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# DALZIEL RATE BEHAVIOUR IN TERNARY-COMPLEX MECHANISMS FOR ENZYME REACTIONS INVOLVING TWO SUBSTRATES

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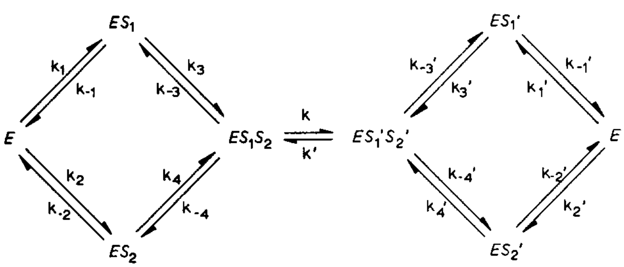
SUMMARY

Rapid-equilibrium and compulsory-order cases are not exclusive in giving rise to a reciprocally bilinear initial rate behaviour in the generalized ternary-complex mechanism for enzymatic two-substrate reactions shown in Scheme 1. General ex-




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.

pressions relating empirical Dalziel coefficients to velocity and equilibrium constants in Scheme 1 are derived and show that rapid-equilibrium and compulsory-order equations may be considered as opposite extremes of a general empirical Dalziel rate equation for the random-order ternary-complex mechanism. Kinetic characteristics of non-equilibrium ternary-complex mechanisms are described and discussed in view of the special case for which the second-degree rate equation corresponding to Scheme 1 becomes mathematically identical with a bilinear Dalziel equation.

Kinetic differences between the bilinear special cases inherent in Scheme 1 primarily concern the Dalziel coefficients $\varphi_1$ and $\varphi_2$. Empirical Dalziel coefficients obtained for enzymes operating by this mechanism should always be interpreted in view of the general Dalziel equation, and any reduction of this relationship to those previously derived for rapid-equilibrium and compulsory-order cases must be supported by experimental evidence. General criteria imposing previously not recognized restrictions on the quotients $\varphi_1/\varphi_{12}$ and $\varphi_2/\varphi_{12}$ in comparison to equilibrium constants for the

formation of binary enzyme–substrate complexes are derived and may be used for such diagnostic purposes. The Haldane relationship $\varphi'_{12}/\varphi_{12} = K_{eq}$ is of general validity within the ternary-complex mechanism and cannot be used to distinguish between different special cases.

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## INTRODUCTION

A large number of enzymes catalyzing reactions involving two substrates operate by mechanisms in which formation of a ternary complex between enzyme and the two substrates is an essential part of the catalytic process. The generalized random-order mechanism shown in Scheme 1 may be considered as fundamental for such enzymatic systems and has, for instance, been much discussed in relation to kinetic studies of a variety of metabolically central dehydrogenases[1].

Dehydrogenases usually give linear Lineweaver–Burk graphs with respect to each substrate at fixed concentrations of the other, and conform to a bilinear reciprocal rate equation of the Dalziel type[2]. The steady-state rate equation for the random-order mechanism in Scheme 1 includes second-degree terms in substrate concentrations and cannot, in general, be reciprocal linearized[3,4]. It has, however, been shown to reduce to a Dalziel rate equation under rapid-equilibrium conditions[3,5], or when one of the binary enzyme–substrate complexes is assumed to be non-existent (the compulsory-order case)[5], and interpretations of kinetic data for dehydrogenases and other enzymes operating by a ternary-complex mechanism have usually been carried out exclusively in view of the relationships derived for these special cases.

In the case of liver alcohol dehydrogenase (alcohol:NAD$^+$ oxidoreductase, EC 1.1.1.1) this has lead to a situation where initial rate studies indicate a strictly compulsory-order mechanism with one single pathway for ternary-complex formation[6,7], whereas isotope-equilibration data suggest the existence of a random-order mechanism in which one of the two alternate pathways carries most of the reaction flux[8]. As was pointed out by Cleland and Wratten[7], this apparent discrepancy might be due to the fact that the complex rate equation for the random-order ternary-complex mechanism has not been analyzed in a sufficiently general way; the rapid-equilibrium and compulsory-order cases have not been shown to be exclusive in giving rise to a reciprocally bilinear rate behaviour. Recent model studies, in fact, provided clear evidence that Scheme 1 includes non-equilibrium random-order cases for which the rate equation becomes identical with or experimentally indistinguishable from a bilinear relationship of the Dalziel type[9]. The corresponding Dalziel coefficients were for some models found to conform to previously derived relationships, but other models showed a rate behaviour which could not be interpreted in terms of rapid-equilibrium or compulsory-order rate equations.

A general analysis and kinetic characterization of the Dalziel rate behaviour inherent in the ternary-complex mechanism is evidently needed and of great interest in view of the biochemical importance of the mechanism. Such an analysis has now been carried out by examination of the asymptotic properties of the second-degree rate equation corresponding to Scheme 1.

## RESULTS

### *The asymptotic Dalziel equation for 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 used.

The steady-state reciprocal rate equation (reciprocal reaction velocity $y$ as a function of reciprocal substrate concentrations $z_i = 1/[S_i]$, $i = 1,2$) for the ternary-complex mechanism in Scheme 1 in absence of products $S'_i$ is given by [3,4]

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

where the coefficients $\alpha_{ij}$ and $\beta_{ij}$ are related to velocity constants in Scheme 1 as indicated in Table I. Velocity constants are restricted by the thermodynamic identity

$$k_1k_{-2}k_3k_{-4} = k_{-1}k_2k_{-3}k_4 \qquad (2)$$

TABLE I

RELATIONSHIPS BETWEEN COEFFICIENTS IN Eqn (1) AND VELOCITY CONSTANTS IN SCHEME 1

| | |
|---|---|
| $\alpha_{21}$ | $= k_1k_3k_4$ |
| $\alpha_{12}$ | $= k_2k_3k_4$ |
| $\alpha_{11}$ | $= k_1k_{-2}k_3+k_{-1}k_2k_4$ |
| $\beta_{21}$ | $= k_1k_3k_4(A+B)$ |
| $\beta_{20}$ | $= k_1k_4(1+k_{-3}A)$ |
| $\beta_{12}$ | $= k_2k_3k_4(A+B)$ |
| $\beta_{11}$ | $= k_3k_4+(k_1k_{-2}k_3+k_{-1}k_2k_4)\ (A+B) + (k_1k_3k_{-4}+k_2k_{-3}k_4)A$ |
| $\beta_{10}$ | $= k_{-1}k_4(1+k_{-3}A) + k_1k_{-2}\ (1+k_{-3}A+k_{-4}A)$ |
| $\beta_{02}$ | $= k_2k_3(1+k_{-4}A)$ |
| $\beta_{01}$ | $= k_{-1}k_2(1+k_{-3}A+k_{-4}A) + k_{-2}k_3(1+k_{-4}A)$ |
| $\beta_{00}$ | $= k_{-1}k_{-2}(1+k_{-3}A+k_{-4}A)$ |
| $A$ | $= (k'_{-3}+k'_{-4}+k')/k(k'_{-3}+k'_{-4})$ |
| $B$ | $= (k'_{-1}k'_{-2}+k'_{-2}k'_{-3}+k'_{-1}k'_{-4})/k'_{-1}k'_{-2}(k'_{-3}+k'_{-4})$ |

Eqn (1) is, in general, non-linear with respect to $z_i$, but approaches a linear asymptote at large values of $z_i$. It follows from the asymptotic properties of higher-degree rate equations that Eqn (1) becomes experimentally indistinguishable from the bilinear Dalziel equation

$$y_{as} = \varphi_0 + \varphi_1z_1 + \varphi_2z_2 + \varphi_{12}z_1z_2 \qquad (3)$$

when both $z_1$ and $z_2$ are sufficiently large[4,10], where

$$\varphi_{12} = \beta_{00}/\alpha_{11} \qquad (4)$$

$$\varphi_1 = (\beta_{01} - \alpha_{12}\varphi_{12})/\alpha_{11} \qquad (5)$$

$$\varphi_2 = (\beta_{10} - \alpha_{21}\varphi_{12})/\alpha_{11} \qquad (6)$$

$$\varphi_0 = (\beta_{11} - \alpha_{21}\varphi_1 - \alpha_{12}\varphi_2)/\alpha_{11} \qquad (7)$$

Inserting the expressions for $\alpha_{ij}$ and $\beta_{ij}$ given in Table I, and using the identity (2), Eqns (4)–(7) may be written

$$\varphi_{12} = \frac{A + R}{K_1K_3} \qquad (8)$$

$$\varphi_1 = \frac{A+R}{K_4} + \frac{(k_{-3}R)^2}{k_1} \tag{9}$$

$$\varphi_2 = \frac{A+R}{K_3} + \frac{(k_{-4}R)^2}{k_2} \tag{10}$$

$$\varphi_0 = A + B - \varepsilon \tag{11}$$

where

$$K_i = k_i/k_{-i} \tag{12}$$

$$R = 1/(k_{-3} + k_{-4}) \tag{13}$$

$$\varepsilon = k_{-3}k_{-4}R^2(\varepsilon_1/k_{-1} + \varepsilon_2/k_{-2}) \tag{14}$$

$$\varepsilon_1 = 1 - k_{-4}Rk_3/k_2 \tag{15}$$

$$\varepsilon_2 = 1 - k_{-3}Rk_4/k_1 \tag{16}$$

$A$ and $B$ are defined in Table I; it may be observed that

$$k(A+R) = 1 + kR + k'R' = k'(A'+R') \tag{17}$$

It has previously been shown that the asymptote relationships (4)–(7) include any exact or approximate bilinear rate behaviour inherent in the ternary-complex mechanism shown in Scheme 1[4]. Eqns (8)–(17) may, therefore, be used for further characterization of the general kinetic properties of the mechanism.

*Exact Dalziel rate behaviour in the ternary-complex mechanism*

It follows from Eqns (1) and (3)–(7) that

$$y - y_{as} = \frac{(\beta_{02} - \alpha_{12}\varphi_1)z_1^2 + (\beta_{20} - \alpha_{21}\varphi_2)z_2^2 + (\beta_{12} - \alpha_{12}\varphi_0)z_1 + (\beta_{21} - \alpha_{21}\varphi_0)z_2}{\alpha_{11}z_1z_2 + \alpha_{12}z_1 + \alpha_{21}z_2} \tag{18}$$

which according to Eqns (8)–(16) and the relationships listed in Table I may be rewritten as

$$y - y_{as} = \frac{\varepsilon_1 k_1 k_4 k_{-4} R z_2^2 + \varepsilon_2 k_2 k_3 k_{-3} R z_1^2 + \varepsilon(\alpha_{12}z_1 + \alpha_{21}z_2)}{\alpha_{11}z_1z_2 + \alpha_{12}z_1 + \alpha_{21}z_2} \tag{19}$$

Eqn (19) shows that $y$ equals $y_{as}$ independently of the concentration variables $z_i$ when and only when $\varepsilon_1 = \varepsilon_2 = 0$ (whence $\varepsilon = 0$), which according to Eqns (2), (15) and (16) is equivalent to putting

$$k_4 = k_1(1 + k_{-4}/k_{-3})\,;\; k_2 = k_{-4}k_3/(k_{-3}+k_{-4})\,;\; k_{-2} = k_{-1} \tag{20}$$

This is the only condition under which the second-degree rate equation for the mechanism in Scheme 1 becomes mathematically identical with a Dalziel equation and will be referred to as the "identity case". The corresponding coefficient relationships can be readily derived from Eqns (8)–(11) and are (*cf.* Table II where they are given in a more condensed form):

$$\varphi_{12} = (1 + (k_{-3}+k_{-4})A)/K_1K_3(k_{-3}+k_{-4}) \tag{21}$$

$$\varphi_1 = (1 + k_{-4}A)/k_1(1 + k_{-4}/k_{-3}) \tag{22}$$

$$\varphi_2 = (1 + k_{-3}A)/k_3 \tag{23}$$

$$\varphi_0 = A + B \tag{24}$$

TABLE II

RELATIONSHIPS BETWEEN VELOCITY CONSTANTS AND DALZIEL COEFFICIENTS FOR DIFFERENT CASES INHERENT IN THE TERNARY-COMPLEX MECHANISM SHOWN IN SCHEME 1

$A$ and $B$ are defined in Table I, and $R$ equals $1/(k_{-3}+k_{-4})$. Relationships for the Dalziel rapid-equilibrium and the compulsory-order cases have been derived previously[3,5]. In the compulsory-order case $k_{-4} = k'_{-4} = 0$ in $A$, $B$ and $R$, and in the identity case velocity constants are restricted by condition (20). $K_i$ stands for $k_i/k_{-i}$.

| *Coefficient* | *Rapid equilibrium* | *Compulsory order* | *General asymptote* | *Identity* |
|---|---|---|---|---|
| $\varphi_0$ | $A+B$ | $A+B$ | $A+B$ | $A+B$ |
| $\varphi_{12}$ | $\frac{A+R}{K_1K_3}$ | $\frac{A+R}{K_1K_3}$ | $\frac{A+R}{K_1K_3}$ | $\frac{A+R}{K_1K_3}$ |
| $\varphi_1$ | $\frac{A+R}{K_4}$ | $\frac{1}{k_1}$ | $\frac{A+R}{K_4}+\frac{(k_{-3}R)^2}{k_1}$ | $\frac{1+k_{-4}A}{k_4}$ |
| $\varphi_2$ | $\frac{A+R}{K_3}$ | $\frac{A+R}{K_3}$ | $\frac{A+R}{K_3}+\frac{(k_{-4}R)^2}{k_2}$ | $\frac{1+k_{-3}A}{k_3}$ |

*Approximate Dalziel rate behaviour in the ternary-complex mechanism*

Eqns (21)–(24) are mainly of theoretical interest as they refer to the restrictive condition (20) under which the second-degree rate equation (1) becomes mathematically identical with its bilinear asymptote. From a practical point of view, however, evaluation of kinetic data for the ternary-complex mechanism in terms of a Dalziel equation is justified as soon as the difference $y - y_{as}$, in view of the actual experimental precision, is insignificantly small in comparison to $y$.

For small values of $z_1$ and $z_2$ Eqns (3) and (19) reduce to, respectively,

$$y_{as} = \varphi_0 \tag{25}$$

$$y - y_{as} = \varepsilon \tag{26}$$

Hence, and from Eqn (11), it can be concluded that approximation of $y$ by $y_{as}$ at substrate concentrations where $\varphi_0$ contributes significantly to the reaction velocity only is justified when

$$\varepsilon \approx y - y_{as} \ll y \approx y_{as} \approx \varphi_0 = A + B - \varepsilon \tag{27}$$

which shows that $\varepsilon$ in such cases cannot be of significant magnitude in comparison to $A + B$.

Empirical $\varphi_0$-values for enzymes operating by the ternary-complex mechanism in Scheme 1 under conditions where approximate bilinear Dalziel kinetics prevail may, consequently, always be interpreted using the simplified Eqn (25) obtained in the identity case. Other Dalziel coefficients should be interpreted in view of the general asymptote relationships expressed by Eqns (8)–(10).

*General diagnostic relationships for the ternary-complex mechanism*

The thermodynamic equilibrium constant $K_{eq}$ for the reaction shown in Scheme 1 is given by[3]

$$K_{eq} = \frac{K_1K_3k}{K_1'K_3'k'} \tag{28}$$

According to Eqn (8) this may be written

$$K_{eq} = \frac{\varphi'_{12}k(A + R)}{\varphi_{12}k'(A' + R')} \tag{29}$$

and it follows from Eqn (17) that the Haldane relationship

$$\varphi'_{12}/\varphi_{12} = K_{eq} \tag{30}$$

must be fulfilled for any reciprocally bilinear special case inherent in the ternary-complex mechanism.

In Eqns (9)–(10) $\varphi_1$ and $\varphi_2$ have been written as the sum of two terms (the quadric term of $R$ will be referred to as the $p$-term). Introducing quantities $p_i \geqslant 0$ describing the relative magnitude of these terms

$$p_1 = K_4(k_{-3}R)^2/k_1(A+R) \tag{31}$$

$$p_2 = K_3(k_{-4}R)^2/k_2(A+B) \tag{32}$$

Eqns (9)–(10) may be written

$$\varphi_1 = (A+R)\ (1+p_1)/K_4 \tag{33}$$

$$\varphi_2 = (A+R)\ (1+p_2)/K_3 \tag{34}$$

Inspection of Table II shows that kinetic differences between previously described special cases of the ternary-complex mechanism mainly concern the magnitude of $p_1$ and $p_2$. In rapid-equilibrium cases we have $p_1 = p_2 = 0$ (both $p$-terms vanish), whereas the compulsory-order case in which, for instance, $ES_2$ is assumed to be non-existent is characterized by $p_2 = 0$ and $p_1 \gg 1$ (the $p$-term vanishes in $\varphi_2$ but dominates in $\varphi_1$). In the general case $p_1$ and $p_2$ may attain any positive value, including unity, which means that $p$-terms must not necessarily dominate or vanish. This can easily be shown by examination of the identity case. Condition (20) implies that Eqns (31)–(32) reduce to

$$p_1 = k_{-3}/k_{-4}\ (1+A/R) \tag{35}$$

$$p_2 = k_{-4}/k_{-3}\ (1+A/R) \tag{36}$$

but does not put any restriction to the magnitudes of $A$ and the quotient $k_{-3}/k_{-4}$. The relative magnitudes of $p_1$ and $p_2$ are, however, restricted by

$$p_2/p_1 = (k_{-4}/k_{-3})^2 \tag{37}$$

$$p_1p_2 = (1 + A/R)^{-2} < 1 \tag{38}$$

which means that $p_1$ and $p_2$ cannot simultaneously be larger than unity.

It follows from Eqns (2), (8), and (33)–(34) that Dalziel coefficients in the ternary-complex mechanism always fulfil the criteria

$$\varphi_1/\varphi_{12} = K_2(1+p_1) \geqslant K_2 \tag{39}$$

$$\varphi_2/\varphi_{12} = K_1(1+p_2) \geqslant K_1 \tag{40}$$

The latter relationships impose previously unrecognized restrictions on the quotients $\varphi_1/\varphi_{12}$ and $\varphi_2/\varphi_{12}$ and are of great diagnostic value in permitting experimental estimates of $p_1$ and $p_2$ to be obtained from a comparison between kinetic data and physical determinations of equilibrium constants for the formation of binary enzyme–substrate complexes.

DISCUSSION

*Reduction of the second-degree Eqn (1) to a Dalziel rate equation*

The question under what conditions the second-degree rate equation for the random-order ternary-complex mechanism may reduce to a bilinear relationship of the Dalziel type has been much discussed[3,6,11–16]. It was early recognized that rapid-equilibrium treatment of the mechanism in Scheme 1 by the Michaelis–Menten method, claimed to be justified when interconversion of ternary complexes is slow in comparison to other reaction steps, directly yields a bilinear reciprocal rate equation[16]. Dalziel later showed[3] that the condition

$$z_1 \gg k_4/k_{-2};\ z_2 \gg k_3/k_{-1} \tag{41}$$

which only implies that binary complexes are in rapid equilibrium with free enzyme and substrates, is sufficient for reduction of Eqn (1) to a Dalziel relationship. The existence of an identity case has, further, been recognized previously[4,13,15], but was considered to be of little interest in view of the restrictions it imposes on velocity constants in Scheme 1.

It was recently established that higher-degree reciprocal rate equations always become asymptotically linear at low substrate concentrations[10], and the Dalziel equilibrium condition (41) can be considered to express the concentration limits below which curvature in Eqn (1) is negligible irrespective of its magnitude at higher substrate concentrations. Curvature in Eqn (1) is, however, not dependent exclusively upon concentration variables, but may for various combinations of values of velocity constants in Scheme 1 be negligible also at substrate concentrations much higher than those for which Dalziel equilibrium conditions prevail[4,9]. The present investigation, in fact, shows that a theoretical rate equation of the Dalziel type only can be obtained under non-equilibrium conditions; according to Eqns (21)–(23), which were derived for the identity case, we have

$$\frac{k_{-1}z_2}{k_3} = \frac{\varphi_{12}z_2}{\varphi_1}\cdot\frac{1 + ck_{-3}A}{1 + (1 + c)k_{-3}A} < \frac{\varphi_{12}z_1z_2}{\varphi_1 z_1} \tag{42}$$

$$\frac{k_{-2}z_1}{k_4} = \frac{\varphi_{12}z_1}{\varphi_2}\cdot\frac{1 + k_{-3}A}{1 + (1 + c)k_{-3}A} < \frac{\varphi_{12}z_1z_2}{\varphi_2 z_2} \tag{43}$$

which means that Dalziel equilibrium conditions (41) cannot be fulfilled in the identity case unless $\varphi_1 z_1$ and $\varphi_2 z_2$ are assumed to be of negligible magnitute in comparison to $\varphi_{12}z_1z_2$ and hence to vanish in the Dalziel equation (3).

Empirical bilinearity in Eqn (1) may thus be a joint effect of two cooperative factors, low substrate concentrations and closeness to the identity case, which only in extreme situations can be separated and related to conditions (41) and (20), respectively. This makes it difficult to state any explicite conditions under which reduction of Eqn (1) to Eqn (3) is justified. The difficulty is further accentuated by the fact that statistical factors such as the experimental precision, the number of observations, and the concentration ranges these observations cover will have a main influence on whether curvature in reciprocal rate plots can be experimentally detected or not. Eqn (19), which gives an exact expression for the difference between Eqn (1) and its bilinear asymptote, is of interest for theoretical considerations but of minor value in the usual experimental situation where most of the parameters in the expression are of unknown magnitude.

From a practical point of view it may suffice to conclude that Eqn (1) can become experimentally indistinguishable from a Dalziel equation under a variety of conditions and, consequently, that coefficients in empirically established Dalziel equations for the ternary-complex mechanism always have to be interpreted in terms of the general asymptote relationships (8)–(10) and (24).

*Kinetic characteristics of the identity case*

Since deviations of Eqn (1) from its linear asymptote in reciprocal rate plots steadily increase with increasing substrate concentrations[4], a bilinear reciprocal rate behaviour in non-equilibrium cases must be due to some degree of closeness to the identity case. The kinetic properties of non-equilibrium cases may, therefore, be illustrated by a discussion of the rate-behaviour inherent in the identity case.

Under condition (20) Dalziel coefficients are given by Eqns (21)–(24) and the relative magnitude of $p$-terms in $\varphi_1$ and $\varphi_2$ by Eqns (35)–(38). When $k_{-3} \approx k_{-4}$, $p_1$ and $p_2$ are of similar magnitude and may vary from zero to unity depending on the magnitude of the quotient $A/R$. If $A \gg R$ a rapid-equilibrium type of rate equation ($p_1 = p_2 = 0$) is obtained (even though the Dalziel equilibrium condition is not fulfilled), otherwise $p_1$ and $p_2$ are of significant magnitude in comparison to unity and the corresponding Dalziel equation will neither be of the rapid-equilibrium nor of the compulsory-order type (*cf.* ref. 9). It may be observed that $A \gg R$ is equivalent to $1 + k'R' \gg kR$, which is valid when $k \ll k_{-3} + k_{-4}$ (slow isomerization of the ternary enzyme–substrate complex in comparison to its dissociation into binary complexes), but also when $k'R' \gg kR$. The appearance of an equilibrium type of rate equation cannot, therefore, be unequivocally related to any rapid-equilibrium conditions whatsoever.

When $k_{-3} \gg k_{-4}$ we have $p_2 \approx 0$, whereas the magnitude of $p_1$ is unrestricted. For $A/R \ll k_{-3}/k_{-4}$ a compulsory-order type of rate equation will be obtained ($p_1 \gg 1$), and for $A/R \gg k_{-3}/k_{-4}$ the rate equation will be of the equilibrium type ($p_1 \approx 0$). If $A/R$ and $k_{-3}/k_{-4}$ are of comparable magnitude $p_1$ will be in the order of unity, and the rate behaviour cannot be reliably interpreted in terms of rapid-equilibrium or compulsory-order equations.

It, finally, follows from the symmetry of the mechanism in Scheme 1 that the case $k_{-3} \ll k_{-4}$ is exactly analogous to the case $k_{-3} \gg k_{-4}$.

*Diagnostic interpretations of empirical Dalziel coefficients*

The asymptote relationships are of great diagnostic value, since they include any exact or approximate bilinear rate behaviour inherent in the ternary-complex mechanism in Scheme 1. This can be seen from Table II, where the coefficient relationships deliberately have been expressed in a form which emphasizes that the kinetic characteristics for different cases mainly concern the coefficients $\varphi_1$ and $\varphi_2$. Diagnostic interpretations of $\varphi_0$ will be essentially the same for all ternary-complex mechanisms adhering to Scheme 1, and have previously been discussed in detail for the rapid-equilibrium case[3]. Similarly, the Haldane relationship (30), which is based exclusively on $\varphi_{12}$-coefficients, is of general validity and cannot be used for discrimination between the different cases inherent in Scheme 1.

As was mentioned above, differences in the interpretation of $\varphi_1$ and $\varphi_2$ for the special cases listed in Table II can be related to magnitudes of the $p$-terms. Evaluation of initial rate data for ternary-complex mechanisms has previously been carried out exclusively in view of relationships where $p$-terms either vanish or completely dominate. The present investigation shows, however, that the equilibrium ($p_1 = p_2 = 0$) and compulsory-order ($p_1 \gg 1$, $p_2 = 0$ or *vice versa*) equations can be regarded as opposite extremes of the general rate equation in which $p_1$ and $p_2$ may attain any positive value including unity, the only restriction being that both p-terms cannot dominate simultaneously. Any reduction of the general expressions for $\varphi_1$ and $\varphi_2$ to those obtained for the extreme cases should, therefore, be justified and supported by experimental determinations of $p_1$ and $p_2$. Estimates of these quantities can be obtained using the relationships (39)–(40), and determinations of stability constants for the binary enzyme–substrate complexes will usually be required as complementary information for the interpretation of initial rate data.

Determination of $p_1$ and $p_2$ will directly establish whether an empirical Dalziel equation is of the rapid-equilibrium, compulsory-order, or mixed type, but such a qualitative characterization of the reaction is of limited diagnostic value. An equilibrium type of rate equation may be obtained also under non-equilibrium conditions, and an effectively ordered reaction can give rise to a rate equation of any of the above three types[4,9]. The information obtained from empirical Dalziel equations is primarily related to the magnitude of $p_1$ and $p_2$ as expressed by Eqns (31)–(32).

Rewriting Eqns (31) and (32) as

$$p_1 = (k_4/k_1)\ (1+k_{-4}/k_{-3})^{-2}(k/k_{-4})\ (1+k/(k_{-3}+k_{-4}) + k'/(k'_{-3}+k'_{-4}))^{-1} \tag{44}$$

$$p_2 = (k_3/k_2)\ (1+k_{-3}/k_{-4})^{-2}(k/k_{-3})\ (1+k/(k_{-3}+k_{-4}) + k'/(k'_{-3}+k'_{-4}))^{-1} \tag{45}$$

it can be seen that the quotients $k_4/k_1$ and $k_3/k_2$ (which deviate from unity when the two substrates bind in a cooperative manner), as well as the relative magnitudes of $k_{-3}$, $k_{-4}$, and k, have a main and predictable influence on $p_1$ and $p_2$; the direction in which these quotients affect $p$-terms is evident from the above expressions and will not be discussed. From a diagnostic point of view, however, the situation is less clear as one observes a joint effect of the above quotients. For example, $k_4 > k_1$ or $k_{-3} > k_{-4}$ which tend to make $p_1$ large can be opposed by $k \ll k_{-4}$ to give $p_1 \approx 0$. A considerable amount of complementary information will usually be required for an explicite and detailed interpretation of empirical $p_i$-values.

On the other hand, as soon as it has been experimentally established that $p_i \approx 0$ the corresponding Dalziel coefficient $\varphi_i$ may be reliably interpreted in view of the relationships previously derived for the rapid-equilibrium case. Similarly, $p_i \gg 1$ implies that $\varphi_i$ may be put equal to the corresponding $p$-term; this and other characteristics of the compulsory-order case will be further discussed below.

*Compulsory-order and Theorell–Chance equations*

Reaction flows through the two alternate pathways in the random-order mechanism in Scheme 1 are under some conditions sufficiently different to make the reaction effectively ordered. An effectively ordered ternary-complex mechanism does not necessarily obey compulsory-order kinetics[4,9] and should be clearly distinguished from a compulsory-order mechanism which must be considered as an unrealistic case of the general ternary-complex mechanism in requiring the assumption that some binary complexes in Scheme 1 are non-existent[5]. The results of the present investigation show that a compulsory-order type of rate behaviour is inherent in the random-order mechanism as an ultimate extreme (which may be approached gradually) of the general empirical Dalziel rate behaviour. The compulsory-order rate equation is obtained when the ratio $k_{-4}/k_{-3}$ approaches zero in Eqns (21)–(23) referring to the restrictive identity case (which, consequently, is more general than the compulsory-order case), and can be formally derived by putting $k_{-4} = 0$ in the general asymptote Eqns (8)–(10) and (24).

A compulsory-order type of rate equation can only be obtained under non-equilibrium conditions (equilibrium assumptions imply that $p_1 = p_2 = 0$), and the identity case may be used as an illustrative example to show that $k_{-4} \ll k_{-3},k$ is a necessary condition for making the $p$-term in $\varphi_1$ dominant. Since $k_{-4} \ll k_{-3}$ implies that $k_{-3}R \approx 1$ it also follows that $\varphi_1 \approx 1/k_1$. In other words, if it has been experimentally established that the empirical Dalziel equation for an enzyme operating by the mechanism in Scheme 1 is of the compulsory-order type ($\varphi_1/\varphi_{12} \gg K_2$ and $\varphi_2/\varphi_{12} \approx K_1$), one may conclude that $k_{-4}$ is negligibly small in comparison to both $k_{-3}$ and $k$, and that $\varphi_1$ equals $1/k_1$. It can, further, be shown that the reaction under these conditions must be effectively ordered; otherwise the difference $y - y_{as}$ cannot be of insignificant magnitude and the enzyme will exhibit non-linear Lineweaver–Burk plots.

What has been stated above is also valid for a Dalziel equation of the Theorell–Chance[17] type, which is known to be a special case of the compulsory-order equation[11]. The rate behaviour characteristic of the Theorell–Chance mechanism is thus inherent in the random-order ternary-complex mechanism, but is only exhibited in non-equilibrium cases and when the $p$-term in $\varphi_1$ or $\varphi_2$ dominates and the reaction, consequently, is effectively ordered.

For these reasons, it may be concluded that the initial rate behaviour of liver alcohol dehydrogenase (see Introduction) and other enzymes conforming to compulsory-order equations is fully consistent with a random-order mechanism in which one of the alternate pathways carries most of the reaction flow; it is not necessary to introduce the assumption that one binary enzyme–susbstrate complex is non-existent. Whether or not product-inhibition data for such enzymes are consistent with a non-equilibrium random-order mechanism remains to be shown.

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