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SUBSTRATE-INHIBITION AND SUBSTRATE-ACTIVATION IN THE RANDOM-ORDER TERNARY-COMPLEX MECHANISM FOR ENZYME REACTIONS INVOLVING TWO SUBSTRATES

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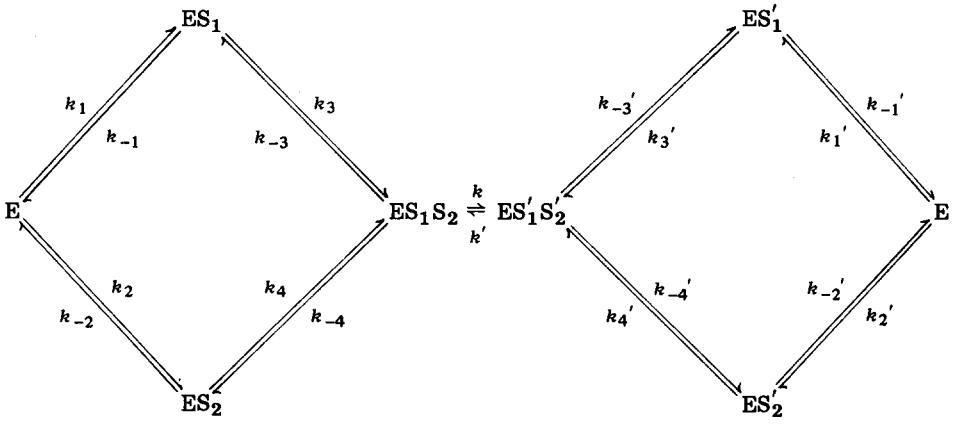
Summary



Scheme 1. The generalized ternary-complex mechanism considered in the present investigation. E, S_i and S_i' stand for enzyme, substrates and products, respectively.

Deviations from Michaelis-Menten kinetics in the random-order ternary-complex mechanism for enzyme reactions involving two substrates (Scheme 1) have been analyzed in view of the asymptote theory for higher-degree rate equations. The patterns of substrate-inhibition or substrate-activation inherent in this mechanism are characterized. Generalized relationships for the evaluations and interpretation of such kinetic patterns in terms of rate constants in the mechanism are derived, and the appropriate reduction of these relationships in some special cases of particular interest is described and discussed.

Introduction

A large number of enzymes catalyzing reactions involving two substrates operate by sequential mechanisms, in which a ternary enzyme-substrate complex is formed as an obligatory intermediate in the catalytic process. The random-order mechanism in Scheme 1 may be considered fundamental for such enzyme systems, and the kinetic characteristics of this mechanism have been much discussed. The steady-state rate-equation corresponding to Scheme 1 is given reciprocally [1,2] by:

$$\frac{c_E}{v} = \frac{\beta_{00} + \beta_{01}[S_2] + \beta_{02}[S_2]^2 + (\beta_{10} + \beta_{11}[S_2] + \beta_{12}[S_2]^2)[S_1] + (\beta_{20} + \beta_{21}[S_2])[S_1]^2}{\alpha_{11}[S_1][S_2] + \alpha_{12}[S_1][S_2]^2 + \alpha_{21}[S_1]^2[S_2]} \quad (1)$$

where S_1 and S_2 denote the two substrates and v/c_E stands for the molar enzymic reaction velocity. At low substrate concentrations Eqn. 1 reduces asymptotically to a Michaelis-Menten type of relationship which, using the Dalziel symbolism [3], may be written [1,2] as:

$$\frac{c_E}{v_{as}} = \phi_0 + \frac{\phi_1}{[S_1]} + \frac{\phi_2}{[S_2]} + \frac{\phi_{12}}{[S_1][S_2]} \quad . \quad (2)$$

Most ternary-complex systems investigated have been found to conform to Eqn. 2 over more or less wide ranges of substrate concentrations. Early analyses of the rate-behaviour inherent in Scheme 1 were, therefore, directed mainly towards some special cases for which Eqn. 2 directly obtains, such as the compulsory-order mechanism [4] and the rapid-equilibrium mechanism [1,3,4]. More recently, generalized relationships for the interpretation of coefficents in Eqn. 2 have been derived by examination of the asymptotic properties of the full rate-equation [5,7], and the Michaelis-Menten behaviour of the random-order ternary-complex mechanism now appears to be well understood.

Deviations from Michaelis-Menten kinetics typical of substrate-inhibition or substrate-activation have frequently been observed in ternary-complex systems. Although such a rate-behaviour can be explained in several different ways, the possibility must always be considered that it is due to random-order addition of substrates in accordance with Scheme 1. Nevertheless, very few attempts have been made to evaluate inhibition and activation data in terms of the full rate-equation for Scheme 1 [8]. This can be ascribed mainly to the fact that Eqn. 1 is too complex to be of any obvious value for the purpose of estimating magnitudes of individual rate and equilibrium constants in the reaction mechanism. The recent characterization of the asymptotic properties of Eqn. 1, however, has made it possible to express deviations from a Michaelis-Menten behaviour in a generalized form apt for theoretical analysis [5].

Such an analysis has now been carried out. The present investigation characterizes the patterns of substrate-inhibition or substrate-activation inherent in the random-order ternary-complex mechanism, and describes the derivation of generalized relationships for evaluation of such kinetic patterns in terms of rate constants in Scheme 1.

Results

Generalized relationships for deviations from Michaelis-Menten kinetics

Eqn. 1 has been shown to approach Eqn. 2 asymptotically at low substrate

concentrations [2]. Deviations from Michaelis-Menten kinetics, i.e. deviations from linearity in Lineweaver-Burk plots with respect to one substrate at fixed concentrations of the other, can be defined as the difference D between Eqn. 1 and its asymptote Eqn. 2:

$$D = \frac{c_E}{v} - \frac{c_E}{v_{as}} \tag{3}$$

For ternary-complex systems operating by Scheme 1, this difference has been shown to be given [15] by:

$$D = \frac{\epsilon_1 \alpha_{21} [S_1]^2 + \epsilon_2 \alpha_{12} [S_2]^2}{\alpha_{11} [S_1][S_2] + \alpha_{12} [S_1][S_2]^2 + \alpha_{21} [S_1]^2 [S_2]} \tag{4}$$

where

$$\alpha_{11} = k_1 k_{-2} k_3 + k_{-1} k_2 k_4 \tag{5}$$

$$\alpha_{12} = k_2 k_3 k_4 \tag{6}$$

$$\alpha_{21} = k_1 k_3 k_4 \tag{7}$$

$$R = \frac{1}{k_{-3} + k_{-4}} \tag{8}$$

$$\epsilon_1 = \frac{k_{-4} R}{k_3} - \frac{(k_{-4} R)^2}{k_2} \tag{9}$$

$$\epsilon_2 = \frac{k_{-3} R}{k_4} - \frac{(k_{-3} R)^2}{k_1} \tag{10}$$

When substrate concentrations are sufficiently low to ensure that Eqn. 2 is obeyed [1,5], the difference D is negligibly small in comparison to c_E/v or c_E/v_{as}. In particular, contributions from the ϵ_2 term in Eqn. 4 are negligibly small and will decrease steadily with increasing values of $[S_1]$. Hence it may be concluded that deviations from Michaelis-Menten kinetics caused by high concentrations of S_1 must derive entirely from the ϵ_1 term in Eqn. 4. Deviations (D_1) from linearity in Lineweaver-Burk plots with respect to S_1 at fixed (low) concentrations of S_2 thus conform to the relationship

$$D_1 = \frac{\epsilon_1 \alpha_{21} [S_1]}{\alpha_{11} [S_2] + \alpha_{12} [S_2]^2 + \alpha_{21} [S_2][S_1]} \tag{11}$$

The sign of ϵ_1 will determine whether D_1 becomes positive or negative. According to Eqn. 9, deviations typical of substrate-inhibition by S_1 ($D_1 > 0$) are obtained when $k_2 > k_3 k_{-4} R$, whereas $k_2 < k_3 k_{-4} R$ results in substrate-activation by S_1. When $k_2 = k_3 k_{-4} R$ we have $\epsilon_1 = 0$, and deviations from linearity in Lineweaver-Burk plots with respect to S_1 will be of insignificant magnitude.

Eqn. 11 prescribes that the absolute magnitude of D_1 decreases with increasing values of $[S_2]$, which confirms previous conclusions that deviations from Michaelis-Menten kinetics with respect to one substrate are most pronounced at low concentrations of the second substrate [2,3]. It can, further, be seen

from Eqn. 11 that D_1 is hyperbolically dependent on $[S_1]$, tending towards the value $\epsilon_1/[S_2]$ as $[S_1]$ approaches infinity. The intercept of the graphs obtained in Lineweaver-Burk plots of c_E/v vs. $1[S_1]$ is thus given by

$$\lim_{[S_1]\to\infty} \frac{c_E}{v} = \phi_0 + \frac{\phi_2 + \epsilon_1}{[S_2]} . \tag{12}$$

Using the expression for ϕ_2 derived by the asymptote theory (Eqn. 20) it can be shown that

$$\phi_2 + \epsilon_1 = \frac{1 + k_{-3}A}{k_3} \tag{13}$$

where

$$A = \frac{1}{k} + \frac{k'}{k(k_{-3'} + k_{-4'})} \tag{14}$$

Eqns. 12—13 may be of diagnostic value under certain conditions, but more useful relationships can be obtained by observing that Eqn. 11 may be written reciprocally as

$$\frac{1}{D_1} = \frac{[S_2]}{\epsilon_1}\left(1 + \frac{K_{app}}{[S_1]}\right) \tag{15}$$

where

$$K_{app} = \frac{\alpha_{11} + \alpha_{12}[S_2]}{\alpha_{21}} \tag{16}$$

Using Eqns. 5—8 and the thermodynamic identity

$$k_1 k_{-2} k_3 k_{-4} = k_{-1} k_2 k_{-3} k_4 \tag{17}$$

Eqn. 16 can be rearranged to give

$$K_{app} = \frac{k_{-1}k_2}{k_1 k_3 k_{-4} R} + \frac{k_2[S_2]}{k_1} \tag{18}$$

Eqn. 15 provides a generalized description of deviations from linearity in Lineweaver-Burk plots of c_E/v vs. $1/[S_1]$ at fixed concentrations of S_2. Eqn. 15 shows that magnitudes of the parameters ϵ_1 and K_{app} can be estimated experimentally from the intercept and slope, respectively, of the straight lines obtained in a replot of $1/D_1$ vs. $1/[S_1]$. Generalized relationships for interpretation of the latter parameters in terms of rate constants in Scheme 1 are given by Eqns. 9 and 18, which can be further simplified in the special cases of particular interest considered below.

Owing to the symmetry of Scheme 1, relationships describing deviations (D_2) from Michaelis-Menten kinetics caused by high concentrations of S_2 are entirely analogous to those reported above and will not be explicitly considered.

The rapid-equilibrium case

Previous analysis of the Michaelis-Menten behaviour inherent in Scheme 1 has established that Dalziel coefficients ϕ_1 and ϕ_2 in Eqn. 2 are related to rate constants in Scheme 1 through [5]:

$$\phi_1 = \frac{k_{-4}(A + R)}{k_4} + \frac{(k_{-3}R)^2}{k_1} \tag{19}$$

$$\phi_2 = \frac{k_{-3}(A + R)}{k_3} + \frac{(k_{-4}R)^2}{k_2} \; . \tag{20}$$

The quadratic terms may be of arbitrary magnitude relatively to the non-quadratic terms, with the restriction that the quadratic terms cannot dominate simultaneously in both Eqn. 19 and Eqn. 20 [5].

When relationships between rate constants in Scheme 1 are such that contributions from the quadratic terms are negligibly small in both Eqn. 19 and Eqn. 20 we have:

$$\phi_1 = \frac{k_{-4}(A + R)}{k_4} \tag{21}$$

$$\phi_2 = \frac{k_{-3}(A + R)}{k_3} \; . \tag{22}$$

This means that the generalized Dalziel equation for the system becomes identical with the rate-equation derived using rapid-equilibrium assumptions as defined by Dalziel [1]. Under such conditions, the corresponding quadratic terms in Eqns. 9—10 cannot contribute significantly to observed deviations from Michaelis-Menten kinetics; it is evident from Eqn. 12 that terms in ϵ_1 contributing significantly to deviations observed at high concentrations of S_1 cannot be of insignificant magnitude in comparison to ϕ_2. By analogy, the same applies to terms in ϵ_2 in comparison to ϕ_1, as concerns deviations caused by high concentrations of S_2. Consequently, deviations possibly observed in the rapid-equilibrium case can be interpreted using:

$$\epsilon_1 = \frac{k_{-4}R}{k_3} \tag{23}$$

$$\epsilon_2 = \frac{k_{-3}R}{k_4} \tag{24}$$

and they will always correspond to a kinetic pattern typical of substrate-inhibition ($D > 0$).

Depending on the magnitude of $1/A$ (the apparent rate constant for forward breakdown of the ternary enzyme-substrate complex to yield free enzyme and products) in comparison to k_{-3} and k_{-4}, such inhibition may or may not be kinetically significant. If $1/A \ll k_{-3}, k_{-4}$, rapid-equilibrium conditions as defined by Alberty [4] prevail. Eqns. 21—24 then prescribe that ϵ_1 (ϵ_2) is negligibly small in comparison to ϕ_2 (ϕ_1), and neither of the substrates will cause any significant deviations from Michaelis-Menten kinetics. If $1/A$ is not much smaller than both k_{-3} and k_{-4}, it can be similarly shown that the condition $k_{-4} \gg k_{-3}$

($k_{-4} \ll k_{-3}$) implies that significant inhibition can be caused by S_1 (S_2), exclusively. Weak inhibition by both substrates is possible when k_{-3} and k_{-4} are of similar magnitude.

The ordered case

If relationships between rate constants in Scheme 1 are such that the quadratic term vanishes in Eqn. 19, but dominates in Eqn. 20, the random-order mechanism in Scheme 1 will become effectively ordered when Michaelis-Menten kinetics prevail, with S_2 adding first to the enzyme [5]. Under such conditions, which only obtain when $k_{-4} \gg k_{-3}$, we have:

$$\phi_1 = \frac{k_{-4}(A + R)}{k_4} \tag{25}$$

$$\phi_2 = \frac{1}{k_2} \tag{26}$$

indicating that the Dalziel equation corresponding to Scheme 1 becomes identical with the rate-equation derived for the compulsory-order mechanism in which S_2 represents the leading substrate [4].

In this case, where $(k_{-3}R)^2/k_1 \ll \phi_1$ and $k_{-4} \gg k_{-3}$, it follows from Eqns. 10 and 25 that ϵ_2 cannot be of significant magnitude in comparison to ϕ_1. This means that deviations from linearity in Lineweaver-Burk plots with respect to S_2 (the leading substrate under Michaelis-Menten conditions) will be of insignificant magnitude. Further, it follows from the definition of R in Eqn. 8 that $k_{-4}R \to 1$ when $k_{-4} \gg k_{-3}$, and Eqns. 9 and 18 reduce to

$$\epsilon_1 = \frac{1}{k_3} - \frac{1}{k_2} \tag{27}$$

$$K_{app} = \frac{k_{-1}k_2}{k_1k_3} + \frac{k_2[S_2]}{k_1} \,. \tag{28}$$

Eqn. 27 prescribes that high concentrations of S_1 will cause substrate-inhibition (substrate-activation) if $k_2 > k_3$ ($k_2 < k_3$), whereas no deviations from Michaelis-Menten kinetics with respect to S_1 will be observed when $k_2 = k_3$.

According to Eqn. 26, the magnitude of k_2 can be estimated from the kinetic behaviour of the system under Michaelis-Menten conditions. Examination of the inhibition or activation pattern produced by S_1 (including determinations of ϵ_1 and of K_{app} as a function of $[S_2]$) can thus be used to calculate k_3 from Eqn. 27. The rate constants k_1 and k_{-1} may then be obtained from Eqn. 28, provided that concentrations of S_2 accessible for experimental variation are such that both terms on the right hand side of Eqn. 28 are of significant magnitude. If such is not the case, determinations of K_{app} will provide estimates only of either k_1 or the quotient k_{-1}/k_1.

Formation of a non-productive binary complex

Eqns. 27—28, referring to the ordered case in which S_2 adds first to the enzyme when Michaelis-Menten kinetics prevail, prescribe that ϵ_1 and K_{app} approach infinity as k_3 approaches zero. It may, therefore, be practically

impossible to estimate the magnitudes of ϵ_1 and K_{app} when $k_3 \ll k_2$. Under such conditions one has to conclude that $k_3 \approx 0$, which means that Eqn. 15 reduces to

$$\frac{1}{D_1} = \frac{k_{-1}k_2[S_2]}{k_1[S_1]} \tag{29}$$

This relationship can be used to obtain an estimate of the dissociation constant k_{-1}/k_1 for the essentially non-productive binary complex ES_1. Eqn. 29 is identical with the relationship previously derived (though presented in a different form) for the corresponding compulsory-order mechanism in which the non-leading substrate forms a dead-end complex with free enzyme [3].

The formation of a productive binary complex ES_1 can be readily distinguished from apparent formation of a dead-end complex by plotting $1/D_1$ vs. $1/[S_1]$. The straight lines obtained in such plots will give an intercept of significant magnitude in the former case, but will pass through the origin in the latter case.

Discussion

The present investigation characterizes the second-degree rate-behaviour inherent in the random-order ternary-complex mechanism shown in Scheme 1. In this mechanism deviations from linearity in Lineweaver-Burk plots with respect to one substrate at fixed concentrations of the other conform to Eqn. 15, and generalized relationships for interpretation of the kinetic parameters (ϵ_1 and K_{app}) defined by Eqn. 15 are given in Eqns. 9 and 18. The analysis presented shows that the evaluation of ϵ_1 and K_{app} in terms of rate constants in Scheme 1 is greatly simplified in the rapid-equilibrium case and the ordered case, which represent typical extremes of the Michaelis-Menten behaviour inherent in Scheme 1 [5]. It may now be noted that very few enzymes operating by a ternary-complex mechanism actually have been established to conform to a Dalziel equation of the rapid-equilibrium type. Ternary-complex systems conforming to a compulsory-order type of rate-equation are common, however, and the mechanistic origin of deviations from Michaelis-Menten kinetics appearing in such systems will be briefly discussed.

Let us consider the ordered case corresponding to the compulsory-order mechanism in which S_2 adds first to the enzyme. In this case, formation of the ternary complex ES_1S_2 in Scheme 1 proceeds almost exclusively via the binary complex ES_2 when Michaelis-Menten kinetics prevail with respect to both substrates [5]. The quotient Q between the reaction flow via ES_1 and the flow via ES_2 is given by [9]

$$Q = \frac{k_1k_{-2}k_3 + k_1k_3k_4[S_1]}{k_{-1}k_2k_4 + k_2k_3k_4[S_2]} \tag{30}$$

In the ordered case considered here we have $k_{-4} \ll k_{-3}$ [5], which implies that $k_1k_{-2}k_3 \ll k_{-1}k_2k_4$ (see Eqn. 17). This means that the term $k_1k_{-2}k_3$ in Eqn. 30 cannot contribute significantly to the magnitude of Q in comparison to unity. Eqn. 30 thus reduces to

$$Q = \frac{k_1k_3[S_1]}{k_{-1}k_2 + k_2k_3[S_2]} \tag{31}$$

which, according to Eqn. 28, may be written as

$$Q = \frac{[S_1]}{K_{app}} \tag{32}$$

Examination of Eqn. 31 shows that the ordered character ($Q \ll 1$) of the reaction will be strengthened at high concentrations of S_2. When the concentration of S_1 is increased, however, there will be an enhanced and ultimately dominating ($Q \gg 1$) utilization of the alternative pathway to ES_1S_2 involving the binary complex ES_1. This suggests that deviations from Michaelis-Menten kinetics (which may be of significant magnitude with respect to S_1, but not with respect to S_2) are attributable to a shift of the pathway preferred for ternary-complex formation. Eqn. 32 establishes that such is the case by showing that deviations observed at high concentrations of S_1 attain half their maximum value when the two pathways are utilized to equal extents ($Q = 1$ for $[S_1] = K_{app}$).

At high concentrations of S_1 the binding of S_2 becomes rate-limiting for formation of the ternary enzyme-substrate complex. If S_2 binds more rapidly to free enzyme than to the binary complex ES_1 ($k_2 > k_3$), high concentrations of S_1 will force the reaction to proceed by a less efficient pathway for ternary-complex formation and substrate-inhibition by S_1 will be observed. The other way round, substrate-activation by S_1 is obtained when S_2 binds more rapidly to ES_1 than to free enzyme ($k_2 < k_3$). This explains why the parameter ϵ_1 provides information about the magnitude of k_3 in comparison to k_2 (Eqn. 27). Similarly, the fact that K_{app} provides information about k_1 and k_{-1} (Eqn. 28) can be well understood in view of a shift of reaction flow towards the pathway involving the binary complex ES_1.

Deviations from Michaelis-Menten kinetics in the random-order Scheme 1 can be considered to derive from a competition between the two substrates for free enzyme. As a consequence of this fact, deviations from linearity in Lineweaver-Burk plots with respect to one substrate decrease with increasing concentrations of the second substrate [2]. This is the most characteristic feature of the second-degree rate-behaviour inherent in the random-order ternary-complex mechanism. Other possible mechanisms for substrate-inhibition or substrate-activation in ternary-complex systems have been discussed by Dalziel [1,8]. None of these alternative mechanisms exhibits such a deviation pattern typical of mutal competition between the substrates. This means that substrate-inhibition or substrate-activation due to a shift of the preferred pathway for ternary-complex formation can be readily distinguished from deviations arising by other plausible mechanisms, e.g. by the formation of a ternary enzyme-substrate-product complex [8]. In the former case, only, non-linearity in Lineweaver-Burk plots with respect to one substrate can be completely eliminated by increasing the concentration of the second substrate.

The applicability and informative value of the relationships derived in the present investigation is illustrated in the following paper, which concerns deviations from Michaelis-Menten kinetics in the citrate synthase system.

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