

Enzymic Mechanisms Involving Concomitant Transfer and Hydrolysis Reactions

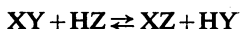
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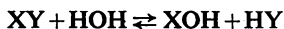
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The kinetic parameters of ten different enzymic mechanisms in which bimolecular transfer reactions occur concomitantly with the hydrolysis of the donor molecule have been studied. The usefulness of these parameters for making a choice of mechanism 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



and the hydrolysis of the XY molecule



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al.*, 1973; J.-M. Ghuysen, M. Leyh-Bouille, J. N. Campbell, R. Moreno, J.-M. Frère, C. Duez, M. Nieto & H. R. Perkins, unpublished work).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. XOH above; P = the product common to both reactions, i.e. HY above; G, F = a modified form of the enzyme (E-X); v_T = initial velocity of

the transfer reaction; v_{Hy} = initial velocity of the hydrolysis reaction; v_P = initial velocity of the total reaction, i.e. the production of P; I = inhibitor.

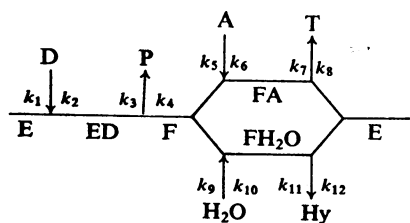
Chemical activity

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).



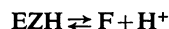
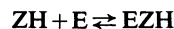
Scheme 1. Ping-Pong Bi Bi mechanism A

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 $XY + ZH \rightleftharpoons XZ + HY$ in the introduction it appears that the only possibility is to assume



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).

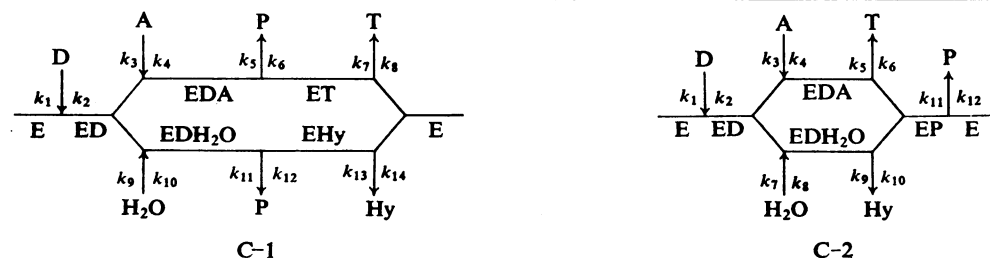
Kinetics of transfer (E_0/v_T), hydrolysis (E_0/v_{Hy}) and total (transfer+hydrolysis: E_0/v_P) reactions

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.



Scheme 2. Theorell–Chance mechanisms B-1 and B-2

D binds first.

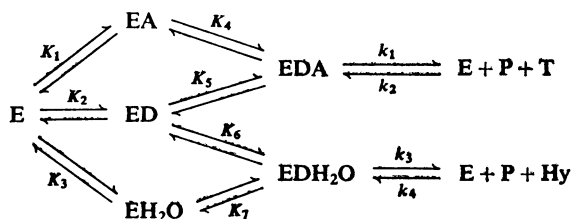


C-1

C-2

Scheme 3. Ordered pathway mechanisms C-1 and C-2

D binds first.



Scheme 4. *Rapid-equilibrium random mechanism D*
 $K_1 K_4 = K_2 K_5$ and $K_2 K_6 = K_3 K_7$.

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 of 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

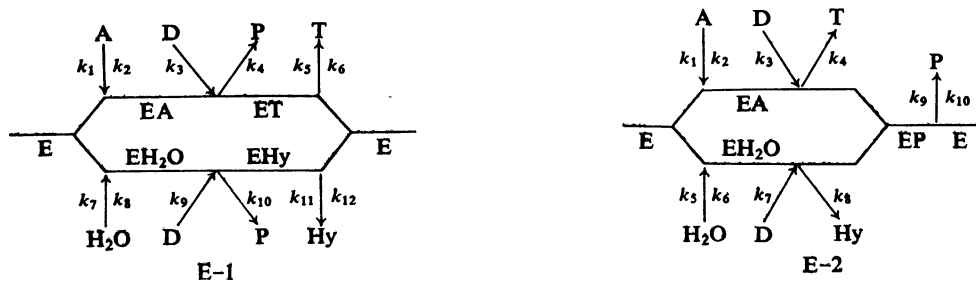
$$\frac{v_T}{v_{Hy}} = \frac{(A)[f + g(D)]}{h + i(D)}$$

i.e. according to

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

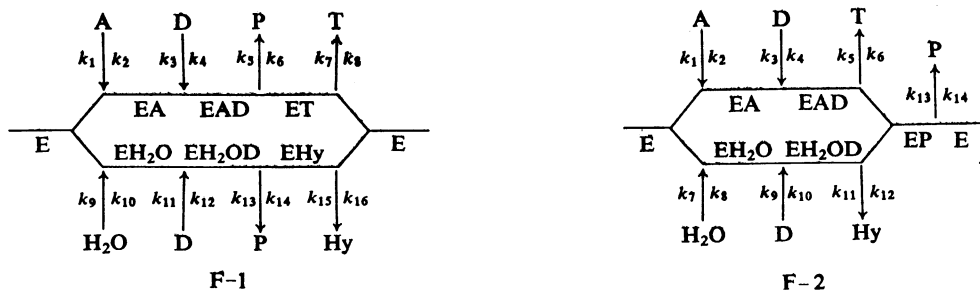
Differentiation of this equation shows that, for $f/h < g/i$, the ratio v_T/v_{Hy} continuously decreases for increasing values of $1/(D)$, whereas for $f/h > g/i$,

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



Scheme 5. *Theorell-Chance mechanisms E-1 and E-2*

A and H₂O bind first.



Scheme 6. *Ordered pathway mechanisms F-1 and F-2*

A and H₂O bind first.

Table 1. Simplified rate equations

For mechanisms, see Schemes 1–6. The values of $\Phi_0 \dots \Phi_3$ and of $a \dots v$ 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_2O). The values of Φ are more complex than those of Dalziel's (1957) formulation. For example, with mechanism A, Φ_0 for transfer = $1/k_7 + 1/k_3$ and Φ_0 for hydrolysis = $1/k_{11} + 1/k_3 + 1/[\alpha k_{11}(H_2O)]$; with mechanism D, Φ_0 for transfer = $1/k_1$ and Φ_0 for hydrolysis = $1/k_3 + K_6/[k_3(H_2O)]$ (see Appendix 1).

Mechanisms	Type of reaction	Equations	No.
D, A, B-1, B-2, C-1, C-2	Transfer	$\frac{E_0}{v_T} = \Phi_0 + \frac{\Phi_1}{(A)} + \frac{\Phi_2}{(D)} + \frac{\Phi_3}{(A)(D)}$	(1)
	Hydrolysis	$\frac{E_0}{v_{Hy}} = \Phi_0 + \Phi_1(A) + \frac{\Phi_2}{(D)} + \frac{\Phi_3(A)}{(D)}$	(2)
E-1, E-2, F-1, F-2	Transfer	$\frac{E_0}{v_T} = \Phi_0 + \frac{\Phi_1}{(A)} + \frac{\Phi_2}{(D)} + \frac{\Phi_3}{(A)(D)} + \frac{a + b(D) + c(D)^2}{(A)(D)[d + e(D)]}$	(3)
	Hydrolysis	$\frac{E_0}{v_{Hy}} = \Phi_0 + \frac{\Phi_1}{(D)} + \frac{(A)[a + b(D) + c(D)^2]}{(D)[d + e(D)]}$	(4)
A	Transfer + hydrolysis	$\frac{E_0}{v_P} = \Phi_0 + \frac{\Phi_1}{(D)} + \frac{a + b(A)}{c + d(A)}$	(5)
B-1, B-2, C-1, C-2, D	Transfer + hydrolysis	$\frac{E_0}{v_P} = \frac{1}{1 + b(A)} \left[\Phi_0 + \Phi_1(A) + \frac{\Phi_2}{(D)} + \frac{\Phi_3(A)}{(D)} \right]$	(6)
E-1, E-2, F-1, F-2	Transfer + hydrolysis	$\frac{E_0}{v_P} = \frac{1 + r(D)}{s + t(D) + u(A) + v(A)(D)} \left\{ \Phi_0 + \frac{\Phi_1}{(D)} + \frac{(A)[a + b(D) + c(D)^2]}{(D)[d + e(D)]} \right\}$	(7)

the ratio v_T/v_{Hy} continuously increases for increasing values of $1/(D)$, with $\lim(v_T/v_{Hy})_{1/(D) \rightarrow \infty} = f(A)/h$.

(3) Since in the equations 1–7 (Table 1), the values of $\Phi_0 \Phi_1 \Phi_2 \dots$ and $a, b, c \dots$ are different for each individual mechanism, it follows that the effects of (A) in the double-reciprocal plots $1/v_P$ versus $1/(D)$ may vary according to the mechanisms. Differentiation of the equations giving $1/v_P$ 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 $1/v_P$ 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 $1/v_P$ 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 $1/v_P$ 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_P were also analysed (Table 4). For non-linear (competitive) [NL(C)] kinetics, the plots are non-linear and $(E_0/v)_{1/(D)=0}$ is not a function of (I). For non-linear (non-competitive) [NL(NC)] kinetics, the plots are non-linear and $(E_0/v)_{1/(D)=0}$ 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

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

No.	Plots	Kinetic patterns			
		Mechanisms A, B-1, B-2, C-1, C-2, D (group I)	Equation no. (Table 1)	Mechanisms E-1, E-2, F-1, F-2 (group II)	Equation no. (Table 1)
(1)	$\frac{1}{v_T}$ vs. $\frac{1}{(A)}$ for different (D)	Converging lines	(1)	Set of lines not meeting at the same point	(3)
(2)	$\frac{1}{v_T}$ vs. $\frac{1}{(D)}$ for different (A)	Converging lines	(1)	Non-linear	(3)
(3)	$\frac{1}{v_{Hy}}$ vs. (A) for different (D)	Converging lines	(2)	Set of lines not meeting at the same point	(4)
(4)	$\frac{1}{v_{Hy}}$ vs. $\frac{1}{(D)}$ for different (A)	Converging lines	(2)	Non-linear	(4)
(5)	$\frac{v_T}{v_{Hy}}$ vs. (A) for different (D)	Directly proportional to (A)*	(5) and (6)	$\left\{ \begin{array}{l} \frac{v_T}{v_{Hy}} = \frac{(A)[f+g(D)]\dagger}{h+i(D)} \end{array} \right.$	
(6)	$\frac{v_T}{v_{Hy}}$ vs. (D) for different (A)	Independent of (D)*			
(7)	$\frac{1}{v_P}$ vs. $\frac{1}{(D)}$ for different (A)	Set of lines not meeting at the same point‡	(5) and (6)	Non-linear	(7)

* 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 $1/v_P$ 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

$$\frac{1}{v_P} = \frac{(1/v_T)(1/v_{Hy})}{1/v_T + 1/v_{Hy}}$$

can be simplified to

$$\frac{1}{v_P} = \frac{1/v_{Hy}}{1 + v_T/v_{Hy}}$$

and finally to the eqn. (7) of Table 1. Since v_T/v_{Hy} is independent of (D) (no. 6 in Table 2), $1/v_P$ versus $1/(D)$ has the same simplified form as $1/v_{Hy}$ 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_{Hy} 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_{Hy} has the general form $f(A)/[h+i(D)]$ and the plots v_{Hy}/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

Mechanism	Intercept $\left(\frac{1}{(D)}\right)$
A	$\frac{\beta[k_3 + \alpha k_{11} + \alpha k_3 + k_3 \gamma(A) + k_7 \gamma(A)]}{k_7 \gamma(A) + k_{11}}$
B-1	$\frac{k_1 [k_3 k_9(A) + k_5 k_9 + k_5 k_7 (H_2O)]}{k_5 k_9 [k_3(A) + k_2 + k_7 (H_2O)]}$
B-2	$\frac{k_1 [k_5 (H_2O) + k_3(A) + k_7]}{k_7 [k_5 (H_2O) + k_3(A) + k_2]}$
C-1	$\frac{k_1 \left[\frac{k_7 k_{13} + \alpha k_7 k_{11} + \alpha k_7 k_{13} + k_7 k_{13} \beta(A) + k_5 k_{13} \beta(A)}{k_2 + \alpha k_{11} + k_5 \beta(A)} \right]}{k_7 k_{13}}$
C-2	$\frac{k_1 \left[\frac{k_{11} + \alpha k_{11} + \alpha k_9 + k_5 \beta(A) + k_{11} \beta(A)}{k_2 + k_5 \beta(A) + k_9 \alpha} \right]}{k_{11}}$
D	$\frac{K_3 [K_6(A) + K_5 K_6 + K_5 (H_2O)]}{K_4 K_6 [K_3(A) + K_1 K_3 + K_1 (H_2O)]}$

Table 4. Influence of competitive inhibitors

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

Mechanism	Competitive inhibitor for	Equilibria to consider	v_T		v_{HY} vs. D	v_P vs. D
			vs. D	vs. A		
A	D	$E + I \rightleftharpoons EI$	C	NC	C	C
	A	$F + I \rightleftharpoons FI$	UC	C	UC	UC
B-1 and B-2	D	$E + I \rightleftharpoons EI$	C	NC	C	C
	A	$ED + I \rightleftharpoons EDI$	UC	C	UC	UC
C-1 and C-2	D	$E + I \rightleftharpoons EI$				
		$EI + A \rightleftharpoons EIA$	C	NL(NC)	C	C
		$EI + H_2O \rightleftharpoons EIH_2O$				
	A	$ED + I \rightleftharpoons EDI$	UC	C	UC	UC
D	D	$E + I \rightleftharpoons EI$				
		$EI + D \rightleftharpoons EID$	C	NC	C	C
		$EI + H_2O \rightleftharpoons EIH_2O$				
	A	$E + I \rightleftharpoons EI$				
E-1 and E-2	D	$EI + D \rightleftharpoons EID$	NC	C	NC	NC
		$EA + I \rightleftharpoons EAI$	NL(C)	NC	NL(C)	NL(C)
F-1 and F-2	A	$EH_2O + I \rightleftharpoons EH_2OI$				
		$E + I \rightleftharpoons EI$	NL(NC)	C	NL(NC)	NL(NC)
	D	$EA + I \rightleftharpoons EAI$	NL(C)	NC	NL(C)	NL(C)
		$EH_2O + I \rightleftharpoons EH_2OI$				
A	$E + I \rightleftharpoons EI$	NL(NC)	C	NL(NC)	NL(NC)	
		$EI + D \rightleftharpoons EID$				

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

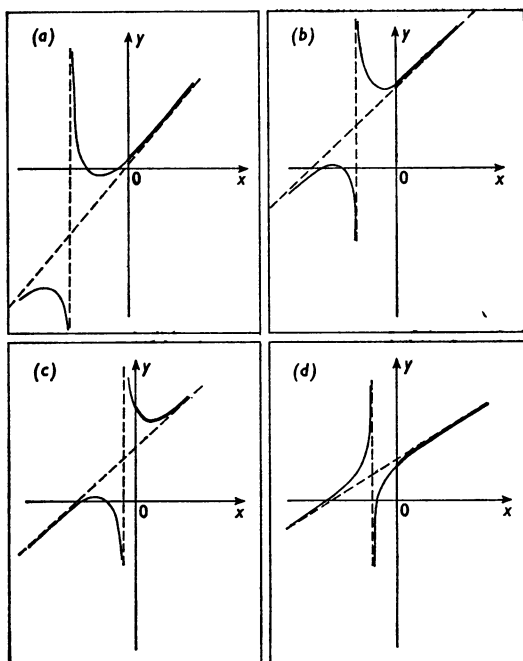


Fig. 1. Graphical representations of the general equation

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

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 $ae^2 > d(be - cd)$.

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

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

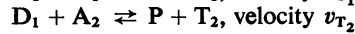
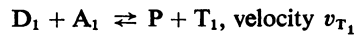
(where x is the inverse of the concentration of the common substrate) also occurs in some equations and therefore the interpretation of the double-reciprocal plots may also be difficult. The results obtained with liver alcohol dehydrogenase (Rudolph & Fromm, 1970) obviously correspond to graph (c) of 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/v_{Hy} appears to be the most useful and dependable parameter. If the ratio v_T/v_{Hy} is independent of (D), the mechanism either is ordered and D binds first to the enzyme or is random. If the ratio v_T/v_{Hy} 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/v_{Hy} 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

Table 5. Simplified rate equations for alternative substrates

The values of $\Phi_0 \dots \Phi_6$ and $a \dots i$ are different for each mechanism. A_2 , alternative substrate of A_1 ; D_1 , common substrate.

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

Table 6. Effects of alternative substrates for A and D

Mechanism	A_2 , alternative for A_1 ; D_1 , common substrate Ratio v_{T_1}/v_{T_2}	D_2 , alternative for D_1 ; A_1 , common substrate Ratio v_{T_1}/v_{T_3}
Random	Proportional to $(A_1)/(A_2)$ Independent of (D_1) Proportional to $(A_1)/(A_2)$	Proportional to $(D_1)/(D_2)$ Independent of (A_1) Proportional to $(D_1)/(D_2)$
Ordered, A binds first	Proportional to $\frac{f + g(D_1)}{h + i(D_1)}$	Independent of (A_1)
Ordered, D binds first	Proportional to $(A_1)/(A_2)$ Independent of (D_1)	Proportional to $(D_1)/(D_2)$ Proportional to $\frac{f' + g'(A_1)}{h' + i'(A_1)}$

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

- Cleland, W. W. (1963) *Biochim. Biophys. Acta* **67**, 104–137
 Dalziel, K. (1957) *Acta Chem. Scand.* **11**, 1706–1723
 Frère, J.-M., Ghuysen, J.-M., Perkins, H. R. & Nieto, M. (1973) *Biochem. J.* **135**, 483–492
 Fromm, H. J. (1964) *Biochim. Biophys. Acta* **81**, 413–417
 Ghuysen, J.-M., Leyh-Bouille, M., Campbell, J. N., Moreno, R., Frère, J.-M., Duez, C., Nieto, M. & Perkins, H. R. (1973) *Biochemistry* **12**, 1243–1251
 Petterson, G. (1969) *Acta Chem. Scand.* **23**, 3243–3247
 Petterson, G. (1972) *Acta Chem. Scand.* **26**, 420–428
 Rudolph, F. B. & Fromm, H. J. (1970) *Biochemistry* **9**, 4460–4465
 Wong, J. T. F. & Hanes, C. S. (1962) *Can. J. Biochem. Physiol.* **40**, 763–804

APPENDIX 1

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

Ping-Pong mechanism A

$$\frac{E_0}{v_T} = \frac{1}{k_7} + \frac{1}{k_3} + \frac{\alpha k_{11} (\text{H}_2\text{O})}{\gamma k_3 k_7 (\text{A})} + \frac{1}{\gamma k_7 (\text{A})} + \frac{\alpha (\text{H}_2\text{O})}{\gamma k_7 (\text{A})} + \frac{1}{k_3 \beta (\text{D})} + \frac{\alpha k_{11} (\text{H}_2\text{O})}{k_3 k_7 \beta \gamma (\text{A})(\text{D})} \quad (1)$$

$$\frac{E_0}{v_{\text{Hy}}} = \frac{1}{k_{11}} + \frac{1}{k_3} + \frac{1}{\alpha k_{11} (\text{H}_2\text{O})} + \frac{\gamma (\text{A})}{\alpha k_{11} (\text{H}_2\text{O})} + \frac{\gamma k_7 (\text{A})}{\alpha k_3 k_{11} (\text{H}_2\text{O})} + \frac{1}{k_3 \beta (\text{D})} + \frac{\gamma k_7 (\text{A})}{k_3 k_{11} \alpha \beta (\text{H}_2\text{O})(\text{D})} \quad (2)$$

$$\frac{E_0}{v_P} = \frac{1}{k_3} + \frac{1}{k_3 \beta (\text{D})} + \frac{1}{\alpha k_{11} (\text{H}_2\text{O}) + k_7 \gamma (\text{A})} [1 + \alpha (\text{H}_2\text{O}) + \gamma (\text{A})] \quad (3)$$

$$\frac{v_T}{v_{\text{Hy}}} = \frac{k_5 k_7 (k_{10} + k_{11}) (\text{A})}{k_9 k_{11} (k_6 + k_7) (\text{H}_2\text{O})} = \frac{k_7 \gamma (\text{A})}{k_{11} \alpha (\text{H}_2\text{O})} \quad (4)$$

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

Theorell-Chance mechanism B-1

$$\frac{E_0}{v_T} = \frac{1}{k_5} + \frac{1}{k_3 (\text{A})} + \frac{k_7 (\text{H}_2\text{O})}{k_3 k_9 (\text{A})} + \frac{1}{k_1 (\text{D})} + \frac{k_2 + k_7 (\text{H}_2\text{O})}{k_1 k_3 (\text{A})(\text{D})} \quad (5)$$

$$\frac{E_0}{v_{\text{Hy}}} = \frac{1}{k_9} + \frac{1}{k_7 (\text{H}_2\text{O})} + \frac{k_3 (\text{A})}{k_5 k_7 (\text{H}_2\text{O})} + \frac{1}{k_1 (\text{D})} + \frac{k_2 + k_3 (\text{A})}{k_1 k_7 (\text{H}_2\text{O})(\text{D})} \quad (6)$$

$$\frac{E_0}{v_P} = \frac{k_7 (\text{H}_2\text{O})}{[k_3 (\text{A}) + k_7 (\text{H}_2\text{O})]} \frac{E_0}{v_{\text{Hy}}} \quad (7)$$

$$\frac{v_T}{v_{\text{Hy}}} = \frac{k_3 (\text{A})}{k_7 (\text{H}_2\text{O})} \quad (8)$$

Theorell-Chance mechanism B-2

$$\frac{E_0}{v_T} = \frac{1}{k_7} + \frac{1}{k_3 (\text{A})} + \frac{k_5 (\text{H}_2\text{O})}{k_3 k_7 (\text{A})} + \frac{1}{k_1 (\text{D})} + \frac{k_2 + k_5 (\text{H}_2\text{O})}{k_1 k_3 (\text{A})(\text{D})} \quad (9)$$

$$\frac{E_0}{v_{\text{Hy}}} = \frac{1}{k_7} + \frac{1}{k_5 (\text{H}_2\text{O})} + \frac{k_3 (\text{A})}{k_5 k_7 (\text{H}_2\text{O})} + \frac{1}{k_1 (\text{D})} + \frac{k_2 + k_3 (\text{A})}{k_1 k_5 (\text{D})(\text{H}_2\text{O})} \quad (10)$$

$$\frac{E_0}{v_P} = \frac{k_5(\text{H}_2\text{O})}{[k_3(\text{A}) + k_5(\text{H}_2\text{O})]} \frac{E_0}{v_{\text{Hy}}} \quad (11)$$

$$\frac{v_T}{v_{\text{Hy}}} = \frac{k_3(\text{A})}{k_5(\text{H}_2\text{O})} \quad (12)$$

Ordered pathway mechanism C-1

$$\frac{E_0}{v_T} = \frac{1}{k_7} + \frac{1}{k_5} + \frac{k_{13} + \alpha k_{13}(\text{H}_2\text{O}) + \alpha k_{11}(\text{H}_2\text{O})}{k_5 k_{13} \beta(\text{A})} + \frac{1}{k_1(\text{D})} + \frac{k_2 + \alpha k_{11}(\text{H}_2\text{O})}{k_1 k_5 \beta(\text{A})(\text{D})} \quad (13)$$

$$\frac{E_0}{v_{\text{Hy}}} = \frac{1}{k_{13}} + \frac{1}{k_{11}} + \frac{k_7 + \beta k_7(\text{A}) + \beta k_5(\text{A})}{k_7 k_{11} \alpha(\text{H}_2\text{O})} + \frac{1}{k_1(\text{D})} + \frac{k_2 + \beta k_5(\text{A})}{k_1 k_{11} \alpha(\text{H}_2\text{O})(\text{D})} \quad (14)$$

$$\frac{E_0}{v_P} = \frac{\alpha k_{11}(\text{H}_2\text{O})}{[\alpha k_{11}(\text{H}_2\text{O}) + \beta k_5(\text{A})]} \frac{E_0}{v_{\text{Hy}}} \quad (15)$$

$$\frac{v_T}{v_{\text{Hy}}} = \frac{k_5 \beta(\text{A})}{k_{11} \alpha(\text{H}_2\text{O})} \quad (16)$$

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

Ordered pathway mechanism C-2

$$\frac{E_0}{v_T} = \frac{1}{k_5} + \frac{1}{k_{11}} + \frac{1}{k_5 \beta(\text{A})} + \frac{\alpha(\text{H}_2\text{O})}{k_5 \beta(\text{A})} + \frac{k_9 \alpha(\text{H}_2\text{O})}{k_5 k_{11} \beta(\text{A})} + \frac{k_2}{k_1 k_5 \beta(\text{A})(\text{D})} + \frac{k_9 \alpha(\text{H}_2\text{O})}{k_1 k_5 \beta(\text{A})(\text{D})} + \frac{1}{k_1(\text{D})} \quad (17)$$

$$\frac{E_0}{v_{\text{Hy}}} = \frac{1}{k_9} + \frac{1}{k_{11}} + \frac{1}{k_9 \alpha(\text{H}_2\text{O})} + \frac{\beta(\text{A})}{k_9 \alpha(\text{H}_2\text{O})} + \frac{k_5 \beta(\text{A})}{k_9 k_{11} \alpha(\text{H}_2\text{O})} + \frac{1}{k_1(\text{D})} + \frac{k_2}{k_1 k_9 \alpha(\text{H}_2\text{O})(\text{D})} + \frac{\beta k_5(\text{A})}{k_1 k_9 \alpha(\text{H}_2\text{O})(\text{D})} \quad (18)$$

$$\frac{E_0}{v_P} = \frac{k_9 \alpha(\text{H}_2\text{O})}{[k_5 \beta(\text{A}) + k_9 \alpha(\text{H}_2\text{O})]} \frac{E_0}{v_{\text{Hy}}} \quad (19)$$

$$\frac{v_T}{v_{\text{Hy}}} = \frac{k_5 \beta(\text{A})}{k_9 \alpha(\text{H}_2\text{O})} \quad (20)$$

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

Rapid-equilibrium random mechanism D

$$\frac{E_0}{v_T} = \frac{1}{k_1} \left[1 + \frac{K_4}{(\text{D})} + \frac{K_5}{(\text{A})} + \frac{K_5(\text{H}_2\text{O})}{K_6(\text{A})} + \frac{K_1 K_4}{(\text{A})(\text{D})} + \frac{K_1 K_4(\text{H}_2\text{O})}{K_3(\text{A})(\text{D})} \right] \quad (21)$$

$$\frac{E_0}{v_{\text{Hy}}} = \frac{1}{k_3} \left[1 + \frac{K_6}{(\text{H}_2\text{O})} + \frac{K_7}{(\text{D})} + \frac{K_2 K_6}{(\text{H}_2\text{O})(\text{D})} + \frac{K_6(\text{A})}{K_5(\text{H}_2\text{O})} + \frac{K_2 K_6(\text{A})}{K_1(\text{H}_2\text{O})(\text{D})} \right] \quad (22)$$

$$\frac{E_0}{v_P} = \frac{k_3 K_5(\text{H}_2\text{O})}{[k_3 K_5(\text{H}_2\text{O}) + k_1 K_6(\text{A})]} \frac{E_0}{v_{\text{Hy}}} \quad (23)$$

$$\frac{v_T}{v_{\text{Hy}}} = \frac{k_1 K_6(\text{A})}{k_3 K_5(\text{H}_2\text{O})} \quad (24)$$

Theorell-Chance mechanism E-1

$$\frac{E_0}{v_T} = \frac{1}{k_5} + \frac{1}{k_3(\text{D})} + \frac{1}{k_1(\text{A})} + \frac{k_2}{k_1 k_3(\text{A})(\text{D})} + \frac{k_7(\text{H}_2\text{O})[k_2 + k_3(\text{D})][k_{11} + k_9(\text{D})]}{k_1 k_3 k_{11}(\text{A})(\text{D})[k_8 + k_9(\text{D})]} \quad (25)$$

$$\frac{E_0}{v_{\text{Hy}}} = \frac{1}{k_{11}} + \frac{1}{k_9(\text{D})} + \frac{1}{k_7(\text{H}_2\text{O})} + \frac{k_8}{k_7 k_9(\text{H}_2\text{O})(\text{D})} + \frac{k_1(\text{A})[k_8 + k_9(\text{D})][k_5 + k_3(\text{D})]}{k_5 k_7 k_9(\text{H}_2\text{O})(\text{D})[k_2 + k_3(\text{D})]} \quad (26)$$

$$\frac{E_0}{v_P} = \frac{k_7 k_9 (\text{H}_2\text{O}) [k_2 + k_3 (\text{D})]}{\{k_7 k_9 (\text{H}_2\text{O}) [k_2 + k_3 (\text{D})] + k_1 k_3 (\text{A}) [k_8 + k_9 (\text{D})]\}} \frac{E_0}{v_{\text{Hy}}} \quad (27)$$

$$\frac{v_T}{v_{\text{Hy}}} = \frac{k_1 k_3 (\text{A}) [k_8 + k_9 (\text{D})]}{k_7 k_9 (\text{H}_2\text{O}) [k_2 + k_3 (\text{D})]} \quad (28)$$

Theorell-Chance mechanism E-2

$$\frac{E_0}{v_T} = \frac{1}{k_9} + \frac{1}{k_1 (\text{A})} + \frac{1}{k_3 (\text{D})} + \frac{k_2}{k_1 k_3 (\text{A}) (\text{D})} + \frac{k_5 (\text{H}_2\text{O}) [k_2 + k_3 (\text{D})] [k_9 + k_7 (\text{D})]}{k_1 k_3 k_9 (\text{A}) (\text{D}) [k_6 + k_7 (\text{D})]} \quad (29)$$

$$\frac{E_0}{v_{\text{Hy}}} = \frac{1}{k_9} + \frac{1}{k_5 (\text{H}_2\text{O})} + \frac{1}{k_7 (\text{D})} + \frac{k_6}{k_5 k_7 (\text{H}_2\text{O}) (\text{D})} + \frac{k_1 (\text{A}) [k_6 + k_7 (\text{D})] [k_9 + k_{13} (\text{D})]}{k_5 k_7 k_9 (\text{H}_2\text{O}) (\text{D}) [k_2 + k_3 (\text{D})]} \quad (30)$$

$$\frac{E_0}{v_P} = \frac{k_5 k_7 (\text{H}_2\text{O}) [k_2 + k_3 (\text{D})]}{\{k_1 k_3 (\text{A}) [k_6 + k_7 (\text{D})] + k_5 k_7 (\text{H}_2\text{O}) [k_2 + k_3 (\text{D})]\}} \frac{E_0}{v_{\text{Hy}}} \quad (31)$$

$$\frac{v_T}{v_{\text{Hy}}} = \frac{k_1 k_3 (\text{A}) [k_6 + k_7 (\text{D})]}{k_5 k_7 (\text{H}_2\text{O}) [k_2 + k_3 (\text{D})]} \quad (32)$$

Ordered pathway mechanism F-1

$$\frac{E_0}{v_T} = \frac{1}{k_7} + \frac{1}{k_5} + \frac{1}{k_5 \beta (\text{D})} + \frac{1}{k_1 (\text{A})} + \frac{k_2}{k_1 k_5 \beta (\text{A}) (\text{D})} + \frac{k_9 (\text{H}_2\text{O}) [k_2 + k_5 \beta (\text{D})] [k_{15} + \alpha k_{15} (\text{D}) + \alpha k_{13} (\text{D})]}{k_1 k_5 k_{15} \beta (\text{A}) (\text{D}) [k_{10} + \alpha k_{13} (\text{D})]} \quad (33)$$

$$\frac{E_0}{v_{\text{Hy}}} = \frac{1}{k_{13}} + \frac{1}{k_{15}} + \frac{1}{k_9 (\text{H}_2\text{O})} + \frac{1}{k_{13} \alpha (\text{D})} + \frac{k_{10}}{k_9 k_{13} \alpha (\text{D}) (\text{H}_2\text{O})} + \frac{k_1 (\text{A}) [k_{10} + k_{13} \alpha (\text{D})] [k_7 + k_7 \beta (\text{D}) + k_5 \beta (\text{D})]}{k_7 k_9 k_{13} \alpha (\text{H}_2\text{O}) (\text{D}) [k_2 + \beta k_5 (\text{D})]} \quad (34)$$

$$\frac{E_0}{v_P} = \frac{k_9 k_{13} \alpha (\text{H}_2\text{O}) [k_2 + k_5 \beta (\text{D})]}{\{k_9 k_{13} \alpha (\text{H}_2\text{O}) [k_2 + k_5 \beta (\text{D})] + k_1 k_5 \beta (\text{A}) [k_{10} + k_{13} \alpha (\text{D})]\}} \frac{E_0}{v_{\text{Hy}}} \quad (35)$$

$$\frac{v_T}{v_{\text{Hy}}} = \frac{k_1 k_5 \beta (\text{A}) [k_{10} + k_{13} \alpha (\text{D})]}{k_9 k_{13} \alpha (\text{H}_2\text{O}) [k_2 + k_5 \beta (\text{D})]} \quad (36)$$

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

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

Ordered pathway mechanism F-2

$$\frac{E_0}{v_T} = \frac{1}{k_5} + \frac{1}{k_{13}} + \frac{1}{k_5 \beta (\text{D})} + \frac{1}{k_1 (\text{A})} + \frac{k_2}{k_1 k_5 \beta (\text{A}) (\text{D})} + \frac{k_7 (\text{H}_2\text{O}) [k_2 + k_5 \beta (\text{D})] [k_{13} + \alpha k_{13} (\text{D}) + \alpha k_{11} (\text{D})]}{k_1 k_5 k_{13} \beta (\text{A}) (\text{D}) [k_8 + k_{11} \alpha (\text{D})]} \quad (37)$$

$$\frac{E_0}{v_{\text{Hy}}} = \frac{1}{k_{11}} + \frac{1}{k_{13}} + \frac{1}{k_7 (\text{H}_2\text{O})} + \frac{1}{k_{11} \alpha (\text{D})} + \frac{k_8}{k_7 k_{11} \alpha (\text{H}_2\text{O}) (\text{D})} + \frac{k_1 (\text{A}) [k_8 + k_{11} \alpha (\text{D})] [k_{13} + k_{13} \beta (\text{D}) + k_5 \beta (\text{D})]}{k_7 k_{11} k_{13} \alpha (\text{H}_2\text{O}) (\text{D}) [k_2 + k_5 \beta (\text{D})]} \quad (38)$$

$$\frac{E_0}{v_P} = \frac{k_7 k_{11} \alpha (\text{H}_2\text{O}) [k_2 + k_5 \beta (\text{D})]}{\{k_7 k_{11} \alpha (\text{H}_2\text{O}) [k_2 + k_5 \beta (\text{D})] + k_1 k_5 \beta (\text{A}) [k_8 + k_{11} \alpha (\text{D})]\}} \frac{E_0}{v_{\text{Hy}}} \quad (39)$$

$$\frac{v_T}{v_{\text{Hy}}} = \frac{k_1 k_5 \beta (\text{A}) [k_8 + k_{11} \alpha (\text{D})]}{k_7 k_{11} \alpha (\text{H}_2\text{O}) [k_2 + k_5 \beta (\text{D})]} \quad (40)$$

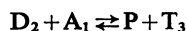
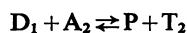
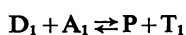
where $\alpha = \frac{k_9}{k_{10} + k_{11}}$

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

APPENDIX 2

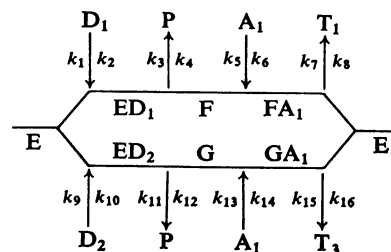
Complete Rate Equations for Enzymic Mechanisms of Transfer Reactions Occurring in the Absence of Hydrolysis but in the Presence of Alternative Substrates

Table 1A of this Appendix show show the complete rate equations were obtained by using the schemes presented in the main text and by replacing accordingly the parameters D, A, H₂O, T and Hy of the equations of Appendix 1 by the parameters D₁, D₂, A₁, A₂, T₁, T₂ and T₃. A₂ is an alternative substrate of A₁ (D₁ being a common substrate) and, alternatively, D₂ is an alternative substrate of D₁ (A₁ being a common substrate). Hence, the following systems were considered:



Where D₂ is an alternative substrate of D₁ (A₁ 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. Ping-Pong Bi Bi mechanism for alternative substrates

D₁ and D₂ bind first.

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

Mechanisms	A ₂ , alternative substrate of A ₁ ; D ₁ , common substrate Replace D by D ₁ , A by A ₁ , H ₂ O by A ₂ , T by T ₁ and Hy by T ₂		D ₂ , alternative substrate of D ₁ ; A ₁ , common substrate Replace D by A ₁ , A by D ₁ , H ₂ O by D ₂ , T by T ₁ and Hy by T ₃	
	In equations of Appendix 1	In schemes of main text	In equations of Appendix 1	In schemes of main text
D binds first				
Ping-Pong	(1)	Scheme 1 (A)	(*)	(*)
Theorell-Chance 1	(5)	Scheme 2 (B-1)	(25)	Scheme 5 (E-1)
Theorell-Chance 2	(9)	Scheme 2 (B-2)	(29)	Scheme 5 (E-2)
Ordered 1	(13)	Scheme 3 (C-1)	(33)	Scheme 6 (F-1)
Ordered 2	(17)	Scheme 3 (C-2)	(37)	Scheme 6 (F-2)
Random	(21)	Scheme 4 (D)	(21)	Scheme 4 (D)
A binds first				
Theorell-Chance 1	(25)	Scheme 5 (E-1)	(5)	Scheme 2 (B-1)
Theorell-Chance 2	(29)	Scheme 5 (E-2)	(9)	Scheme 2 (B-2)
Ordered 1	(33)	Scheme 6 (F-1)	(13)	Scheme 3 (C-1)
Ordered 2	(37)	Scheme 6 (F-2)	(17)	Scheme 3 (C-2)

* This scheme differs from all the schemes presented in the main text. The relevant scheme is the adjacent Scheme 1A (mechanism G) and equations are as follows:

$$\frac{E_0}{v_{T_1}} = \frac{1}{k_3} + \frac{1}{k_7} + \frac{k_6 + k_7}{k_3 k_7 (A_1)} + \frac{k_2 + k_3}{k_1 k_3 (D_1)} + \frac{k_9 (k_2 + k_3)}{k_1 k_3 (k_{10} + k_{11})} \frac{(D_2)}{(D_1)} \left[1 + \frac{k_{11}}{k_{15}} \right] + \frac{k_9 k_{11} (k_2 + k_3) (k_{14} + k_{15})}{k_1 k_3 k_{13} k_{15} (k_{10} + k_{11})} \frac{(D_2)}{(A_1) (D_1)}$$

and

$$\frac{v_{T_1}}{v_{T_3}} = \frac{k_1 k_3 (k_{10} + k_{11}) (D_1)}{k_9 k_{11} (k_2 + k_3) (D_2)}$$

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→T*+P and v the velocity of the reaction A+D→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 H₂O by A* in Scheme 6 (F-1) of the main paper.

The equations giving v*/v and E₀/v* are obtained by replacing H₂O by A* in eqn. (36) and eqn. (34) of Appendix 1 respectively.

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

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. k₁ = k₉, k₂ = k₁₀... Hence, eqn. (36*) simplifies to:

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

and equation (34*) simplifies to:

$$\frac{E_0}{v^*} = \frac{1}{k_5} + \frac{1}{k_7} + \frac{1}{k_5 \beta(D)} + \frac{1}{(A^*)} \left[\frac{1}{k_1} + \frac{(A)}{k_5} + \frac{(A)}{k_7} \right] + \frac{1}{k_5 \beta(A^*)(D)} \left[\frac{k_2}{k_1} + (A) \right] \quad (34^*)$$

Ordered mechanism in which D binds first

The Cleland scheme and eqns. (16*) and (14*) are obtained by replacing H₂O by A* in Scheme 3 (C-1)

of the main paper and in eqns. (16) and (14) respectively. Since k₃ = k₉, k₄ = k₁₀..., eqns. (16*) and (14*) simplify to

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

and

$$\frac{E_0}{v^*} = \frac{1}{k_5} + \frac{1}{k_7} + \frac{1}{(A^*)} \left[\frac{1}{k_5 \beta} + \frac{(A)}{k_5} + \frac{(A)}{k_7} \right] + \frac{1}{k_1 (A^*)(D)} \left[\frac{k_2}{k_5 \beta} + (A) \right] \quad (14^*)$$

Rapid-equilibrium random mechanism

The Cleland scheme and eqns. (24*) and (22*) are obtained by replacing H₂O by A* in Scheme 4 of the main paper and in eqns. (24) and (22) respectively. Since k₁ = k₃, k₂ = k₄, K₁ = K₃, K₅ = K₆ and K₄ = K₇, eqns. (24*) and (22*) simplify to

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

$$\frac{E_0}{v^*} = \frac{1}{k_1} + \frac{1}{k_1 (A^*)} [K_5 + (A)] + \frac{K_4}{k_1 (D)} + \frac{K_2 K_5}{k_1 K_1 (A^*)(D)} [K_1 + (A)] \quad (22^*)$$

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.