Enzymic Mechanisms Involving Concomitant Transfer and Hydrolysis Reactions

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The kinetic parameters of ten different enzymic mechanisms in which bimolecular transfer reactions occur concomitantly with the hydrolysis of the donor molecule have been studied. The usefulness of these parameters for making a choice of mechanism is discussed. The analysis has been extended to the use of alternative substrates in bimolecular transfer reactions that proceed without the hydrolysis of the donor molecule.

In aqueous solution, some enzymes catalyse both a transfer reaction

\[ XY + HZ \rightarrow XZ + HY \]

and the hydrolysis of the XY molecule

\[ XY + HOH \rightarrow XO + HY \]

i.e. the transfer reaction is in competition with an unavoidable hydrolysis reaction, both reactions being catalysed by the same enzyme and yielding a common product HY. This situation makes it very difficult to select, from among the several possible mechanisms, the one through which the two reactions proceed. The purpose of this paper is to present an analysis of the kinetic parameters of enzymic systems in which such concomitant reactions occur and to offer an approach that may be useful in making a choice of mechanism. These theoretical analyses have been applied to the study of the mechanism of action of the exocellular DD-carboxypeptidases-transpeptidases from *Streptomyces* strains R61 (Frère *et al.*, 1973) and R39 (Ghuysen *et al.*, 1973; J.-M. Ghuysen, M. Leyh-Bouille, J. N. Campbell, R. Moreno, J.-M. Frère, C. Duez, M. Nieto & H. R. Perkins, unpublished work).

Nomenclature

**Symbols**

The following symbols are used: \( E = \) enzyme; \( A = \) acceptor, i.e. \( HZ \) above; \( D = \) donor, i.e. \( XY \) above; \( T = \) product of the transfer reaction, i.e. \( XZ \) above; \( Hy = \) product of the hydrolysis reaction, i.e. \( XO + H \) above; \( P = \) the product common to both reactions, i.e. \( HY \) above; \( G, F = a \) modified form of the enzyme (E-X); \( v_r = \) initial velocity of the transfer reaction; \( v_{HY} = \) initial velocity of the hydrolysis reaction; \( v_T = \) initial velocity of the total reaction, i.e. the production of \( P \); \( I = \) inhibitor.

**Chemical activity**

The chemical activity of the acceptor and that of the donor are considered as being equal to their respective concentrations. The chemical activities are represented by the use of parentheses: (A) and (D).

The concentration of water is 55M. Under these conditions, the chemical activity of water is not equal to its concentration. This factor has not been used as a variable for the following reasons. (1) Although the amount of water in a reaction mixture can be experimentally modified, the corresponding water activity is exceedingly difficult to estimate. (2) The variations in the reaction velocities that can be induced by altering the amount of water in the reaction mixtures are complex and factors other than the water activity are involved, such as, for example, the viscosity of the solution and the changes that may occur in the quaternary and tertiary structures of the enzyme molecule.

**Mechanisms**

Ten different mechanisms have been analysed, i.e. the mechanisms in which the donor D binds first to the enzyme (mechanisms A, B-1, B-2, C-1 and C-2), the rapid-equilibrium random mechanism (mechanism D) and, finally, the mechanisms in which the acceptor A and water bind first to the enzyme (mechanisms E-1, E-2, F-1 and F-2). These mechanisms are described by using the graphical representations of Cleland (1963).
Mechanisms in which D binds first to the enzyme. 
(A) Ping-Pong Bi Bi mechanism (see Scheme 1:A).
(B) Theorell–Chance mechanisms: depending upon the order of release of the products (P, T and Hy) two possibilities exist (see Scheme 2: B-1 and B-2). 
(C) Ordered pathway mechanisms: depending upon the order of release of the products (P, T and Hy), again two possibilities exist (see Scheme 3: C-1 and C-2).

Random pathways. (D) Rapid-equilibrium random mechanism (see Scheme 4: D): the general sequential random-order mechanism was not analysed. In such a mechanism, the steady-state rate equations are of the second degree with respect to both substrates (Wong & Hanes, 1962). Only the rapid-equilibrium mechanism, in which the trans-
formation of the EDA complex is the rate-limiting step, has been considered.

Mechanisms in which A and water bind first to the enzyme. A Ping-Pong Bi Bi mechanism, in which A would bind first to the enzyme, is difficult to visualize. From the equation XY + ZH ⇌ XZ + HY in the introduction it appears that the only possibility is to assume

$$ZH + E \rightleftharpoons EZH$$
$$EZH \rightleftharpoons F + H^+$$

Since the reaction is taking place in a buffered solution, the proton is not usually considered as a reaction product. Hence this mechanism has not been analysed.

(E) Theorell–Chance mechanisms: depending upon the order of release of the products (P, T and Hy), two possibilities exist (see Scheme 5: E-1 and E-2).

(F) Ordered pathway mechanisms: depending upon the order of release of the products (P, T and Hy), two possibilities exist (see Scheme 6: F-1 and F-2).

Kinetics of transfer ($E_0/v_T$), hydrolysis ($E_0/v_H$) and total (transfer+hydrolysis: $E_0/v_P$) reactions

The complete rate equation for the ten mechanisms that have been envisaged are presented in Appendix 1 and the relevant simplified rate equations are presented in Table 1.
Based on the simplified rate equations of Table 1, the main characteristic features of the kinetics of these reactions are summarized in Table 2. These features allow two groups of mechanisms to be distinguished. Group I includes the mechanisms D, A, B-1, B-2 and C-1, C-2, i.e. the rapid-equilibrium random mechanism and those mechanisms in which D binds first to the enzyme. Group II includes the mechanisms E-1, E-2 and F-1, F-2, i.e. those mechanisms in which A binds first to the enzyme. It should be noted that the double-reciprocal plots $1/v_T$ versus $1/(A)$ and $1/v_T$ versus $1/(D)$ for the mechanisms of group I (plots nos. 1 and 2 in Table 2) are similar to those obtained through an ordered Bi Bi mechanism, which occurs in the absence of a secondary reaction. In the present cases, however, the apparent Michaelis constants for A (at saturating concentration of D) and for D (at saturating concentration of A) are complex values. These values depend upon the individual constants of both the hydrolysis and the transfer pathways.

The effects of (A) or (D) or both are of particular interest in several cases.

1. With mechanisms of group I, A behaves as a non-competitive inhibitor of the hydrolysis reactions.

2. With the mechanisms of group II (Table 2, nos. 5-6), the ratio $v_T/v_{HY}$ is a function of (A) and (D) according to

$$
\frac{v_T}{v_{HY}} = \frac{(A)[f + g(D)]}{h + i(D)}
$$

i.e. according to

$$
\frac{v_T}{v_{HY}} = \frac{(A)[f/(D)] + g}{[h/(D)] + i}
$$

Differentiation of this equation shows that, for $f/h < g/i$, the ratio $v_T/v_{HY}$ continuously decreases for increasing values of $1/(D)$, whereas for $f/h > g/i$, ...
Table 1. Simplified rate equations

For mechanisms, see Schemes 1–6. The values of $\Phi_1$...$\Phi_3$ and of a...v are different for each equation and, when the same equation is relevant to various mechanisms, they also differ depending upon the mechanism. Their meaning can be obtained from the complete equations in Appendix 1. They group several constants and the water activity (H$_2$O). The values of $\Phi$ are more complex than those of Dalziel’s (1957) formulation. For example, with mechanism A, $\Phi_0$ for transfer $=1/k_7+1/k_3$ and $\Phi_0$ for hydrolysis $=1/k_{11}+1/k_3+1/[ak_{11}(H_2O)]$; with mechanism D, $\Phi_0$ for transfer $=1/k_4$ and $\Phi_0$ for hydrolysis $=1/k_5+K_d/[k_5(H_2O)]$ (see Appendix 1).

<table>
<thead>
<tr>
<th>Mechanisms</th>
<th>Type of reaction</th>
<th>Equations</th>
</tr>
</thead>
<tbody>
<tr>
<td>D, A, B-1, B-2, C-1, C-2</td>
<td>Transfer</td>
<td>$\frac{E_0}{v_T} = \Phi_0 + \frac{\Phi_1}{(A)} + \frac{\Phi_2}{(D)} + \frac{\Phi_3}{(A)(D)}$ (1)</td>
</tr>
<tr>
<td></td>
<td>Hydrolysis</td>
<td>$\frac{E_0}{v_{hy}} = \Phi_0 + \frac{\Phi_1}{(A)} + \frac{\Phi_2}{(D)} + \frac{\Phi_3}{(A)(D)}$ (2)</td>
</tr>
<tr>
<td>E-1, E-2, F-1, F-2</td>
<td>Transfer</td>
<td>$\frac{E_0}{v_T} = \Phi_0 + \frac{\Phi_1}{(A)} + \frac{\Phi_2}{(D)} + \frac{\Phi_3}{(A)(D)} + \frac{a+b(D)+c(D)^2}{(D)(d+e(D))}$ (3)</td>
</tr>
<tr>
<td></td>
<td>Hydrolysis</td>
<td>$\frac{E_0}{v_{hy}} = \Phi_0 + \frac{\Phi_1}{(A)} + \frac{\Phi_2}{(D)} + \frac{\Phi_3}{(A)(D)(d+e(D))}$ (4)</td>
</tr>
<tr>
<td>A</td>
<td>Transfer + hydrolysis</td>
<td>$\frac{E_0}{v_T} = \Phi_0 + \frac{\Phi_1}{(D)} + \frac{a+b(A)}{c+d(A)}$ (5)</td>
</tr>
<tr>
<td>B-1, B-2, C-1, C-2, D</td>
<td>Transfer + hydrolysis</td>
<td>$\frac{E_0}{v_T} = \frac{1}{1+b(A)} \left[ \frac{\Phi_0 + \Phi_1(A)}{(D)} + \frac{\Phi_2}{(D)(D)} + \frac{\Phi_3}{(A)(D)(d+e(D))} \right]$ (6)</td>
</tr>
<tr>
<td>E-1, E-2, F-1, F-2</td>
<td>Transfer + hydrolysis</td>
<td>$\frac{E_0}{v_T} = \frac{1+\tau(D)}{s+t(D)+u(A)+v(A)(D)} \left[ \frac{\Phi_0 + \Phi_1}{(D)} + \frac{\Phi_2}{(D)(d+e(D))} + \frac{\Phi_3}{(A)(d+e(D))} \right]$ (7)</td>
</tr>
</tbody>
</table>

the ratio $v_T/v_{hy}$ continuously increases for increasing values of $1/(D)$, with $\lim(c_T/c_{hy})_{1/(D)|_{\to 0}} = f(A)/h$.

(3) Since in the equations 1–7 (Table 1), the values of $\Phi_0$, $\Phi_1$, $\Phi_2$... and a, b, c... are different for each individual mechanism, it follows that the effects of (A) in the double-reciprocal plots $1/v_T$ versus $1/(D)$ may vary according to the mechanisms. Differentiation of the equations giving $1/v_T$ with respect to (A) gives rise to the following conclusions: with mechanism A, A is either an uncompetitive inhibitor [if $k_7<k_{11}/(1+1/\alpha)$; equation no. 3 in Appendix 1] or an activator [if $k_7>k_{11}/(1+1/\alpha)$]; with mechanism B-2, A is always an activator; with the other mechanisms B-1, C-1, C-2, D, E-1, E-2 and F-1, F-2, A may be an activator or an inhibitor depending upon the values of various constants and of the concentration of D.

The intercepts on the abscissa in the double-reciprocal plots $1/v_T$, $1/v_{hy}$ and $1/v_T$ versus $1/(D)$ for a given concentration of A, are also useful parameters for making a choice of mechanism. For any given mechanism of group I, the three reciprocal plots $1/v_T$, $1/v_{hy}$ and $1/v_T$ versus $1/(D)$, obtained at a fixed concentration of A, yield lines intercepting the abscissa at the same $1/(D)$ value. The $1/(D)$ value varies according to the concentration of A and according to the mechanism (Table 3). On the contrary, with the mechanisms of group II, the three double-reciprocal plots $1/v_T$, $1/v_{hy}$ and $1/v_T$ versus $1/(D)$ do not intercept the abscissa at the same $1/(D)$ value.

The effects of an inhibitor competing with A or of an inhibitor competing with D upon the velocity of each of the reactions $v_T$, $v_{hy}$ and $v_T$ were also analysed (Table 4). For non-linear (competitive) [NL(C)] kinetics, the plots are non-linear and $(E_0/b)_{1/(D)}$ is not a function of (I). For non-linear (non-competitive) [NL(NC)] kinetics, the plots are non-linear and $(E_0/b)_{1/(D)}$ is a function of (I).

Finally, it should be emphasized that in practice the kinetics may sometimes be very difficult to interpret. Indeed, within experimental limits, curves that are characterized by general non-linear equations may present themselves as straight lines. The analysis of the general equation

$$y = \frac{ax^2 + bx + c}{dx + e}$$

where $x = 1/(D)$, shows that, depending upon the cases, the curve may be indistinguishable from a straight line for $x$ values $>0$ (Fig. 1). A similar case of 'asymptotic linearity' was discussed by Petterson.
ENZYME TRANSFER AND HYDROLYSIS MECHANISMS

Table 2. Characteristic features of transfer, hydrolysis and total reactions

<table>
<thead>
<tr>
<th>No.</th>
<th>Plots</th>
<th>Kinetic patterns</th>
<th>Equation no. (Table 1)</th>
<th>Mechanisms A, B-1, B-2, C-1, C-2, D (group I)</th>
<th>Equation no. (Table 1)</th>
<th>Mechanisms E-1, E-2, F-1, F-2 (group II)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)</td>
<td>$\frac{1}{v_T}$ vs. $\frac{1}{v_{ty}}$ for different (D)</td>
<td>Converging lines</td>
<td>(1)</td>
<td>Set of lines not meeting at the same point</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(2)</td>
<td>$\frac{1}{v_T}$ vs. $\frac{1}{v_{ty}}$ for different (A)</td>
<td>Converging lines</td>
<td>(1)</td>
<td>Non-linear</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(3)</td>
<td>$\frac{1}{v_{ty}}$ vs. (A) for different (D)</td>
<td>Converging lines</td>
<td>(2)</td>
<td>Set of lines not meeting at the same point</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(4)</td>
<td>$\frac{1}{v_{ty}}$ vs. (D) for different (A)</td>
<td>Non-linear</td>
<td>(2)</td>
<td>Non-linear</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(5)</td>
<td>$\frac{v_T}{v_{ty}}$ vs. (A) for different (D)</td>
<td>Independent of (A)*</td>
<td>(3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(6)</td>
<td>$\frac{v_T}{v_{ty}}$ vs. (D) for different (A)</td>
<td>Independent of (D)*</td>
<td>(4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(7)</td>
<td>$\frac{1}{v_T}$ vs. $\frac{1}{v_{ty}}$ for different (A)</td>
<td>Set of lines not meeting at the same point†‡</td>
<td>(5) and (6)</td>
<td>Non-linear</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* See eqns. (4), (8), (12), (16), (20) and (24) in Appendix 1.
† See eqns. (28), (32), (36) and (40) in Appendix 1.
‡ The equations (6) and (7) of Table 1 are similar to those obtained by Fromm (1964) for the mechanisms B-2, C-2 and D. However, Fromm’s proposal was that $1/v_T$ versus $1/(D)$ for different (A) in the case of mechanism D was non-linear, whereas it is proposed here that these double-reciprocal plots give rise to non-converging lines. One should note that (with the presently used nomenclature) Fromm’s equation

$$\frac{1}{v_T} = \frac{1}{v_{ty}} = \frac{1}{v_T} + 1/v_{ty}$$

can be simplified to

$$\frac{1}{v_T} = \frac{1/v_{ty}}{1 + v_T/v_{ty}}$$

and finally to the eqn. (7) of Table 1. Since $v_T/v_{ty}$ is independent of (D) (no. 6 in Table 2), $1/v_T$ versus $1/(D)$ has the same simplified form as $1/v_{ty}$ versus $1/(D)$, i.e. gives rise for different (A) to a set of lines not meeting at the same point.

(1969, 1972). Graph (c) in Fig. 1 is the only one that shows an important deviation from linearity at low values of $x$. When the values of the coefficients $a$, $b$, $c$, $d$ and $e$ are such that graphs of the type (a), (b) or (d) (Fig. 1) are obtained, the relation between $y$ and $x$ is virtually linear even for low values of $x$.

Non-symmetrical mechanisms

The above analysis has been restricted to ‘symmetrical’ mechanisms, i.e. mechanisms in which both the transfer and the hydrolysis reactions follow similar pathways. Mechanisms in which the two reactions have non-symmetrical pathways can also be visualized, as, for example, ordered mechanisms in which A would bind first to the enzyme in the transfer reaction whereas D would bind first in the hydrolysis reaction, or vice versa. In such non-symmetrical mechanisms one observes that, irrespective of the pathway of the hydrolysis and of the order of the binding of the substrates (D or water) in this latter reaction, the ratio $v_T/v_{ty}$ is independent of (D) if in the transfer reaction D binds first to the enzyme, and is a function of (D) if in the transfer reaction A binds first to the enzyme. For example, for an ordered transfer reaction with A binding first, the ratio $v_T/v_{ty}$ has the general form $f(A)/[h+i(D)]$ and the plots $v_{ty}/v_T$ versus (D) are linear whether the
Table 3. Common intercept with the abscissa axis when 1/\(v_T\), 1/\(v_{Hy}\), and 1/\(v_p\) are plotted versus 1/(\(D\)) at a given concentration of \(A\)

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Intercept (\left(\frac{1}{D}\right))</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>(\frac{\beta[k_3 + \alpha k_{11} + \alpha k_2 + k_7 \gamma(A) + k_7 \gamma(A)]}{k_7 \gamma(A) + k_{11}})</td>
</tr>
<tr>
<td>B-1</td>
<td>(\frac{k_1 [k_3 k_9 (A) + k_3 k_9 + k_7 (H_2 O)]}{k_3 k_9 [k_3 (A) + k_2 + k_7 (H_2 O)]})</td>
</tr>
<tr>
<td>B-2</td>
<td>(\frac{k_1 [k_5 (H_2 O) + k_3 (A) + k_2]}{k_7 [k_5 (H_2 O) + k_3 (A) + k_2]})</td>
</tr>
<tr>
<td>C-1</td>
<td>(\frac{k_1 \left[\frac{k_9 k_{13} + \alpha k_7 k_{11} + \alpha k_7 k_{13} + k_7 k_{13} \beta(A) + k_5 k_{13} \beta(A)}{k_2 + \alpha k_{11} + k_9 \beta(A)}\right]}{k_{11} \left[\frac{k_{11} + \alpha k_{11} + \alpha k_9 + k_5 \beta(A) + k_{11} \beta(A)}{k_2 + k_5 \beta(A) + k_9 \alpha}\right]})</td>
</tr>
<tr>
<td>C-2</td>
<td>(\frac{k_1 \left[\frac{k_9 k_{13} + \alpha k_7 k_{11} + \alpha k_7 k_{13} + k_7 k_{13} \beta(A) + k_5 k_{13} \beta(A)}{k_2 + \alpha k_{11} + k_9 \beta(A)}\right]}{k_{11} \left[\frac{k_{11} + \alpha k_{11} + \alpha k_9 + k_5 \beta(A) + k_{11} \beta(A)}{k_2 + k_5 \beta(A) + k_9 \alpha}\right]})</td>
</tr>
<tr>
<td>D</td>
<td>(\frac{K_4 [K_3 (A) + K_3 K_6 + K_1 (H_2 O)]}{K_4 K_6 [K_3 (A) + K_1 K_3 + K_1 (H_2 O)]})</td>
</tr>
</tbody>
</table>

Table 4. Influence of competitive inhibitors

C = Competitive; NC = non-competitive; UC = uncompetitive; NL = non-linear.

<table>
<thead>
<tr>
<th>Competitive inhibitor</th>
<th>Equilibria to consider</th>
<th>(v_T) vs. D</th>
<th>(v_{Hy}) vs. D</th>
<th>(v_P) vs. D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mechanism for A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>E+I ⇋ EI</td>
<td>C (NC)</td>
<td>C (C)</td>
<td>C (C)</td>
</tr>
<tr>
<td>A</td>
<td>F+I ⇋ FI</td>
<td>UC (C)</td>
<td>UC (UC)</td>
<td>UC (UC)</td>
</tr>
<tr>
<td>B-1 and B-2</td>
<td>D</td>
<td>C (NC)</td>
<td>C (C)</td>
<td>C (C)</td>
</tr>
<tr>
<td>A</td>
<td>ED+I ⇋ EDI</td>
<td>UC (C)</td>
<td>UC (UC)</td>
<td>UC (UC)</td>
</tr>
<tr>
<td>C-1 and C-2</td>
<td>D</td>
<td>C (NC)</td>
<td>C (C)</td>
<td>C (C)</td>
</tr>
<tr>
<td>D</td>
<td>E+I ⇋ EI</td>
<td>C (NC)</td>
<td>C (C)</td>
<td>C (C)</td>
</tr>
<tr>
<td>D</td>
<td>E+D ⇋ EID</td>
<td>C (NC)</td>
<td>C (C)</td>
<td>C (C)</td>
</tr>
<tr>
<td>D</td>
<td>D+H_2O ⇋ EH_2O</td>
<td>UC (C)</td>
<td>UC (UC)</td>
<td>UC (UC)</td>
</tr>
<tr>
<td>A</td>
<td>E+I ⇋ EI</td>
<td>C (NC)</td>
<td>C (C)</td>
<td>C (C)</td>
</tr>
<tr>
<td>A</td>
<td>E+D ⇋ EID</td>
<td>C (NC)</td>
<td>C (C)</td>
<td>C (C)</td>
</tr>
<tr>
<td>E-1 and E-2</td>
<td>D</td>
<td>NC (NC)</td>
<td>NC (NC)</td>
<td>NC (NC)</td>
</tr>
<tr>
<td>D</td>
<td>EA+I ⇋ EAI</td>
<td>NL(C) (NC)</td>
<td>NL(C) (NC)</td>
<td>NL(C) (NC)</td>
</tr>
<tr>
<td>D</td>
<td>EH_2O+I ⇋ EH_2O</td>
<td>NC (NC)</td>
<td>NC (NC)</td>
<td>NL(C) (NC)</td>
</tr>
<tr>
<td>A</td>
<td>E+I ⇋ EI</td>
<td>NL(NC) (C)</td>
<td>NL(NC) (NC)</td>
<td>NL(NC) (NC)</td>
</tr>
<tr>
<td>F-1 and F-2</td>
<td>D</td>
<td>NL(NC) (C)</td>
<td>NL(NC) (NC)</td>
<td>NL(NC) (NC)</td>
</tr>
<tr>
<td>A</td>
<td>E+I ⇋ EI</td>
<td>NL(NC) (C)</td>
<td>NL(NC) (NC)</td>
<td>NL(NC) (NC)</td>
</tr>
</tbody>
</table>

hydrolysis reaction is random or ordered with \(D\) binding first.

Utilization of alternative substrates

The above analysis has been generalized to transfer reactions in which an unavoidable secondary reaction does not occur but in which either an alternative substrate for \(A\) or an alternative substrate for \(D\) is added to the reaction mixture. The complete rate equations were obtained as indicated in Appendix 2. The simplified rate equations are given in Table 5. Based on these simplified rate equations, the main
characteristic features of the kinetics are summarized in Table 6. These features make it possible to distinguish between random and ordered mechanisms and, for the ordered mechanisms, to determine which of the two substrates A or D binds first to the enzyme. Equations (7), (11), (15) and (19) and equations (1), (13), (17), (21), (33) and (37) are similar to those derived by Fromm (1964) and Rudolph & Fromm (1970).

When mechanisms with alternative substrates are analysed, the term
\[
\frac{ax^2 + bx + c}{dx + e}
\]
(where \(x\) is the inverse of the concentration of the common substrate) also occurs in some equations and therefore the interpretation of the double-reciprocal plots may also be difficult. The results obtained with liver alcohol dehydrogenase (Rudolph & Fromm, 1970) obviously correspond to graph (c) of Fig. 1. Where results give rise to graphs (a), (b) or (d) (Fig. 1), the ratios \(v_{T_1}/v_{T_2}\) and \(v_{T_1}/v_{T_3}\) are more useful parameters. These ratios depend upon the concentration of the common substrate if the reaction follows an ordered pathway in which the common substrate binds last to the enzyme (Table 6).

**Discussion**

When a transfer reaction is in competition with an unavoidable hydrolysis reaction that is catalysed by the same enzyme, the usual kinetic parameters are so complex that it is difficult to extract useful information from them. Furthermore, ambiguity can also arise from practical limitations. For example, curves that are characterized by general non-linear equations may present themselves as straight lines, and furthermore distinguishing between converging and non-converging lines may be very difficult. For these reasons, the ratio \(v_{T_1}/v_{T_2}\) appears to be the most useful and dependable parameter. If the ratio \(v_{T_1}/v_{T_2}\) is independent of \((D)\), the mechanism either is ordered and \(D\) binds first to the enzyme or is random. If the ratio \(v_{T_1}/v_{T_2}\) is a function of \((D)\) of the general form \([f+g(D)]/[h+i(D)]\). A binds first to the enzyme. Moreover, this ratio \(v_{T_1}/v_{T_2}\) remains a valuable parameter even if 'non-symmetrical' mechanisms are involved in the enzymic system. Finally, a choice of mechanism can also be approached through the use of competitive inhibitors.

With transfer reactions occurring in the absence of hydrolysis, the use of alternative substrates is a useful means for the unravelling of the enzymic mechanism that is involved in the reaction. The effects of the concentrations of the substrates \(\{A_1\}, \{A_2\}\) and \(\{D_1\}, \{D_2\}\) on the ratios \(v_{T_1}/v_{T_2}\) and \(v_{T_1}/v_{T_3}\) are also especially instructive. In one particular case, however, the ratios \(v_{T_1}/v_{T_2}\) and \(v_{T_1}/v_{T_3}\) are independent of the concentration of the common substrate even when the common substrate is fixed last to the enzyme. This situation occurs when \(f/h = g/i = n\) and \(f'/h' = g'/i' = n'\) (Table 6). It should be understood that in such an instance the usual double-reciprocal plots do not, however, allow us to determine which of the substrates is bound first to the

---

**Fig. 1. Graphical representations of the general equation**

\[
y = \frac{ax^2 + bx + c}{dx + e}
\]

From eqns. (25), (26), (29), (30), (33), (34), (37), (38), it follows that \(ax^2 + bx + c\) can be decomposed into \((a_1x + b_1)(a_2x + b_2)\). The coefficients \(a, b, c, d, e, a_1, a_2, b_1, b_2\) are products and/or sums of kinetic constants and hence are always positive. From the foregoing, it follows that (1) the vertical asymptote \(x = -e/d\) has a negative abscissa; (2) the intercept of the curve with the \(y\) axis is always positive: \(y = c/e\); (3) there are two intercepts of the curve with the \(x\) axis \((x = -b_1/a_1; x = -b_2/a_2)\) and both have a negative abscissa; (4) the slope of the oblique asymptote \((a/d)\) is always positive. Experimental data only fall in the area where \(x\) values are positive, i.e. the thickened part of the curves. The four graphs represent different proportions for the coefficients. Graph (d) is obtained for \(ae^2 < d(be - cd)\) and graphs (a), (b), (c) are obtained for \(ae^2 > d(be - cd)\).
Table 5. Simplified rate equations for alternative substrates

The values of \( \Phi_0 \ldots \Phi_6 \) and \( a \ldots i \) are different for each mechanism.

A₂, alternative substrate of A₁; D₁, common substrate.

- \( D_1 + A_1 \rightleftharpoons P + T_1 \), velocity \( v_{T_1} \)
- \( D_1 + A_2 \rightleftharpoons P + T_2 \), velocity \( v_{T_2} \)

\[
\begin{align*}
\text{Mechanism} & \quad \frac{E_0}{v_{T_1}} & \quad \frac{v_{T_1}}{v_{T_2}} \\
D_1 \text{ binds first} & \quad \Phi_0 + \frac{\Phi_1}{(D_1)} + \frac{\Phi_2}{(A_1)} + \frac{\Phi_3(A_2)}{(D_1)(A_1)} + \Phi_4(A_2) + \Phi_5(A_3) & \quad \Phi_6(A_1) \\
\text{Ping-Pong} & \quad \Phi_6(A_2) \\
\text{Theorell-Chance} & \quad \Phi_0 + \frac{\Phi_1}{(D_1)} + \frac{\Phi_2}{(A_1)} + \frac{\Phi_3}{(D_1)(A_1)} + \frac{\Phi_4(A_2)}{(D_1)(A_1)} + \Phi_5(A_3) & \quad \Phi_6(A_1) \\
\text{ordered} & \quad \Phi_6(A_2) \\
\text{Random} & \quad \Phi_0 + \frac{\Phi_1}{(D_1)} + \frac{\Phi_2}{(A_1)} + \frac{\Phi_3}{(D_1)(A_1)} + \frac{(A_2)[c(D_1)^2 + b(D_1) + a]}{(A_1)[e(D_1)^2 + d(D_1)]} & \quad (A_1)[f + g(D_1)] \\
A_1 \text{ and } A_2 \text{ bind first} & \quad (A_2)[h + i(A_1)] \\
\text{Theorell-Chance} & \quad \Phi_0 + \frac{\Phi_1}{(D_1)} + \frac{\Phi_2}{(A_1)} + \frac{\Phi_3}{(D_1)(A_1)} + \frac{(D_1)[e(A_1)^2 + d(A_1)]}{(D_1)[e(A_1)^2 + d(A_1)]} & \quad (A_1)[f + g(D_1)] \\
\text{ordered} & \quad (A_2)[h + i(A_1)] \\
D_2, \text{ alternative substrate of } D_1; A_1, \text{ common substrate} & \quad D_1 + A_1 \rightleftharpoons P + T_1 \), velocity \( v_{T_1} \)
- \( D_2 + A_1 \rightleftharpoons P + T_3 \), velocity \( v_{T_3} \)

\[
\begin{align*}
\text{Mechanism} & \quad \frac{E_0}{v_{T_1}} & \quad \frac{v_{T_1}}{v_{T_3}} \\
D_1 \text{ and } D_2 \text{ bind first} & \quad \Phi_0 + \frac{\Phi_1}{(A_1)} + \frac{\Phi_2}{(D_1)} + \frac{\Phi_3}{(D_1)(A_1)} + \frac{\Phi_4(D_2)}{(D_1)} + \Phi_5(D_3) & \quad \Phi_6(D_1) \\
\text{Ping-Pong} & \quad \Phi_6(D_2) \\
\text{Theorell-Chance} & \quad \Phi_0 + \frac{\Phi_1}{(A_1)} + \frac{\Phi_2}{(D_1)} + \frac{\Phi_3}{(D_1)(A_1)} + \frac{(D_1)[e(A_1)^2 + d(A_1)]}{(D_1)[e(A_1)^2 + d(A_1)]} & \quad (A_1)[f + g(D_1)] \\
\text{ordered} & \quad (A_2)[h + i(A_1)] \\
\text{Random} & \quad \Phi_0 + \frac{\Phi_1}{(A_1)} + \frac{\Phi_2}{(D_1)} + \frac{\Phi_3}{(D_1)(A_1)} + \frac{(D_1)[e(A_1)^2 + d(A_1)]}{(D_1)[e(A_1)^2 + d(A_1)]} & \quad (A_1)[f + g(D_1)] \\
A_1 \text{ binds first} & \quad (A_2)[h + i(A_1)] \\
\text{Theorell-Chance} & \quad \Phi_0 + \frac{\Phi_1}{(A_1)} + \frac{\Phi_2}{(D_1)} + \frac{\Phi_3}{(D_1)(A_1)} + \frac{(D_1)[e(A_1)^2 + d(A_1)]}{(D_1)[e(A_1)^2 + d(A_1)]} & \quad (A_1)[f + g(D_1)] \\
\text{ordered} & \quad (A_2)[h + i(A_1)]
\end{align*}
\]

Table 6. Effects of alternative substrates for A and D

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Ratio ( v_{T_1}/v_{T_2} )</th>
<th>Ratio ( v_{T_1}/v_{T_3} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Random</td>
<td>Independent of ((A_1)/(A_2))</td>
<td>Proportional to ((D_1)/(D_2))</td>
</tr>
<tr>
<td>Ordered, A binds first</td>
<td>Proportional to (f + g(D_1))</td>
<td>Independent of ((A_1)/(A_2))</td>
</tr>
<tr>
<td>Ordered, D binds first</td>
<td>Proportional to (f + g(A_1))</td>
<td>Proportional to (h + i(A_1))</td>
</tr>
</tbody>
</table>

enzyme. It has been suggested by Rudolph & Fromm (1970) that, when obtaining a good alternative substrate is a difficult undertaking, the problem can be circumvented by using a radioactive substrate and the non-radioactive compound as the alternative substrate. It can be demonstrated (Appendix 3) that, under these circumstances, the above condition \(f/h = g/i\) is fulfilled. Therefore the isotope competition appears not to be a useful means for selecting from among several possible mechanisms.

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References


APPENDIX 1

Complete Rate Equations for Enzymic Mechanisms Involving Concomitant Transfer and Hydrolysis Reactions

Ping-Pong mechanism A

\[ \frac{E_0}{v_T} = \frac{1}{k_7} + \frac{1}{k_5} + \frac{\alpha}{k_3 k_7} \frac{(H_2O)}{(A)} + \frac{1}{\gamma k_7(A)} + \frac{1}{k_3 \beta(D)} + \frac{\alpha k_{11}(H_2O)}{k_3 k_7 \beta(A)(D)} \]  

\[ \frac{E_0}{v_{tr}} = \frac{1}{k_9} + \frac{1}{k_3} + \frac{1}{\alpha k_11(H_2O)} + \frac{\gamma(A)}{k_2 k_11(H_2O)} + \frac{\gamma k_2(A)}{\alpha k_1 k_11(H_2O)} + \frac{1}{k_3 \beta(D)} + \frac{1}{k_3 k_1 \alpha \beta(H_2O)(D)} \]  

where \( \alpha = \frac{k_9}{k_{10} + k_{11}}, \beta = \frac{k_1}{k_2 + k_3}, \) and \( \gamma = \frac{k_5}{k_6 + k_7}. \)

Théorell–Chance mechanism B-1

\[ \frac{E_0}{v_T} = \frac{1}{k_5} + \frac{1}{k_3(A)} + \frac{k_3 k_7(A)}{k_1(D)} + \frac{k_2 + k_7(H_2O)}{k_1 k_3(A)(D)} \]  

\[ \frac{E_0}{v_{tr}} = \frac{k_3 k_7(k_{10} + k_{11})}{k_9 k_5 k_7(k_6 + k_7)(H_2O)} \]  

\[ \frac{E_0}{v_{tr}} = \frac{k_9 k_5 k_7(k_{10} + k_{11})}{k_9 k_5 k_7(k_6 + k_7)(H_2O)} = \frac{k_7(A)}{k_5(A)} \frac{k_7(A)}{k_7(H_2O)} \]  

\[ \frac{E_0}{v_T} = \frac{k_3(A)}{k_7(H_2O)} \]  

Theorell–Chance mechanism B-2

\[ \frac{E_0}{v_T} = \frac{1}{k_7} + \frac{1}{k_3(H_2O)} + \frac{k_3 k_7(A)}{k_1(D)} + \frac{k_2 + k_3(H_2O)}{k_1 k_3(A)(D)} \]  

\[ \frac{E_0}{v_{tr}} = \frac{k_3(A)}{k_7(H_2O)} \]  

\[ \frac{E_0}{v_{tr}} = \frac{k_3(A)}{k_3 k_7(H_2O)} + \frac{k_3 k_7(H_2O)}{k_1(D)} + \frac{k_2 + k_3(A)}{k_1 k_3(D)(H_2O)} \]  

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Ordered pathway mechanism C-1

\[
\frac{E_0}{v_T} = \frac{1}{k_7 + k_5} \frac{1}{k_{13} + \alpha k_{13} (H_2O) + \alpha k_{11} (H_2O) + k_2 + \alpha k_{11}(H_2O)} \frac{1}{k_1} \frac{1}{k_7 k_5 \beta(A)} \frac{1}{k_1 k_5} \frac{1}{k_9 k_3 \beta(A)(D)}
\]

\[
\frac{E_0}{v_H} = \frac{1}{k_7 + k_5} \frac{1}{k_{13} + \alpha k_{13} (H_2O) + \alpha k_{11} (H_2O) + k_2 + \alpha k_{11}(H_2O)} \frac{1}{k_1} \frac{1}{k_7 k_5 \beta(A)} \frac{1}{k_1 k_5} \frac{1}{k_9 k_3 \beta(A)(D)}
\]

where \( \alpha = \frac{k_9}{k_{10} + k_{11}} \) and \( \beta = \frac{k_3}{k_4 + k_5} \).

Ordered pathway mechanism C-2

\[
\frac{E_0}{v_T} = \frac{1}{k_7 + k_5} \frac{1}{k_{13} + \alpha k_{13} (H_2O) + \alpha k_{11} (H_2O) + k_2 + \alpha k_{11}(H_2O)} \frac{1}{k_1} \frac{1}{k_7 k_5 \beta(A)} \frac{1}{k_1 k_5} \frac{1}{k_9 k_3 \beta(A)(D)}
\]

\[
\frac{E_0}{v_H} = \frac{1}{k_7 + k_5} \frac{1}{k_{13} + \alpha k_{13} (H_2O) + \alpha k_{11} (H_2O) + k_2 + \alpha k_{11}(H_2O)} \frac{1}{k_1} \frac{1}{k_7 k_5 \beta(A)} \frac{1}{k_1 k_5} \frac{1}{k_9 k_3 \beta(A)(D)}
\]

where \( \beta = \frac{k_3}{k_4 + k_5} \) and \( \alpha = \frac{k_7}{k_8 + k_9} \).

Rapid-equilibrium random mechanism D

\[
\frac{E_0}{v_T} = \frac{1}{k_7 + k_5} \frac{1}{k_{13} + \alpha k_{13} (H_2O) + \alpha k_{11} (H_2O) + k_2 + \alpha k_{11}(H_2O)} \frac{1}{k_1} \frac{1}{k_7 k_5 \beta(A)} \frac{1}{k_1 k_5} \frac{1}{k_9 k_3 \beta(A)(D)}
\]

\[
\frac{E_0}{v_H} = \frac{1}{k_7 + k_5} \frac{1}{k_{13} + \alpha k_{13} (H_2O) + \alpha k_{11} (H_2O) + k_2 + \alpha k_{11}(H_2O)} \frac{1}{k_1} \frac{1}{k_7 k_5 \beta(A)} \frac{1}{k_1 k_5} \frac{1}{k_9 k_3 \beta(A)(D)}
\]

\[
\frac{E_0}{v_T} = \frac{1}{k_7 + k_5} \frac{1}{k_{13} + \alpha k_{13} (H_2O) + \alpha k_{11} (H_2O) + k_2 + \alpha k_{11}(H_2O)} \frac{1}{k_1} \frac{1}{k_7 k_5 \beta(A)} \frac{1}{k_1 k_5} \frac{1}{k_9 k_3 \beta(A)(D)}
\]

\[
\frac{E_0}{v_H} = \frac{1}{k_7 + k_5} \frac{1}{k_{13} + \alpha k_{13} (H_2O) + \alpha k_{11} (H_2O) + k_2 + \alpha k_{11}(H_2O)} \frac{1}{k_1} \frac{1}{k_7 k_5 \beta(A)} \frac{1}{k_1 k_5} \frac{1}{k_9 k_3 \beta(A)(D)}
\]

Theorell-Chance mechanism E-1

\[
\frac{E_0}{v_T} = \frac{1}{k_7 + k_5} \frac{1}{k_{13} + \alpha k_{13} (H_2O) + \alpha k_{11} (H_2O) + k_2 + \alpha k_{11}(H_2O)} \frac{1}{k_1} \frac{1}{k_7 k_5 \beta(A)} \frac{1}{k_1 k_5} \frac{1}{k_9 k_3 \beta(A)(D)}
\]

\[
\frac{E_0}{v_H} = \frac{1}{k_7 + k_5} \frac{1}{k_{13} + \alpha k_{13} (H_2O) + \alpha k_{11} (H_2O) + k_2 + \alpha k_{11}(H_2O)} \frac{1}{k_1} \frac{1}{k_7 k_5 \beta(A)} \frac{1}{k_1 k_5} \frac{1}{k_9 k_3 \beta(A)(D)}
\]

\[
\frac{E_0}{v_T} = \frac{1}{k_7 + k_5} \frac{1}{k_{13} + \alpha k_{13} (H_2O) + \alpha k_{11} (H_2O) + k_2 + \alpha k_{11}(H_2O)} \frac{1}{k_1} \frac{1}{k_7 k_5 \beta(A)} \frac{1}{k_1 k_5} \frac{1}{k_9 k_3 \beta(A)(D)}
\]

\[
\frac{E_0}{v_H} = \frac{1}{k_7 + k_5} \frac{1}{k_{13} + \alpha k_{13} (H_2O) + \alpha k_{11} (H_2O) + k_2 + \alpha k_{11}(H_2O)} \frac{1}{k_1} \frac{1}{k_7 k_5 \beta(A)} \frac{1}{k_1 k_5} \frac{1}{k_9 k_3 \beta(A)(D)}
\]

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\[ E_0 = \frac{k_7k_9(H_2O)[k_2+k_3(D)]}{k_1k_5k_9(A)[k_4+k_7(D)]} \]

\[ v_T = \frac{k_1k_3(A)[k_4+k_7(D)]}{k_7k_9(H_2O)[k_2+k_3(D)]} \]

Theorell–Chance mechanism E-2

\[ E_0 = \frac{1 + \frac{1}{k_9} + \frac{1}{k_1(A)} + \frac{1}{k_3(D)} + \frac{k_2}{k_1k_3(A)(D)} + \frac{k_3(H_2O)[k_2+k_3(D)]}{k_1k_3k_9(A)(D)[k_4+k_7(D)]}}{v_T} \]

\[ E_0 = \frac{1 + \frac{1}{k_9} + \frac{1}{k_3(H_2O)} + \frac{1}{k_7(D)} + \frac{k_6}{k_5k_7(H_2O)(D)} + \frac{k_1(A)[k_4+k_7(D)]}{k_5k_7k_9(H_2O)(D)[k_2+k_3(D)]}}{v_H} \]

\[ E_0 = \frac{k_9k_7(H_2O)[k_3+k_4(D)]}{k_9k_7(H_2O)[k_3+k_4(D)] + k_9k_7(H_2O)[k_3+k_4(D)]} \]

Ordered pathway mechanism F-1

\[ E_0 = \frac{1 + \frac{1}{k_9} + \frac{1}{k_3(H_2O)} + \frac{1}{k_13(D)} + \frac{k_2}{k_1k_3(\alpha)(D)} + \frac{k_3(H_2O)[k_2+k_3(\beta)(D)]}{k_1k_3(\beta)(A)(D)[k_10+\alpha k_13(D)]}}{v_T} \]

\[ E_0 = \frac{k_9k_13(\alpha)(H_2O)[k_2+k_3(\beta)(D)]}{k_9k_13(\alpha)(H_2O)[k_2+k_3(\beta)(D)] + k_9k_13(\alpha)(H_2O)[k_2+k_3(\beta)(D)]} \]

\[ v_T = \frac{k_1k_2(\alpha)[k_10+k_13(\alpha)(D)]}{k_9k_13(\alpha)(H_2O)[k_2+k_3(\beta)(D)]} \]

where \( \alpha = \frac{k_{11}}{k_{12}+k_{13}} \)

and \( \beta = \frac{k_3}{k_4+k_5} \).

Ordered pathway mechanism F-2

\[ E_0 = \frac{1 + \frac{1}{k_9} + \frac{1}{k_3(\beta)(D)} + \frac{1}{k_1(\alpha)} + \frac{k_2}{k_1k_3(\beta)(A)(D)} + \frac{k_7(H_2O)[k_2+k_3(\beta)(D)]}{k_1k_3(\beta)(A)(D)[k_10+\alpha k_13(\alpha)(D)]}}{v_T} \]

\[ E_0 = \frac{k_7k_13(\alpha)(H_2O)[k_2+k_3(\beta)(D)]}{k_7k_13(\alpha)(H_2O)[k_2+k_3(\beta)(D)] + k_7k_13(\alpha)(H_2O)[k_2+k_3(\beta)(D)]} \]

\[ v_T = \frac{k_1k_2(\alpha)[k_8+k_11(\alpha)(D)]}{k_7k_13(\alpha)(H_2O)[k_2+k_3(\beta)(D)]} \]

where \( \alpha = \frac{k_9}{k_{10}+k_{11}} \)

and \( \beta = \frac{k_3}{k_4+k_5} \).

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APPENDIX 2

Complete Rate Equations for Enzymic Mechanisms of Transfer Reactions Occurring in the Absence of Hydrolysis but in the Presence of Alternative Substrates

Table 1A of this Appendix show the complete rate equations were obtained by using the schemes presented in the main text and by replacing accordingly the parameters $D$, $A$, $H_2O$, $T$ and $Hy$ of the equations of Appendix 1 by the parameters $D_1$, $D_2$, $A_1$, $A_2$, $T_1$, $T_2$ and $T_3$. $A_2$ is an alternative substrate of $A_1$ ($D_1$ being a common substrate) and, alternatively, $D_2$ is an alternative substrate of $D_1$ ($A_1$ being a common substrate). Hence, the following systems were considered:

$$D_1 + A_1 \rightleftharpoons P + T_1$$
$$D_1 + A_2 \rightleftharpoons P + T_2$$
$$D_2 + A_1 \rightleftharpoons P + T_3$$

Where $D_2$ is an alternative substrate of $D_1$ ($A_1$ being a common substrate), the scheme for a Ping-Pong mechanism with $D$ binding first differs from among all the schemes presented in the main text and is given in Scheme 1A.

![Scheme 1A](image)

Table 1A. Method of obtaining complete rate equations for systems with alternative substrates

<table>
<thead>
<tr>
<th>Mechanisms</th>
<th>In equations of Appendix 1</th>
<th>In schemes of main text</th>
<th>In equations of Appendix 1</th>
<th>In schemes of main text</th>
</tr>
</thead>
<tbody>
<tr>
<td>D binds first</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ping-Pong</td>
<td>(1)</td>
<td>Scheme 1 (A)</td>
<td>(*)</td>
<td>(*)</td>
</tr>
<tr>
<td>Theorell–Chance 1</td>
<td>(5)</td>
<td>Scheme 2 (B-1)</td>
<td>(25)</td>
<td>Scheme 5 (E-1)</td>
</tr>
<tr>
<td>Theorell–Chance 2</td>
<td>(9)</td>
<td>Scheme 2 (B-2)</td>
<td>(29)</td>
<td>Scheme 5 (E-2)</td>
</tr>
<tr>
<td>Ordered 1</td>
<td>(13)</td>
<td>Scheme 3 (C-1)</td>
<td>(33)</td>
<td>Scheme 6 (F-1)</td>
</tr>
<tr>
<td>Ordered 2</td>
<td>(17)</td>
<td>Scheme 3 (C-2)</td>
<td>(37)</td>
<td>Scheme 6 (F-2)</td>
</tr>
<tr>
<td>Random</td>
<td>(21)</td>
<td>Scheme 4 (D)</td>
<td>(21)</td>
<td>Scheme 4 (D)</td>
</tr>
<tr>
<td>A binds first</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Theorell–Chance 1</td>
<td>(25)</td>
<td>Scheme 5 (E-1)</td>
<td>(5)</td>
<td>Scheme 2 (B-1)</td>
</tr>
<tr>
<td>Theorell–Chance 2</td>
<td>(29)</td>
<td>Scheme 5 (E-2)</td>
<td>(9)</td>
<td>Scheme 2 (B-2)</td>
</tr>
<tr>
<td>Ordered 1</td>
<td>(33)</td>
<td>Scheme 6 (F-1)</td>
<td>(13)</td>
<td>Scheme 3 (C-1)</td>
</tr>
<tr>
<td>Ordered 2</td>
<td>(37)</td>
<td>Scheme 6 (F-2)</td>
<td>(17)</td>
<td>Scheme 3 (C-2)</td>
</tr>
</tbody>
</table>

* This scheme differs from all the schemes presented in the main text. The relevant scheme is the adjacent Scheme IA (mechanism G) and equations are as follows:

$$\frac{E_0}{v_{T_1}} = \frac{1}{k_6} + \frac{1}{k_7} + \frac{k_6 + k_7}{k_7 k_9} + \frac{k_3 + k_1}{k_5 (D_1)} + \frac{k_4 (k_1 + k_2)}{k_5 k_3 (k_1 + k_2) (D_1)} + \frac{k_6 (k_2 + k_3)}{k_5 k_3 (k_1 + k_2) (D_1)} + \frac{k_6 (k_2 + k_3)}{k_5 k_3 (k_1 + k_2) (D_1)} + \frac{k_4 (k_1 + k_2)}{k_5 k_3 (k_1 + k_2) (D_1)}$$

and

$$\frac{v_{T_3}}{v_{T_1}} = \frac{k_1 k_3 (k_1 + k_2) (D_1)}{k_9 k_{11} k_2 (D_2)}$$

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APPENDIX 3

Simplification of the Rate Equations in Isotope Competition

Let $A^*$ be the labelled substrate, $A$ the non-radioactive compound, $D$ the common substrate, $v^*$ the velocity of the reaction $A^* + D \rightarrow T^* + P$ and $v$ the velocity of the reaction $A + D \rightarrow T + P$.

Ordered and rapid-equilibrium random mechanisms are considered here.

**Ordered mechanism in which $A^*$ and $A$ bind first**

The Cleland scheme is obtained by replacing $H_2O$ by $A^*$ in Scheme 6 (F-1) of the main paper.

The equations giving $v^*/v$ and $E_0/v^*$ are obtained by replacing $H_2O$ by $A^*$ in eqn. (36) and eqn. (34) of Appendix 1 respectively.

$$\frac{v^*}{v} = \frac{k_9 k_{13} \alpha(A^*) [k_2 + k_5 \beta(D)]}{k_1 k_5 \beta(A) [k_{10} + k_{13} \alpha(D)]} \tag{36*}$$

Assuming the absence of any strong isotopic effect, all the kinetic constants in the pathway with $A^*$ are equal to the corresponding constants in the pathway with $A$, i.e. $k_1 = k_9$, $k_2 = k_{10}$... Hence, eqn. (36*) simplifies to:

$$\frac{v^*}{v} = \frac{(A^*)}{(A)} \tag{36*}$$

and equation (34*) simplifies to:

$$\frac{E_0}{v^*} = \frac{1}{k_5} + \frac{1}{k_7} + \frac{1}{k_5 \beta(D)} + \left(\frac{A^*}{(A)}\right) \frac{1}{k_1 + \frac{1}{k_5}} + \frac{1}{k_5 \beta(A^*)[k_1 + (A)]} \tag{34*}$$

**Ordered mechanism in which $D$ binds first**

The Cleland scheme and eqns. (16*) and (14*) are obtained by replacing $H_2O$ by $A^*$ in Scheme 3 (C-1) of the main paper and in eqns. (16) and (14) respectively. Since $k_3 = k_9$, $k_4 = k_{10}$..., eqns. (16*) and (14*) simplify to

$$\frac{v^*}{v} = \frac{(A^*)}{(A)} \tag{16*}$$

and

$$\frac{E_0}{v^*} = \frac{1}{k_5} + \frac{1}{k_7} + \frac{1}{k_5 \beta(D)} + \left(\frac{A^*}{(A)}\right) \frac{1}{k_1 + \frac{1}{k_5}} + \frac{1}{k_5 \beta(A^*)[k_1 + (A)]} \tag{14*}$$

**Rapid-equilibrium random mechanism**

The Cleland scheme and eqns. (24*) and (22*) are obtained by replacing $H_2O$ by $A^*$ in Scheme 4 of the main paper and in eqns. (24) and (22) respectively. Since $k_1 = k_3$, $k_2 = k_4$, $K_1 = K_3$, $K_5 = K_6$ and $K_4 = K_7$, eqns. (24*) and (22*) simplify to

$$\frac{v^*}{v} = \frac{(A^*)}{(A)} \tag{24*}$$

$$\frac{E_0}{v^*} = \frac{1}{k_5} + \frac{1}{k_7} + \frac{1}{k_5 \beta(D)} + \left(\frac{A^*}{(A)}\right) \frac{1}{k_1 + \frac{1}{k_5}} + \frac{K_2 K_5}{k_1 k_1 (A^*)[k_1 + (A)]} \tag{22*}$$

Eqns. (36*), (16*) and (24*) are identical and equations (34*), (14*) and (22*) have the same general form. These latter equations are linear in 1/(D), 1/(A*) and (A). In the three cases examined, $A$ behaves as a competitive inhibitor of $A^*$ and as a non-competitive inhibitor of $D$. Thus it is not possible to make a choice among different mechanisms from isotope-competition experiments.