

EQUATIONS FOR PROGRESS CURVES OF SOME KINETIC MODELS OF ENZYME–SINGLE SUBSTRATE–SINGLE SLOW BINDING MODIFIER SYSTEM

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A procedure is described by means of which the equations for progress curves for the kinetic models that include fast and slow reaction steps can be derived. It is based on combined assumptions of equilibrium and steady-state and uses Laplace transformation for solving the systems of differential equations. The progress curve equations and the significance of the corresponding parameters are given for some most frequently occurring models describing the influence of a slow binding modifier on a single substrate enzyme reaction.

Keywords: Kinetic models; Progress curves; Slow-binding

INTRODUCTION

Recent developments in the kinetic equipment available for the study of fast chemical reactions, together with modern computer technology, makes it possible to obtain additional kinetic information on the action of various compounds to macromolecules, such as enzymes, drug or hormone receptors, carrier proteins etc. The progress of the reactions becomes “visible” in milli-, micro- and even nanosecond time ranges, and the distinction between rapid and slow reactions depends, actually, only on the capability of the equipment used in the experiments.¹

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The theory of the interactions between macromolecules with small ligands which include rapid and slow steps has been extensively discussed by several authors (cf. references [2], [3]). Mathematical modeling has become a powerful tool for establishing the background of the mechanisms⁴ in such processes.

The analysis of the kinetic data, in particular, consists of several steps, where the result of the precedent step determines a subsequent one:¹ data reduction, analog data plot inspection, kinetic model construction, derivation of kinetic equations and the regression analysis for the determination of all relevant kinetic parameters. Of course, the principal decision with the most fundamental consequences, during the course of this analysis, is the selection of the correct model out of several possible kinetic models. The criteria were discussed in depth by Mannervik⁵ but the quantitative evaluation (statistical analysis) of kinetic models seems to introduce a certain degree of objectivity in the discrimination between rival models. In order to be able to fit them to the experimental data, the corresponding kinetic equations for each of the considered models should be derived.

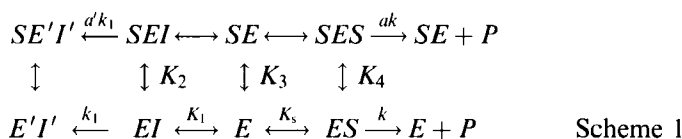
The purpose of this paper is to present a simple procedure for derivation of explicit equations for progress curves for the systems that include fast and slow reaction steps. Accordingly, the integrated rate equations (progress curve equations) with the significance of all corresponding parameters, are added for some in the enzyme kinetics most frequently occurring models, such as those describing the influence of a slow acting modifier on a single substrate enzyme reaction.

A PROCEDURE FOR DERIVATION OF EQUATIONS

The proposed procedure foresees certain conditions that must be fulfilled for the equations to be valid: (a) the ligands concentrations are much greater than the enzyme concentration; (b) the depletion of free ligands during the progress of the reaction is negligible; (c) the extent of product formation in the reaction under the influence of the modifier must be within the range of linearity of the control reaction in the absence of the modifier (i.e. steady state conditions in the reaction between the enzyme and the substrate); (d) the progress of the reaction is followed until steady-state conditions in the reaction between the enzyme and the inhibitor are reached. All these presume that the amount of product formed through the progress of the reaction is small, such that the inhibition by the product and the reverse reaction are negligible.

The described conditions are, in general, easily complied with. If they are not fulfilled, the derivation of explicit integrated rate equations become complex⁶ or frequently even impossible calling for some other usually numerical integration method.^{7,8} The kinetic processing of the acquired data in accordance with these assumptions should result in one or more reaction schemes (kinetic models), for which the corresponding equations for the time course of product formation must be derived.

For the purpose of illustration of such a derivation an example for the reaction between the enzyme, the active site directed irreversible modifier and the substrate which binds also to a second binding site was chosen. Scheme 1 is a kinetic model of the described reaction in which the release of the product and the formation of irreversibly inactivated enzyme forms are considered as slow steps:



In this scheme *E* is free enzyme, *S* is substrate, *I* is inhibitor and *P* is the product of substrate decomposition. *ES*, *SE*, *SES*, *EI* and *SEI* are the “instantaneously” formed enzyme–substrate, enzyme–inhibitor and enzyme–substrate–inhibitor complexes. *E'I'* and *SE'I'*, are slowly formed irreversibly inactivated enzyme–inhibitor and substrate–enzyme–inhibitor complexes, respectively. *K_s*, *K₁*, *K₂*, *K₃*, *K₄*, represent the equilibrium constants for instantaneous steps in the reaction scheme, *k*, *k₁* are the rate constants in slow steps; *a* and *a'* are the corresponding proportional factors (see also Table III, mechanism 2).

According to Cha², Scheme 1 can be written in the form where only slow steps are included:



Here *X* contains the enzyme forms with instantaneously formed equilibria (*E*, *ES*, *SE*, *SEI*, *EI*, *SEI*) and *X'* contains the slowly formed irreversibly inactivated enzyme forms *E'I'* and *SE'I'*. So:

$$(X) = (E) + (ES) + (SE) + (SES) + (EI) + (SEI) \quad (1)$$

$$(X') = (E'I') + (SE'I'). \quad (2)$$

According to Scheme 1 and Eqs. (1) and (2), the fractional concentrations may be expressed as,

$$(ES) = \frac{(S)/K_s}{1 + (S)/K_s + (S)/K_3 + (S)^2/(K_s K_4) + (I)/K_1 + (S)(I)/(K_2 K_3)} (X) \quad (3)$$

$$(SES) = \frac{(S)^2/(K_s K_4)}{1 + (S)/K_s + (S)/K_3 + (S)^2/(K_s K_4) + (I)/K_1 + (S)(I)/(K_2 K_3)} (X) \quad (4)$$

$$(EI) = \frac{(I)/K_1}{1 + (S)/K_s + (S)/K_3 + (S)^2/(K_s K_4) + (I)/K_1 + (S)(I)/(K_2 K_3)} (X) \quad (5)$$

$$(SEI) = \frac{(S)(I)/(K_2 K_3)}{1 + (S)/K_s + (S)/K_3 + (S)^2/(K_s K_4) + (I)/K_1 + (S)(I)/(K_2 K_3)} (X) \quad (6)$$

Differential equations for the system described by Scheme 1 are:

$$\frac{d(P)}{dt} = k(ES) + ak(SES) \quad (7)$$

$$\frac{d(X)}{dt} = -k_1(EI) - a'k_1(SEI). \quad (8)$$

Putting Eqs. (3), (4) and (5), (6) into Eqs. (7) and (8), respectively, gives

$$\frac{d(P)}{dt} = \frac{k(S)/K_s + ak(S)^2/(K_s K_4)}{1 + (S)/K_s + (S)/K_3 + (S)^2/(K_s K_4) + (I)/K_1 + (S)(I)/(K_2 K_3)} (X) \quad (9)$$

$$\frac{d(X)}{dt} = \frac{k_1(I)/K_1 + a'k_1(S)(I)/(K_2 K_3)}{1 + (S)/K_s + (S)/K_3 + (S)^2/(K_s K_4) + (I)/K_1 + (S)(I)/(K_2 K_3)} (X) \quad (10)$$

and

$$\frac{d(P)}{dt} = \varphi(X) \quad (11)$$

$$\frac{d(X)}{dt} = -\beta(X) \quad (12)$$

In order to solve the system of differential equations (Eqs. (11) and (12)) Laplace transformation is employed (cf. references [9], [10]). Since the differential rate equations are in the form of first derivatives, replacing differentials by “s” operators gives:

$$s(P)_s - (P)_0 = \varphi(X)_s \tag{13}$$

$$s(X)_s - (X)_0 = -\beta(X)_s. \tag{14}$$

$(P)_0$ and $(X)_0$ represent the initial concentration of P and X , respectively. At time zero (P) is zero and (X) is $(E)_0$. $(E)_0$ is the total concentration of the enzyme. So,

$$(P)_s = \frac{(E)_0\varphi}{s(s + \beta)}. \tag{15}$$

Eq. (15) can be retransformed according to Laplace, giving:

$$(P)_t = \frac{(E)_0\varphi}{\beta} [1 - e^{-\beta t}]. \tag{16}$$

Since $(E)_0\varphi$ is defined as initial velocity v_0 , and $(E)_0k$ as V_{\max} , it follows from Eqs. (9)–(12) and Eq. (16) that,

$$(P)_t = v_0 \frac{[1 - e^{-\beta t}]}{\beta} \tag{17}$$

$$v_0 = \frac{V_{\max}(S)[1 + a(S)/K_4]}{K_s[1 + (S)/K_3 + (I)/K_1 + (S)(I)/(K_2K_3)] + (S)[1 + (S)/K_4]} \tag{18}$$

$$\beta = \frac{k_1K_s[(I)/K_1 + a'(S)(I)/(K_2K_3)]}{K_s[1 + (S)/K_3 + (I)/K_1 + (S)(I)/(K_2K_3)] + (S)[1 + (S)/K_4]}. \tag{19}$$

In this way, the progress curve equation (Eq. (17)) and the meaning of all relevant kinetic parameters (Eqs. (18), (19)) for the mechanism represented by Scheme 1 are defined in terms of 9 relevant kinetic constants (Table III, mechanism 2).

EQUATIONS AND THE MEANING OF THE RELEVANT PARAMETERS FOR SOME MECHANISTIC MODELS OF ENZYME–SINGLE SUBSTRATE–SINGLE SLOW BINDING MODIFIER SYSTEM

Tables I–IV show the kinetic equations and the significance of all relevant parameters for the time course of some most common enzymic reactions in the presence of slow binding competitive, noncompetitive and partial modifier. Each reaction is treated by means of two mechanistic patterns: as a single slow step process (odd numbers in each Table) and as a double fast-slow process (even numbers). The latter case is usually explained by an “instantaneous binding” of the ligand to the enzyme that subsequently undergoes a slow isomerization reaction. All enzyme–substrate complexes are considered to form instantaneously. In Tables I and III the modifier is irreversible, while in Tables II and IV it is reversible. With reversible inhibitors a different initial velocity can be observed if the reaction is started by the addition of enzyme or if the enzyme is preincubated with the modifier before the addition of the substrate. In the case of irreversible inhibitors, however, the initial velocity after preincubation of the enzyme with the modifier is the same as steady-state velocity of the reaction if started by the addition of enzyme. Tables III and IV include the binding of a second modulating molecule of the same substrate (for example, instantaneous inhibition or activation by the excess of substrate from an allosteric site).

DISCUSSION

The prolonged presteady-state in the processes which include slow reaction steps makes the study of such processes by classical steady-state kinetics difficult. This can be overcome by using an appropriate rapid kinetic data acquisition technique. Progress curves, obtained from such measurements, provide much more information about the studied reaction and, besides, in comparison with initial rate experiments, give much higher accuracy of the kinetic parameters which had to be determined.¹¹ However, when analyzing progress curves, difficulties are encountered which are avoided when analyzing the initial rate experiments.¹² The crucial difficulty, unavoidable in progress curve analysis is the integration of the corresponding rate equations, i.e. the derivation of the mathematical expressions for the time course of product formation. The procedure for the derivation which is presented here extends Cha's method for derivation of rate equations² to a level of progress

TABLE I Mechanistic models for enzyme–single substrate–single slow binding irreversible modifier system, together with the equations for progress curves and the significance of the corresponding kinetic parameters

Mechanism – parameters to fit	P_t	V_0	β	V_s
$ \begin{array}{l} E \xrightarrow{K_s} ES \xrightarrow{k} E + P \\ k_1 \downarrow \\ E' I \quad (3) \end{array} $	$\frac{v_0(1 - e^{-\beta t})}{\beta}$	$\frac{V_{\max}(S)}{K_s + (S)}$	$\frac{k_1 K_s(I)}{K_s + (S)}$	
$ \begin{array}{l} E \xrightarrow{K_s} ES \xrightarrow{k} E + P \\ K_1 \downarrow \\ EI \xrightarrow{k_1} E' I \quad (4) \end{array} $	$\frac{v_0(1 - e^{-\beta t})}{\beta}$	$\frac{V_{\max}(S)}{K_s \left[1 + \frac{(I)}{K_i} \right] + (S)}$	$\frac{k_1 K_s \left[\frac{(I)}{K_i} \right]}{K_s \left[1 + \frac{(I)}{K_i} \right] + (S)}$	
$ \begin{array}{l} E \xrightarrow{K_s} ES \xrightarrow{k} E + P \\ k_1 \downarrow \quad a'k_1 \downarrow \\ IE' \longleftrightarrow IE' S \quad (4) \end{array} $	$\frac{v_0(1 - e^{-\beta t})}{\beta}$	$\frac{V_{\max}(S)}{K_s + (S)}$	$\frac{k_1(I)[K_s + a'(S)]}{K_s + (S)}$	
$ \begin{array}{l} E \xrightarrow{K_s} ES \xrightarrow{k} E + P \\ K_1 \downarrow \quad \quad \quad \downarrow K_2 \\ IE \longleftrightarrow IES \xrightarrow{bk} IE + P \\ k_1 \downarrow \quad a'k_1 \downarrow \\ IE' \longleftrightarrow IE' S \quad (7) \end{array} $	$\frac{v_0(1 - e^{-\beta t})}{\beta}$	$\frac{V_{\max}(S) \left[1 + b \frac{(I)}{K_2} \right]}{K_s \left[1 + \frac{(I)}{K_1} \right] + (S) \left[1 + \frac{(I)}{K_2} \right]}$	$\frac{k_1(I) \left[\frac{K_s}{K_1} + a' \frac{(S)}{K_2} \right]}{K_s \left[1 + \frac{(I)}{K_1} \right] + (S) \left[1 + \frac{(I)}{K_2} \right]}$	

TABLE I (Continued)

Mechanism – parameters to fit	P_t	V_0	β	V_s
5 $E \xrightleftharpoons{K_s} ES \xrightarrow{k} E + P$ $k_1 \downarrow \quad d'k_1 \downarrow$ $IE' \xrightleftharpoons{K_s} IE'S \xrightarrow{ck} IE' + P \quad (6)$	$v_s t + \frac{(v_0 - v_s)(1 - e^{-\beta t})}{\beta}$	$\frac{V_{\max}(S)}{K_s + (S)}$	$\frac{k_1(I)[K_s + d'(S)]}{K_s + (S)}$	$\frac{cV_{\max}(S)}{K_s + (S)}$
6 $E \xrightleftharpoons{K_s} ES \xrightarrow{k} E + P$ $K_1 \downarrow \quad \quad \downarrow K_2$ $IE \rightleftharpoons IES \xrightarrow{bk} IE + P$ $k_1 \downarrow \quad d'k_1 \downarrow$ $IE' \xrightleftharpoons{K_s} IE'S \xrightarrow{ck} IE' + P \quad (9)$	$v_s t + \frac{(v_0 - v_s)(1 - e^{-\beta t})}{\beta}$	$\frac{V_{\max}(S) \left[1 + b \frac{(I)}{K_2} \right]}{K_s \left[1 + \frac{(I)}{K_1} \right] + (S) \left[1 + \frac{(I)}{K_2} \right]}$	$\frac{k_1(I) \left[\frac{K_s}{K_1} + d' \frac{(S)}{K_2} \right]}{K_s \left[1 + \frac{(I)}{K_1} \right] + (S) \left[1 + \frac{(I)}{K_2} \right]}$	$\frac{cV_{\max}(S)}{K_s + (S)}$

TABLE II Mechanistic models for enzyme–single substrate–single slow binding reversible modifier system, together with the equations for progress curves and the significance of the corresponding kinetic parameters. (Note the significance of v_0 if the reaction is started by the addition of enzyme or if the enzyme is preincubated with the modifier before the addition of the substrate)

	<i>Mechanism – parameters to fit</i>	P_t	V_0	β	V_s	α	$V_{0,preincubation}$
1	$E \xrightleftharpoons[k_1]{K_s} ES \xrightarrow{k} E + P$ $E'I \xrightleftharpoons[k_2]{k_1} E$ <p style="text-align: center;">(4)</p>	$v_s t + \frac{(v_0 - v_s)(1 - e^{-\beta t})}{\beta}$	$\frac{V_{max}(S)}{K_s + (S)}$	$\frac{k_1 K_s (I)}{K_s + (S)} + \alpha$	$v_0 \frac{\alpha}{\beta}$	k_2	$v_0 \frac{k_2}{k_1(I) + k_2}$
2	$E \xrightleftharpoons[K_1]{K_s} ES \xrightarrow{k} E + P$ $EI \xrightleftharpoons[k_1]{k_1} E$ $E'I \xrightleftharpoons[k_2]{k_1} E$ <p style="text-align: center;">(5)</p>	$v_s t + \frac{(v_0 - v_s)(1 - e^{-\beta t})}{\beta}$	$\frac{V_{max}(S)}{K_s \left[1 + \frac{(I)}{K_1}\right] + (S)}$	$\frac{k_1 K_s \left[\frac{(I)}{K_1}\right]}{K_s \left[1 + \frac{(I)}{K_1}\right] + (S)} + \alpha$	$v_0 \frac{\alpha}{\beta}$	k_2	$v_0 \frac{K_1 + (I)}{K_1 + (I) + \frac{k_1}{k_2}(I)}$
3	$E \xrightleftharpoons[k_1]{K_s} ES \xrightarrow{k} E + P$ $IE \xrightleftharpoons[k_2]{k_1} E$ $IES \xrightleftharpoons[k_2]{d'k_1} IE$ <p style="text-align: center;">(7)</p>	$v_s t + \frac{(v_0 - v_s)(1 - e^{-\beta t})}{\beta}$	$\frac{V_{max}(S)}{K_s + (S)}$	$\frac{k_1(I)[K_s + a'(S)]}{K_s + (S)} + \alpha$	$v_0 \frac{\alpha}{\beta}$	$\frac{k_2 K_s + b'k_2(S)}{K_s + (S)}$	$v_0 \frac{k_2}{k_1(I) + k_2}$
4	$E \xrightleftharpoons[K_1]{K_s} ES \xrightarrow{k} E + P$ $IE \xrightleftharpoons[k_1]{k_1} E$ $IES \xrightleftharpoons[k_2]{d'k_1} IE$ $IE \xrightleftharpoons[k_2]{k_1} E$ <p style="text-align: center;">(10)</p>	$v_s t + \frac{(v_0 - v_s)(1 - e^{-\beta t})}{\beta}$	$\frac{V_{max}(S) \left[1 + b \frac{(I)}{K_2}\right]}{K_s \left[1 + \frac{(I)}{K_1}\right] + (S) \left[1 + \frac{(I)}{K_2}\right]}$	$\frac{k_1(I) \left[\frac{K_s}{K_1} + a'(S)\right]}{K_s \left[1 + \frac{(I)}{K_1}\right] + (S) \left[1 + \frac{(I)}{K_2}\right]} + \alpha$	$v_0 \frac{\alpha}{\beta}$	$\frac{k_2 K_s + b'k_2(S)}{K_s + (S)}$	$v_0 \frac{K_1 + (I)}{K_1 + (I) + \frac{k_1}{k_2}(I)}$

TABLE II (Continued)

Mechanism - parameters to fit		P_i	V_0	β	V_s	α	V_0 -precipitation
5	$E \xrightleftharpoons{K_1} ES \xrightleftharpoons{k} E + P$ $k_1 \uparrow \downarrow k_2 \quad d'k_1 \uparrow \downarrow b'k_2$ $IE \xrightleftharpoons{K_5} IES \xrightleftharpoons{ck} IE + P \quad (8)$	$v_3 f + \frac{(v_0 - v_3)(1 - e^{-\beta t})}{\beta}$	$\frac{V_{\max}(S)}{K_5 + (S)}$	$\frac{k_1(I)K_5 + d'(S)}{K_5 + (S)} + \alpha$	$v_0 \frac{\alpha}{\beta} + \frac{cV_{\max}(S) \left[1 - \frac{\beta}{\alpha} \right]}{K_5 + (S)}$	$\frac{k_2 K_5 + b'k_2(S)}{K_5 + (S)}$	$v_0 \frac{k_2}{k_1(I) + k_2} + \frac{cV_{\max}(S) \left[1 - \frac{\beta}{\alpha} \right]}{K_5 + (S)}$
6	$E \xrightleftharpoons{K_1} ES \xrightleftharpoons{k} E + P$ $K_1 \downarrow \quad \uparrow K_2$ $IE \xrightleftharpoons{K_5} IES \xrightleftharpoons{ck} IE + P$ $k_1 \uparrow \downarrow k_2 \quad d'k_1 \uparrow \downarrow b'k_2$ $IE \xrightleftharpoons{K_1} IES \xrightleftharpoons{ck} IE + P \quad (11)$	$v_3 f + \frac{(v_0 - v_3)(1 - e^{-\beta t})}{\beta}$	$\frac{V_{\max}(S) \left[1 + b \frac{d'}{K_2} \right]}{K_5 \left[1 + \frac{d'}{K_1} \right] + (S) \left[1 + \frac{d'}{K_2} \right]}$	$\frac{k_1(I) \left[\frac{K_5 + d'(S)}{K_1} \right]}{K_5 \left[1 + \frac{d'}{K_1} \right] + (S) \left[1 + \frac{d'}{K_2} \right]} + \alpha$	$v_0 \frac{\alpha}{\beta} + \frac{cV_{\max}(S) \left[1 - \frac{\beta}{\alpha} \right]}{K_5 + (S)}$	$\frac{k_2 K_5 + b'k_2(S)}{K_5 + (S)}$	$v_0 \frac{(K_1 + I)}{K_1 + (I) + \frac{k_2}{k_1}(I)} + \frac{cV_{\max}(S) \left[1 - \frac{\beta}{\alpha} \right]}{K_5 + (S)}$

TABLE III Mechanistic models for enzyme–single substrate–single slow binding irreversible modifier system which include the enzyme activity modulation by the second molecule of the same substrate, together with the equations for progress curves and the significance of the corresponding kinetic parameters

Mechanism – parameters to fit	P_t	V_0	β	V_s
$ \begin{array}{c} SE'I \xrightleftharpoons{a'k_1} SE \longleftrightarrow SES \xrightarrow{ak} SE + P \\ \downarrow \quad \quad \downarrow K_3 \quad \downarrow K_4 \\ E'I \xrightleftharpoons{k_1} E \xrightleftharpoons{k_s} ES \xrightarrow{k} E + P \quad (7) \end{array} $	$\frac{v_0(1 - e^{-\beta t})}{\beta}$	$\frac{V_{\max}(S) \left[1 + a \frac{(S)}{K_4} \right]}{K_s \left[1 + \frac{(S)}{K_3} \right] + (S) \left[1 + \frac{(S)}{K_4} \right]}$	$\frac{k_1 K_s (I) \left[1 + a' \frac{(S)}{K_3} \right]}{K_s \left[1 + \frac{(S)}{K_3} \right] + (S) \left[1 + \frac{(S)}{K_4} \right]}$	
$ \begin{array}{c} SE'I \xrightleftharpoons{a'k_1} SEI \xrightleftharpoons{K_2} SE \longleftrightarrow SES \xrightarrow{ak} SE + P \\ \downarrow \quad \quad \downarrow \quad \quad \downarrow K_3 \quad \downarrow K_4 \\ E'I \xrightleftharpoons{k_1} EI \xrightleftharpoons{K_1} E \xrightleftharpoons{K_s} ES \xrightarrow{k} E + P \quad (9) \end{array} $	$\frac{v_0(1 - e^{-\beta t})}{\beta}$	$\frac{V_{\max}(S) \left[1 + a \frac{(S)}{K_4} \right]}{K_s \left[1 + \frac{(S)}{K_3} + \frac{(I)}{K_1} + \frac{(S)(I)}{K_2 K_3} \right] + (S) \left[1 + \frac{(S)}{K_4} \right]}$	$\frac{k_1 K_s \left[\frac{(I)}{K_1} + a' \frac{(S)(I)}{K_2 K_3} \right]}{K_s \left[1 + \frac{(S)}{K_3} + \frac{(I)}{K_1} + \frac{(S)(I)}{K_2 K_3} \right] + (S) \left[1 + \frac{(S)}{K_4} \right]}$	
$ \begin{array}{c} SE \longleftrightarrow SES \xrightarrow{ak} SE + P \\ K_3 \downarrow \quad \quad \downarrow K_4 \\ E \xrightleftharpoons{K_s} ES \xrightarrow{k} E + P \\ k_1 \downarrow \quad \quad a'k_1 \downarrow \\ IE' \longleftrightarrow IE'S \quad (7) \end{array} $	$\frac{v_0(1 - e^{-\beta t})}{\beta}$	$\frac{V_{\max}(S) \left[1 + a \frac{(S)}{K_4} \right]}{K_s \left[1 + \frac{(S)}{K_3} \right] + (S) \left[1 + \frac{(S)}{K_4} \right]}$	$\frac{k_1 (I) [K_s + a'(S)]}{K_s \left[1 + \frac{(S)}{K_3} \right] + (S) \left[1 + \frac{(S)}{K_4} \right]}$	
$ \begin{array}{c} SE \longleftrightarrow SES \xrightarrow{ak} SE + P \\ K_3 \downarrow \quad \quad \downarrow K_4 \\ E \xrightleftharpoons{K_s} ES \xrightarrow{k} E + P \\ K_1 \downarrow \quad \quad \downarrow K_2 \\ IE \longleftrightarrow IES \xrightarrow{bk} IE + P \\ k_1 \downarrow \quad \quad a'k_1 \downarrow \\ IE' \longleftrightarrow IE'S \quad (10) \end{array} $	$\frac{v_0(1 - e^{-\beta t})}{\beta}$	$\frac{V_{\max}(S) \left[1 + a \frac{(S)}{K_4} + b \frac{(I)}{K_2} \right]}{K_s \left[1 + \frac{(S)}{K_3} + \frac{(I)}{K_1} \right] + (S) \left[1 + \frac{(S)}{K_4} + \frac{(I)}{K_2} \right]}$	$\frac{k_1 (I) \left[1 + \frac{(K_s)}{K_1} + a' \frac{(S)}{K_2} \right]}{K_s \left[1 + \frac{(S)}{K_3} + \frac{(I)}{K_1} \right] + (S) \left[1 + \frac{(S)}{K_4} + \frac{(I)}{K_2} \right]}$	

TABLE III (Continued)

Mechanism – parameters to fit	P_i	V_0	β	V_s
5 $SE \xleftrightarrow{k_1} SES \xrightleftharpoons{a_k} SE + P$ $K_3 \downarrow \quad \quad \quad \downarrow K_4$ $E \xrightleftharpoons{k_5} ES \xrightarrow{k} E + P$ $k_1 \downarrow \quad d'k_1 \downarrow$ $IE' \xrightleftharpoons{k_5} IE'S \xrightarrow{c_k} IE' + P$ (9)	$v_s t + \frac{(v_0 - v_s)(1 - e^{-\beta t})}{\beta}$	$\frac{V_{\max}(S) \left[1 + a \frac{(S)}{K_4} \right]}{K_5 \left[1 + \frac{(S)}{K_3} \right] + (S) \left[1 + \frac{(S)}{K_4} \right]}$	$\frac{k_1(I) [K_5 + d'(S)]}{K_5 \left[1 + \frac{(S)}{K_3} \right] + (S) \left[1 + \frac{(S)}{K_4} \right]}$	$\frac{c V_{\max}(S)}{K_5 + (S)}$
6 $SE \xleftrightarrow{k_1} SES \xrightleftharpoons{a_k} SE + P$ $K_3 \downarrow \quad \quad \quad \downarrow K_4$ $E \xrightleftharpoons{k_5} ES \xrightarrow{k} E + P$ $K_1 \downarrow \quad \quad \quad \downarrow K_2$ $IE \xleftrightarrow{k_5} IES \xrightleftharpoons{b_k} IE + P$ $k_1 \downarrow \quad d'k_1 \downarrow$ $IE' \xrightleftharpoons{k_5} IE'S \xrightarrow{c_k} IE' + P$ (12)	$v_s t + \frac{(v_0 - v_s)(1 - e^{-\beta t})}{\beta}$	$\frac{V_{\max}(S) \left[1 + a \frac{(S)}{K_4} + b \frac{(I)}{K_2} \right]}{K_5 \left[1 + \frac{(S)}{K_3} + \frac{(I)}{K_1} \right] + (S) \left[1 + \frac{(S)}{K_4} + \frac{(I)}{K_2} \right]}$	$\frac{k_1(I) \left[1 + \frac{d'}{K_1} + d' \frac{(S)}{K_2} \right]}{K_5 \left[1 + \frac{(S)}{K_3} + \frac{(I)}{K_1} \right] + (S) \left[1 + \frac{(S)}{K_4} + \frac{(I)}{K_2} \right]}$	$\frac{c V_{\max}(S)}{K_5 + (S)}$

curve analysis. The treated mechanistic models of one substrate–one modifier–one slow step reactions, might seem an oversimplification of the actual enzymic processes, but more complex systems can often be reduced to this level by choosing adequate experimental conditions.¹³ A direct applicability of the derived equations for progress curves of presented mechanistic models is plausible since, in practice, such systems are frequently encountered (reference [14], mechanism 4 in Table IV; reference [15], slightly modified mechanism 1 in Table I; reference [16], modified mechanism 2 in Table IV; reference [17], extended mechanism 2 in Table III; reference [18], mechanism 5 in Table IV). In addition, the systematic display in Tables I–IV makes it possible to get a feeling of what takes place as more and more complex systems are being studied. Moreover, if the conditions specified above are fulfilled, it is possible to derive progress curve equations and the meaning of the corresponding parameters for almost any mechanistic model, including those with more than one slow step (*cf.* reference [19]) or more than one modifier. As in the case of reversible modifiers, the equations are valid, no matter whether the reaction is started by the addition of the enzyme, or the enzyme is preincubated with the modifier before the addition of the substrate (note the significance of v_0 in each case in Tables II and IV). The rate of the reaction with the enzyme preincubated with the irreversible modifiers exerting partial inhibitory effect (Tables I and III, mechanisms 5 and 6) equals steady-state velocity.

Finally, it seems important to emphasize the applicability of the models, worked out and summarized in Tables I–IV, in the evaluation of kinetic data. It appears, at first sight, that the derived equations support the misleading thesis, that with several disposable parameters any curve can be fitted, since all treated mechanisms can be described by only two types of progress curve equations. The detailed examination of the relevance of all parameters in the Tables, however, shows that there is only one combination of parameters which is unique for only one of the treated mechanistic models. So, the inspection of the dependence of various kinetic parameters on the individual ligand's concentrations (*cf.* reference [2]), with subsequent simultaneous analysis of all experimental data²⁰ seems to be a good criterion for the discrimination between the rival kinetic models.

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TABLE IV Mechanistic models for enzyme–single substrate–single slow binding reversible modifier system which include the enzyme activity modulation by the second molecule of the same substrate, together with the equations for progress curves and the significance of the corresponding kinetic parameters. (Note the significance of v_0 if the reaction is started by the addition of enzyme or the enzyme is preincubated with the modifier before the addition of the substrate)

	Mechanism – parameters to fit	P_t	V_0
1	$SE'I \xrightleftharpoons[k_2]{a'k_1} SE \xleftrightarrow{K_3} SES \xrightarrow{ak} SE + P$ $K_5 \downarrow \quad \quad \quad \downarrow K_3 \quad \quad \downarrow K_4$ $E'I \xrightleftharpoons[k_2]{k_1} E \xrightarrow{k_4} ES \xrightarrow{k} E + P \quad (10)$	$v_s t + \frac{(v_0 - v_s)(1 - e^{-\beta t})}{\beta}$	$\frac{V_{\max}(S) \left[1 + a \frac{(S)}{K_4} \right]}{K_s \left[1 + \frac{(S)}{K_1} \right] + (S) \left[1 + \frac{(S)}{K_4} \right]}$
2	$SE'I \xrightleftharpoons[k_2]{a'k_1} SEI \xleftrightarrow{K_2} SE \xleftrightarrow{K_3} SES \xrightarrow{ak} SE + P$ $K_5 \downarrow \quad \quad \quad \downarrow \quad \quad \quad \downarrow K_3 \quad \quad \downarrow K_4$ $E'I \xrightleftharpoons[k_2]{k_1} EI \xrightarrow{K_1} E \xrightarrow{K_4} ES \xrightarrow{k} E + P \quad (12)$	$v_s t + \frac{(v_0 - v_s)(1 - e^{-\beta t})}{\beta}$	$\frac{V_{\max}(S) \left[1 + a \frac{(S)}{K_4} \right]}{K_s \left[1 + \frac{(S)}{K_1} + \frac{(I)}{K_1} + \frac{(S)(I)}{K_2 K_1} \right] + (S) \left[1 + \frac{(S)}{K_4} \right]}$
3	$SE \xleftrightarrow{K_3} SES \xrightarrow{ak} SE + P$ $K_3 \downarrow \quad \quad \quad \downarrow K_4$ $E \xrightarrow{K_4} ES \xrightarrow{k} E + P$ $k_1 \updownarrow k_2 \quad a'k_1 \updownarrow b'k_2$ $IE \xleftrightarrow{K_5} IES \quad (10)$	$v_s t + \frac{(v_0 - v_s)(1 - e^{-\beta t})}{\beta}$	$\frac{V_{\max}(S) \left[1 + a \frac{(S)}{K_4} \right]}{K_s \left[1 + \frac{(S)}{K_1} \right] + (S) \left[1 + \frac{(S)}{K_4} \right]}$
4	$SE \xleftrightarrow{K_3} SES \xrightarrow{ak} SE + P$ $K_3 \downarrow \quad \quad \quad \downarrow K_4$ $E \xrightarrow{K_4} ES \xrightarrow{k} E + P$ $K_1 \downarrow \quad \quad \quad \downarrow K_2$ $IE \xleftrightarrow{K_5} IES \xrightarrow{hk} IE + P$ $k_1 \updownarrow k_2 \quad a'k_1 \updownarrow b'k_2$ $IE \xleftrightarrow{K_5} IES \quad (13)$	$v_s t + \frac{(v_0 - v_s)(1 - e^{-\beta t})}{\beta}$	$\frac{V_{\max}(S) \left[1 + a \frac{(S)}{K_4} + b \frac{(I)}{K_2} \right]}{K_s \left[1 + \frac{(S)}{K_1} + \frac{(I)}{K_1} \right] + (S) \left[1 + \frac{(S)}{K_4} + \frac{(I)}{K_2} \right]}$
5	$SE \xleftrightarrow{K_3} SES \xrightarrow{ak} SE + P$ $K_3 \downarrow \quad \quad \quad \downarrow K_4$ $E \xrightarrow{K_4} ES \xrightarrow{k} E + P$ $k_1 \updownarrow k_2 \quad a'k_1 \updownarrow b'k_2$ $IE \xleftrightarrow{K_5} IES \xrightarrow{ck} IE + P \quad (11)$	$v_s t + \frac{(v_0 - v_s)(1 - e^{-\beta t})}{\beta}$	$\frac{V_{\max}(S) \left[1 + a \frac{(S)}{K_4} \right]}{K_s \left[1 + \frac{(S)}{K_1} \right] + (S) \left[1 + \frac{(S)}{K_4} \right]}$
6	$SE \xleftrightarrow{K_3} SES \xrightarrow{ak} SE + P$ $K_3 \downarrow \quad \quad \quad \downarrow K_4$ $E \xrightarrow{K_4} ES \xrightarrow{k} E + P$ $K_1 \downarrow \quad \quad \quad \downarrow K_2$ $IE \xleftrightarrow{K_5} IES \xrightarrow{hk} IE + P$ $k_1 \updownarrow k_2 \quad a'k_1 \updownarrow b'k_2$ $IE' \xleftrightarrow{K_5} IE'S \xrightarrow{ck} IE' + P \quad (14)$	$v_s t + \frac{(v_0 - v_s)(1 - e^{-\beta t})}{\beta}$	$\frac{V_{\max}(S) \left[1 + a \frac{(S)}{K_4} + b \frac{(I)}{K_2} \right]}{K_s \left[1 + \frac{(S)}{K_1} + \frac{(I)}{K_1} \right] + (S) \left[1 + \frac{(S)}{K_4} + \frac{(I)}{K_2} \right]}$

β	V_s	α	$V_{0\text{-preincubation}}$
$\frac{k_1 K_s(I) \left[1 + a' \frac{(S)}{K_3} \right]}{K_s \left[1 + \frac{(S)}{K_3} \right] + (S) \left[1 + \frac{(S)}{K_4} \right]} + \alpha$	$v_0 \frac{\alpha}{\beta}$	$\frac{k_2 K_5 + b' k_2 (S)}{K_5 + (S)}$	$v_0 \frac{k_2}{k_1(I) + k_2}$
$\frac{k_1 K_s \left[\frac{(I)}{K_1} + a' \frac{(S)(I)}{K_2 K_3} \right]}{K_s \left[1 + \frac{(S)}{K_3} + \frac{(I)}{K_1} + \frac{(S)(I)}{K_2 K_3} \right] + (S) \left[1 + \frac{(S)}{K_4} \right]} + \alpha$	$v_0 \frac{\alpha}{\beta}$	$\frac{k_2 K_5 + b' k_2 (S)}{K_5 + (S)}$	$v_0 \frac{K_1 + (I)}{K_1 + (I) + \frac{k_1}{k_2} (I)}$
$\frac{k_1(I) [K_s + a'(S)]}{K_s \left[1 + \frac{(S)}{K_3} \right] + (S) \left[1 + \frac{(S)}{K_4} \right]} + \alpha$	$v_0 \frac{\alpha}{\beta}$	$\frac{k_2 K_5 + b' k_2 (S)}{K_5 + (S)}$	$v_0 \frac{k_2}{k_1(I) + k_2}$
$\frac{k_1(I) \left[1 + \frac{(K_s)}{K_1} + a' \frac{(S)}{K_2} \right]}{K_s \left[1 + \frac{(S)}{K_3} + \frac{(I)}{K_1} \right] + (S) \left[1 + \frac{(S)}{K_4} + \frac{(I)}{K_2} \right]} + \alpha$	$v_0 \frac{\alpha}{\beta}$	$\frac{k_2 K_5 + b' k_2 (S)}{K_5 + (S)}$	$v_0 \frac{k_1 + (I)}{K_1 + (I) + \frac{k_1}{k_2} (I)}$
$\frac{k_1(I) [K_s + a'(S)]}{K_s \left[1 + \frac{(S)}{K_3} \right] + (S) \left[1 + \frac{(S)}{K_4} \right]} + \alpha$	$v_0 \frac{\alpha}{\beta} + \frac{cV_{\max}(S) \left[1 - \frac{\alpha}{\beta} \right]}{K_5 + (S)}$	$\frac{k_2 K_5 + b' k_2 (S)}{K_5 + (S)}$	$v_0 \frac{k_2}{k_1(I) + k_2} + \frac{cV_{\max}(S) \left[1 - \frac{\alpha}{\beta} \right]}{K_5 + (S)} * \frac{k_1(I)}{k_1(I) + k_2}$
$\frac{k_1(I) \left[\frac{K_s}{K_1} + a' \frac{(S)}{K_2} \right]}{K_s \left[1 + \frac{(S)}{K_3} + \frac{(I)}{K_1} \right] + (S) \left[1 + \frac{(S)}{K_4} + \frac{(I)}{K_2} \right]} + \alpha$	$v_0 \frac{\alpha}{\beta} + \frac{cV_{\max}(S) \left[1 - \frac{\alpha}{\beta} \right]}{K_5 + (S)}$	$\frac{k_2 K_5 + b' k_2 (S)}{K_5 + (S)}$	$v_0 \frac{K_1 + (I)}{K_1 + (I) + \frac{k_1}{k_2} (I)}$ $+ \frac{cV_{\max}(S) \left[1 - \frac{\alpha}{\beta} \right]}{K_5 + (S)} * \frac{k_1(I)}{k_2 [K_1 + (I) + \frac{k_1}{k_2} (I)]}$

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