

The Kinetics of Product Inhibition in the Ternary-Complex Mechanism for Enzyme Reactions Involving Two Substrates

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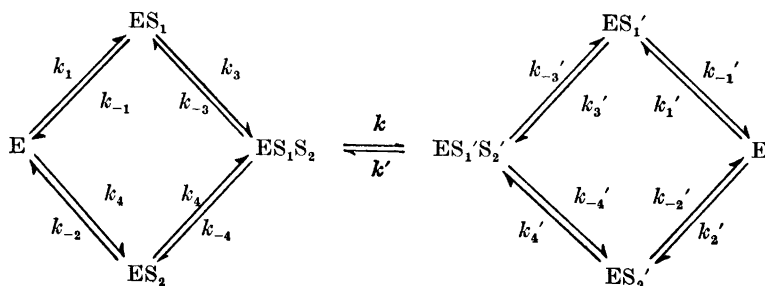
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Relationships for the effects of product-inhibition on steady-state kinetic Dalziel coefficients obtained for enzymes operating by the random-order ternary-complex mechanism for two-substrate reactions have been derived. They show that Dalziel coefficients, in general, are non-linear functions of the concentration of the product used as inhibitor. At low product concentrations Dalziel coefficients become linearly dependent upon the inhibitor concentration. Product-linear Dalziel coefficients can be related to velocity constants in the random-order mechanism through diagnostically valuable equations which are of general applicability within the mechanism, and which permit the formulation of some useful kinetic criteria.

A large number of enzymes catalyzing reactions involving two substrates operate by a sequential mechanism in which a ternary enzyme-substrate complex is formed as an obligatory intermediate in the catalytic process. Kinetic data for such systems have usually been interpreted in view of relationships derived for two special cases of the generalized ternary-complex mechanism shown in Scheme 1, the rapid-equilibrium random-order case^{1,2} and the compulsory-order case.¹ Alberty has shown that these cases can be differentiated by examination of the kinetics obtained in presence of either of the reaction products.³ The product-inhibition technique also makes it possible to identify the leading substrate in a compulsory-order sequence, and has been adopted as a standard kinetic method for the elucidation of reaction mechanisms in ternary-complex systems.

The product-inhibition pattern has, however, only been established for symmetric cases of the ternary-complex mechanism, and cases in which substrates combine to the enzyme in compulsory order while products dissociate in random order (or *vice versa*) have not been considered. Furthermore, recent investigations have established that rate-equations derived under assumptions of rapid-equilibrium or a compulsory order of addition of substrates only represent opposite extremes of the general Dalziel rate-behaviour inherent in Scheme 1.^{4,5} This means that the existence of non-equilibrium random-order

cases conforming to a Dalziel type of rate-equation has also to be considered in diagnostic interpretations of initial-velocity and product-inhibition data for enzymatic ternary-complex systems.



Scheme 1. The generalized ternary-complex mechanism considered in the present investigation. E, S₁ and S₁' stand for enzyme, substrates, and products, respectively.

Due to the biochemical importance of the ternary-complex mechanism, it is of great interest to investigate and describe the corresponding reaction kinetics in a sufficiently general way to permit a conclusive evaluation of kinetic data within the limits given by the fundamental reaction sequences shown in Scheme 1. The steady-state kinetic asymptote theory^{6,7} has made it possible to analyze the initial-velocity kinetics of Scheme 1 in such a generalized way,⁵ and has now been used to establish product-inhibition patterns in the mechanism. The analysis has been restricted to cases giving rise to a Dalziel type of rate-equation with respect to the substrates, and general relationships describing the effect of product concentrations on Dalziel coefficients are derived and discussed.

RESULTS

The asymptotic Dalziel equation for the mechanism in Scheme 1. The reaction shown in Scheme 1 will only be explicitly considered in the forward direction, complementary relationships for the reverse reaction being obtained by insertion or deletion of primes on velocity constants and other kinetic symbols.

The full steady-state reciprocal rate-equation (reciprocal molar reaction velocity y as a function of reciprocal substrate concentrations $z_i = 1/[S_i]$, $i = 1, 2$) for the mechanism in Scheme 1 in the absence of S₂' (product inhibition by S₁') was derived using a computer program based upon the method described by Schultz and Fisher,⁸ and is given by

$$y = \frac{(\beta_{00}z_2^2 + \beta_{01}z_2 + \beta_{02})z_1^2 + (\beta_{10}z_2^2 + \beta_{11}z_2 + \beta_{12})z_1 + \beta_{20}z_2^2 + \beta_{21}z_2}{\alpha_{11}z_1z_2 + \alpha_{12}z_1 + \alpha_{21}z_2} \quad (1)$$

where the coefficients α_{ij} and β_{ij} are polynomials of the first or second degree in $[S_1']$ as indicated in Table 1. Relationships between coefficients α_{ijk} and

Table 1. Functional dependence of coefficients in eqn. (1) on the concentration of the product used as inhibitor.

$$\begin{aligned}
 \alpha_{11} &= \alpha_{110} + \alpha_{111}[S_1'] \\
 \alpha_{12} &= \alpha_{120} + \alpha_{121}[S_1'] \\
 \alpha_{21} &= \alpha_{210} + \alpha_{211}[S_1'] \\
 \beta_{00} &= \beta_{000} + \beta_{001}[S_1'] + \beta_{002}[S_1']^2 \\
 \beta_{01} &= \beta_{010} + \beta_{011}[S_1'] + \beta_{012}[S_1']^2 \\
 \beta_{02} &= \beta_{020} + \beta_{021}[S_1'] \\
 \beta_{10} &= \beta_{100} + \beta_{101}[S_1'] + \beta_{102}[S_1']^2 \\
 \beta_{11} &= \beta_{110} + \beta_{111}[S_1'] + \beta_{112}[S_1']^2 \\
 \beta_{12} &= \beta_{120} + \beta_{121}[S_1'] \\
 \beta_{21} &= \beta_{200} + \beta_{201}[S_1'] \\
 \beta_{21} &= \beta_{210} + \beta_{211}[S_1']
 \end{aligned}$$

Table 2. Relationships between coefficients α_{ijk} and β_{ijk} in Table 1 and velocity constants in Scheme 1.

$$\begin{aligned}
 \alpha_{110} &= (k_1 k_{-2} k_3 + k_{-1} k_2 k_4) k k_{-1}' k_{-2}' (k_{-3}' + k_{-4}') \\
 \alpha_{111} &= (k_1 k_{-2} k_3 + k_{-1} k_2 k_4) k k_{-1}' k_{-3}' k_4' \\
 \alpha_{120} &= k_2 k_3 k_4 k k_{-1}' k_{-2}' (k_{-3}' + k_{-4}') \\
 \alpha_{121} &= k_2 k_3 k_4 k k_{-1}' k_{-3}' k_4' \\
 \alpha_{210} &= k_1 k_3 k_4 k k_{-1}' k_{-2}' (k_{-3}' + k_{-4}') \\
 \alpha_{211} &= k_1 k_3 k_4 k k_{-1}' k_{-3}' k_4' \\
 \beta_{000} &= k_{-1} k_{-2} k_{-1}' k_{-2}' [(k_{-3} + k_{-4})(k' + k_{-3}' + k_{-4}') + k(k_{-3}' + k_{-4}')] \\
 \beta_{001} &= k_{-1} k_{-2} k_{-1}' k_{-2}' [(k_{-3} + k_{-4})(k' + k_{-3}' + k_{-4}') + k(k_{-3}' + k_{-4}')] + \\
 &\quad + k_{-1} k_{-2} k_{-1}' k_4' [(k_{-3} + k_{-4})(k' + k_{-3}') + k k_{-3}'] \\
 \beta_{002} &= k_{-1} k_{-2} k_{-1}' k_4' [(k_{-3} + k_{-4})(k' + k_{-3}') + k k_{-3}'] \\
 \beta_{010} &= k_{-1} k_2 k_{-1}' k_{-2}' [(k_{-3} + k_{-4})(k' + k_{-3}' + k_{-4}') + k(k_{-3}' + k_{-4}')] + \\
 &\quad + k_{-2} k_3 k_{-1}' k_{-2}' [(k_{-3} + k_{-4})(k' + k_{-3}' + k_{-4}') + k(k_{-3}' + k_{-4}')] \\
 \beta_{011} &= k_{-2} k_3 k_{-1}' k_{-2}' [(k_{-3} + k_{-4})(k' + k_{-3}' + k_{-4}') + k(k_{-3}' + k_{-4}')] + \\
 &\quad + k_{-2} k_3 k_{-1}' k_4' [(k_{-3} + k_{-4})(k' + k_{-3}') + k k_{-3}'] + \\
 &\quad + k_{-1} k_2 k_{-1}' k_4' [(k_{-3} + k_{-4})(k' + k_{-3}') + k k_{-3}'] \\
 \beta_{012} &= k_{-2} k_3 k_{-1}' k_4' [(k_{-3} + k_{-4})(k' + k_{-3}') + k k_{-3}'] \\
 \beta_{020} &= k_2 k_3 k_{-1}' k_{-2}' [(k_{-3} + k_{-4})(k' + k_{-3}' + k_{-4}') + k(k_{-3}' + k_{-4}')] \\
 \beta_{021} &= k_2 k_3 k_{-1}' k_4' [(k_{-3} + k_{-4})(k' + k_{-3}') + k k_{-3}'] \\
 \beta_{100} &= k_1 k_{-2} k_{-1}' k_{-2}' [(k_{-3} + k_{-4})(k' + k_{-3}' + k_{-4}') + k(k_{-3}' + k_{-4}')] + \\
 &\quad + k_{-1} k_4 k_{-1}' k_{-2}' [(k_{-3} + k_{-4})(k' + k_{-3}' + k_{-4}') + k(k_{-3}' + k_{-4}')] \\
 \beta_{101} &= k_{-1} k_4 k_{-1}' k_{-2}' [(k_{-3} + k_{-4})(k' + k_{-3}' + k_{-4}') + k(k_{-3}' + k_{-4}')] + \\
 &\quad + k_{-1} k_4 k_{-1}' k_4' [(k_{-3} + k_{-4})(k' + k_{-3}') + k k_{-3}'] + \\
 &\quad + k_1 k_{-2} k_{-1}' k_4' [(k_{-3} + k_{-4})(k' + k_{-3}') + k k_{-3}'] \\
 \beta_{102} &= k_{-1} k_4 k_{-1}' k_4' [(k_{-3} + k_{-4})(k' + k_{-3}') + k k_{-3}'] \\
 \beta_{110} &= (k_1 k_{-2} k_3 + k_{-1} k_2 k_4) [k_{-1}' k_{-2}' (k + k' + k_{-3}' + k_{-4}') + k(k_{-1}' k_{-4}' + k_{-2}' k_{-3}')] + \\
 &\quad + (k_1 k_3 k_{-4} + k_2 k_{-3} k_4) k_{-1}' k_{-2}' (k' + k_{-3}' + k_{-4}') + \\
 &\quad + k_3 k_4 k k_{-1}' k_{-2}' (k_{-3}' + k_{-4}') \\
 \beta_{111} &= (k_1 k_{-2} k_3 + k_{-1} k_2 k_4) [k_{-1}' k_4' (k + k' + k_{-3}') + k k_{-3}' k_4'] + \\
 &\quad + (k_1 k_3 k_{-4} + k_2 k_{-3} k_4) k_{-1}' k_4' (k' + k_{-3}') + \\
 &\quad + k_3 k_4 k [k_{-1}' k_{-2}' (k_{-3}' + k_{-4}') + k_{-1}' k_{-3}' k_4'] \\
 \beta_{112} &= k_3 k_4 k k_{-1}' k_{-3}' k_4' \\
 \beta_{120} &= k_2 k_3 k_4 [k_{-1}' k_{-2}' (k' + k_{-3}' + k_{-4}') + k(k_{-1}' k_{-2}' + k_{-1}' k_{-4}' + k_{-2}' k_{-3}')] \\
 \beta_{121} &= k_2 k_3 k_4 k_4' [k_{-1}' (k + k' + k_{-3}') + k k_{-3}'] \\
 \beta_{200} &= k_1 k_4 k_{-1}' k_{-2}' [(k_{-3} + k_{-4})(k' + k_{-3}' + k_{-4}') + k(k_{-3}' + k_{-4}')] \\
 \beta_{201} &= k_1 k_4 k_{-1}' k_4' [(k_{-3} + k_{-4})(k' + k_{-3}') + k k_{-3}'] \\
 \beta_{210} &= k_1 k_3 k_4 [k_{-1}' k_{-2}' (k' + k_{-3}' + k_{-4}') + k(k_{-1}' k_{-2}' + k_{-1}' k_{-4}' + k_{-2}' k_{-3}')] \\
 \beta_{211} &= k_1 k_3 k_4 k_4' [k_{-1}' (k + k' + k_{-3}') + k k_{-3}']
 \end{aligned}$$

β_{ijk} in these polynomials and velocity constants in Scheme 1 are listed in Table 2. Velocity constants are restricted by the thermodynamic identity

$$k_1 k_{-2} k_3 k_{-4} = k_{-1} k_2 k_{-3} k_4 \quad (2)$$

and using the notation

$$K_i = k_i/k_{-i} \quad (3)$$

the thermodynamic equilibrium constant K_{eq} for the reaction in Scheme 1 may be written ²

$$K_{\text{eq}} = K_1 K_3 k / K_1' K_3' k' \quad (4)$$

Eqn. (1) is, in general, non-linear with respect to z_1 and z_2 , but may under certain conditions become experimentally indistinguishable from a reciprocally bilinear relationship of the Dalziel type, which will always be approached asymptotically at low substrate concentrations:⁶

$$y_{\text{as}} = \Delta_0 \phi_0 + \Delta_1 \phi_1 z_1 + \Delta_2 \phi_2 z_2 + \Delta_{12} \phi_{12} z_1 z_2 \quad (5)$$

ϕ_i refer to the Dalziel coefficients obtained in absence of products, and Δ_i stand for the perturbation factors by which the Dalziel coefficients are modified in presence of S_1' .

According to the asymptote theory, Dalziel coefficients $\Delta_i \phi_i$ in eqn. (5) are related to coefficients in eqn. (1) through ⁵⁻⁷

$$\Delta_{12} \phi_{12} = \beta_{00} / \alpha_{11} \quad (6)$$

$$\Delta_1 \phi_1 = (\beta_{01} - \alpha_{12} \Delta_{12} \phi_{12}) / \alpha_{11} \quad (7)$$

$$\Delta_2 \phi_2 = (\beta_{10} - \alpha_{21} \Delta_{12} \phi_{12}) / \alpha_{11} \quad (8)$$

$$\Delta_0 \phi_0 = (\beta_{11} - \alpha_{21} \Delta_1 \phi_1 - \alpha_{12} \Delta_2 \phi_2) / \alpha_{11} \quad (9)$$

It follows from eqn. (6) and the relationships listed in Table 1 that

$$\Delta_{12} \phi_{12} = (\beta_{000} + \beta_{001}[S_1'] + \beta_{002}[S_1']^2) / (\alpha_{110} + \alpha_{111}[S_1']) \quad (10)$$

and it is, similarly, evident from Table 1 that the expressions on the right hand side of eqns. (7)–(9) represent non-linear functions of $[S_1']$. The presence of a product will thus, in general, affect Dalziel coefficients for the ternary-complex mechanism non-linearly, and eqns. (6)–(9) may be applied as soon as deviations from a linear dependence of Dalziel coefficients on $[S_1']$ are significant.

Asymptote relationships for the reaction in Scheme 1 in absence of S_1' product inhibition by S_2' are, due to the symmetry of the mechanism, entirely analogous to those given for inhibition by S_1' and will not be considered here.

General relationships between product-linear Dalziel coefficients and coefficients in eqn. (1). Product-inhibition data in ternary-complex mechanisms are usually evaluated in terms of theoretical or empirical relationships in which Dalziel coefficients are linearly dependent on the concentration of the inhibitory product, and the detailed analysis of eqns. (6)–(9) will be restricted to such product-linear cases. Rewriting eqn. (10) as

$$\begin{aligned} \Delta_{12}\phi_{12} = & \frac{\beta_{000}}{\alpha_{110}} + \frac{\alpha_{110}\beta_{001} - \alpha_{111}\beta_{000}}{\alpha_{110}^2} [S_1'] + \\ & + \frac{(\alpha_{110}^2\beta_{002} + \alpha_{111}^2\beta_{000} - \alpha_{110}\alpha_{111}\beta_{001})[S_1']^2}{\alpha_{110}^2(\alpha_{110} + \alpha_{111}[S_1'])} \end{aligned} \quad (11)$$

and observing that $\phi_{12} = \beta_{000}/\alpha_{110}$,⁵ it can be seen that deviations from the linear relationship

$$\Delta_{12}\phi_{12} = \phi_{12} + [S_1']\{\alpha_{110}\beta_{001} - \alpha_{111}\beta_{000}\}/\alpha_{110}^2 \quad (12)$$

are negligible at low concentrations of the product S_1' . It, similarly, follows from eqns. (7)–(9) and Table 1 that all of the Dalziel coefficients become approximately linear functions of $[S_1']$ at low product concentrations, and the following relationships are obtained:

$$\begin{aligned} \Delta_1\phi_1 = \phi_1 + [S_1']\{\alpha_{110}(\alpha_{110}\beta_{011} - \alpha_{111}\beta_{010} - \alpha_{120}\beta_{001} - \alpha_{121}\beta_{000}) + \\ + 2\alpha_{111}\alpha_{120}\beta_{000}\}/\alpha_{110}^3 \end{aligned} \quad (13)$$

$$\begin{aligned} \Delta_2\phi_2 = \phi_2 + [S_1']\{\alpha_{110}(\alpha_{110}\beta_{101} - \alpha_{111}\beta_{100} - \alpha_{210}\beta_{001} - \alpha_{211}\beta_{000}) + \\ + 2\alpha_{111}\alpha_{210}\beta_{000}\}/\alpha_{110}^3 \end{aligned} \quad (14)$$

$$\begin{aligned} \Delta_0\phi_0 = \phi_0 + [S_1']\{\alpha_{110}^3\beta_{111} - 6\alpha_{111}\alpha_{120}\alpha_{210}\beta_{000} - \\ - \alpha_{110}^2(\alpha_{111}\beta_{110} + \alpha_{120}\beta_{101} + \alpha_{121}\beta_{100} + \alpha_{210}\beta_{011} + \alpha_{211}\beta_{010}) + \\ + 2\alpha_{110}(\alpha_{111}\alpha_{120}\beta_{100} + \alpha_{111}\alpha_{210}\beta_{010} + \alpha_{120}\alpha_{210}\beta_{001} + \\ + \alpha_{120}\alpha_{211}\beta_{000} + \alpha_{121}\alpha_{210}\beta_{000})\}/\alpha_{110}^4 \end{aligned} \quad (15)$$

General relationships between product-linear Dalziel coefficients and velocity constants in Scheme 1. Inserting the expressions for α_{ijk} and β_{ijk} listed in Table 2, and using the identity given in eqn. (2), eqn. (12) may be written

$$\Delta_{12}\phi_{12} = \phi_{12} + [S_1'] \left\{ \frac{(A + R)K_1'}{K_1K_3} + \frac{k_4'k_{-4}'R'k'R'}{k_{-2}'K_1K_3k} \right\} \quad (16)$$

The quantities A , A' , R , and R' have been defined elsewhere,⁵ and are inter-related through

$$k(A + R) = k'(A' + R') \quad (17)$$

Hence eqn. (16) may be written

$$\Delta_{12}\phi_{12} = \phi_{12} + [S_1'] \left\{ \frac{A' + R'}{K_3'} + \frac{(k_{-4}'R')^2}{k_2'} \right\} / K_{eq} \quad (18)$$

which is identical to⁵

$$\Delta_{12}\phi_{12} = \phi_{12} + [S_1']\phi_2'/K_{eq} \quad (19)$$

It has previously been shown that $K_{eq} = \phi_{12}'/\phi_{12}$,⁵ and insertion of this into eqn. (19) yields

$$\Delta_{12}\phi_{12} = \phi_{12}(1 + [S_1']\phi_2'/\phi_{12}') \quad (20)$$

From a diagnostic point of view, different special cases of the general ternary-complex mechanism in Scheme 1 can be characterized by positive quantities p_1 , p_2 , p_1' , and p_2' which have been defined and discussed elsewhere.⁵ Introducing these quantities into eqn. (20), the perturbation factor Δ_{12} becomes

$$\Delta_{12} = 1 + K_1'[\text{S}_1'](1 + p_2') \quad (21)$$

A similar treatment of eqns. (13)–(14) yields

$$\Delta_1 = 1 + K_1'[\text{S}_1'](p_2' + p_1)/(1 + p_1) \quad (22)$$

$$\Delta_2 = 1 + K_1'[\text{S}_1'](p_2' + p_2)/(1 + p_2) \quad (23)$$

whence it follows that

$$1 \leq \Delta_1, \Delta_2 \leq \Delta_{12} \quad (24)$$

Examination of eqn. (15) under conditions where Dalziel kinetics prevail, *i.e.* where the difference $y - y_{\text{as}}$ defined by eqns. (1) and (5) is negligibly small in comparison to y , shows that the presence of S_1' will affect the zero-order Dalziel coefficient ϕ_0 only when $k_{-3}' \ll k_{-4}'$. In such cases eqn. (15) reduces to

$$\Delta_0 \phi_0 = \phi_0 + K_4'[\text{S}_1'](1 + k'/k + k_3'/k_1')/k_{-2}' \quad (25)$$

DISCUSSION

Basic restrictions for the asymptote relationships. The full rate-equation for the random-order ternary-complex mechanism in Scheme 1 is of the second degree with respect to both substrates and both products,² whereas kinetic data for ternary-complex systems usually are evaluated in terms of empirical first-degree relationships of the Dalziel type. This has previously made it difficult to correlate empirical data to kinetic parameters referring to Scheme 1, except in a number of special cases for which the theoretical rate-equation reduces to a first-degree relationship. The recently developed asymptote theory,^{5–7} however, provides a general method for relating parameters in empirical first-degree equations to coefficients in higher-degree theoretical rate-equations, and has been successfully applied to Scheme 1 for the derivation of generalized relationships for Dalziel coefficients obtained in the absence of products.⁵ The present investigation shows that empirical Dalziel coefficients obtained in the presence of one of the reaction products, similarly, can be related to velocity constants in Scheme 1 in a unique way through relationships which include any possible case of Dalziel rate-behaviour inherent in the mechanism. Conditions justifying reduction of a higher-degree theoretical rate-equation to an empirical Dalziel equation have previously been discussed in detail and will not be considered here.^{5,6}

Eqns. (6)–(9) imply that Dalziel coefficients, in general, are non-linear functions of the concentration of the product used as inhibitor, which is a consequence of the fact that the full rate-equation is of the second degree with respect to each product. Explicit general expressions for the non-linear de-

pendence of Dalziel coefficients on product concentrations can be derived from eqns. (6) – (9) using the relationships listed in Tables 1 and 2, but are too complex to be of any evident diagnostic value. The product-linear eqns. (21) – (25), on the other hand, provide a valuable basis for the interpretation of product-inhibition data in the usual experimental situation. Application of these equations is justified as soon as it has been experimentally established that Dalziel coefficients vary linearly with the concentration of the inhibitory product. They can also be applied in cases of product non-linearity, provided that perturbation factors A_1 are estimated from the initial slope in plots of Dalziel coefficients *versus* the product concentration.

Product-inhibition data for the forward reaction in Scheme 1 must, evidently, show some kind of correlation to initial-velocity data for the reverse reaction. Eqn. (20) gives a very obvious, previously not recognized, expression for such a correlation in stating that the proportionality factor for the effect of $[S_1']$ on $A_{12} - 1$ always equals the quotient ϕ_2'/ϕ_{12}' . This means that the magnitude of A_{12} (and hence according to eqn. (24) upper limits for the magnitude of A_1 and A_2) can be anticipated for any product concentration as soon as initial-velocity Dalziel coefficients for the reverse reaction have been determined. Since eqns. (20) and (24) are of general validity for product-linear Dalziel cases inherent in Scheme 1, they also provide useful criteria on the validity of the assumption of product-linearity, and on the consistence of product-inhibition data with Scheme 1.

Diagnostic value of the product-linear asymptote relationships. It has previously been shown for the mechanism in Scheme 1 that the information obtained on steady-state kinetic studies in absence of products primarily concerns the magnitude of the mechanism-characterizing p -values, which can be estimated from quotients of Dalziel coefficients by comparison with equilibrium constants for the combination of substrates and products to the enzyme.⁵ p_1 and p_2 (as well as p_1' and p_2' for the reverse reaction) may attain any positive value, although both cannot simultaneously be larger than unity. A mechanism of the rapid-equilibrium type corresponds to the ultimate case in which $p_1 = p_2 = 0$, compulsory-order mechanisms to the extremes $p_1 = 0$ and $p_2 \gg 1$ or *vice versa*, while a mixed type of mechanism is at hand when either p_1 or p_2 or both are in the order of unity.

The present investigation shows that product-inhibition studies in ternary-complex mechanisms primarily yield the same kind of information, and thus may be a valuable complement to initial-velocity studies for the purpose of establishing magnitudes of p -values and hence the type of the mechanism. The perturbation factor A_{12} is, according to eqn. (21), dependent exclusively upon the kinetic character of the product-side of the mechanism and may be used to estimate the magnitude of p_2' by a comparison with K_1' (p_1' may be estimated similarly from kinetic data obtained on product-inhibition by S_2'). A_1 and A_2 , on the other hand, are dependent upon the kinetic character of both the substrate-side (p_1, p_2) and the product-side (p_2') of the mechanism, as expressed by eqns. (22) and (23). The latter perturbation factors have previously been interpreted exclusively in terms of relationships prescribing that they equal either unity or A_{12} , but the present investigation shows that A_1 and A_2 may attain any value between unity and A_{12} . This means that product-

inhibition experiments in ternary-complex mechanisms preferably should be evaluated in terms of a complete Dalziel equation. Evaluation of effects of the inhibitory product on apparent K_m -values and maximum activities with respect to one substrate at a single fixed concentration of the other will be of less informative value, and may lead to the neglectance of kinetic terms which become of significant magnitude at higher concentrations of the fixed substrate.

As was mentioned above, eqns. (21)–(25) are of general validity within Scheme 1. Not only do they include the product-inhibition patterns previously described for symmetric rapid-equilibrium or compulsory-order cases inherent in Scheme 1, but they can also be used for evaluation of product-inhibition data in asymmetric mechanisms and in mechanisms of a mixed type on either or both sides of the ternary complexes. Product-inhibition data for ternary-complex systems should, therefore, always be interpreted in terms of the generalized eqns. (21)–(25), and any simplification of these relationships to those obtained for extreme cases, such as the symmetric compulsory-order mechanism, should be justified and supported by experimental evidence.

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REFERENCES

1. Alberty, R. A. *J. Am. Chem. Soc.* **75** (1953) 1928.
2. Dalziel, K. *Trans. Faraday Soc.* **54** (1958) 1247.
3. Alberty, R. A. *J. Am. Chem. Soc.* **80** (1958) 1777.
4. Pettersson, G. and Nylén, U. *Acta Chem. Scand.* **26** (1972) 420.
5. Pettersson, G. *Biochim. Biophys. Acta* **276** (1972) 1.
6. Pettersson, G. *Acta Chem. Scand.* **23** (1969) 2717.
7. Pettersson, G. *Acta Chem. Scand.* **24** (1970) 2171.
8. Schultz, A. R. and Fisher, D. D. *Can. J. Biochem.* **47** (1969) 889.

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