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REVERSIBLE INHIBITION OF ENZYMATIC SYSTEMS INVOLVING COVALENT INTERMEDIATES

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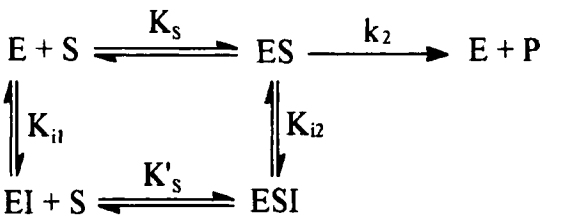
Reversible inhibition phenomena are analyzed for enzymatic systems involving covalent intermediates, where the inhibitor can bind to the pure enzyme, the Henri-Michaelis complex or the covalent intermediate, or to two or three of these enzyme forms. Classical competitive, non-competitive or un-competitive phenomena can be observed in some cases but unexpected features are also observed. Complex phenomena sometimes prevail where increased k_{cat}/K_m values can be accompanied by decreased or increased k_{cat} values.

Keywords: Mechanism; reversible inhibition.

INTRODUCTION

Reversible inhibition phenomena are commonly discussed on the basis of the simple Henri-Michaelis Scheme[1–4] (Scheme A):

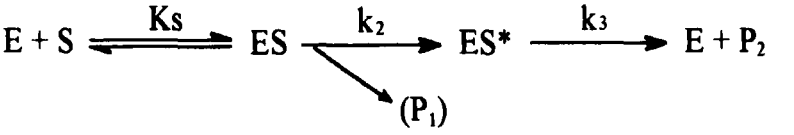


SCHEME A

*Correspondence.

If the inhibitor binds to both E and ES, the inhibition is non-competitive, "Pure" non-competitive inhibition (unchanged K_m) occurs when binding of the substrate does not affect that of the inhibitor and vice-versa, that is: $K_{i1} = K_{i2}$ and $K'_S = K_S$, (Scheme A). If the inhibitor only binds to E or ES, the inhibition is, respectively, competitive (unchanged V_{max}) or un-competitive (unchanged V_{max}/K_m).

Numerous enzymatic mechanisms however involve the formation of a covalent intermediate ES^* (active-site Serine peptidases and esterases, β-lactamases, etc....) as in Scheme B:



SCHEME B

The inhibitor can bind to E, ES and/or ES^*. This results in a large number of possible inhibition patterns, with some unexpected effects. These are discussed in the following contribution.

THEORY

The discussion is based on the rapid equilibrium hypothesis for the formation of ES and the steps involving the inhibitor, i.e. all the reversible steps. If the steady-state conditions were applied to the binding of substrate only, the equations would be more complex in the cases of Models I, II, III and VI. A general treatment with non-equilibrium binding of Model I would yield exceedingly complicated equations. However, this latter case might sometimes be diagnosed by monitoring a time-dependent decrease of the enzyme activity.[5]

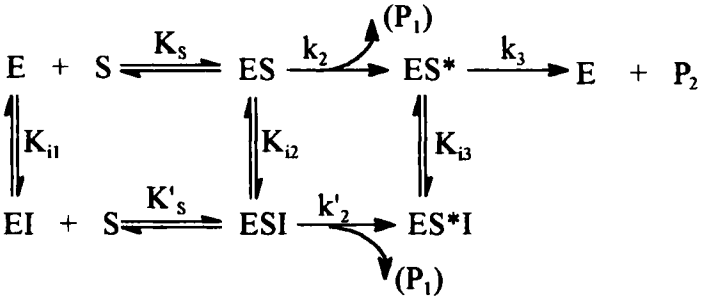
In the absence of inhibitor, the well-known Equations (1–3) derive from Scheme B:

$$(k_{cat})_0 = k_2k_3/(k_2 + k_3) \quad (1)$$

$$(K_m)_0 = k_3K_S/(k_2 + k_3) \quad (2)$$

$$(k_{cat}/K_m)_0 = k_2/K_S. \quad (3)$$

When the inhibitor is present, the general model is represented by Scheme C:



SCHEME C

Table I shows the various possible models based on Scheme C. The complete, general model I involves the 3 complexes EI, ESI and ES^*I, whose dissociation constants are K_{i1}, K_{i2} and K_{i3} respectively. Hence, $K_S K_{i2} = K'_S K_{i1}$. Various more simple cases are possible. They are presented in Table I.

EQUATIONS AND DISCUSSIONS

The complete equations for k_{cat}, K_m and k_{cat}/K_m are given in Appendix 1. In several cases, the Dixon plots (1/v vs. [I]) are not linear and, although the Lineweaver-Burk, Hanes and Eadie-Hofstee plots remain linear, the lines do not converge. By contrast, classical competitive, non-competitive or un-competitive patterns can also be obtained.

Table II summarizes the characteristics of the various models. Table III details the co-ordinates of the convergence point $P(x, y)$ when applicable, i.e. when linear Dixon plots are observed. The complex patterns of Models I and VI can result in activation phenomena. For the analysis of the 4 typical plots, see Appendix 2.

The following points deserve attention.

1. In models I.a. and I.b., both k_{cat} and K_m can increase with increasing [I], and k_{cat}/K_m increases if $k'_2 K_{i1} > k_2 K_{i2}$ (Model I.a.), or if $k'_2 > k_2$ (Model I.b.) (Figures 1, 2).
2. In Model I.c., k_{cat} is always $< (k_{cat})_0$, but $k_{cat}/K_m > (k_{cat}/K_m)_0$ if $K_{i1} > K_{i2}$. If $k_3 \gg k_2(1 + [I]/K_{i3})$, then, $k_{cat} \to (k_{cat})_0$, and $K_m \to K_S(1 + [I]/K_{i1})/(1 + [I]/K_{i2})$, the pattern becomes competitive, but I can behave as an activator. In the same model, if $K_{i1} = K_{i2}, k_{cat}/K_m = k_2/K_S = (k_{cat}/K_m)_0$ and un-competitive inhibition is observed.

TABLE I Summary of reversible inhibition models

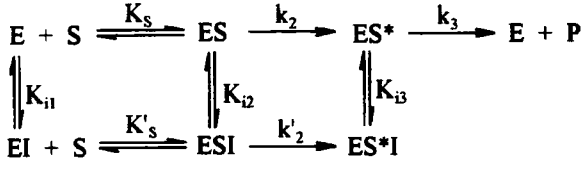
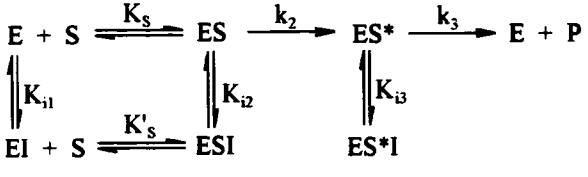
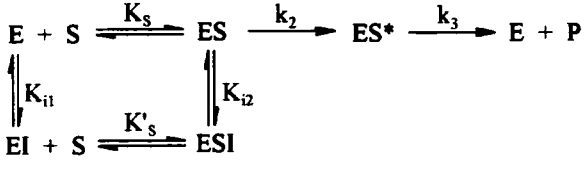
Models	*Schemes*	*Sub-Models*	*Conditions*
Model I		I.a.	$K_{i1} \neq K_{i2} \neq K_{i3}, k'_2 \neq k_2$
		I.b.	$K_{i1} = K_{i2} = K_{i3} = K_i, k'_2 \neq k_2$
		I.c.	$K_{i1} \neq K_{i2} \neq K_{i3}, k'_2 = k_2$
		I.d.	$K_{i1} = K_{i2} = K_{i3}, k'_2 = k_2$
Model II		II.a.	$K_{i1} \neq K_{i2} \neq K_{i3}, k'_2 = 0$
		II.b.	$K_{i1} = K_{i2} = K_{i3} = K_i, k'_2 = 0$
Model III		III.a.	$K_{i1} \neq K_{i2}, K_{i3} \to \infty, k'_2 = 0$
		III.b.	$K_{i1} = K_{i2} = Ki, K_{i3} \to \infty, k'_2 = 0$

TABLE I *Continued*

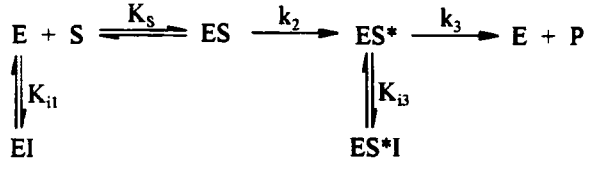
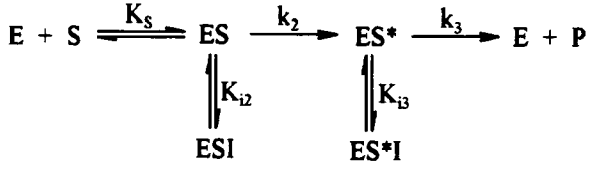
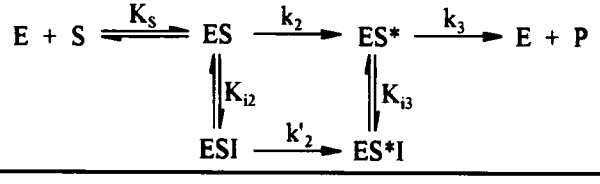
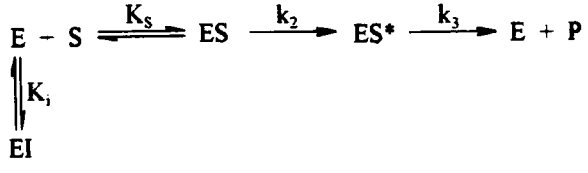
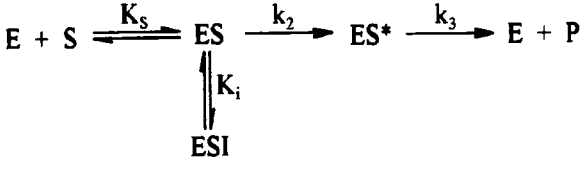
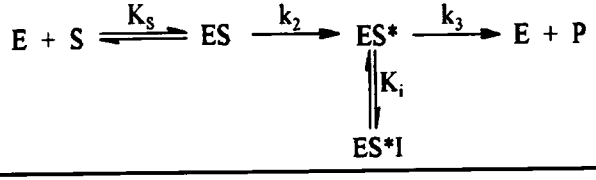
Models	*Schemes*	*Sub-Models*	*Conditions*
Model IV		IV.a. IV.b.	$K_{i1} \neq K_{i3}, K_{i2} \to \infty, k'_2 = 0$ $K_{i1} = K_{i3} = K_i, K_{i2} \to \infty, k'_2 = 0$
Model V		V.a. V.b.	$K_{i2} \neq K_{i3}, K_{i1} \to \infty, k'_2 = 0$ $K_{i2} = K_{i3} = K_i, K_{i1} \to \infty, k'_2 = 0$
Model VI		VI.a. VI.b. VI.c. VI.d.	$K_{i2} \neq K_{i3}, K_{i1} \to \infty, k'_2 \neq k_2$ $K_{i2} = K_{i3} = K_i, K_{i1} \to \infty, k'_2 \neq k_2$ $K_{i2} \neq K_{i3}, K_{i1} \to \infty, k'_2 = k_2$ $K_{i2} = K_{i3} = K_i, K_{i1} \to \infty, k'_2 = k_2$

TABLE I *Continued*

Models	*Schemes*	*Sub-Models*	*Conditions*
Model VII			$K_{i1} = K_i, K_{i2} \to \infty, K_{i3} \to \infty, k_2' = 0$
Model VIII			$K_{i2} = K_i, K_{i1} \to \infty, K_{i3} \to \infty, k_2' = 0$
Model IX			$K_{i3} = K_i, K_{i1} \to \infty, K_{i2} \to \infty, k_2' = 0$

E = free enzyme, ES = Henri-Michaelis complex, ES* = covalent intermediate, I = inhibitor, ES*I = acylenzyme-inhibitor complex, P = (second) product. K_S, K_S', K_i, K_{i1}, K_{i2} and K_{i3} are dissociation constants, k_2, k_2' and k_3 are first order rate constants.

TABLE II Characteristics of three classical plots for each model[a]

Model	*L-B Plots*[b]	*Hanes Plots*	*Dixon Plots*	*Inhibition Type*
I.a.	No convergence	No convergence	Non-linear	Complex. General situation
I.b.	No convergence	No convergence	Non-linear	Complex
I.c.	No convergence	No convergence	Non-linear	Complex
I.d.	Parallel	Converge	Parallel	Un-competitive
II.a.	Converge	Converge	Converge	Complex
II.b.	Converge	Converge	Converge	Non-competitive
III.a.	Converge	Converge	Converge	Complex
III.b.	Converge	Converge	Converge	Complex
IV.a.	Converge	Converge	Converge	Complex
IV.b.	Converge	Converge	Converge	Complex
V.a.	Parallel	Converge	Parallel	Un-competitive
V.b.	Parallel	Converge	Parallel	Un-competitive
VI.a.	No convergence	No convergence	Non-linear	Complex, k_{cat}/K_m increases vs. [I]
VI.b.	No convergence	No convergence	Non-linear	Complex, k_{cat}/K_m increases vs. [I]
VI.c.	No convergence	No convergence	Non-linear	Complex, k_{cat}/K_m increases vs. [I]
VI.d.	No convergence	No convergence	Non-linear	Complex, k_{cat}/K_m increases vs. [I]
VII	Converge	Parallel	Converge	Competitive
VIII	Parallel	Converge	Parallel	Un-competitive
IX	Parallel	Converge	Parallel	Un-competitive

[a] For schemes, see Table I. [b] L-B = Lineweaver-Burk.

3. In Model I.a., an unmodified k_{cat}/K_m value can be observed if $k'_2 K_{i1} = k_2 K_{i2}$. The Lineweaver-Burk and Hanes plots suggest an un-competitive inhibition, but the Dixon plots are not linear.
4. In Model III.a., the non-competitive inhibition can become apparently competitive if $k_2 \gg k_3(1 + [I]/K_{i2})$ (Figure 3). Similarly, in Model III.b., the non-competitive inhibition can become apparently "pure" non-competitive or competitive if $k_3 \gg k_2$ or if $k_2 \gg k_3(1 + [I]/K_i)$ respectively.
5. In Model IV.a. and IV.b., the non-competitive inhibition becomes apparently competitive if $k_3 \gg k_2(1 + [I]/K_{i3})$, and in Model IV.b., "pure" non-competitive if $k_2 \gg k_3$.
6. In Model VI, k_{cat}/K_m is always $> (k_{cat}/K_m)_0$, and k_{cat} can increase or decrease (VI.a., VI.b.), or always decrease (VI.c., VI.d.) with increasing [I] (Figures 4, 5). With Model VI.c., an apparently "competitive activation" can be obtained if $k_3 \gg k_2(1 + [I]/K_{i3})$. In that case, $k_{cat} \to (k_{cat})_0 = k_2$, but the Dixon plot is not linear and k_{cat}/K_m increases with [I] in a linear fashion.

TABLE III Coordinates of the convergence points $P(x, y)$[a] for each model in the three classical "linearized" plots

	Lineweaver-Burk Plots		*Hanes Plots*		*Dixon Plots*	
Models	$P(x, y)$, $(Z = k_2/(k_2 + k_3))$	*Position*	$P(x, y)$, $(Z = k_2/(k_2 + k_3))$	*Position*	$P(x, y)$, $(Z = k_2/(k_2 + k_3))$	*Position*
I.a,I.b,I.c	No convergence	—	No convergence	—	Curve	—
I.d	Parallel	—	$0, (K_m/k_{cat})_0^c$	$+Y^e$	Parallel	—
II.a	$-(1/K_m)_0(ZK_{i1}/K_{i3} + (1 - Z)K_{i1}/K_{i2})$,	$Q2$ or	$-(K_m)_0K_{i2}K_{i3}/(K_{i1}(ZK_{i2} + (1 - Z)K_{i3}))$,	$Q2$ or	$-K_{i1}$,	$Q2$ or
	$(1/k_{cat})_0(1 - ZK_{i1}/K_{i3} - (1 - Z)K_{i1}/K_{i2})$	$Q3$[b]	$(K_m/k_{cat})_0(1 - K_{i2}K_{i3}/(K_{i1}(ZK_{i2} + (1 - Z)K_{i3})))$	$Q3$	$(1/k_{cat})_0(1 - ZK_{i1}/K_{i3} - (1 - Z)K_{i1}/K_{i2})$	$Q3$
II.b	$-1/(K_m)_0, 0$	$-X$[d]	$-(K_m)_0, 0$	$-X$	$-K_i, 0$	$-X$
III.a	$-(1 - Z)K_{i1}/((K_m)_0K_{i2})$,	$Q2$ or	$-(K_m)_0K_{i2}/((1 - Z)K_{i1})$,	$Q2$ or	$-K_{i1}$,	$Q2$ or
	$(K_{i2} - (1 - Z)K_{i1})/((k_{cat})_0K_{i1})$	$Q3$	$(1 - K_{i2}/((1 - Z)K_{i1}))(K_m/k_{cat})_0$	$Q3$	$(K_{i2} - (1 - Z)K_{i1})/((k_{cat})_0K_{i1})$	$Q3$
III.b	$(1 - Z)/(K_m)_0, Z/(k_{cat})_0$	$Q2$	$-(K_m)_0/(1 - Z), -(Z/(1 - Z))(K_m/k_{cat})_0$	$Q3$	$-K_i, 1/k_3$	$Q2$
IV.a	$-ZK_{i1}/((K_m)_0K_{i3})$,	$Q2$ or	$-(K_m)_0K_{i3}/ZK_{i1}$,	$Q2$ or	$-K_{i1}$,	$Q2$ or
	$(K_{i3} - ZK_{i1})/((k_{cat})_0K_{i3})$	$Q3$	$(1 - K_{i3}/ZK_{i1})(K_m/k_{cat})_0$	$Q3$	$(K_{i3} - ZK_{i1})/((k_{cat})_0K_{i3})$	$Q3$
IV.b	$-Z/(K_m)_0, 1/k_2$	$Q2$	$-(K_m)_0/Z, -(k_3/k_2)(K_m/k_{cat})_0$	$Q3$	$-K_i, 1/k_2$	$Q2$
V.a	Parallel	—	$0, (K_m/k_{cat})_0$	$+Y$	Parallel	—
V.b	Parallel	—	$0, (K_m/k_{cat})_0$	$+Y$	Parallel	—
VI.a-VI.d	No convergence	$Q1$[f]	No convergence	$Q1$[f]	Curve	—
VII	$0, 1/(k_{cat})_0$	$+Y$	Parallel	—	$-K_i, 1/(k_{cat})_0$	$Q2$
VIII	Parallel	—	$0, (K_m/k_{cat})_0$	$+Y$	Parallel	—
IX	Parallel	—	$0, (K_m/k_{cat})_0$	$+Y$	Parallel	—

(a) $P(x, y)$ is the convergence point of the lines corresponding to the different concentrations of inhibitor (for Lineweaver-Burk or Hanes plots) or substrate (for Dixon plot). $v/[E_0]$ was used instead of v (initial reaction rate) where $[E_0]$ is total enzyme concentration. (b) $Q1$ = Quadrant 1, $Q2$ = Quadrant 2, $Q3$ = Quadrant 3. (c) $(k_{cat})_0$, $(K_m)_0$ and $(k_{cat}/K_m)_0$ are the values in the absence of inhibitor. (d) $-X = P(x, y)$ is on the negative side of the x-axis ($1/[S]$-axis in L-B plots, $[S]$-axis in Hanes plots or $[I]$-axis in Dixon plots). (e) $+Y = P(x, y)$ is on the positive side of the Y-axis ($[E_0]/v$ axis in L-B plots and Dixon plots, or $[E_0][S]/v$ axis in Hanes plots). (f) No general convergence, but the convergence point for any pair of lines is in Quadrant 1.

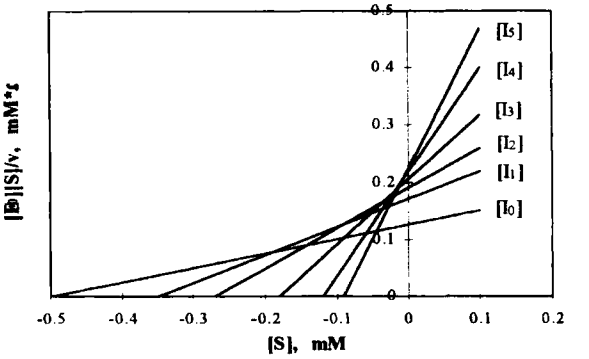


FIGURE 1 Hanes plots of Model I.b. with $k_{cat} = 4\ s^{-1}$, $K_m = 0.5$ mM, $Z = k_2/(k_2 + k_3) = 0.86$, $\lambda = k_2'/k_2 = 0.5$, $K_i = 10$ mM, and $[I_0] = 0$, $[I_1] = 10$ mM, $[I_2] = 20$ mM, $[I_3] = 40$ mM, $[I_4] = 70$ mM, $[I_5] = 100$ mM.

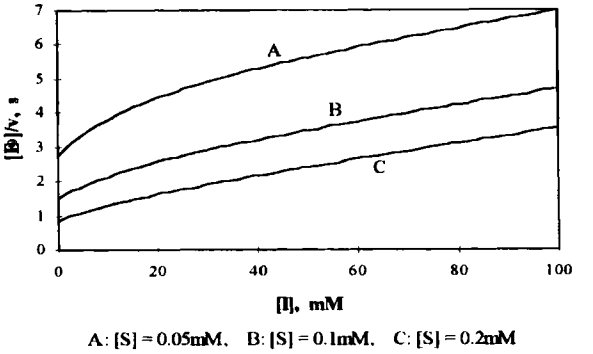


FIGURE 2 Dixon plots pattern of Model I.b. For the values of parameters, see the legend of Figure 1. The substrate concentrations [S] are given on the figure.

CONCLUSIONS

This analysis discussed a few unexpected features of inhibition phenomena when the enzyme follows a catalytic pathway involving a covalent intermediate. Complex phenomena, sometimes involving activation, can be observed, and increased k_{cat}/K_m values can be accompanied by decreased k_{cat} values. It becomes difficult to distinguish between the various models in the absence of additional (e.g. binding) data. For example, Model I.d. can be distinguished from Models VIII and IX if the inhibitor I binds to free enzyme E. A similar experiment would also help distinguishing between Model I and Model VI.

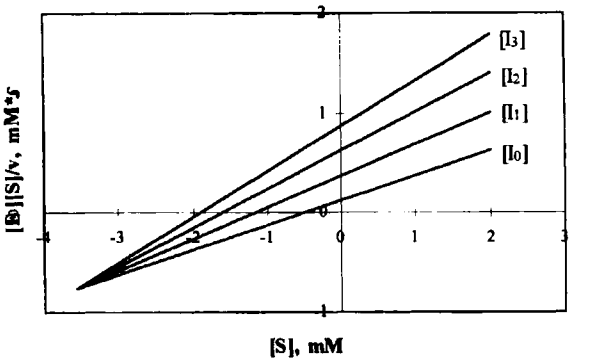


FIGURE 3A Hanes plots of Model III. (for Model IV, similar plots are obtained). The convergence points $P(x, y)$ are in Quadrants 2 or 3, depending on the relative values of Z, K_{i1} and K_{i2}. When $K_{i1} = K_{i2}$ (Model III.b.), $P(x, y)$ is in Quadrant 3. The parameter values are: $k_{cat} = 4\ s^{-1}$, $K_m = 0.5$ mM, $Z = 0.86$, (see the legend of Figure 1), $K_{i1} = K_{i2} = 10$ mM $[I_0] = 0$, $[I_1] = 20$ mM, $[I_2] = 40$ mM, $[I_3] = 60$ mM.

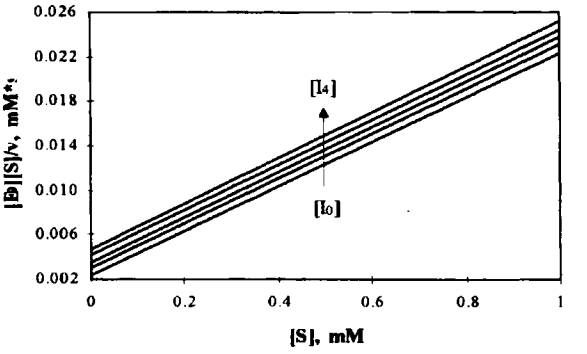


FIGURE 3B Hanes plots of Model III.b. when $k_2 \gg k_3(1 + [I]/K_i)$. The parameter values are: $k_{cat} = 50\ s^{-1}$, $K_m = 0.12$ mM, $Z > 0.98(Z = k_2/(k_2 + k_3))$, $K_i = 10$ mM. The $[I]$ values are 0, 2.5, 5.0, 7.5 and 10.0 mM ($[I_0]$ to $[I_4]$) respectively.

APPENDIX 1

Inhibition Type and Complete Expressions of the Kinetic Parameters (k_{cat}, K_m and k_{cat}/K_m) in Various Models and Sub-models

Scheme C represents the general case. For identification of the different models, see Table I. In the following expressions, $(k_{cat})_0$, $(K_m)_0$ and $(k_{cat}/K_m)_0$ are the values in the absence of inhibitor. The "Classical (non-/un-)competitive inhibition" means that the equations have the same forms as those derived from Scheme A.

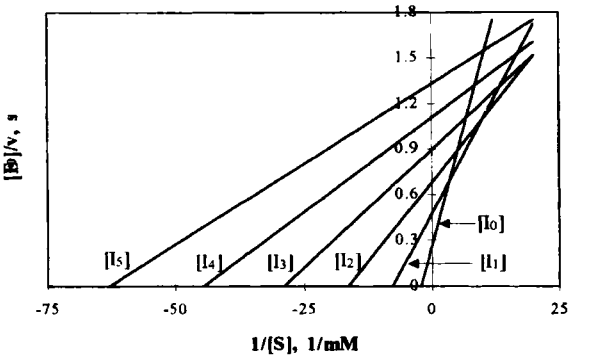


FIGURE 4 Lineweaver-Burk plots for Model VI.d. For the parameter values, see the legend of Figure 1. $[I_0] = 0$, $[I_1] = 10$ mM, $[I_2] = 20$ mM, $[I_3] = 30$ mM, $[I_4] = 40$ mM, $[I_5] = 50$ mM. All crossing points are in Quadrant 1.

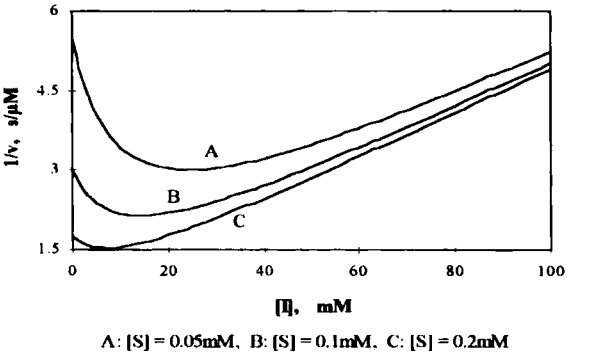


FIGURE 5 Dixon plots for Model VI.d. For the values of parameters, see the legend of Figure 1. The substrate concentrations $[S]$ are given in the figure.

Model I. The Inhibitor Binds to All Three Enzyme Forms (E, ES and ES*), and ESI Can Yield ES*I (Scheme C).

Model I.a. (general situation) $K_{i1} \neq K_{i2} \neq K_{i3}, k_2' \neq k_2$

$$k_{cat} = \frac{k_3 k_2 (1 + \lambda[I]/K_{i2})}{k_2(1 + \lambda[I]/K_{i2})(1 + [I]/K_{i3}) + k_3(1 + [I]/K_{i2})} \qquad \text{(A1-1)}$$

$$K_m = \frac{k_3 K_S(1 + [I]/K_{i1})}{k_2(1 + \lambda[I]/K_{i2})(1 + [I]/K_{i3}) + k_3(1 + [I]/K_{i2})} \quad \text{(A1-2)}$$

$$k_{cat}/K_m = \frac{k_2(1 + \lambda[I]/K_{i2})}{K_S(1 + [I]/K_{i1})} = \frac{1 + \lambda[I]/K_{i2}}{1 + [I]/K_{i1}}(k_{cat}/K_m)_0 \quad \text{(A1-3)}$$

where $\lambda = k_2'/k_2$

Model I.b. $K_{i1} = K_{i2} = K_{i3} = K_i, k_2' \neq k_2$

$$k_{cat} = \frac{k_3(k_2 + k_2'[I]/K_i)}{(k_2 + k_2'[I]/K_i)(1 + [I]/K_i) + k_3(1 + [I]/K_i)} = \frac{1 + \lambda[I]/K_i}{1 + [I]/K_i}\alpha_1(k_{cat})_0 \quad \text{(A1-4)}$$

$$K_m = \frac{k_3 K_S}{k_2 + k_2'[I]/K_i + k_3} = \alpha_1(K_m)_0 \quad \text{(A1-5)}$$

$$k_{cat}/K_m = \frac{k_2 + k_2'[I]/K_i}{K_S(1 + [I]/K_i)} = \frac{1 + \lambda[I]/K_i}{1 + [I]/K_i}(k_{cat}/K_m)_0 \quad \text{(A1-6)}$$

where $\alpha_1 = \frac{k_2 + k_3}{k_2(1 + \lambda[I]/K_i) + k_3}$ and $\lambda = k_2'/k_2$

Model I.c. $K_{i1} \neq K_{i2} \neq K_{i3}, k_2' = k_2$

$$k_{cat} = \frac{k_2 k_3}{k_2(1 + [I]/K_{i3}) + k_3} = \alpha_2(k_{cat})_0 \quad \text{(A1-7)}$$

$$K_m = \frac{k_3 K_S(1 + [I]/K_{i1})}{(1 + [I]/K_{i2})(k_2(1 + [I]/K_{i3}) + k_3)} = \frac{1 + [I]/K_{i1}}{1 + [I]/K_{i2}}\alpha_2(K_m)_0 \quad \text{(A1-8)}$$

$$k_{cat}/K_m = \frac{k_2}{K_S} * \frac{1 + [I]/K_{i2}}{1 + [I]/K_{i1}} = \frac{1 + [I]/K_{i2}}{1 + [I]/K_{i1}}(k_{cat}/K_m)_0 \quad \text{(A1-9)}$$

where $\alpha_2 = \frac{k_2 k_3}{k_2(1 + [I]/K_{i3}) + k_3}$

Model I.d. $K_{i1} = K_{i2} = K_{i3} = K_i, k_2' = k_2$ (un-competitive inhibition)

$$k_{cat} = \frac{k_2 k_3}{k_2(1 + [I]/K_i) + k_3} = \alpha_1 (k_{cat})_0 \tag{A1-10}$$

$$K_m = \frac{k_3 K_S}{k_2(1 + [I]/K_i) + k_3} = \alpha_1 (K_m)_0 \tag{A1-11}$$

$$k_{cat}/K_m = k_2/K_S = (k_{cat}/K_m)_0 \tag{A1-12}$$

$$\text{where } \alpha_1 = \frac{k_2 + k_3}{k_2(1 + [I]/K_i) + k_3}$$

Model II. The Inhibitor Binds to the Three Enzyme Forms, but ESI Does Not Yield ES*I ($k_2' = 0$)

Model II.a. $K_{i1} \neq K_{i2} \neq K_{i3}$

$$k_{cat} = \frac{k_2 k_3}{k_2(1 + [I]/K_{i3}) + k_3(1 + [I]/K_{i2})} = \beta (k_{cat})_0 \tag{A2-1}$$

$$K_m = \frac{k_3 K_S (1 + [I]/K_{i1})}{k_2(1 + [I]/K_{i3}) + k_3(1 + [I]/K_{i2})} = (1 + [I]/K_{i1}) \beta (K_m)_0 \tag{A2-2}$$

$$k_{cat}/K_m = (k_2/K_S)/(1 + [I]/K_{i1}) = (k_{cat}/K_m)_0/(1 + [I]/K_{i1}) \tag{A2-3}$$

$$\text{where } \beta = \frac{k_2 + k_3}{k_2(1 + [I]/K_{i3}) + k_3(1 + [I]/K_{i2})}$$

Model II.b. $K_{i1} = K_{i2} = K_{i3} = K_i$ (classical non-competitive inhibition)

$$k_{cat} = \frac{k_2 k_3}{(k_2 + k_3)(1 + [I]/K_i)} = (k_{cat})_0/(1 + [I]/K_i) \tag{A2-4}$$

$$K_m = \frac{k_3 K_S}{k_2 + k_3} = (K_m)_0 \tag{A2-5}$$

$$k_{cat}/K_m = (k_2/K_S)/(1 + [I]/K_i) = (k_{cat}/K_m)_0/(1 + [I]/K_{i1}) \tag{A2-6}$$

Model III. The Inhibitor Only Binds to the Free Enzyme E and the Henri-Michaelis Complex ES ($K_{i3} \to \infty, k_2' = 0$)

Model III.a. $K_{i1} \neq K_{i2}$

$$k_{cat} = \frac{k_2 k_3}{k_2 + k_3(1 + [I]/K_{i2})} = \gamma_1 (k_{cat})_0 \tag{A3-1}$$

$$K_m = \frac{k_3 K_S (1 + [I]/K_{i1})}{k_2 + k_3(1 + [I]/K_{i2})} = (1 + [I]/K_{i1}) \gamma_1 (K_m)_0 \tag{A3-2}$$

$$k_{cat}/K_m = (k_2/K_S)/(1 + [I]/K_{i1}) = (k_{cat}/K_m)_0/(1 + [I]/K_{i1}) \tag{A3-3}$$

$$\text{where } \gamma_1 = \frac{k_2 + k_3}{k_2 + k_3(1 + [I]/K_{i2})}$$

Model III.b. $K_{i1} = K_{i2}$

$$k_{cat} = \frac{k_2 k_3}{k_2 + k_3(1 + [I]/K_i)} = \gamma_2 (k_{cat})_0 \tag{A3-4}$$

$$K_m = \frac{k_3 K_S (1 + [I]/K_i)}{k_2 + k_3(1 + [I]/K_i)} = (1 + [I]/K_i) \gamma_2 (K_m)_0 \tag{A3-5}$$

$$k_{cat}/K_m = (k_2/K_S)/(1 + [I]/K_i) = (k_{cat}/K_m)_0/(1 + [I]/K_i) \tag{A3-6}$$

$$\text{where } \gamma_2 = \frac{k_2 + k_3}{k_2 + k_3(1 + [I]/K_i)}$$

Model IV. The Inhibitor Only Binds to the Free Enzyme E and the Covalent Intermediate ES* ($K_{i2} \to \infty, K_S' \to \infty, k_2' = 0$)

Model IV.a. $K_{i1} \neq K_{i3}$

$$k_{cat} = \frac{k_2 k_3}{k_2(1 + [I]/K_{i3}) + k_3} = \alpha_2 (k_{cat})_0 \tag{A4-1}$$

$$K_m = \frac{k_3 K_S (1 + [I]/K_{i1})}{k_2(1 + [I]/K_{i3}) + k_3} = (1 + [I]/K_{i1}) \alpha_2 (K_m)_0 \tag{A4-2}$$

$$k_{cat}/K_m = (k_2/K_S)/(1 + [I]/K_{i1}) = (k_{cat}/K_m)_0/(1 + [I]/K_{i1}) \tag{A4-3}$$

$$\text{where } \alpha_2 = \frac{k_2 + k_3}{k_2(1 + [I]/K_{i3}) + k_3}$$

Model IV.b. $K_{i1} = K_{i3}$

$$k_{cat} = \frac{k_2 k_3}{k_2(1 + [I]/K_i) + k_3} = \alpha_3 (k_{cat})_0 \tag{A4-4}$$

$$K_m = \frac{k_3 K_S (1 + [I]/K_i)}{k_2(1 + [I]/K_i) + k_3} = (1 + [I]/K_i)\alpha_3 (K_m)_0 \tag{A4-5}$$

$$k_{cat}/K_m = (k_2/K_S)/(1 + [I]/K_i) = (k_{cat}/K_m)_0/(1 + [I]/K_i) \tag{A4-6}$$

where $\alpha_3 = \dfrac{k_2 + k_3}{k_2(1 + [I]/K_i) + k_3}$

Model V. The Inhibitor Only Binds to the Henri-Michaelis Complex ES and the Covalent Intermediate ES*. ESI Does Not Yield ES*I ($K_{i1} \to \infty, k_2' = 0$)

Model V.a. $K_{i2} \neq K_{i3}$ (un-competitive inhibition)

$$k_{cat} = \frac{k_2 k_3}{k_2(1 + [I]/K_{i3}) + k_3(1 + [I]/K_{i2})} = \beta (k_{cat})_0 \tag{A5-1}$$

$$K_m = \frac{k_3 K_S}{k_2(1 + [I]/K_{i3}) + k_3(1 + [I]/K_{i2})} = \beta (K_m)_0 \tag{A5-2}$$

$$k_{cat}/K_m = k_2/K_S = (k_{cat}/K_m)_0 \tag{A5-3}$$

where $\beta = \dfrac{k_2 + k_3}{k_2(1 + [I]/K_{i3}) + k_3(1 + [I]/K_{i2})}$

Model V.b. $K_{i2} = K_{i3}$ (classical un-competitive inhibition)

$$k_{cat} = \frac{k_2 k_3}{(k_2 + k_3)(1 + [I]/K_i)} = (k_{cat})_0/(1 + [I]/K_i) \tag{A5-4}$$

$$K_m = \frac{k_3 K_S}{(k_2 + k_3)(1 + [I]/K_i)} = (K_m)_0/(1 + [I]/K_i) \tag{A5-5}$$

$$k_{cat}/K_m = k_2/K_S = (k_{cat}/K_m)_0 \tag{A5-6}$$

Model VI. The Inhibitor Only Binds to the Henri-Michaelis Complex ES and the Covalent Intermediate ES*, ESI Yields ES*I ($K_{i1} \to \infty, k_2' \neq 0$)

Model VI.a. $K_{i2} \neq K_{i3}, k_2' \neq k_2$

$$k_{cat} = \frac{k_2 k_3 (1 + \lambda[I]/K_{i2})}{k_2(1 + \lambda[I]/K_{i2})(1 + [I]/K_{i3}) + k_3(1 + [I]/K_{i2})} \tag{A6-1}$$

$$K_m = \frac{k_3 K_S}{k_2(1 + \lambda[I]/K_{i2})(1 + [I]/K_{i3}) + k_3(1 + [I]/K_{i2})} \tag{A6-2}$$

$$k_{cat}/K_m = k_2(1 + \lambda[I]/K_{i2})/K_S = (1 + \lambda[I]/K_{i2})(k_{cat}/K_m)_0 \tag{A6-3}$$

where $\lambda = k_2'/k_2$

Model VI.b. $K_{i2} = K_{i3}, k_2' \neq k_2$

$$k_{cat} = \frac{k_3(k_2 + k_2'[I]/K_i)}{(k_2 + k_2'[I]/K_i + k_3)(1 + [I]/K_i)} = \frac{1 + \lambda[I]/K_i}{1 + [I]/K_i}\alpha_1(k_{cat})_0 \tag{A6-4}$$

$$K_m = \frac{k_3 K_S}{(k_2 + k_2'[I]/K_i + k_3)(1 + [I]/K_i)} = \alpha_1(K_m)_0/(1 + [I]/K_i) \tag{A6-5}$$

$$k_{cat}/K_m' = k_2(1 + \lambda[I]/K_i)/K_S = (1 + \lambda[I]/K_i)(k_{cat}/K_m)_0 \tag{A6-6}$$

where $\alpha_1 = \dfrac{k_2 + k_3}{k_2(1 + \lambda[I]/K_i) + k_3}$ and $\lambda = k_2'/k_2$

Model VI.c. $K_{i2} \neq K_{i3}, k_2' = k_2$

$$k_{cat} = \frac{k_2 k_3}{k_2(1 + [I]/K_{i3}) + k_3} = \alpha_2(k_{cat})_0 \tag{A6-7}$$

$$K_m = \frac{k_3 K_S}{k_2(1 + [I]/K_{i3}) + k_3} * \frac{1}{1 + [I]/K_{i2}} = \alpha_2(K_m)_0/(1 + [I]/K_{i2}) \tag{A6-8}$$

$$k_{cat}/K_m = (1 + [I]/K_{i2})k_2/K_S = (1 + [I]/K_{i2})(k_{cat}/K_m)_0 \tag{A6-9}$$

where $\alpha_2 = \dfrac{k_2 + k_3}{k_2(1 + [I]/K_{i3}) + k_3}$

Model VI.d. $K_{i2} = K_{i3} = K_i, k_2' = k_2$

$$k_{cat} = \frac{k_2 k_3}{k_2(1 + [I]/K_i) + k_3} = \alpha_3 (k_{cat})_0 \tag{A6-10}$$

$$K_m = \frac{k_3 K_S}{k_2(1 + [I]/K_i) + k_3} * \frac{1}{1 + [I]/K_i}$$

$$= \alpha_3 (K_m)_0 / (1 + [I]/K_i) \tag{A6-11}$$

$$k_{cat}/K_m = (1 + [I]/K_i) k_2 / K_S = (1 + [I]/K_i)(k_{cat}/K_m)_0 \tag{A6-12}$$

where $\alpha_3 = \dfrac{k_2 + k_3}{k_2(1 + [I]/K_i) + k_3}$

Model VII. The Inhibitor Only Binds to the Free Enzyme E. (classical competitive inhibition)

$$k_{cat} = k_2 k_3 / (k_2 + k_3) = (k_{cat})_0 \tag{A7-1}$$

$$K_m = (1 + [I]/K_i) k_3 K_S / (k_2 + k_3) = (1 + [I]/K_i)(K_m)_0 \tag{A7-2}$$

$$k_{cat}/K_m = k_2 / (K_S(1 + [I]/K_i)) = (k_{cat}/K_m)_0 / (1 + [I]/K_i) \tag{A7-3}$$

Model VIII. The Inhibitor Only Binds to the Henri-Michaelis Complex ES. (un-competitive inhibition)

$$k_{cat} = \frac{k_2 k_3}{k_2 + k_3(1 + [I]/K_i)} = \gamma_2 (k_{cat})_0 \tag{A8-1}$$

$$K_m = \frac{k_3 K_S}{k_2 + k_3(1 + [I]/K_i)} = \gamma_2 (K_m)_0 \tag{A8-2}$$

$$k_{cat}/K_m = k_2/K_S = (k_{cat}/K_m)_0 \tag{A8-3}$$

where $\gamma_2 = \dfrac{k_2 + k_3}{k_2 + k_3(1 + [I]/K_i)}$

Model IX. The Inhibitor Only Binds to the Covalent Intermediate ES*. (un-competitive inhibition)

$$k_{cat} = \frac{k_2 k_3}{k_2(1 + [I]/K_i) + k_3} = \alpha_3 (k_{cat})_0 \quad \text{(A9-1)}$$

$$K_m = \frac{k_3 K_S}{k_2(1 + [I]/K_i) + k_3} = \alpha_3 (K_m)_0 \quad \text{(A9-2)}$$

$$k_{cat}/K_m = k_2/K_S = (k_{cat}/K_m)_0 \quad \text{(A9-3)}$$

$$\text{where } \alpha_3 = \frac{k_2 + k_3}{k_2(1 + [I]/K_i) + k_3}$$

APPENDIX 2

Lineweaver-Burk, Hanes, Eadie-Hofstee Equations and Dixon Plots in the Various Models

In the general situation (Scheme C, Model I.a.), the expressions of these four typical plots are shown below, where $(k_{cat})_0$, $(K_m)_0$ and $(k_{cat}/K_m)_0$ or $(K_m/k_{cat})_0$ are the values in the absence of inhibitor. The constants Z and λ are $k_2/(k_2 + k_3)$ and k'_2/k_2 respectively, $[E_0]$ is the total enzyme concentration. Note that the classical Dixon plot is $1/v$ vs. $[I]$. Using $1/v$ only results in the replacement of $(k_{cat})_0$ by $(V_{max})_0$ in the right-hand term.

Lineweaver-Burk Plot ($[E_0]/v$ vs. $1/[S]$)

$$\frac{[E_0]}{v} = \frac{Z\left(1 + \lambda\frac{[I]}{K_{i2}}\right)\left(1 + \frac{[I]}{K_{i3}}\right) + (1 - Z)\left(1 + \frac{[I]}{K_{i2}}\right)}{(k_{cat})_0\left(1 + \lambda\frac{[I]}{K_{i2}}\right)}$$

$$+ \left(\frac{K_m}{k_{cat}}\right)_0 * \frac{1 + \frac{[I]}{K_{il}}}{1 + \lambda\frac{[I]}{K_{i2}}} * \frac{1}{[S]} \quad \text{(A10)}$$

Hanes Plot ($[S]([E_0]/v)$ vs. $[S]$)

$$\frac{[E_0][S]}{v} = \left(\frac{K_m}{k_{cat}}\right)_0 * \frac{1 + \frac{[I]}{K_{i1}}}{1 + \lambda\frac{[I]}{K_{i2}}} + \frac{Z\left(1 + \lambda\frac{[I]}{K_{i2}}\right)\left(1 + \frac{[I]}{K_{i3}}\right) + (1 - Z)\left(1 + \frac{[I]}{K_{i2}}\right)}{(k_{cat})_0\left(1 + \lambda\frac{[I]}{K_{i2}}\right)} * [S] \quad \text{(A11)}$$

Eadie-Hofstee Plot ($v/[E_0]$ vs. $(v/[E_0])/[S]$)

$$\frac{v}{[E_0]} = \frac{(1 + \lambda[I]/K_{i2})(k_{cat})_0}{Z(1 + \lambda[I]/K_{i2})(1 + [I]/K_{i3}) + (1 - Z)(1 + [I]/K_{i2})} - \frac{(1 + [I]/K_{i1})(K_m)_0}{Z(1 + \lambda[I]/K_{i2})(1 + [I]/K_{i3}) + (1 - Z)(1 + [I]/K_{i2})} * \frac{v}{[S][E_0]} \quad \text{(A12)}$$

Dixon Plot ($[E_0]/v$ vs. $[I]$)

$$\frac{[E_0]}{v} = \frac{Z(1 + [I]/K_{i3})}{(k_{cat})_0} + \frac{1 - Z}{(k_{cat})_0} * \frac{1 + [I]/K_{i2}}{1 + \lambda[I]/K_{i2}} + \frac{(K_m/k_{cat})_0}{[S]} * \frac{1 + [I]/K_{i1}}{1 + \lambda[I]/K_{i2}} \quad \text{(A13)}$$

These complex expressions can be simplified as mentioned in Table I for the other models (see also Appendix 1). It is easy to see that, for all models, the Lineweaver-Burk, Hanes and Eadie-Hofstee plots are always linear, but the Dixon plots are not linear if $\lambda \neq 0 (k_2' \neq 0$, that is, in Models I and VI). An exception is Model I.d., where, since $\lambda = 1 (k_2' = k_2)$ and $K_{i1} = K_{i2} (= K_{i3}) = K_i$, the Dixon plot reduces to a straight line although $\lambda \neq 0$.

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