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A Comparative Analysis of Strict Kinetic Equations for the Enzyme Systems and Equations Obtained by the Rapid Equilibrium Approach

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Abstract

The derivation of equations, which give the concentration of enzyme or ligand species in both the steady-state and the transient phase for a given mechanism of an enzyme reaction,is an unavoidable task for most kinetic analyses of any enzyme reaction. The above-mentioned equations can be obtained either under strict conditions, i.e. without the assumption of reversible steps being in rapid equilibrium, or by assuming a rapid equilibrium of one of thereversible step, or more,involved in the reaction mechanism (partial rapid equilibrium approach), or them all(total rapid equilibrium approach). Both equation types, strict ones and those obtained by assuming arapid equilibrium, which formally differ for the same reaction mechanismin many cases, have advantages and limitations, which are discussed herein with specific examples.

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1. Introduction

Strict equationsare those obtained bya minimum set of assumptions whichallow the linearization of the set of algebraic (for steady-state) or differential (for the transient phase) equations that provide the kinetic behavior of a given enzyme system;e.gunder free enzyme limiting conditions. In transient phase equations, another condition is a reaction time, usedfor the initial concentration of the ligand species (substrates, inhibitors, activators, etc.) thatbind to an enzyme species to be consideredapproximately constant during this time.The rapid equilibrium approach adds another assumption: that some or all of the reversible steps involved in the reaction mechanism reach theequilibrium practically uponthe onset of the reaction [1]. This would mean that all the rate constants of the first or pseudo-first orders involved in the reversible steps assumed in the rapid equilibrium would be much larger than all the others involved in the mechanism, andwould not differ much mutually [2-5]. Both equationtypes, strict ones and those obtained by assuming a rapid equilibrium,which formally differ for thesame situationin most cases [5], have their advantages and drawbacks. The present work uses specific examples to clarify such conflicting attributes.

Several contributions exist, e.g. references [1,3,5-9], on the strict steady-state of enzyme reactions, although the presented equations may be too complex to be of practical interest [10]. Thus more simplified steady-state equations arefrequently obtained byassuming that one of the reversible steps or more is/are in rapid equilibrium [2,3,5-13]. The steady-state equations of an enzyme reaction mechanismcan be obtained in five different ways when assumingrapid equilibrium: (1)directly from the reaction scheme byassuming that some or all of the reversible steps are in rapid equilibrium, and thenfrom the corresponding set of algebraic equations [1,2]; (2) from the strict steady state in which some rate constants must be much larger (or much smaller) than others [3,5,6,9];(3) using appropriate software, some of which have been implemented by our research group, to allow these equationsto be rapidly and safely obtained [3,5,6,9,14,15]; (4) from the equation of another mechanism of which the first can be considered a particular case [16];(5) as a particular case of the transient phase equations according tothe assumption of rapid equilibrium when time obtains high values; i.e. when $t \to \infty$ [3,17].

The more recent method forobtaining transient phase equations by assuming the rapid equilibrium of some or all the reversible steps is analogous to the above [3]. Transient phase equations,together with the experimental time progress curves of enzyme species throughout

the reaction course, are the most commonly used tools to characterize enzyme systems. The transient phase analysis identifies experimental designs and kinetic data analyses, which can provide more information about the kinetic parameters involved than a steady-state analysisalone[3,18,19]. This is why more and more analyses of the transient phase of enzyme reactions are becoming increasingly popular.

The transient phase kinetic equations of an enzyme reaction mechanism can be acquired in four waysby assuming a rapid equilibrium: (1)directly from the scheme of the reaction by assuming some or all the reversible steps to be in rapid equilibrium, and then from the corresponding set of differential equations [20,21]; (2) by setting some rate constants that are much larger or smaller than others in the strict transient phase equations [22-24]; (3) using software, some of which have been implemented by our work group[3,25]; (4) from the equations of another mechanism of which the first can be considered a particular case [16]. By obtaining transient phase equations, steady-state equations can also be obtained as a particular case of the former[3,19,25,26].

The main purpose of this contribution is to carry out a cursory comparative analysis of the advantages and disadvantages of using steady-state and transient phase equationsby the rapid equilibrium approach comparedwith the corresponding strict equations.

2. Material and Methods

In order to obtain the steady-state equations, we used the software ALBASS [9], wREFERAS[27] or WinStes [4], while we used the software SKEE-w2013 [25] or TRAPHAER [3] to obtain the transient phase equations for the strict ones, and the software TRAPHAER for the rapid equilibrium ones. All these software packages are available viahttp://oretano.iele-ab.uclm.es/~BioChem-mg/software.php. Some basic elementary mathematicsconcepts were also necessary.

3. Rapid Equilibrium Kinetic Equations versus Strict Equations

Figure 1 schematically represents the four equation typesdiscussed in this work and the relationshipsamong them. Rapid equilibrium equations can be consideredthose of the strict equations in which one reversible step or more is/are assumed to be in rapid equilibrium. Thus it could be stated that rapid equilibrium equations emerge from strict ones. The contrary is not

observed. Both strict and rapid equilibrium steady-state equations can be considered a particular case of the corresponding transient phase equations when time obtains high values; e.g. when $t \to \infty$.

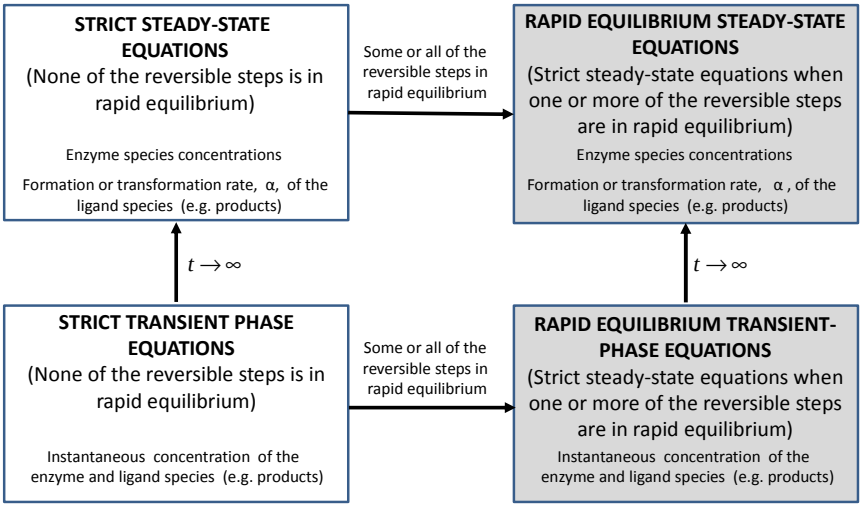


Figure 1.The different kinetic equations treated in this work and the relationships between them.

Let us assume an enzyme system that consists in *n* enzyme species and *g* ligand speciesin which the interconversions between the different enzyme species are first- or pseudo first-order. Most specific enzyme systems fit this general system type. If we denote the enzyme species as X_i (i=1,2,…,n) and the ligand species involved in any reaction mechanism as Y_s(s=1,2,…,g), the strict steady state and transient phase for the concentrations of X_i and Y_s, $[X_i]$ and $[Y_s]$ are[3,5,6,9,19,25]:

3.1. Strict equations

Strict steady-state equations

$$[X_i] = A_{i,0} \qquad (i = 1,2,\ldots,n) \qquad (1)$$

$$[Y_s] - [Y_s]_0 = \beta + \alpha t \quad (\beta \text{or} \alpha \text{ could be zero in some cases}) \quad (s=1,2,\ldots,g) \qquad (2)$$

where $A_{i,0}$, β and α are the expressions that contain the rate constants and the initial concentrations of the enzyme and ligand species present upon the onset of the reaction. In Eq. (2) α is the so-called initial rate, or steady-state rate of ligand species Y_s,and $[Y_s]$ is the initial concentration of Y_s.

Strict transient phase equations

$$[X_i] = A_{i,0} + \sum_{h=1}^{u} A_{i,h} e^{\lambda_h t} \qquad (i = 1,2,\ldots,n) \tag{3}$$

$$[Y_s] - [Y_s]_0 = \beta + \alpha t + \sum_{h=1}^{u} \gamma_h e^{\lambda_h t} \qquad (s=1,2,\ldots,g) \tag{4}$$

In Eqs. (3) and (4),u is an integer number less than n. The u-value depends on the mechanism of the enzyme reaction. $A_{i,0}$, α and β are the same as in Eqs. (1) and (2).Arguments $\lambda_1, \lambda_2, \ldots, \lambda_u$ are functions of the rate constants and the initial concentrations of the ligand species, and are real and negative or complex with a negative real part [28].Amplitudes $A_{i,1}$, $A_{i,2}$,…,$A_{i,u}$ in Eq. (3) and amplitudes γ_1, γ_2, …, γ_u in Eq. (4) are functions of arguments λ_1, λ_2,…, λ_u, the rate constants and the initial concentration of the species present upon the onset of the reaction.

Note that steady-state Eqs. (1) and (2) can be obtained from Eqs. (3) and (4) by setting $t \to \infty$ since arguments are real and negative, or complex with a negative real part, and all the exponential terms vanish at $t \to \infty$.

3.2. Rapid equilibrium equations

Rapid equilibrium equations result when it is assumed that one reversible step or more is/arein rapid equilibriumin strict equations, as so:

$$\text{Rapid equilibrium equations} = \lim_{\substack{\text{one or more}\\ \text{reversible steps}\\ \text{assumed to be}\\ \text{in rapid equilibrium}}} \boxed{\text{Strict equations}} \tag{5}$$

Their general mathematical forms are similar to these of strictequations,but are generally more reduced. Below we provide these general forms, and the equilibrium equations are shaded in grayfor clarity.

Rapid equilibrium steady-state equations

$$[X_i] = A_{i,0} \qquad (i = 1,2,\ldots,n) \tag{6}$$

$$[Y_s] - [Y_s]_0 = \beta + \alpha t \quad (\beta \text{or} \alpha \text{ could be zero in some cases}) \qquad (s=1,2,\ldots,g) \tag{7}$$

where $A_{i,0}$, β and α are the expressions that contain rate constants, equilibrium constantsand the initial concentrations of the enzyme and ligand species present upon the onset of the reaction. These expressions can be formally equal, identical or simpler than those that correspond to strict equations, as we will see below.

Rapid equilibrium transient phase equations

$$[X_i] = A_{i,0} + \sum_{h=1}^{w} A_{i,h} e^{\lambda_h t} \qquad (w<u) \tag{8}$$

$$[Y_s] - [Y_s]_0 = \beta + \alpha t + \sum_{h=1}^{w} \gamma_h e^{\lambda_h t} \qquad (w<u) \tag{9}$$

In Eqs. (8) and (9),*w* is an integer number less than *u*. The *w*-value depends on the mechanism of the enzyme reaction and on the number of reversible steps in rapid equilibrium. Generally, if there are *m* reversible steps in rapid equilibrium, the *w*-value equals*u*-*m*, but there are exceptions to this rule; when there are loops in the reaction mechanism, all the steps in the loops are reversible and assumed to be in rapid equilibrium. In these cases, the *w*-value is higher than *u*-*m*, but is always less than*u*[3,19]. $A_{i,0}$, α and β are the same as in Eqs. (6) and (7). Arguments λ_1, λ_2,…, λ_w in Eqs. (8) and (9) are functions of the rate constants, equilibrium constants and the initial concentrations of the ligand species, and they are real and negative or complex with a negative real part [28]. Amplitudes $A_{i,1}$, $A_{i,2}$,…,$A_{i,w}$ in Eq. (8) and amplitudes γ_1, γ_2, …, γ_w in Eq. (9) are functions of arguments λ_1, λ_2,…, λ_w, the rate and equilibrium constants and the initial concentration of the species present upon the onset of the reaction.

4. Results and Discussion

The use of kinetic equations,both those that correspond to steady-state and transition phase equations in rapid equilibrium, can be justified by the following:

(1) They are generally easier to obtain than those without these assumptions.

(2) They are mostly simpler than strict equations. The assumption of a rapid equilibrium mechanism usually results in much simpler equations compared with those that correspond to the strict steady-state conditions, particularly when different assumptions are applied to systems with many reaction steps.

(3) As a result of (2), they are more suitable for fitting experimental data and for a kinetic data analysis than strict equations. There are several contributions in the literature where,once a strict kinetic equation is obtained,it is quickly replaced with the corresponding equation obtained by the rapid equilibrium approach [22-24]. Yet despite these apparent advantages, their use has its limitations, e.g.:

(a) When one of these equations is simpler than the strict one, fewer kinetic parameters can be evaluated.

(b) Since these equations are rather artificialgiven the lower probability of a rapid equilibrium being fulfilled (and also because it is unclear whether anequilibrium exists or not), the resulting values of the kinetic parameters canvastly differ fromtheir true values[29].However, the better the rapid equilibrium conditions are fulfilled, the more similar they will be [4].

(c) As regards transient phase equations, and sometimes also steady-state equations, the mathematical form of a strict equation differsfrom the corresponding equations by assuming a rapid equilibrium,which underlines the considerations made in point (b) above.

(d) Only by means of numerical integration, in which arbitrary values are assigned to rate constants and initial concentrations, can strict results be compared with those obtained by the rapid equilibrium assumption [4,30].

(e) There is good reason to believe that the rapid equilibrium approach does not prevail in the enzyme systems of living cells [31-34].Sostudies that do not use this approach better reflect the physiology of cells.

One can express a rule equivalent to the well-known *leverrule* as: “the more reduced the kinetic equation becomes, the fewer the kinetic parameters that can be evaluated and the less accurate the evaluations”. Put more simply, “what is gained in simplicity is lost in the accuracy of the kinetic analysis”,as already pointed out by Yago et al. [4]

We chose five enzyme reaction mechanism schemes (summarized below), together with their corresponding kinetic equations, todiscuss their advantages and disadvantages. For all the schemes, both the strict and rapid equilibrium steady-state equations are provided, and the form of the transient phase is also provided for Schemes 3-5 only. These equations can be obtained in any of the ways indicated in the Introduction. For those cases in which kinetic equations were not found in the literature, we used the software implemented by our research group for the steadystate alone [3,4,6,9,25,27], and for both the transient phase and the steady state either with [3] or without reversiblesteps [3,25] in rapid equilibrium. Next we go on todistinguish between steady-state and transient phase equations.

4.1 Steady-state equations

Scheme 1(A) indicates no rapid equilibrium in the only reversible step. In Scheme 1(B) the reversible step is in rapid equilibrium. Note that Eqs. (1A.1) and (1B.1) are formally identical. Eq. (1B.1) differs from Eq. (1A.1) in that the latter contains equilibrium constant K_1instead of Michaelis constant K_m. Thus if one assumes rapid equilibrium,K_1rather than $K_m$is obtained,e.g.$K_m$is taken as if it were K_1 and the error becomes very large if k_2 is much lower than $k_{-1}$is not verified. We believethat the rapid equilibrium assumption is unnecessaryin this case because the effort made tofit the experimental data to Eqs. (1A.1) and (1B.1) is the same. Perhaps the only advantage of assuminga rapid equilibrium is thatEq. (1B.1) is slightly easier to obtain than Eq. (1A.1).

Scheme 2(A) shows no rapid equilibrium in the only reversible step. The reversible stepin Scheme 2(B) is in rapid equilibrium. Exactly the same considerations as for Schemes 1 may be made here. Otherwise Eqs. (2A.1) and (2B.1) are formally equal, as in Scheme 1, and global parameter $K_m$differs rather thanK_1. If we use Eq. (2B.1) for fitting purposes, we obtain K_1, which is really K_m. It would be senseless to use the rapid equilibrium approach here, except that Eq. (2B.1) is slightly easier to obtain than Eq. (2A.1).

Scheme 3(A) includes no rapid equilibrium in the reversible steps of inhibitor binding from the onset of the reaction, but equilibrium is necessarily reached in the steady state of the reaction, which is why Eqs. (3A.1) and (3B.1) are identicalin this particular case.Using the rapid equilibrium approach offers no advantagehere.

In Scheme 4(A) no rapid equilibrium is found in any of the three reversible steps, and the three reversible steps in Scheme 4(B) are in rapid equilibrium. In Scheme 4 we can

observe a huge difference between Eqs. (4A.1)[33] and (4B.1). Eq. (4A.1) is a rational equation of five parameters, whose numerator and denominator aresecond-order in $[S]_0$, whereas Eq. (4B.1) is a rational equation with three parameters of first-order in $[S]_0$ in both the numerator and the denominator. Therefore, the rapid equilibrium equation is much simpler, as is the expression of the coefficients involved in it. In this case, usingEq. (4B.1) is justified because strict Eq. (4A.1) is untreatable. Yet we must not forget that the values obtained for k_3, K_2 and $K_5+K_1K_2$ do not coincide with the true values of these rate and equilibrium constants. Another advantage of Eq. (4B.1) is that it is very easy to obtain manually from the three equilibria and the enzyme mass balance; e.g. $[E]_0 = [E]+[ES]+[E'S]+[E']$.

Finally in Scheme 5, the rapid equilibrium Eq. (5B.1) is considerably simpler than Eq. (5A.1). From the latter, and by means of the following steps, we find that: (a) different fits by non-linear regression of the experimental α-values *vs.* $[A]_0$ at boththe fixed$[B]_0$ and $[C]_0$; (b) the corresponding replots *vs.* $[B]_0$ atthe fixed $[C]_0$; (c) finally, replots *vs.* $[C]_0$allow the evaluation of all the rate and equilibrium constants involved in Eq. (5B.1).

In Schemes 1-5 the steady-state equations that are referred to are only specific examples of the following general results:

- Rapid equilibrium steady-state equations are never more complicated than strict ones [compare Eq. (*r*A.1) with (*r*B.1) (*r*=1,2,3,4,5)].

- Rapid equilibrium equations involve always one equilibrium constant or more[see Eqs. (*r*B.1) (*r*=1,2,3,4,5)].

- Strict steady-state equations may sometimes involve equilibrium constants [see Eq. (3A.1)].

- Rapid equilibrium steady-state equations may sometimes involve Michaelis constants [see Eq. (3B.1)].

- Rapid equilibrium steady-state equations may formally equal strict ones, but involve one different kinetic parameter or more [compare Eqs. (1A.1) with (1B.1) and Eq. (2A.1) with (2B.1)].

- In some cases, both strict and rapid equilibrium steady-state equations are identical [compare Eqs. (3A.1) with (3B.1)].

- Equilibrium steady-state equations are usually simpler than the corresponding strict ones and are, therefore, more suitable for designing experimentalkinetic data analyses [compare Eqs. (4A.1) with (4B.1), and Eq. (5A.1) with (5B.1)].

- Strict and rapid equilibrium steady-state equations may be identical, different but formally equal,or totally different depending on the reaction mechanism, and also from the steps assumed to be in rapid equilibrium, as indicated above.

- Both strict steady-state and rapid equilibrium equations can be easily obtained in a very short time and with no human errors by using the software valid only for strict equations [18,25], or for both strict and rapid equilibrium equations [3].

- One of the reasons for using steady-state equations with one reversible step or more in rapid equilibrium was because obtaining rapid equilibrium equations is manually easier than obtaining strict ones. Nowadays, this difference is no longer important if suitable softwareis used.

- Based on the above, the use of rapid equilibrium steady-state equations would only be justified in those cases in which the equation is simpler thanthe strict one and is, therefore, more suitable for suggesting a convenient experimental design and a kinetic data analysis.

4.2 Transient phase equations

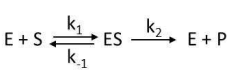
Transient phase equations, together with the time course monitoring of the concentration of the corresponding species, allow more kinetic parameters to be evaluated than a simple study of steady-state behavior [3,18,22-25]. These equations can correspond to strict conditions, e.g.without assuming that any reversible step is in rapid equilibrium, or that the rapid equilibrium assumption of one reversible step or more is involved in the reaction mechanism. Our work group has implemented software [3,18,25] that allows the computerized acquisition of transient phase equations, both according to a strict and rapid equilibrium assumption, which circumvents the laborious and prone-to-human error manual acquisition of these equations by using any of the procedures discussed in the Introduction.

As a support, we used the same five reaction schemes as used for the steady-state equations. The complete transient phase equations are providedfor Schemes 1 and 2, and only the mathematical form (sufficient for our purpose) of these equations is provided for Schemes 3, 4 and 5. As particular cases,transient phase equations contain steady-state equations. The following statements are valid for strict and rapid equilibrium transient phase equations, some of which are easy to confirm from the equations that correspond to Schemes 1-5.

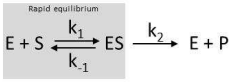
- Rapid equilibrium transient phase equations are always simpler than strict ones.
- Equations according to rapid equilibrium assumptions always have fewer exponential terms that strict ones.
- Each reversible step assumed to be in rapid equilibrium reduces one exponential term from the corresponding strict equation [3].
- The amplitudes and arguments in rapid equilibrium assumptions are always reduced compared with strict ones.
- The remaining coefficients in the rapid equilibrium equation are generally simpler than the corresponding onesin strict equations.
- Rapid equilibrium equations always involve one equilibrium constant or more.
- In some cases, both strict and the rapid equilibrium steady-state equations are totally identical or formally equal.
- Strict transient phase equations may sometimes involve equilibrium constants.
- Rapid equilibrium equations may sometimes involve Michaelis constants.
- Rapid equilibrium equations are more suitable than strict equations for designing experiments and for kinetic data analyses.
- Both strict steady-state and rapid equilibrium equations can be easily obtained in a very short time and with no human errors by using the software valid either for only strict equations [25,18] or for both strict and rapid equilibrium equations [3].
- One of the reasons for using transient phase equations with one reversible step or more in rapid equilibrium was that obtaining rapid equilibrium equations was manually easier than obtaining strict ones. Nowadays, this difference no longer exists thanks to the software available.

- Due to the above, using rapid equilibrium transient phase equationsis justified only in those cases in which the equation is simpler than the strict one and is, therefore, more suitable for suggesting a convenient experimental design and kinetic data analyses.

- When w =0, e.g. when no exponential terms are in rapid equilibrium, both rapid equilibrium steady-state equations coincide according to Eqs. (1)-(4).



1(A)



1(B)

Strict steady-state equations

$$\alpha = \frac{k_2[S]_0[E]_0}{[S]_0 + K_m} \quad (1A.1)$$

$$K_m = \frac{k_{-1} + k_2}{k_1} \quad (1A.2)$$

Steady-state equations with rapid equilibrium

$$\alpha = \frac{k_2[S]_0[E]_0}{[S]_0 + K_1} \quad (1B.1)$$

$$K_1 = k_{-1}/k_1 \quad (1B.2)$$

Strict transient phase equations

$$[P] = \alpha t + \beta\left(1 - e^{\lambda t}\right) \quad (1A.3)$$

$$\lambda = -(k_{-1} + k_2 + k_1[S]_0) \quad (1A.4)$$

α is given by Eq. (1A.1)

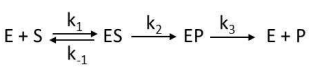
$$\beta = -\frac{k_1 k_2 [S]_0 [E]_0}{\left(k_{-1} + k_2 + k_1[S]_0\right)^2} \quad (1A.5)$$

Strict transient phase equations with rapid equilibrium

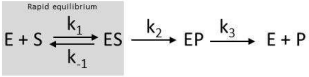
$$[P] = \alpha t \quad (1B.3)$$

α is given by Eq. (1B.1)

Schemes 1.A simple Michaelis Menten mechanism and the corresponding kinetic equations. $[E]_0$ and $[S]_0$ are the initial concentrations of E and S [1,19].



2(A)



2(B)

Strict steady-state equations

$$\alpha = \frac{k_2 k_3 [S]_0 [E]_0}{(k_2 + k_3)[S]_0 + k_3 K_m} \quad (2A.1)$$

$$K_m = \frac{k_{-1} + k_2}{k_1} \quad (2A.2)$$

Strict transient phase equations

$$[P] = \beta + \alpha t + \gamma_1 e^{\lambda_1 t} + \gamma_2 e^{\lambda_2 t} \quad (2A.3)$$

λ_1 and λ_2 being the roots of equation:

$$\lambda^2 + F_1 \lambda + F_2 = 0 \quad (2A.4)$$

where F_1 and F_2 are:

$$F_1 = k_1 [S]_0 + k_{-1} + k_2 + k_3 \quad (2A.5)$$

$$F_2 = k_1 (k_2 + k_3)[S]_0 + k_3 (k_{-1} + k_2) \quad (2A.6)$$

$$\gamma_1 = -\frac{k_1 k_2 k_3 [S]_0}{\lambda_1^2 (\lambda_2 - \lambda_1)} \quad (2A.7)$$

$$\gamma_2 = -\frac{k_1 k_2 k_3 [S]_0}{\lambda_2^2 (\lambda_1 - \lambda_2)} \quad (2A.8)$$

α is given by Eq. (2A.1)

$$\beta = -\frac{k_1 [S]_0 + k_{-1} + k_2 + k_3}{k_1 (k_2 + k_3)[S]_0 + k_3 (k_{-1} + k_2)} \quad (2A.9)$$

Steady-state equations with rapid equilibrium

$$\alpha = \frac{k_2 k_3 [S]_0 [E]_0}{(k_2 + k_3)[S]_0 + k_3 K_1} \quad (2B.1)$$

$$K_1 = k_{-1}/k_1 \quad (2B.2)$$

Strict transient phase equations with rapid equilibrium

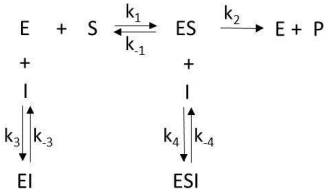
$$[P] = \alpha t + \beta \left(1 - e^{\lambda_1 t}\right) \quad (2B.3)$$

$$\lambda_1 = -\frac{(k_2 + k_3)[S]_0 + k_3 K_1}{[S]_0 + K_1} \quad (2B.4)$$

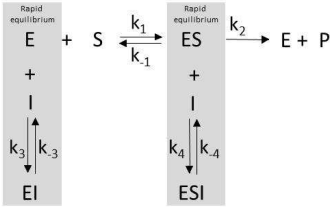
α is given by Eq. (2B.1)

$$\beta = -\frac{k_2 k_3 ([S]_0 + K_1)[S]_0 [E]_0}{\left\{(k_2 + k_3)[S]_0 + k_3 K_1\right\}^2} \quad (2B.5)$$

Schemes 2.The Michaelis Menten mechanism with two intermediates.$[E]_0$ and $[S]_0$ are the initial concentrations of *E* and *S* [36-39].



3(A)



3(B)

Strict steady-state equations

$$\alpha = \frac{k_2 K_3 K_4 [S]_0 [E]_0}{K_m K_3 K_4 + K_3 K_4 [S]_0 + K_m K_4 [I]_0 + K_3 [I]_0 [S]_0} \quad (3A.1)$$

$$K_m = \frac{k_{-1} + k_2}{k_1} \quad (3A.2)$$

$$K_3 = k_{-3}/k_3 \quad (3A.3)$$

$$K_4 = k_{-4}/k_4 \quad (3A.4)$$

Strict transient phase equations

$$[P] = \beta + \alpha t + \sum_{h=1}^{3} \gamma_h e^{\lambda_h t} \quad (3A.5)$$

α is given by Eqs. (3A.1)

Steady-state equations with rapid equilibrium

$$\alpha = \frac{k_2 K_3 K_4 [S]_0 [E]_0}{K_m K_3 K_4 + K_3 K_4 [S]_0 + K_m K_4 [I]_0 + K_3 [I]_0 [S]_0} \quad (3B.1)$$

$$K_m = \frac{k_{-1} + k_2}{k_1} \quad (3B.2)$$

$$K_3 = k_{-3}/k_3 \quad (3B.3)$$

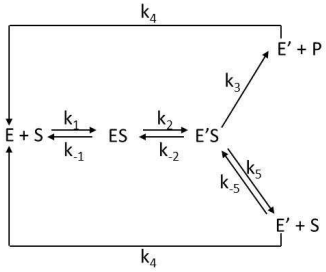
$$K_4 = k_{-4}/k_4 \quad (3B.4)$$

Strict transient phase equations with rapid equilibrium

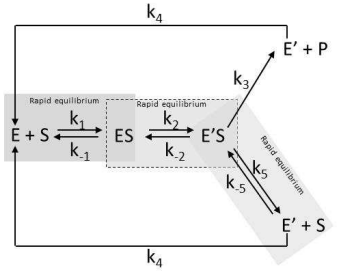
$$[P] = \alpha t + \beta \left(1 - e^{\lambda t}\right) \quad (3B.5)$$

α is given by Eq. (3B.1)

Schemes 3.The Michaelis Menten mechanism with linear mixed inhibition.$[E]_0$, $[S]_0$ and $[I]_0$ are the initial concentrations of E, S and I [35].



4(A)



4(B)

Strict steady-state equations

$$\alpha = \frac{k_1 k_2 k_3 \left(k_4 + k_{-5}[S]_0\right)[S]_0[E]_0}{a + b[S]_0 + c[S]_0^2} \qquad (4A.1)$$

$$a = k_4\left\{k_{-1}k_{-2} + \left(k_{-1} + k_2\right)\left(k_3 + k_5\right)\right\} \qquad (4A.2)$$

$$b = k_1 k_2\left(k_3 + k_4 + k_5\right) + k_1 k_4\left(k_{-2} + k_3 + k_5\right) + k_{-1}k_{-2}k_{-5} \qquad (4A.3)$$

$$c = k_1 k_{-5}\left(k_{-2} + k_2\right) \qquad (4A.4)$$

Strict transient phase equations

$$[P] = \beta + \alpha t + \sum_{h=1}^{3} \gamma_h e^{\lambda_h t} \qquad (4A.5)$$

α is given by eq. (4A.1)

Steady-state equations with rapid equilibrium

$$\alpha = \frac{k_3[S]_0[E]_0}{K_5 + K_1K_2 + (1 + K_2)[S]_0} \qquad (4B.1)$$

$$K_1 = k_{-1}/k_1 \qquad (4B.2)$$

$$K_2 = k_{-2}/k_2 \qquad (4B.3)$$

$$K_5 = k_5/k_{-5} \qquad (4B.4)$$

Strict transient phase equations with rapid equilibrium

$$[P] = \alpha t + \beta\left(1 - e^{\lambda_3 t}\right) \qquad (4B.5)$$

α is given by eq. (4B.1)

Schemes 4.The Rabin cooperativity model. $[E]_0$ and $[S]_0$ are the initial concentrations of *E* and *S* [40,41].

5(A)

Strict steady-state equations

$$\alpha = \frac{k_1k_2k_3k_4k_5[A]_0[B]_0[C]_0[E]_0}{a + b[A]_0 + c[C]_0 + d[A]_0[B]_0 + e[A]_0[C]_0 + f[B]_0[C]_0 + g[A]_0[B]_0[C]_0} \quad (5A.1)$$

$$a = k_{-1}k_{-2}k_4k_5 \quad (5A.2)$$

$$b = k_1k_{-2}k_4k_5 \quad (5A.3)$$

$$c = k_{-1}k_3k_4k_5 \quad (5A.4)$$

$$d = k_1k_2k_4k_5 \quad (5A.5)$$

$$e = k_1k_3k_4k_5 \quad (5A.6)$$

$$f = k_2k_3k_4k_5 \quad (5A.7)$$

$$g = k_1k_2k_3(k_4 + k_5) \quad (5A.8)$$

Strict transient phase equations

$$[P] = \beta + \alpha t + \sum_{h=1}^{4} \gamma_h e^{\lambda_h t} \quad (5A.9)$$

α is given by Eq. (5A.1)

5(B)

Steady-state equations with rapid equilibrium

$$\alpha = \frac{k_3k_4k_5[A]_0[B]_0[C]_0[E]_0}{K_1K_2k_4k_5 + K_2k_4k_5[A]_0 + k_4k_5[A]_0[B]_0 + k_3(k_4 + k_5)[A]_0[B]_0[C]_0} \quad (5B.1)$$

$$K_1 = k_{-1}/k_1 \quad (5B.2)$$

$$K_2 = k_{-2}/k_2 \quad (5B.3)$$

$$K_5 = k_5/k_{-5} \quad (5B.4)$$

Strict transient phase equations with rapid equilibrium

$$[P] = \beta + \alpha t + \gamma_1 e^{\lambda_1 t} + \gamma_2 e^{\lambda_2 t} \quad (5B.5)$$

α is given by Eq. (5B.1)

Schemes 5.The sequential ordered Ter-TerTheorell-Chance mechanism. $[E]_0$, $[A]_0$, $[B]_0$ and $[C]_0$ are the initial concentrations of E, A, B and C. [1,42].

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