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Reversible Inhibition in Bimolecular Rapid Equilibrium Random Order Enzyme Systems

THE EFFECT OF SUBSTRATE–SUBSTRATE AND INHIBITOR–SUBSTRATE INTERACTIONS

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A model is presented that accounts for all types of reversible inhibition by a single inhibitor molecule in bimolecular rapid-equilibrium random-order enzyme systems. The characterization of inhibition mechanisms by graphical methods is examined, and a system of nomenclature is suggested.

Inhibition kinetics in bimolecular rapid-equilibrium random-order enzyme systems have been studied in some detail by Friedenwald & Maengwyn-Davies (1954) and Webb (1963) and is included in the more general approach by Cleland (1963). The first-named authors proposed a general equation that would account for all types of inhibition except those in which the rate of breakdown of the ternary complex is partially inhibited. Webb (1963) examined some of the simpler cases in greater detail, characterizing each by means of the curves expected when any of six different methods of graphical analysis was used. Webb (1963) recognized that subsidiary effects such as the reciprocal effect on each other's affinity for the enzyme, of inhibitor and substrate and of substrate and substrate (hereafter termed inhibitor–substrate interaction and substrate–substrate interactions) would affect the value of the experimentally determined inhibition constant. The examples chosen by Webb (1963) did not, however, illustrate the fact that such interactions would also, in many cases, alter the nature of curves obtained by the graphical methods used. Interpretation of data obtained in such cases would thus be incorrect unless allowance were made for such effects, a point made, but not developed, by Frieden (1964) in his examination of the effects of modifiers on single-substrate enzyme systems.

The work of Cleland (1963) is concerned primarily with systems in which sequential reaction mechanisms take place. Substrate–substrate interactions do not occur in such systems, and therefore Cleland's (1963) methods of interpretation and system of nomenclature are likely to be misleading when dealing with systems in which such effects do arise.

THEORETICAL

Two systems have been examined. The first, shown in Scheme 1, is general and the second, shown in Scheme 2, is for cases where competitive inhibition can take place at either site of a two-substrate enzyme with different molecules of the same inhibitor.

The symbols V, a, b, i, K_a, K_b, K'_a, K'_b and K_t are those defined by Dixon & Webb (1964, p. 786) and α, β and γ are interaction constants defined by Webb (1963, p. xv). Thus:

$$K_a = \frac{[E][A]}{[EA]} \quad (1)$$

$$K'_a = \frac{[EB][A]}{[EAB]} \quad (2)$$

$$K_t = \frac{[E][I]}{[EI]} \quad (3)$$

$$\alpha K_a = \frac{[EI][A]}{[EIA]} \quad (4)$$

$$\alpha K'_a = \frac{[EIB][A]}{[EIAB]} \quad (5)$$

$$\alpha K_t = \frac{[EA][I]}{[EIA]} \quad (6)$$

and the constants pertaining to substrate B are similarly derived.

The ratio:

$$\frac{K'_a}{K_a} = \frac{K'_b}{K_b} = \frac{[EA][EB]}{[E][EAB]} \quad (7)$$

represents the degree of substrate–substrate interaction and the constants α and β represent the degree of inhibitor–substrate interaction between inhibitor I and substrates A and B respectively. The constant γ represents the extent to which the inhibitor affects the rate of breakdown of the EAB complex.

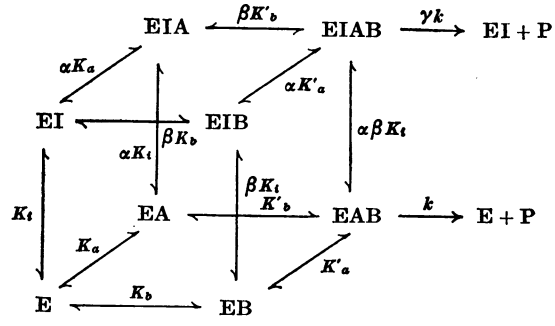
General system

The model for the general system is shown in Scheme 1.

The uninhibited reaction velocity is given by:

$$v=\frac{V}{1+\frac{K'_a}{a}+\frac{K'_b}{b}+\frac{K_aK'_b}{ab}} \tag{8}$$

and the inhibited velocity by:



Scheme 1. Reversible inhibition by a single inhibitor molecule in a bimolecular rapid-equilibrium random-order enzyme system.

$$v_i=\frac{V\left(1+\frac{\gamma}{\alpha\beta}\cdot\frac{i}{K_i}\right)}{1+\frac{i}{\alpha\beta K_i}+\frac{K'_a}{a}\left(1+\frac{i}{\beta K_i}\right)+\frac{K'_b}{b}\left(1+\frac{i}{\alpha K_i}\right)+\frac{K_aK'_b}{ab}\left(1+\frac{i}{K_i}\right)} \tag{9}$$

The six plotting methods used by Webb (1963) to characterize the mechanism of the inhibition are considered here and are as follows: I, $1/v_i$ against $1/s$; II, s/v_i against s (Lineweaver & Burk, 1934); III, v_i against v_i/s (Augustinson, 1948; Hofstee, 1956); IV, $1/v_i$ against i (Dixon, 1953); V, $v/(v-v_i)$ against $1/i$ (Webb 1963, p. 153; VI $iv_i/(v-v_i)$ against s (Hunter & Downs, 1945). The corresponding equations are shown below:

I
$$\frac{1}{v_i}=\frac{K'_a\left(1+\frac{i}{\beta K_i}\right)+a\left(1+\frac{i}{\alpha\beta K_i}\right)}{V\cdot a\left(1+\frac{\gamma i}{\alpha\beta K_i}\right)}\left\{1+\frac{K'_b}{b}\left[\frac{K_a\left(1+\frac{i}{K_i}\right)+a\left(1+\frac{i}{\alpha K_i}\right)}{K'_a\left(1+\frac{i}{\beta K_i}\right)+a\left(1+\frac{i}{\alpha\beta K_i}\right)}\right]\right\} \tag{10}$$

II
$$\frac{b}{v_i}=\frac{K'_b\left[K_a\left(1+\frac{i}{K_i}\right)+a\left(1+\frac{i}{\alpha K_i}\right)\right]+b\left[K'_a\left(1+\frac{i}{\beta K_i}\right)+a\left(1+\frac{i}{\alpha\beta K_i}\right)\right]}{V\cdot a\left(1+\frac{\gamma i}{\alpha\beta K_i}\right)} \tag{11}$$

III
$$v_i=\frac{V\cdot a\left(1+\frac{\gamma i}{\alpha\beta K_i}\right)-\frac{v_i}{b}\left[K'_bK_a\left(1+\frac{i}{K_i}\right)+K'_b\cdot a\left(1+\frac{i}{\alpha K_i}\right)\right]}{K'_a\left(1+\frac{i}{\beta K_i}\right)+a\left(1+\frac{i}{\alpha\beta K_i}\right)} \tag{12}$$

IV
$$\frac{1}{v_i}=\frac{\frac{i}{K_i}\left[\frac{\beta}{b}\left(K'_a+\frac{a}{\alpha}\right)+K'_b\left(K_a+\frac{a}{\alpha}\right)\right]+K'_b(K_a+a)+b(K'_a+a)}{V\cdot ab\left(1+\frac{\gamma i}{\alpha\beta K_i}\right)} \tag{13}$$

V
$$\frac{v}{v-v_i}=\frac{\frac{K_i}{i}\left[K'_b(K_a+a)+b(K'_a+a)\right]+K'_b\left(K_a+\frac{a}{\alpha}\right)+\frac{b}{\beta}\left(K'_a+\frac{a}{\alpha}\right)}{K'_b\left[K_a\left(1-\frac{\gamma}{\alpha\beta}\right)+\frac{a}{\alpha}\left(1-\frac{\gamma}{\beta}\right)\right]+\frac{b}{\beta}\left[K'_a\left(1-\frac{\gamma}{\alpha}\right)+\frac{a}{\alpha}(1-\gamma)\right]} \tag{14}$$

$$\text{VI} \quad i\cdot\frac{v_i}{v-v_i}=\frac{\gamma i+\alpha\beta K_i}{\alpha\beta}\left[\frac{K'_b(K_a+a)+b(K'_a+a)}{K'_b\left[K_a\left(1-\frac{\gamma}{\alpha\beta}\right)+\frac{a}{\alpha}\left(1-\frac{\gamma}{\beta}\right)\right]+\frac{b}{\beta}\left[K'_a\left(1-\frac{\gamma}{\alpha}\right)+\frac{a}{\alpha}(1-\gamma)\right]}\right] \tag{15}$$

For a full analysis of an inhibition mechanism, three sets of data will be required. These concern substrate–substrate interactions and inhibitor–substrate interactions with both substrates. Substrate–substrate interactions may be examined by the method of Florini & Vestling (1957), which will enable the substrate constants to be derived.

The inhibition with respect to substrate A may be examined by measuring the initial velocities at different concentrations of this substrate at a fixed inhibitor concentration, for several different concentrations of the inhibitor. The initial concentration of substrate B should be constant throughout the experiment. Similarly, the kinetics of the inhibition with respect to substrate B may be examined at constant initial concentrations of substrate A, by varying the concentrations of the inhibitor and of substrate B. If possible, the concentration of the unvaried substrate should be of the order of the lower of the two substrate constants pertaining to that substrate. When attempting to characterize an inhibition mechanism, saturating concentrations of the unvaried substrate should not be used, as this will suppress the interactions and may thus lead to an incorrect characterization. It may, however, be necessary to use saturating concentrations when determining inhibition and interaction constants.

Results obtained as above may be analysed by any of the graphical methods quoted. In accord with Webb (1963, p. 190), it is recommended that all six should be used as a routine. This minimizes the possibility of overlooking anomalous kinetic behaviour, and may permit several independent determinations of the inhibition and interaction constants.

The general characteristics of these plotting methods, as related to this model, are examined below.

Plots of types I, II or III will consist of pencils of straight lines intersecting at points defined by:

$$v_i=\frac{V\cdot a[a(\beta-\gamma)+K_a(\alpha\beta-\gamma)]}{a[a(\beta-1)+K_a(\alpha\beta-1)]+K'_a[a(\beta-\alpha)+K_a(\beta-1)]} \tag{16}$$

$$b=-K'_b\left[\frac{K_a(\alpha\beta-\gamma)+a(\beta-\gamma)}{K'_a(\alpha-\gamma)+a(1-\gamma)}\right] \tag{17}$$

Similar expressions may be derived with respect to substrate A.

Curves obtained by plotting method IV will be linear if $\gamma/\alpha\beta=0$ and hyperbolic if $\gamma/\alpha\beta>0$.

If linear, the curves will intersect at a point defined by v_i (eqn. 16) and by:

$$i=-K_i\cdot\frac{\beta(K_b+b)}{\beta K_b+b} \tag{18}$$

if A is being varied or

$$i=-K_i\cdot\frac{\alpha(K_a+a)}{\alpha K_a+a} \tag{19}$$

if B is being varied.

If the curves are hyperbolic they will share a common vertical asymptote at $i=-\alpha\beta K_i/\gamma$.

Curves obtained by plotting method V will always be linear and will intersect at a point defined by $v/(v-v_i)=1$ and $1/i=-\gamma/\alpha\beta K_i$.

Data plotted by method VI will yield a single curve if $\gamma/\alpha\beta=0$, and a family of curves if $\gamma/\alpha\beta>0$. Straight lines parallel to the horizontal axis will be obtained if $\alpha=\beta=1$. When $iv_i/(v-v_i)$ is plotted against a, a single straight line of positive gradient will be obtained if $\alpha=\infty$. If α takes any other positive value the curves will be hyperbolic. The horizontal asymptote will form the upper or lower limit of the curve for positive values of a, depending on the relative values of α and K_a/K'_a and on the concentration of substrate B.

As it stands, the model can be used to represent either activation or inhibition, depending on the values of α, β and γ. Where $\gamma<\alpha$, $\gamma<\beta$ and $\gamma<1$ the system will be one of inhibition. Where $\gamma>\alpha$, $\gamma>\beta$ and $\gamma>1$ the system will be one of activation. Cases in which the values of α, β and γ do not fall within these constraints will lead to either activation or inhibition, depending on the substrate concentrations.

This paper is concerned primarily with the case where $\gamma\leqslant\alpha$, $\gamma\leqslant\beta$ and $0\leqslant\gamma\leqslant1$. Competitive, non-competitive and mixed-inhibition mechanisms will be produced when $0\leqslant\gamma\leqslant1$, $1\leqslant\alpha\leqslant\infty$, $1\leqslant\beta\leqslant\infty$. In this category there will be 11 mechanistically distinguishable cases. These are listed in Table 1, and a system of classification is suggested based on that used by Dixon & Webb (1964, pp. 318–325) and Webb (1963, pp. 157–165) for the unimolecular case.

Table 1. *Types of reversible inhibition in bimolecular Michaelis–Menten enzyme systems*

The constants α and β represent the degree of inhibitor–substrate interaction as defined in the text. The constant γ represents the extent to which the inhibitor affects the rate of breakdown of the EAB complex.

Type of inhibition	$\gamma = 1$	$0 < \gamma < 1$	$\gamma = 0$
Fully non-competitive			$\alpha = 1, \beta = 1$
Partially non-competitive		$\alpha = 1, \beta = 1$	
Fully competitive	$\alpha = \infty, \beta = \infty$	$\alpha = \infty, \beta = \infty$	$\alpha = \infty, \beta = \infty$
Partially competitive (I)	$\alpha = 1, 1 < \beta < \infty$		
(II)	$1 < \alpha < \infty, 1 < \beta < \infty$		
Non-competitive mixed (I)			$\alpha = 1, 1 < \beta < \infty$
(II)			$1 < \alpha < \infty, 1 < \beta < \infty$
Partially mixed (I)		$\alpha = 1, 1 < \beta < \infty$	
(II)		$1 < \alpha < \infty, 1 < \beta < \infty$	
Competitive mixed (I)	$\alpha = 1, \beta = \infty$	$\alpha = 1, \beta = \infty$	$\alpha = 1, \beta = \infty$
(II)	$1 < \alpha < \infty, \beta = \infty$	$1 < \alpha < \infty, \beta = \infty$	$1 < \alpha < \infty, \beta = \infty$

Plotting patterns expected from these mechanisms are summarized in Table 2. In theory, it is possible to characterize all mechanisms by means of plotting methods I and IV. In practice, however, depending on the accuracy of the experimental methods used, it may be difficult to determine without ambiguity whether a common intersection point in these plots is axial or non-axial. Use of methods V and VI should furnish additional, independent, criteria, such as multiplicity or non-linearity of curves, sufficient to permit a definite characterization. In cases where the position of the intersection point in a type I plot is in doubt, secondary plots in which derived data such as gradients or intercepts are plotted against inhibitor concentration should be avoided, as these data will themselves be dependent on the position of the intersection point.

Table 2 also indicates that some inhibition mechanisms may not be distinguished from each other by the inspection of plotting patterns alone. In these cases the mechanism can be fully characterized only by evaluation of the inhibitor constant and the interaction constants. The most direct methods for determining these constants in each case are considered below.

Fully non-competitive inhibition. The inhibitor has no effect on the binding of either substrate ($\alpha = \beta = 1$), but prevents the breakdown of the complex ($\gamma = 0$). The inhibition constant may be determined from a type IV plot, in which the lines intersect on the horizontal axis at $i = -K_i$, or from a type VI plot, in which the vertical intercept of the horizontal line is at $iv_i/(v-v_i) = K_i$.

Partially non-competitive inhibition. In this case the affinity of the enzyme for the substrates is again unaffected, but the breakdown of the complex is only partially inhibited ($\alpha = \beta = 1$, $0 < \gamma < 1$). The inhibition constant and the constant γ are most easily derived from a type VI plot, in which the horizontal straight lines intersect the vertical axis at $iv_i/(v-v_i) = \gamma i + K_i$. Thus a secondary plot of these vertical intercepts against the inhibitor concentration will yield a straight line of gradient γ and vertical intercept K_i.

Fully competitive inhibition. The presence of the inhibitor on the enzyme completely prevents the attachment of both substrates ($\alpha = \beta = \infty$). The formation of an enzyme–inhibitor–substrate complex is thus prevented, so that a meaningful value cannot be assigned to the constant γ. The inhibition constant may be obtained from a type IV plot, in which, if substrate B is being held constant, the lines intersect at a point defined by $i = -K_i(1+b/K_b)$ and $1/v_i = 1/V$, or from a type VI plot in which the single straight line intersects the vertical axis at $iv_i/(v-v_i) = K_i$.

Partially competitive inhibition. Three cases may be defined. In all three the attachment of one substrate is partially inhibited while the breakdown of the complex is unaffected ($1 < \beta < \infty$, $\gamma = 1$). In this, as in the cases of mixed inhibition discussed below, the three subsidiary cases are defined by the effect of the inhibitor on the second substrate. Thus in the first case the affinity of the enzyme for the second substrate is unaffected ($\alpha = 1$), whereas in the second case it is decreased ($1 < \alpha < \infty$). A third, restricted, case is found when the second substrate is affected by neither the inhibitor nor by the first substrate ($\alpha = K'_a/K_a = 1$). Determination of the inhibitor constant and the interaction constants is most practicable by the method described below for the case of partially mixed inhibition.

Competitive mixed inhibition. In this case the presence of the inhibitor on the enzyme completely prevents the attachment of one of the substrates to the enzyme ($\beta = \infty$). The three subsidiary cases are defined with respect to the second substrate as above. α may be determined from a type I plot made with respect to substrate A, the common point of intersection being defined by $1/a = -1/\alpha K_a$

Table 2. *Characterization of type of inhibition by graphical analysis*

Nature of curve: ML, linear, multiple; SL, linear, single line; MH, multiple, hyperbolic; SH, hyperbolic, single curve. Common intersection point: X, on horizontal axis; Y, on vertical axis; NA, non-axial; P, parallel lines. Gradient: +, positive; −, negative; 0, parallel to horizontal axis.

Plotting method	I		II		III		IV		V		VI	
Varied substrate	A	B	A	B	A	B	A	B	A	B	A	B
Type of inhibition												
Fully non-competitive	MLX+	MLX+	MLX+	MLX+	MLP−	MLP−	MLX+	MLX+	SL+	SL+	SL0	SL0
Partially non-competitive	MLX+	MLX+	MLX+	MLX+	MLP−	MLP−	MH	MH	SL+	SL+	MLP0	MLP0
Fully competitive	MLY+	MLY+	MLP+	MLP+	MLY−	MLY−	MLNA+	MLNA+	MLY+	MLY+	SL+	SL+
Partially competitive (I)	MLNA+	MLY+	MLNA+	MLP+	MLNA−	MLY−	MH	MH	MLNA+	MLNA+	MH	MLX+
(II)	MLNA+	MLNA+	MLNA+	MLNA+	MLNA−	MLNA−	MH	MH	MLNA+	MLNA+	MH	MH
(III)	MLX+	MLY+	MLX+	MLP+	MLP−	MLY−	MH	MH	SL+	MLNA+	MLP0	MLX+
Non-competitive mixed (I), (II)	MLNA+	MLNA+	MLNA+	MLNA+	MLNA−	MLNA−	MLNA+	MLNA+	MLY+	MLY+	SH	SH
(III)	MLX+	MLNA+	MLX+	MLNA+	MLP−	MLNA−	MLX+	MLNA+	SL+	MLY+	SL0	SH
Competitive mixed (I), (II)	MLNA+	MLY+	MLNA+	MLP+	MLNA−	MLY−	MLNA+	MLNA+	MLY+	MLY+	SH	SL+
(III)	MLX+	MLY+	MLX+	MLP+	MLP−	MLY−	MLX+	MLNA+	SL+	MLY+	SL0	SL+
Partially mixed (I), (II)	MLNA+	MLNA+	MLNA+	MLNA+	MLNA−	MLNA−	MH	MH	MLNA+	MLNA+	MH	MH
(III)	MLX+	MLNA+	MLX+	MLNA+	MLP−	MLNA−	MH	MH	SL+	MLNA+	MLP0	MH

and $1/v_i = (1-K'_b/\alpha K_b)/V$. The inhibitor constant may be derived from a type IV plot made with respect to substrate A, the common intersection point occurring at $i = -K_i(1+b/K_b)$, $1/v_i$ being defined as above.

Only in the third subsidiary case, in which $\alpha = K'_a/K_a = 1$, will the kinetics of the inhibition with respect to the non-competing substrate be classically non-competitive in character. That is to say, only in this third case will the pencils of lines obtained in type I and type IV plots intersect on the horizontal axis. Thus, even if the inhibitor competing with substrate B has no direct effect on the affinity of substrate A ($\alpha = 1$), if there is significant substrate–substrate interaction the inhibition kinetics with respect to substrate A will have the character of mixed inhibition in the unimolecular case. This illustrates the point that the observation of apparently mixed-inhibition kinetics does not necessarily imply that the inhibitor acts at more than one site in the reaction sequence.

Non-competitive mixed inhibition. Three cases may again be distinguished. In all three the breakdown of the complex is completely prevented and the affinity of the enzyme for the substrate is decreased ($\gamma = 0$, $1<\beta<\infty$). The subsidiary cases are defined with respect to the second substrate as above. In all cases the interaction constants may be obtained from type I plots if the concentration of the unvaried substrate is held at a saturating value. Thus, if substrate B is present in saturating concentration, then a type I plot made with respect to substrate A will have a common intersection point defined by $1/a = -1/\alpha K_a$ and $1/v_i = [(1-1/\alpha)/V]$. β can be determined in a similar fashion. The inhibition constant may then be obtained by replotting the data on type IV axes where, if substrate A is being held at saturating concentration, the pencil of lines obtained will have a common intersection point at $i = -\alpha K_i$.

Partially mixed inhibition. Again, three classes of inhibition occur within this category. In all cases the breakdown of the complex is partially inhibited and the affinity of one substrate is reduced ($0<\gamma<1$, $1<\beta<\infty$). The subsidiary cases are defined as before. As is the case for partially competitive inhibition, the interaction constants are best derived by the determination, by the method of Florini & Vestling (1957), of the apparent substrate constants and the apparent maximum velocity at saturating inhibitor concentration. These will be αK_a, $\alpha K'_a$, βK_b, $\beta K'_b$ and γV. The values of α, β and γ thus obtained may then be used to derive the inhibition constant from a type V plot in which the intersection point will be defined by $1/i = -\gamma/\alpha\beta K_i$.

Uncompetitive inhibition. In the unimolecular model inhibition by an uncompetitive mechanism

is defined as taking place when the inhibitor combines only with the enzyme–substrate complex (Ebersole, Guttentag & Wilson, 1944). In the bimolecular case such inhibition could take place by combination of the inhibitor with either the ternary complex or with one of the binary complexes. (It seems improbable *a priori* that the inhibitor would combine with both binary complexes but not with the free enzyme.) Appropriate equations can be obtained from the general equation by postulating a very large value for K_i and a very small value for α or β or for both. When the inhibitor combines only with the ternary complex both α and β take small values, so that the only inhibition term significantly greater than unity is $1+i/\alpha\beta K_i$. In this case plots made with respect to either substrate will be as for the classical case of uncompetitive inhibition. Thus plots I and IV will consist of series of parallel straight lines of positive gradient. Plots II and V will consist of pencils of straight lines intersecting on the vertical axis, and the lines obtained in a type III plot will intersect on the horizontal axis. Plotting data on type VI axes will give a hyperbola whose horizontal asymptote forms the lower limit of the curve, regardless of substrate concentration. Replotting the vertical intercepts from a type I plot will yield a straight line intersecting the horizontal axis at $i = -\alpha\beta K_i(1+K'_a/a)$.

If α is very small and $\beta = \infty$, plots made with respect to substrate A will be uncompetitive in character, whereas plots made with respect to substrate B will be competitive in character. If α is very small, while $1<\beta<\infty$, then plots made with respect to substrate A will be uncompetitive in character, whereas plots made with respect to substrate B will be as for non-competitive mixed inhibition.

Uncompetitive kinetics will be observed in the case in which $\alpha = 1$, $1<\beta<\infty$, $\beta = \gamma$, when plots are made with respect to substrate B. The mechanism in this case cannot, however, be described as uncompetitive.

Doubly competitive inhibition

The model for this system is shown in Scheme 2. The inhibitor may compete at both substrate sites on the enzyme. It is to be distinguished from the fully competitive case defined above where a single inhibitor molecule is able to block both sites simultaneously.

The general equation is:

$$v_i = \frac{\dfrac{V\cdot a}{a+K'_a\left(1+\dfrac{i}{\beta' K'_i}\right)}}{1+\dfrac{K'_b}{b}\left[\dfrac{\left(1+\dfrac{i}{K_i}+\dfrac{i}{K'_i}+\dfrac{i^2}{\delta K_i K'_i}\right)K_a+a\left(1+\dfrac{i}{\alpha' K_i}\right)}{a+K'_a\left(1+\dfrac{i}{\beta' K'_i}\right)}\right]} \tag{20}$$

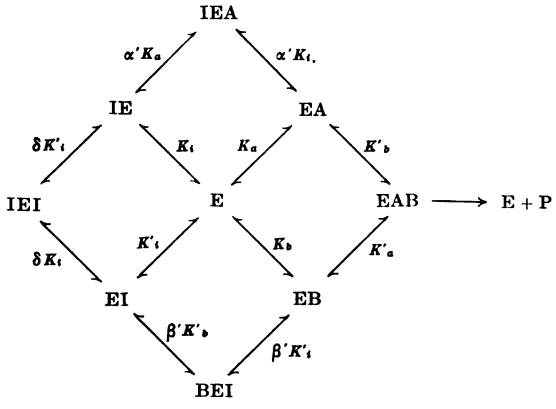
and the equations for the plotting methods are:

$$\text{I–IV}\qquad \frac{1}{v_i} = \frac{1}{V\cdot a}\left\{a+K'_a\left(1+\frac{i}{\beta' K'_i}\right)+\frac{K'_b}{b}\left[1+\frac{i}{K_i}+\frac{i}{K'_i}+\frac{i^2}{\delta K_i K'_i}+a\left(1+\frac{i}{\alpha' K_i}\right)\right]\right\} \tag{21}$$

$$\text{V}\qquad \frac{v}{v-v_i} = 1+\frac{1}{i}\left[\frac{1+\dfrac{K'_a}{a}+\dfrac{K'_b}{b}+\dfrac{K_a K'_b}{ab}}{\dfrac{K'_a}{a}\cdot\dfrac{1}{\beta' K_i}+\dfrac{K'_b}{b}\cdot\dfrac{1}{\alpha' K_i}+\dfrac{K_a K'_b}{ab}\left(1+\dfrac{1}{K_i}+\dfrac{1}{K'_i}+\dfrac{i}{\delta K_i K'_i}\right)}\right] \tag{22}$$

$$\text{VI}\qquad \frac{iv_i}{v-v_i} = \frac{a(b+K'_b)+K'_a(b+K_b)}{\dfrac{aK'_b}{\alpha' K_i}+K'_a\left[\dfrac{b}{\beta' K'_i}+K_b\left(\dfrac{1}{K_i}+\dfrac{1}{K'_i}+\dfrac{i}{\delta K_i K'_i}\right)\right]} \tag{23}$$

In general, plots on type I, II or III axes will be linear but with no common point of intersection. If the inhibitor is fully competitive at either site ($\alpha' = \beta' = \infty$) the lines will be as for fully competitive inhibition, intersecting on the vertical axis on a type I plot. In the type IV plot, however, the curves will be parabolic unless the unvaried substrate is present in saturating concentration. If this is the case, the term containing i^2 will vanish and the inhibition will appear to be fully competitive. With the unvaried substrate present in saturating concentration it will be possible to



Scheme 2. Doubly competitive inhibition by an inhibitor that competes at both sites of a two-substrate enzyme.

derive values for $\alpha' K_i$ and $\beta' K'_i$, as for fully competitive inhibition. The other constants may not be derived by the use of any simple graphical methods.

Type V plots will be parabolic and type VI plots will be hyperbolic in the general case.

DISCUSSION

The 'doubly competitive' model described above seems likely to occur experimentally with some of the GSH transferases and DDT* dehydrochlorinase, since there is reason to believe that some compounds may combine with either of the receptor sites on these enzymes. Bromsulphalein competes with GSH in grass-grub GSH *S*-aryltransferase (Clark, Darby & Smith, 1967); this compound is also a substrate for the insect enzyme (Cohen, Turbert & Smith, 1964) and should therefore be a competitor for substrates like 1-chloro-2,4-dinitrobenzene. DDT dehydrochlorinase is inhibited by a range of diphenylmethane derivatives that are GSH competitors in GSH transferases and are also structural analogues of DDT. The data quoted by Cohen *et al.* (1964) and by Balabaskaran & Smith (1970) do not, however, show the parabolic form taken by the type IV plot in doubly competitive inhibition. A related enzyme examined by Boyland & Chasseaud (1968) may, however, be an experimental case of a parabolic type IV plot, and it is not unreasonable to believe that their inhibitor, *S* - $\alpha\beta$ - diethoxycarbonylethylglutathione, could compete with both the GSH site and the second substrate site of GSH *S*-alkenetransferase.

In the general model described above the anomalous patterns of plots arise when substrate interactions occur. The equations derived from the general model also describe mixed activation–inhibition systems, and it is noteworthy that some combinations of α, β and γ can yield values of b or v from eqns. (16) and (17) corresponding to positive values of b on a type I plot as was demonstrated for the single-substrate case by Frieden (1964).

* Abbreviation: DDT, 1,1,1-trichloro-2,2-bis-(*p*-chlorophenyl)ethane.

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