ACTA CHEMICA SCANDINAVICA 23 (1969) 3243–3247

Relationships between Rapid Equilibrium Conditions and Linearization of the Reciprocal Rate Equation for the Sequential Random Two-substrate Enzyme Mechanism

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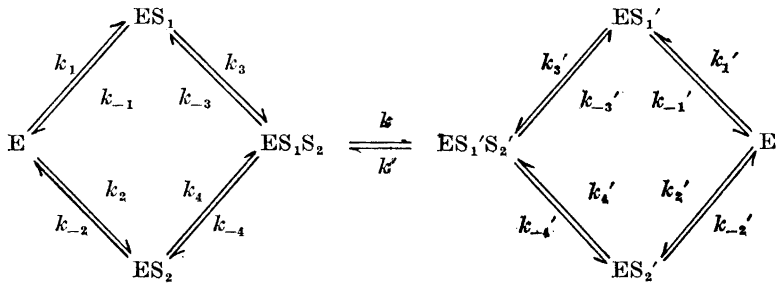
Functions describing steady-state deviations from equilibrium conditions at different reaction steps in the sequential random two-substrate mechanism [scheme (1) below] have been derived and used to show that linearization of the reciprocal rate equation for this mechanism cannot be generally related to whether rapid equilibrium conditions prevail or not. It is pointed out that the rapid equilibrium (Michaelis-Menten) method for derivation of rate equations may fail to predict the rate behaviour of more complicated enzyme systems, also when Michaelis-Menten conditions prevail.

The sequential random two-substrate mechanism [scheme (1)] is of great importance in enzyme kinetics and has been discussed by several authors. Using the terminology of Wong and Hanes,[1] this mechanism is of the second degree with respect to both substrates, *i.e.* steady-state reciprocal rate equations (reciprocal rate $1/v$ as a function of $1/[S_1]$ or $1/[S_2]$) are, in general, non-linear.



Linear relationships are obtained asymptotically at "comparatively low" substrate concentrations,[2] when the full steady-state rate equation reduces to a Dalziel type of equation:[3]

$$1/v = \varphi_0 + \varphi_1/[S_1] + \varphi_2/[S_2] + \varphi_{12}/[S_1][S_2] \quad (2)$$

Application of the rapid equilibrium (Michaelis-Menten) method for derivation of rate equations to mechanism (1) directly gives a relationship of the Dalziel type, and has been claimed to be justified in two different situations:

A. Substrate concentrations are sufficiently low to ensure that reaction steps 1 and 2 are virtually at equilibrium.[4]

B. The breakdown of the ternary complex ES_1S_2 is "rate-limiting", "kinetically significant", or sufficiently slow to ensure that substrates remain in equilibrium with binary complexes and/or the ternary complex.[5,6]

The purpose of the present investigation is to determine whether linearization of the full steady-state reciprocal rate equation for mechanism (1) can be reliably related to such rapid equilibrium conditions.

THEORETICAL

The equilibrium equation for reaction step 1 in mechanism (1) is given by

$$\frac{[E]_{eq}[S_1]_{eq}}{[ES_1]_{eq}} = K_1 = \frac{k_{-1}}{k_1} \quad (3)$$

and as a measure of the relative deviation from equilibrium conditions at this reaction step in the steady state we introduce the quantity D_1 defined by

$$D_1 = \frac{([E][S_1]/[ES_1]) - K_1}{K_1} \quad (4)$$

which may be recast as

$$D_1 = \frac{k_1[E][S_1]}{k_{-1}[ES_1]} - 1 \quad (5)$$

D_1 obviously approaches zero when steady-state concentrations of E, S_1, and ES_1 approach equilibrium concentrations. We may, similarly, define functions D_2—D_4 describing deviations from equilibrium conditions at reaction steps 2—4:

$$D_2 = \frac{k_2[E][S_2]}{k_{-2}[ES_2]} - 1 \quad (6)$$

$$D_3 = \frac{k_3[ES_1][S_2]}{k_{-3}[ES_1S_2]} - 1 \quad (7)$$

$$D_4 = \frac{k_4[ES_2][S_1]}{k_{-4}[ES_1S_2]} - 1 \quad (8)$$

Expressions for steady-state concentrations of free enzyme and of enzyme-substrate complexes in absence of products S_1' and S_2' (we will only consider rate equations for the forward reaction in mechanism (1) and in absence of products, as complementary relationships for the reverse reaction may be

obtained by insertion or deletion of primes on rate constants and reactants) can easily be calculated by the usual determinant method,[1] and substitution of these expressions into eqns. (5)—(8) yields

$$D_1 = \frac{k_3[S_2]}{k_{-1}} \frac{D_3}{1+D_3} \tag{9}$$

$$D_2 = \frac{k_4[S_1]}{k_{-2}} \frac{D_4}{1+D_4} \tag{10}$$

$$D_3 = \frac{C(1+k_3[S_1]/k_{-2})}{k_{-3}(1+k_4[S_1]/k_{-2})+k_{-4}(1+k_3[S_2]/k_{-1})} \tag{11}$$

$$D_4 = \frac{C(1+k_3[S_2]/k_{-1})}{k_{-3}(1+k_4[S_1]/k_{-2})+k_{-4}(1+k_3[S_2]/k_{-1})} \tag{12}$$

where

$$C = k(k_{-3}'+k_{-4}')/k' \tag{13}$$

Conditions claimed to justify application of rapid equilibrium treatment of mechanism (1) (see introduction) may be explicitly expressed as [4,5]

$$\text{A. } [S_1] \ll k_{-2}/k_4 \text{ and } [S_2] \ll k_{-1}/k_3 \tag{14}$$

$$\text{B. } C/k_{-3} \ll 1 \text{ and } C/k_{-4} \ll 1 \tag{15}$$

Condition (14) has been shown to be sufficient for linearization [4] of the full steady-state rate equation for mechanism (1) and, according to eqns. (9) and (10), also implies that $D_1 \approx 0$ and $D_2 \approx 0$. Condition (14) is, however, not equivalent to the assumption that reaction steps 1 and 2 are virtually at equilibrium, as was stated by Dalziel;[4] inspection of eqns. (9) and (10) shows that D_1 and D_2 may be approximately equal to zero also when substrate concentrations are higher than stated in condition (14), provided only that D_3 and D_4 are sufficiently small. Consequently, the condition $D_1 \approx 0$ and $D_2 \approx 0$ does not imply linearization of the reciprocal rate equation unless it can be shown that the condition $D_3 \approx 0$ and $D_4 \approx 0$ does so.

We will therefore, now examine eqns. (11) and (12). It can be seen that $D_3 \approx 0$ and $D_4 \approx 0$ follows from condition (15); also D_1 and D_2 will, in general become very small. Condition (15) does not, however, impose any evident restrictions on the curvature of the reciprocal rate equation; the curvature of $y = 1/v$ as a function of $x = 1/[S_1]$ (or $1/[S_2]$) is proportional to d^2y/dx^2, which has been shown to be independent of C, k_{-3}, and k_{-4}.[2]

Let us, for example, assume that condition (15) requires that $C/k_{-3} \leq 10^{-4}$ and $C/k_{-4} \leq 10^{-4}$, which implies that deviations from equilibrium conditions at reaction steps 3 and 4 are less than 0.01 % (if not substrate concentrations are extremely high, also reaction steps 1 and 2 will be virtually at equilibrium). Condition (15) is thus fulfilled when $k_{-3}/C = 10^5$, but the rate equation may still be of the non-linear type IIIb (exhibition of a minimum value) as the only requirement for exhibition of type IIIb kinetics is that k_2 is sufficiently large in comparison to k_3;[2] in this case a sufficient condition for IIIb kinetics

is that $k_2/k_3 > 1 + 10^5 + k_{-3}/k$ when $k_4 \geq k_1$ and $k_2/k_3 > (1 + 10^5 + k_{-3}/k)k_1/k_4$ when $k_4 < k_1$.

The conclusion must be that the reciprocal rate equation for mechanism (1) may be non-linear (*e.g.* of type IIIb) under condition (14), *i.e.* when reaction steps 3 and 4, and hence probably also steps 1 and 2, are virtually at equilibrium.

On the other hand, reciprocal rate plots for mechanism (1) may be approximately linear (the difference between the rate function and its linear asymptote being negligible [2]) when deviations from rapid equilibrium conditions are most significant. This can be illustrated by examination of a simple model, obtained by putting all rate constants equal to unity with exception for $k_{-1} = k_{-2} = k_{-3} = k_{-4} = 10^{-6}$. Varying $[S_1]$ from 0.1 to 0.001 at constant $[S_2] = 1$ (rate constants and concentrations have not been given any dimensions, but any consistent set of dimensions would be acceptable) we get $D_1 \approx D_3 \approx D_4 \approx 10^6$, while D_2 varies from about 10^3 to 10^5 [see eqns. (9)—(13)]. Deviations from equilibrium conditions are thus very extreme at reaction steps 1—4. Nevertheless, reciprocal rate plots calculated using the full steady-state rate equation [2,4] will appear linear, as shown in Fig. 1.

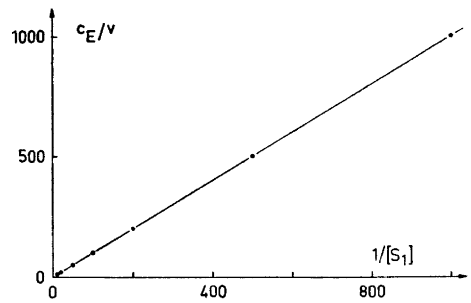


Fig. 1. Steady-state reciprocal rate plot for the model described in the text. c_E stands for the total concentration of enzyme.

DISCUSSION

The results presented in the theoretical section seem to establish that linearization of the steady-state reciprocal rate equation for mechanism (1) cannot be generally related to any rapid equilibrium conditions. The reciprocal rate function may show most significant non-linearity, such as exhibition of a minimum, also when all of the reaction steps involving substrates are virtually at equilibrium. Linearization of the rate equation in the mathematical sense can only be related to the magnitude of substrate concentrations, as has been discussed in detail elsewhere,[2] and reciprocal rate plots may be asymptotically linear at "comparatively low" substrate concentrations independently of whether rapid equilibrium conditions prevail or not (Fig. 1).

It is of particular interest to note that the Dalziel [4] condition (14) is sufficient, but not necessary, for reduction of the full steady-state rate equation to the linear Dalziel relationship (2). Asymptotically linear reciprocal rate plots may be obtained at much higher substrate concentration than stated in condition (14). The model described above exhibits linear kinetics at the sub-

strate concentrations indicated in Fig. 1, even though $[S_2] \gg k_{-1}/k_3 = 10^{-6}$ and $[S_1] \gg k_{-2}/k_4 = 10^{-6}$. A consequence of this fact is that expressions for the different coefficients φ in eqn. (2) which have been derived under the Dalziel assumption (14) are less general than those derived under the "low concentration" assumption recently described.[2] The latter rate expressions, which include rapid equilibrium equations as special cases, should preferably be used for interpretation of the kinetics of enzyme systems operating by mechanism (1) and conforming to a Dalziel type of rate equation.

The rapid equilibrium (Michaelis-Menten) method for derivation of enzyme kinetic rate equations is open to serious criticisms of several kinds.[7] Nevertheless, it is frequently used for analysis of more complicated reaction mechanisms such as (1),[8] where a steady-state treatment by the method of Briggs and Haldane becomes very laborious.[1] The rapid equilibrium *assumption* is logically contradictory, as no reaction can take place when reactants are at equilibrium, and is only meaningful when being considered as an *approximation* which may be of possible value for expression of relationships between concentrations of reactants when these approach equilibrium concentrations. The present investigation shows that errors introduced into the rate equation for mechanism (1) (neglection of non-linearity) by application of rapid equilibrium approximations do not necessarily become small in a mathematical sense when deviations from rapid equilibrium conditions become negligible. It must be emphasized, however, that it may be very difficult, or even practically impossible, to demonstrate non-linearity in the rate equation under rapid equilibrium conditions, since the range over which substrate concentrations have to be varied in order to establish curvature becomes extremely wide. Nevertheless, it appears that rate equations derived for more complicated reaction mechanisms under rapid equilibrium assumptions, or under combined assumptions of equilibrium and steady-state,[8] should be treated with great caution. It will always be preferable to simplify a rate equation by imposing appropriate restrictions on the full steady-state equation.

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Received April 14, 1969.