance to the equilibrium reaction mixture.

The following sections present:

- (1) the mechanisms discussed in this paper;
- (2) a summary of the equations and procedures usually used for analysing isotope-exchange kinetic data;
- (3) the steady-state isotope-exchange rate equations for the various mechanisms considered in this paper;
- (4) a graphical procedure for deciding whether isotope-exchange kinetic data show first-order behaviour.

Throughout, it is assumed that:

- (1) the isotopic components are chemically equivalent to their corresponding unlabelled components, i.e. the mechanism is the same for labelled and unlabelled components, and the effect of isotopic substitution on the rate constants of the mechanism is negligible;
- (2) only trace amounts of radioactively labelled components are present relative to the amounts of unlabelled components;
- (3) the total concentration of all components

containing enzyme is negligible compared with the concentrations of unlabelled substrate and product;
(4) the reactions are at chemical equilibrium.

Enzyme Mechanisms

The general mechanism (Mechanism I) considered in this paper may be written as shown in Scheme 1.

Mechanism I would apply to an enzyme which consisted of two different subunits, each containing a catalytic site; the binding of a substrate or product molecule at one catalytic site would influence the binding and reaction rate at the second site; i.e. there is an interaction between the two catalytic sites.

Three special cases of Mechanism I (Mechanisms II, III and IV) are also discussed. These are listed in Table 1.

Scheme 1. General mechanism for enzyme reactions discussed in the present paper

k_{+1} , k_{-1} , k_{+2} , k_{-2} ... denote the velocity constants for the various steps of Mechanism I; S, P and E denote substrate, product and enzyme; X_1 , X_2 and X_3 denote various enzyme intermediates.

Equations Usually Used for Analysing Data

For enzyme reactions in which only one molecule of a radioactively labelled substrate (or product) binds to an enzyme molecule at the one time, the steady-state rate of isotopic exchange, v^* , for a reaction at chemical equilibrium, may be written (Alberty *et al.*, 1962; Cole & Schimmel, 1970) as:

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

R is the rate of exchange of labelled atoms between S and P at equilibrium; $[S^*]$ and $[P^*]$ are the steady-state concentrations of radioactively labelled S and P (denoted by S^* and P^* , respectively) at time t ; $[\bar{S}]$ and $[\bar{P}]$ are the equilibrium concentrations of S and P.

If the initial concentrations of S^* and P^* present in the reaction mixture are:

$$\left. \begin{aligned} [S^*]_{t=0} &= [\bar{S}] \\ [P^*]_{t=0} &= 0 \end{aligned} \right\} \quad (2)$$

then, the conservation equation for isotopic label is:

$$[S] \approx [S^*] + [P^*] \quad (3)$$

By using eqn. (3), one can integrate eqn. (1) to obtain the familiar expression (Boyer, 1959; Alberty *et al.*, 1962) for the extent of attainment of isotopic equilibrium:

$$F = 1 - \exp \left(- \frac{[\bar{S}] + [\bar{P}]}{[\bar{S}][\bar{P}]} R t \right) \quad (4)$$

F is the fraction of isotopic equilibrium attained for species S or P at time t , i.e.:

$$F = \frac{[S] - [S^*]}{[\bar{S}] - [\bar{S}^*]} = \frac{[P^*]}{[\bar{P}]} \quad (5)$$

Table 1. Mechanisms for one substrate-one product enzyme reactions in which two molecules of substrate or product can combine with an enzyme molecule at the one time

Mechanism	Conditions imposed on the velocity constants of Mechanism I	Nature of the subunits	Nature of the catalytic sites
I	—	Different	Interacting
II	$k_{+1} = k_{+5}$, $k_{-1} = k_{-5}$ $k_{+2} = k_{+6}$, $k_{-2} = k_{-6}$ $k_{+3} = k_{+7}$, $k_{-3} = k_{-7}$ $k_{+4} = k_{+8}$, $k_{-4} = k_{-8}$	Identical	Interacting
III	$k_{+1} = k_{+7}$, $k_{-1} = k_{-7}$ $k_{+2} = k_{+8}$, $k_{-2} = k_{-8}$ $k_{+3} = k_{+5}$, $k_{-3} = k_{-5}$ $k_{+4} = k_{+6}$, $k_{-4} = k_{-6}$	Different	Independent
IV	$k_{+1} = k_{+3} = k_{+5} = k_{+7}$ $k_{-1} = k_{-3} = k_{-5} = k_{-7}$ $k_{+2} = k_{+4} = k_{+6} = k_{+8}$ $k_{-2} = k_{-4} = k_{-6} = k_{-8}$	Identical	Independent

$[\tilde{S}^*]$ and $[\tilde{P}^*]$ are the concentrations of S^* and P^* at isotopic equilibrium.

Eqns. (1) and (4) are the differential and integrated forms of the rate expressions for isotope exchange at equilibrium. They have been widely used for analysing data for isotope-exchange kinetics in enzyme reactions.

Procedures Usually Used for Computation of R

(i) From the differential rate expression

In using eqn. (1), R is computed from a measurement of the initial slope of a plot of $[S^*]$ against t , or of $[P^*]$ against t (Morrison & Cleland, 1966; Cleland, 1967, 1970; Cole & Schimmel, 1970; Plowman, 1972). If S^* is added to an equilibrium mixture of S , P and E , so that its concentration on addition to the reaction mixture is $[S_0^*]$, then R is given by

$$R = \frac{v_0^*}{[S_0^*]/[\tilde{S}]} \quad (6)$$

v_0^* is the initial slope of a plot of $[S^*]$ against t or of $[P^*]$ against t . The term $[S_0^*]/[\tilde{S}]$ in the denominator of eqn. (6) represents the specific radioactivity of S present in the reaction mixture at the start of the reaction.

(ii) From the integrated rate expression

The use of eqn. (4) requires measurements of $[S^*]$ or $[P^*]$ over the whole time-course of the exchange reaction. F is computed for various values of t from eqn. (5). Boyer and his co-workers (Boyer *et al.*, 1959; Graves & Boyer, 1962; Silverstein & Boyer, 1964a,b; Fromm *et al.*, 1964; Hansen *et al.*, 1969; Dinovo & Boyer, 1971) obtained R by averaging values of R computed from:

$$R = - \frac{[\tilde{S}][\tilde{P}]}{[\tilde{S}] + [\tilde{P}]} \frac{\ln(1-F)}{t} \quad (7)$$

at different times.

Alternatively, R can be obtained from the slope of a plot of $\ln(1-F)$ against t (Yagil & Hoberman, 1969).

Comparison of procedures

Cleland (1967, 1970) has stated that the procedures based on measuring the initial rates of isotope exchange may be more accurate and precise than those based on eqn. (4). According to Plowman (1972), the procedure based on the integrated equation 'suffers from the handicap of allowing possible errors of some magnitude to become involved'.

It would therefore seem that, unless highly accurate data are available, it may be difficult to decide, by the use of integrated equations, whether isotope-exchange data fit the first-order rate equation.

In neither of the procedures does one examine whether different values of $[S_0^*]$ have any effect on the computed values of R . Usually, once R has been computed for various values of $[\tilde{S}]$ or $[\tilde{P}]$, expressions relating R and $[\tilde{S}]$ or $[\tilde{P}]$ are tested for distinguishing between different mechanisms.

Steady-State Rate Equations for Isotope-Exchange Kinetics of Mechanisms I–IV

(i) Differential rate expressions

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

$$v^* = - \frac{d[S^*]}{dt} = \frac{d[P^*]}{dt} = \left(\frac{[S^*]}{[\tilde{S}]} - \frac{[P^*]}{[\tilde{P}]} \right) [\tilde{P}][E] \times \frac{a_0 + a_s[S^*] + a_p[P^*] + a_{ss}[S^*]^2 + a_{pp}[P^*]^2 + a_{sp}[S^*][P^*]}{b_0 + b_s[S^*] + b_p[P^*]} \quad (8)$$

The coefficients may be expressed in terms of the velocity constants of the reaction as shown in Table 2.

Table 2. Expressions for the coefficients appearing in eqn. (8) for Mechanism I

Coefficient	Expression in terms of velocity constants
a_0	$[k_{-1}k_{-2}(k_{-5}+k_{+6})+k_{-5}k_{-6}(k_{-1}+k_{+2})](k_{-3}+k_{+4}+k_{-7}+k_{+8})$
a_s	$(\bar{K})^{-1}\{[k_{+1}k_{+3}(k_{-5}+k_{+6})+k_{+5}k_{+7}(k_{-1}+k_{+2})](k_{+4}+k_{+8})+$ $[k_{+2}k_{+7}(k_{-3}+k_{+4})+k_{+3}k_{+6}(k_{-7}+k_{+8})](k_{+1}+k_{+5})\}$
a_p	$[k_{-2}k_{-4}(k_{-5}+k_{+6})+k_{-6}k_{-8}(k_{-1}+k_{+2})](k_{-3}+k_{-7})+$ $[k_{-1}k_{-8}(k_{-3}+k_{+4})+k_{-4}k_{-5}(k_{-7}+k_{+8})](k_{-2}+k_{-6})$
a_{ss}	$(\bar{K})^{-1}k_{+3}k_{+7}(k_{+1}+k_{+5})(k_{+4}+k_{+8})$
a_{pp}	$k_{-4}k_{-8}(k_{-2}+k_{-6})(k_{-3}+k_{-7})$
a_{sp}	$(\bar{K})^{-1}k_{+3}k_{+7}(k_{-2}+k_{-6})(k_{+4}+k_{+8})+k_{-4}k_{-8}(k_{+1}+k_{+5})(k_{-3}+k_{-7})$
b_0	$(k_{-1}+k_{+2})(k_{-5}+k_{+6})(k_{-3}+k_{+4}+k_{-7}+k_{+8})$
b_s	$k_{+3}(k_{-5}+k_{+6})(k_{-7}+k_{+8})+k_{+7}(k_{-1}+k_{+2})(k_{-3}+k_{+4})$
b_p	$k_{-4}(k_{-5}+k_{+6})(k_{-7}+k_{+8})+k_{-8}(k_{-1}+k_{+2})(k_{-3}+k_{+4})$

$\bar{K}$ is the equilibrium constant for the reaction $S \rightleftharpoons P$; i.e.

$$\bar{K} = \frac{k_{+1}k_{+2}}{k_{-1}k_{-2}} = \frac{k_{+3}k_{+4}}{k_{-3}k_{-4}} = \frac{k_{+5}k_{+6}}{k_{-5}k_{-6}} = \frac{k_{+7}k_{+8}}{k_{-7}k_{-8}} = \frac{[P]}{[S]} \quad (9)$$

$[E]$ is the equilibrium concentration of E in the reaction mixture. It can be expressed in terms of $[E_T]$ (the total concentration of E added to the reaction mixture) and either $[S]$ or $[P]$. Expressions for $[E_T]/[E]$ in terms of $[S]$ or $[P]$ are given in Table 3.

For the three special cases of Mechanism I (i.e. Mechanisms II–IV) eqn. (8) simplifies to:

$$v^* = \left(\frac{[S^*]}{[S]} - \frac{[P^*]}{[P]} \right) [P][E](C_0 + C_s[S^*] + C_p[P^*]) \quad (10)$$

Expressions for $[E_T]/[E]$ for Mechanisms II–IV are given in Table 3; expressions for the coefficients C_0 , C_s and C_p for these three mechanisms in terms of velocity constants are given in Table 4.

(ii) Integrated rate expressions

The integrated rate equation for Mechanism I, which can be obtained from eqns. (3) and (8), is cumbersome and probably contains too many parameters to be of any practical use. For Mechanisms

II–IV, eqn. (10) can be integrated, after elimination of either $[S^*]$ or $[P^*]$ by using eqn. (3), to give:

$$F = \frac{1 - e^{-\gamma}}{1 + \mu e^{-\gamma}} \quad (11)$$

where:

F is defined by eqn. (5);

$$\gamma = \{(C_s + C_p\bar{K})[S_0^*] + C_0(1 + \bar{K})\}[E]$$

$$\mu = \frac{\bar{K}(C_p - C_s)[S_0^*]}{1 + \bar{K}C_s[S_0^*] + C_0}$$

In contrast with eqn. (4), whose right-hand side is independent of $[S_0^*]$, the right-hand side of eqn. (11) depends on $[S_0^*]$. However, as with eqn. (4), it may be difficult to obtain data of sufficient accuracy to fit eqn. (11) uniquely. Therefore, no further consideration will be given to integrated rate expressions.

Graphical Procedure for Analysing Data Based on Initial Rates of Isotope Exchange

In this section a graphical procedure is presented which allows one to decide whether isotope-exchange data follow first-order kinetics. The procedure is based on measuring the initial slopes of plots of $[S^*]$ against t or of $[P^*]$ against t at various values of $[S_0^*]$.

Table 3. Expressions for $[E_T]/[E]$ in terms of $[S]$ and $[P]$ for Mechanisms I–IV

Mechanism	$[E_T]/[E]$ in terms of $[S]$	$[E_T]/[E]$ in terms of $[P]$
I	$1 + \left(\frac{k_{+1}}{k_{-1}} + \frac{k_{+5}}{k_{-5}} \right) [S] + \frac{k_{+1}k_{+3}}{k_{-1}k_{-3}} [S]^2$	$1 + \left(\frac{k_{-2}}{k_{+2}} + \frac{k_{-6}}{k_{+6}} \right) [P] + \frac{k_{-2}k_{-4}}{k_{+2}k_{+4}} [P]^2$
II	$1 + 2 \frac{k_{+1}}{k_{-1}} [S] + \frac{k_{+1}k_{+3}}{k_{-1}k_{-3}} [S]^2$	$1 + 2 \frac{k_{-2}}{k_{+2}} [P] + \frac{k_{-2}k_{-4}}{k_{+2}k_{+4}} [P]^2$
III	$\left(1 + \frac{k_{+1}}{k_{-1}} [S] \right) \left(1 + \frac{k_{+3}}{k_{-3}} [S] \right)$	$\left(1 + \frac{k_{-2}}{k_{+2}} [P] \right) \left(1 + \frac{k_{-4}}{k_{+4}} [P] \right)$
IV	$\left(1 + \frac{k_{+1}}{k_{-1}} [S] \right)^2$	$\left(1 + \frac{k_{-2}}{k_{+2}} [P] \right)^2$

Table 4. Expressions for the coefficients appearing in eqn. (10) for Mechanisms II–IV

Mechanism	C_0	C_s	C_p
II	$\frac{2k_{-1}k_{-2}}{k_{-1} + k_{+2}}$	$\frac{2k_{+1}k_{-3}k_{-4}}{(k_{-1} + k_{+2})(k_{-3} + k_{+4})}$	$\frac{2k_{-2}k_{-3}k_{-4}}{(k_{-1} + k_{+2})(k_{-3} + k_{+4})}$
III	$\frac{k_{-1}k_{-2}}{k_{-1} + k_{+2}} + \frac{k_{-3}k_{-4}}{k_{-3} + k_{+4}}$	$\frac{(\bar{K})^{-1}k_{+1}k_{+3}(k_{+2} + k_{+4})}{(k_{-1} + k_{+2})(k_{-3} + k_{+4})}$	$\frac{k_{-2}k_{-4}(k_{-1} + k_{-3})}{(k_{-1} + k_{+2})(k_{-3} + k_{+4})}$
IV	$\frac{2k_{-1}k_{-2}}{k_{-1} + k_{+2}}$	$\frac{2k_{+1}k_{-1}k_{-2}}{(k_{-1} + k_{+2})^2}$	$\frac{2k_{-1}k_{-2}^2}{(k_{-1} + k_{+2})^2}$

Table 5. Expressions for v_0^* and nature of plots of v_0^* and $v_0^*/[S_0^*]$ against $[S_0^*]$ for various mechanisms

Expression or plot	Mechanisms where only one molecule of S or P combines with an enzyme molecule at the one time	Mechanisms II–IV	Mechanism I
v_0^*	$\frac{R}{[S]} [S^*]$	$\bar{K}[\bar{E}](C_0 + C_s[S_0^*])[S_0^*]$	$\frac{\bar{K}[\bar{E}](a_0 + a_s[S_0^*] + a_{ss}[S_0^*]^2)[S_0^*]}{b_0 + b_s[S_0^*]}$
v_0^* against $[S_0^*]$	Straight line with positive slope passing through the origin	Parabola passing through the origin	Non-linear curve passing through the origin
$v_0^*/[S_0^*]$ against $[S_0^*]$	Straight line parallel to the $[S_0^*]$ axis	Straight line with positive slope and positive intercept on the $v_0^*/[S_0^*]$ axis	Non-linear curve with a positive intercept on the $v_0^*/[S_0^*]$ axis.

Expressions for v_0^* in terms of $[S_0^*]$ for Mechanisms I–IV are given in Table 5, together with the corresponding expression for mechanisms where only one molecule of S or P can combine with an enzyme molecule at the one time. A comparison of these expressions shows that, although a plot of v_0^* against $[S_0^*]$ should be linear for mechanisms where only one molecule of S or P can combine with an enzyme molecule at the one time, such plots are non-linear for Mechanisms I–IV. The shape of the plots obtained for v_0^* against $[S_0^*]$ for the mechanisms considered in this paper are given in Table 5.

One can also examine expressions for $v_0^*/[S_0^*]$ which are related to those usually used for computing R (eqn. 6). For mechanisms where only one molecule of S or P can combine with an enzyme molecule at the one time $v_0^*/[S_0^*]$ is independent of $[S_0^*]$. However, for Mechanisms I–IV, $v_0^*/[S_0^*]$ depends on $[S_0^*]$. Table 5 lists the shapes of plots obtained for $v_0^*/[S_0^*]$ against $[S_0^*]$ for the mechanisms considered in this paper. It is clear that such plots also allow one to distinguish between Mechanism I and Mechanisms II–IV.

Quantitative Analysis of Proposed Graphical Procedure

The graphical procedure of the previous section is relatively simple, and in theory appears useful. However, one must test whether it will yield unequivocal interpretations when applied to experimental data; i.e. if typical values for the velocity constants are inserted into the equations relating v_0^* and $[S_0^*]$ for the various mechanisms, will plots of v_0^* against $[S_0^*]$ for the different classes of mechanisms be unmistakably distinct?

The equation relating v_0^* and $[S_0^*]$ for Mechanisms II–IV can be rewritten as:

$$v_0^* = \bar{K}[\bar{E}]C_s(C_0/C_s + [S_0^*])[S_0^*] \quad (12)$$

If $[S_0^*] \ll C_0/C_s$, eqn. (12) simplifies to:

$$v_0^* = \bar{K}[\bar{E}]C_0[S_0^*] \quad (13)$$

Under this condition, if the concentrations of all unlabelled species are held constant, plots of v_0^* against $[S_0^*]$ are linear. However, for mechanisms where only one molecule of S or P combines with an enzyme molecule at the one time, plots of v_0^* against $[S_0^*]$ are also linear (see Table 5). Thus, when $[S_0^*] \ll C_0/C_s$, these two classes of mechanisms cannot be distinguished by plots of v_0^* against $[S_0^*]$.

Only if $[S_0^*]$ is of the same order of magnitude as C_0/C_s , will the quadratic term $[S_0^*]^2$ of eqn. (12) make a significant contribution to the value of v_0^* , so that plots of v_0^* against $[S_0^*]$ will be non-linear. Thus, under this condition, the graphical procedure distinguishes Mechanisms II–IV from mechanisms where only one molecule of S or P combines with an enzyme molecule at the one time.

This is illustrated in Fig. 1 where plots of v_0^* against $[S_0^*]$ are shown for $[S_0^*]$ values less than $25\mu\text{M}$ and C_0/C_s values in the range $1\mu\text{M}$ – 1mM . (The ordinate axis is actually a scaled value of v_0^* ; the scaling factor $1/(v_0^*)_{[S_0^*]=25\mu\text{M}}$ is the reciprocal of the value of v_0^* at the maximum value of $[S_0^*]$ chosen in the plots in Fig. 1.) As the value of C_0/C_s increases, deviations from linear behaviour in plots of v_0^* against $[S_0^*]$ become more difficult to discern.

The values chosen for C_0/C_s in Fig. 1 are typical of those obtained for some enzyme reactions when appropriate values of the velocity constants are inserted into expressions relating C_0/C_s and the velocity constants for various mechanisms. This is most conveniently illustrated for Mechanism IV, since it contains the smallest number of velocity constants; it is likely that the results are also applicable to Mechanisms II and III. For Mechanism IV, it follows from Table 4 that:

$$\frac{C_0}{C_s} = \frac{k_{-1} + k_{+2}}{k_{+1}}$$

Fig. 1. $v_0^*/(v_0^*)_{[S_0]=2.5\mu\text{M}}$ against $[S_0^*]$ for Mechanisms II-IV when C_0/C_s takes on values: (i) $1\mu\text{M}$, (ii) $10\mu\text{M}$, (iii) $100\mu\text{M}$ and (iv) 1mM

For details see the text.

i.e. for this mechanism, C_0/C_s is the Michaelis constant for S.

One can conclude that the graphical procedure will distinguish between Mechanism IV and mechanisms where only one molecule of S or P can combine with an enzyme molecule at the one time, provided that concentrations of labelled S are used that are of the same order of magnitude as the Michaelis constant for S.

Discussion

It seems that, in most of the theoretical and experimental papers on steady-state isotope-exchange kinetics of enzyme reactions, no one has examined what effect changes in the initial concentration of the labelled species added to the reaction mixture have on the rate of isotope exchange. The graphical procedures, described in the previous sections, are based on an analysis of these effects. They allow one to decide whether isotope-exchange kinetic data of enzyme reactions show first-order behaviour. In these procedures, values of v_0^* are obtained at various values of $[S_0^*]$ but at a fixed value of $[S]$ or $[P]$. By using these

graphical procedures (or, alternatively, a least-squares fitting procedure) one can obtain estimates for the various parameters (e.g. C_0 [E] and C_s [E] for Mechanisms II-IV) in the expressions listed in Table 5. By repeating this procedure at different values of $[S]$ or $[P]$, one can test for relationships between these various parameters and $[S]$ or $[P]$. This last step is analogous to the procedures normally used in isotope-exchange kinetic studies when the relationships between R and $[S]$ or $[P]$ are examined for different enzyme mechanisms.

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