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Multiple Intermediates in Steady-state Enzyme Kinetics. II. Systems Involving Two Reactants and Two Products[1a]

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General steady-state rate laws valid for an arbitrary number of intermediates are developed for reversible enzyme-catalyzed reactions involving two reactants and two products. Two distinct types of rate laws applicable to dehydrogenase- and transaminase-catalyzed reactions are discussed. These rate laws involve a set of kinetic parameters which are coefficients of terms which depend in a unique manner on the concentrations of the various reactants and products. Certain dependency relationships are shown to exist between these observable kinetic parameters. Lower limits are formulated for the bimolecular and unimolecular rate constants in terms of these parameters. For the dehydrogenase systems, a kinetic criterion requiring the consideration of more than one binary isomer in the reaction sequence is presented together with a kinetic means of distinguishing the order of combination of the two reactant and two product species with the enzyme. For the transaminase systems, a criterion is presented in terms of the kinetic parameters necessitating consideration of more than one aminated enzyme.

Introduction

The kinetics of most enzyme systems have been investigated under steady state conditions where the rates of change of concentration of the intermediates, the various enzyme–substrate complexes, are small compared to the rate of change of concentration of reactants and products. In the majority of such studies these elusive intermediates escape direct detection.

The prevailing steady state conditions place severe restrictions on the information which can be derived from such kinetic studies. In the absence of direct observation of intermediates, it is clear that discussions of the kinetics in terms of a specific number of these species with the concomitant introduction of a definite set of rate constants is often highly tendentious.

An analysis of the steady state kinetic behavior of an enzyme-catalyzed reversible reaction where no assumption was introduced concerning the number of intermediates has been presented previously.[2] It is the purpose of this article to extend some of the arguments appearing there to enzymatic reactions involving more than one reactant and product. The chief virtue of this approach resides in the fact that from the steady state rate law certain general conclusions can be derived which are not restricted to arbitrary reaction mechanisms. A number of other general discussions of steady state enzyme kinetics have been presented along lines similar to ours.[3-7] It should be emphasized that the kinetic parameters which characterize the treatment we propose are all susceptible to direct measurement *under steady state conditions*. In the following development the mechanisms introduced will be purely formal in nature and imply no detailed chemical description of the intermediates.

"Dehydrogenase" Mechanisms

In this article and the succeeding one,[8] we shall be concerned with reactions whose stoichiometry is typified by the reaction catalyzed by the alcohol dehydrogenases, namely

$$DPN^+ + \text{alcohol} \rightleftharpoons \text{aldehyde} + DPNH + H^+$$

where DPN^+ and DPNH represent the oxidized and reduced forms of the diphosphopyridine nucleotides.

Our kinetic analysis will be developed in detail for a reaction whose stoichiometry is represented by

$$A + B \rightleftharpoons Q + R \qquad (1)$$

where, for example, A = DPN^+, B = alcohol, Q = aldehyde and R = DPNH. Kinetic studies of the alcohol dehydrogenases where the *hydrogen ion concentration is maintained constant* by added buffers would be expected to conform to an analysis for a reaction with a stoichiometry given in eq. 1. We shall briefly consider in the following manuscript[8] the effect of inclusion of a third species, *e.g.*, H^+, on the kinetics. It should be remarked that the dependence of the kinetic parameters on *p*H for enzyme-catalyzed reactions in which the hydrogen ion does not participate in the over-all reaction gives rise to certain complications in the analysis.[2,9,10]

Mechanisms Involving Ternary Complexes

We can formulate a quite general mechanism corresponding to a reaction with a stoichiometry given by eq. 1 as

$$E + A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} X_1 \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \cdots \underset{k_{-\alpha}}{\overset{k_\alpha}{\rightleftharpoons}} X_\alpha \underset{k_{-(\alpha+1)}}{\overset{k_{(\alpha+1)}}{\rightleftharpoons}} \cdots \underset{k_{-(f-1)}}{\overset{k_{(f-1)}}{\rightleftharpoons}} X_{f-1}$$

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(2) L. Peller and R. A. Alberty, *J. Am. Chem. Soc.*, **81**, 5907 (1959).

(3) (a) J. A. Christiansen, *Acta Chem. Scand.*, **3**, 493 (1949); (b) J. Z. Hearon, *Physiol. Rev.*, **32**, 499 (1952).

(4) R. Lumry, *Disc. Faraday Soc.*, **20**, 257 (1955).

(5) E. L. King and C. Altman, *J. Phys. Chem.*, **60**, 1375 (1956).

(6) J. Z. Hearon, S. A. Bernhard, S. L. Friess, D. F. Botts and M. F. Morales in "The Enzymes," Vol. I, 2nd ed., Academic Press, Inc., New York, N. Y., 1959, pp. 89–108.

(7) H. D. Ohlenbusch, "Die Kinetik der Effektorwirkuug auf Stationäre Fermentsysteme," Habilitationschrift, Universität Kiel, 1959.

(8) V. Bloomfield, L. Peller and R. A. Alberty, *J. Am. Chem. Soc.*, **84**, 4375 (1962).

(9) K. J. Laidler, *Trans. Faraday Soc.*, **51**, 528 (1955).

(10) R. A. Alberty, *J. Cell. and Comp. Physiol.*, **47**, Suppl. 1, 245 (1956).

$$B + X_{f-1} \underset{k_{-f}}{\overset{k_f}{\rightleftarrows}} X_f \underset{k_{-(f+1)}}{\overset{k_{(f+1)}}{\rightleftarrows}} \cdots \underset{k_{-\beta}}{\overset{k_\beta}{\rightleftarrows}} X_\beta \underset{k_{-(\beta+1)}}{\overset{k_{(\beta+1)}}{\rightleftarrows}} \cdots \underset{k_{-g}}{\overset{k_g}{\rightleftarrows}} X_g + Q \quad (2)$$

$$X_g \underset{k_{-(g+1)}}{\overset{k_{(g+1)}}{\rightleftarrows}} X_{g+1} \underset{k_{-(g+2)}}{\overset{k_{(g+2)}}{\rightleftarrows}} \cdots \underset{k_{-\gamma}}{\overset{k_\gamma}{\rightleftarrows}} X_\gamma \underset{k_{-(\gamma+1)}}{\overset{k_{(\gamma+1)}}{\rightleftarrows}} \cdots \underset{k_{-n}}{\overset{k_n}{\rightleftarrows}} X_n \underset{k_{-(n+1)}}{\overset{k_{(n+1)}}{\rightleftarrows}} E + R$$

In eq. 2 there are a total of n intermediates and $2(n + 1)$ rate constants. The ternary intermediates are labeled X_β where $f \leqslant \beta \leqslant g - 1$. The other binary intermediates would be expected to form in reactions between E and A alone, for example, in the absence of any over-all enzymatic reaction. The above scheme involves an obligatory order of combination of substrates with enzyme, *i.e.*, A before B.

Following a procedure developed previously[1] based on some earlier work of Christiansen,[11] a steady rate law of the following form can be derived

$$v = \frac{(V_{AB}/K_{AB})(A)(B) - (V_{QR}/K_{QR})(Q)(R)}{1 + \frac{(A)}{K_A} + \frac{(B)}{K_B} + \frac{(Q)}{K_Q} + \frac{(R)}{K_R} + \frac{(A)(B)}{K_{AB}} + \frac{(Q)(R)}{K_{QR}} + \frac{(A)(Q)}{K_{AQ}} + \frac{(B)(R)}{K_{BR}} + \frac{(A)(B)(Q)}{K_{ABQ}} + \frac{(B)(Q)(R)}{K_{BQR}}} \quad (3)$$

where $v = -d(A)/dt = -d(B)/dt = d(Q)/dt = d(R)/dt$ is the steady state velocity of the reaction. Equation 3 is valid irrespective of the number of binary and ternary intermediates involved.

The kinetic parameters introduced in equation (3) have the following definitions in terms of the rate constants appearing in equation (2).

$$V_{AB} = (E)_0 \Big/ \left[\sum_{\alpha=1}^{f-1} \sum_{s=\alpha}^{f-2} \frac{K_\alpha^s}{k_{(s+1)}} + \sum_{\beta=f}^{g-1} \sum_{s=\beta}^{g-1} \frac{K_\beta^s}{k_{(s+1)}} + \sum_{\gamma=g}^{n} \sum_{s=\gamma}^{n} \frac{K_\alpha^s}{k_{(s+1)}} \right] \quad (4a)$$

$$V_{QR} = (E)_0 \Big/ \left[\sum_{\alpha=1}^{f-1} \sum_{s=0}^{\alpha-1} \frac{K_\alpha^s}{k_{(s+1)}} + \sum_{\beta=f}^{g-1} \sum_{s=f-1}^{\beta-1} \frac{K_\beta^s}{k_{(s+1)}} + \sum_{\gamma=g}^{n} \sum_{s=g}^{\gamma-1} \frac{K_\gamma^s}{k_{(s+1)}} \right] \quad (4b)$$

$$K_A = \frac{\sum_{s=f-1}^{g-1} \frac{K_0^s}{k_{(s+1)}}}{\sum_{\alpha=1}^{f-1} \sum_{s=f-1}^{g-1} \frac{K_\alpha^s}{k_{(s+1)}}} \quad (5a)$$

$$K_B = \frac{\sum_{s=f-1}^{g-1} \frac{K_0^s}{k_{(s+1)}}}{\sum_{s=0}^{f-2} \frac{K_0^s}{k_{(s+1)}}} \quad (5b)$$

$$K_R = \frac{\sum_{s=f-1}^{g-1} \frac{K_0^s}{k_{(s+1)}}}{K_0^{n+1} \sum_{\gamma=g}^{n} \sum_{s=f-1}^{g-1} \frac{K_\gamma^s}{k_{(s+1)}}} \quad (5c)$$

$$K_Q = \frac{\sum_{s=f-1}^{g-1} \frac{K_0^s}{k_{(s+1)}}}{\sum_{s=g}^{n} \frac{K_0^s}{k_{(s+1)}}} \quad (5d)$$

$$K_{AB} = \frac{\sum_{s=f-1}^{g-1} \frac{K_0^s}{k_{(s+1)}}}{\sum_{\alpha=1}^{f-1} \sum_{s=\alpha}^{f-2} \frac{K_\alpha^s}{k_{(s+1)}} + \sum_{\beta=f}^{g-1} \sum_{s=\beta}^{g-1} \frac{K_\beta^s}{k_{(s+1)}} + \sum_{\gamma=g}^{n} \sum_{s=\gamma}^{n} \frac{K_\gamma^s}{k_{(s+1)}}} \quad (6a)$$

$$K_{QR} = \frac{\sum_{s=f-1}^{g-1} \frac{K_0^s}{k_{(s+1)}}}{K_0^{n+1} \left[\sum_{\alpha=1}^{f-1} \sum_{s=0}^{\alpha-1} \frac{K_\alpha^s}{k_{(s+1)}} + \sum_{\beta=f}^{g-1} \sum_{s=f-1}^{\beta-1} \frac{K_\beta^s}{k_{(s+1)}} + \sum_{\gamma=g}^{n} \sum_{s=g}^{\gamma-1} \frac{K_\gamma^s}{k_{(s+1)}} \right]} \quad (6b)$$

$$K_{AQ} = \frac{\sum_{s=f-1}^{g-1} \frac{K_0^s}{k_{(s+1)}}}{\sum_{\alpha=1}^{f-1} \sum_{s=g}^{n} \frac{K_\alpha^s}{k_{(s+1)}}} \quad (6c)$$

$$K_{BR} = \frac{\sum_{s=f-1}^{g-1} \frac{K_0^s}{k_{(s+1)}}}{K_0^{n+1} \sum_{\gamma=g}^{n} \sum_{s=0}^{f-2} \frac{K_\gamma^s}{k_{(s+1)}}} \quad (6d)$$

$$K_{ABQ} = \frac{\sum_{s=f-1}^{g-1} \frac{K_0^s}{k_{(s+1)}}}{\sum_{\beta=f}^{g-1} \sum_{s=g}^{n} \frac{K_\beta^s}{k_{(s+1)}}} \quad (7a)$$

$$K_{BQR} = \frac{K_0^{n+1} \sum_{\beta=f}^{g-1} \sum_{s=0}^{f-2} \frac{K_\beta^s}{k_{(s+1)}}}{K_0^{n+1} \sum_{\gamma=g}^{n} \sum_{s=0}^{f-2} \frac{K_\beta^s}{k_{(s+1)}}} \quad (7b)$$

In the above expressions $(E)_0$ is the total molar concentration of enzymatic sites.

$$K_{is} = \prod_{r=i+1}^{s} (k_{-r}/k_r) \text{ if } s > i \text{ and } K_i^s = 1/K_i^s \text{ if } s < i$$

while $K_i^i = 1$ with $i = \alpha, \beta, \gamma$. K_i^s is defined as in an earlier paper[2] and is plainly an equilibrium constant, *i.e.*, $(X_i)_{eq}/(X_s)_{eq} = K_i^s$.

The twelve kinetic parameters defined in eq. 4–7 are not all independent. From the above expressions the following four relations can be obtained

(11) J. A. Christiansen, *Z. physik. Chem.*, **28B**, 303 (1935); **33B**, 145 (1936).

$$K_{AQ} = K_A K_Q \quad (8a) \qquad K_{BR} = K_B K_R \quad (8b)$$

$$K_{ABQ} = \frac{V_{QR}K_R K_Q^2 K_{AB} K_A K_B}{V_{AB}K_A K_B(K_Q K_R - K_{QR}) + V_{QR}K_Q K_R(K_A K_B - K_{AB})} \quad (9a)$$

$$K_{BQR} = \frac{V_{AB}K_A K_B^2 K_{QR} K_Q K_R}{V_{AB}K_A K_B(K_Q K_R - K_{QR}) + K_{QR}K_Q K_R(K_A K_B - K_{AB})} \quad (9b)$$

At equilibrium ($v = 0$), it follows from eq. 3 that

$$V_{AB}K_{QR}/V_{QR}K_{AB} = 1/K_0^{n+1} = K_{eq} = (Q)_{eq}(R)_{eq}/(A)_{eq}(B)_{eq} \quad (10)$$

which is a relation of the type first noted by Haldane.[12]

Equations 8 and 9 reduce the number of independent kinetic parameters to eight. These suffice to determine the eight rate constants in a reaction scheme involving two binary intermediates (X_1 and X_3) and one ternary intermediate (X_2) as depicted below.

$$\begin{gathered} E + A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} X_1 \\ B + X_1 \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} X_2 \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} X_3 + Q \\ X_3 \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} E + R \end{gathered} \quad (11)$$

The above is clearly the minimal mechanism derivable from the general scheme presented in eq. 2.

It is of considerable interest that lower limits for the various types of rate constants appearing in eq. 2 can be given in terms of the kinetic parameters defined above. The arguments are similar to those outlined previously.[2] We will consider first k_1 and $k_{-(n+1)}$, the bimolecular rate constants applying to the combination of E with A and R, respectively. From eq. 4a, 5b and 6a, it follows that

$$\frac{V_{AB}K_B}{K_{AB}(E)_0} = \frac{1}{\sum_{s=0}^{f-2} \frac{K_0^s}{k_{(s+1)}}} = \frac{1}{\frac{1}{k_1}\left(1 + \sum_{s=1}^{f-2} \frac{k_{-1}K_1^s}{k_{(s+1)}}\right)}$$

This leads to the relation

$$k_1 \geqslant V_{AB}K_B/K_{AB}(E)_0 \quad (12a)$$

The above expression becomes an equality if $f = 2$, or only one binary intermediate need be considered in the combination of A with E. The symmetric relation of A to R in eq. 2 requires that

$$k_{-(n+1)} \geqslant V_{QR}K_Q/K_{QR}(E)_0 \quad (12b)$$

with the equality holding when only one binary complex arises from the combination of E with R, *i.e.*, $g = n$.

Lower limits can also be given for k_f and k_{-g}, the bimolecular rate constants for the formation of ternary complexes by the combination of B with X_{f-1} and Q with X_g, respectively. From eq. 4a, 5a and 6a, we obtain

$$\frac{V_{AB}K_A}{K_{AB}(E)_0} = \frac{1}{\sum_{\alpha=1}^{f-1}\sum_{s=f-1}^{g-1} \frac{K_\alpha^s}{k_{(s+1)}}} < \frac{1}{(K_{f-1}{}^{f-1}/k_f)}$$

(12) I. B. S. Haldane, "Enzymes," Longmans, Green, London, 1930.

As $K_{f-1}{}^{f-1} = 1$, it follows that

$$k_f > V_{AB}K_A/K_{AB}(E)_0 \quad (13a)$$

The symmetric relation of B to Q ensures that

$$k_{-g} > V_{QR}K_R/K_{QR}(E)_0 \quad (13b)$$

For the minimal mechanism in eq. 11 with $f + 1 = g = n = 3$, we have the relations

$$k_f = k_2 = \frac{V_{AB}K_A}{K_{AB}(E)_0}\left[1 + \frac{V_{QR} - (K_{QR}V_{AB}/K_Q K_R)}{V_{AB} - (K_{AB}V_{QR}/K_A K_B)}\right] \quad (14a)$$

$$k_{-g} = k_{-3} = \frac{V_{QR}K_R}{K_{QR}(E)_0}\left[1 + \frac{V_{AB} - (K_{AB}V_{QR}/K_A K_B)}{V_{QR} - (K_{QR}V_{AB}/K_Q K_R)}\right] \quad (14b)$$

For *all* the unimolecular rate constants applying to the reaction in the forward direction, a general lower limit of $V_{AB}/(E)_0$ exists as can be seen from eq. 4a.

$$k_{(\alpha+1)}, k_{(\beta+1)}, k_{(\gamma+1)} > V_{AB}/(E)_0 \quad (15a)$$

Similarly from eq. 4b, we have for the unimolecular rate constants for the reaction in the reverse direction

$$k_{-\alpha}, k_{-\beta}, k_{-\gamma} > V_{QR}/(E)_0 \quad (15b)$$

These limits are identical to those given previously for the unimolecular rate constants for the reaction involving a single reactant and product.[2] They comprise simple statements of the fact that the maximum velocity in a given direction cannot exceed any unimolecular rate constant for reaction in that direction.

In addition to these general lower limits for the unimolecular rate constants some further limits can be found for those rate constants involved in the dissociation of substrate and isomerization of the binary complexes. For example, from eq. 4a, 5a, 5b and 6a we have

$$\left.\begin{aligned} \frac{V_{AB}K_A K_B}{K_{AB}(E)_0} = \frac{1}{\sum_{\alpha=1}^{f-1}\sum_{s=0}^{f-2} \frac{K_\alpha^s}{k_{(s+1)}}} \end{aligned}\right\} \begin{aligned} &\leqslant \frac{1}{(K_\alpha{}^{\alpha-1}/k_\alpha)} \\ &< \frac{1}{(K_\alpha{}^{\alpha}/k_{(s+1)})} \end{aligned}$$

which lead to the two relations

$$k_{-\alpha} \geqslant V_{AB}K_A K_B/K_{AB}(E)_0 \quad 1 \leqslant \alpha \leqslant f - 1 \quad (16a)$$

$$k_{(\alpha+1)} > V_{AB}K_A K_B/K_{AB}(E)_0 \quad 1 \leqslant \alpha \leqslant f - 2 \quad (16b)$$

The equality in 16a holds if $f = 2$. In this circumstance the inequality 16b is inapplicable. The symmetry manifest in the general mechanism of equation 2 requires that

$$k_{(\gamma+1)} \geqslant V_{QR}K_Q K_R/K_{QR}(E)_0 \quad g \leqslant \gamma \leqslant n \quad (16c)$$

$$k_{-\gamma} > V_{QR}K_Q K_R/K_{QR}(E)_0 \quad g + 1 \leqslant \gamma \leqslant n \quad (16d)$$

Again, if there is only one binary complex to be considered in the combination of E with R, *i.e.*, $g = n$, the equality sign in 16c applies and the inequality 16d is not applicable. For the minimal mechanism of eq. 11, we have the results

$$k_g = k_3 = \frac{V_{AB}}{(E)_0}\left[\frac{1}{1 - (K_{QR}V_{AB}/V_{QR}K_Q K_R)}\right] \quad (17a)$$

$$k_{-f} = k_{-2} = \frac{V_{QR}}{(E)_0}\left[\frac{1}{(1 - (K_{AB}V_{QR}/V_{AB}K_A K_B)}\right] \quad (17b)$$

We can employ two expressions for lower limits on the unimolecular rate constants to develop an

interesting kinetic criterion for the necessity of considering more than one binary complex derived from the combination of E and A. If we suppose there is only one binary complex of this type, then from eq. 16a with $\alpha = f - 1 = 1$

$$k_{-1} = \frac{V_{AB}K_AK_B}{K_{AB}(E)_0}$$

However, for any number of binary and ternary intermediates

$$k_{-1} > V_{QR}/(E)_0$$

Provided that the kinetics are consistent with one binary intermediate, it follows from these two relations that

$$V_{AB}K_AK_B/V_{QR}K_{AB} > 1$$

If these experimentally determinable kinetic parameters satisfy the *reverse* inequality

$$V_{QR}K_{AB}/V_{AB}K_AK_B \geqslant 1 \qquad (18a)$$

then there is *prima facie* evidence for the necessity of considering *more than one* binary complex in the reaction of E and A. In a like fashion the inequality

$$V_{AB}K_{QR}/V_{QR}K_QK_R \geqslant 1 \qquad (18b)$$

is a criterion for the implication of more than one binary complex in the reaction of E and R.

The above two inequalities represent *sufficient* conditions for the requirement of the consideration of isomers of the binary complexes. They are, however, not *necessary* conditions. In point of fact both of these inequalities cannot be satisfied simultaneously. The necessity that K_{ABQ} and K_{BQR} be positive quantities ensures that if inequality 18a is met then the inequality 18b is reversed. Examination of the denominators of eq. 9a and 9b for K_{ABQ} and K_{BQR}, respectively, demonstrates this requirement. Values of these parameters computed from experimental data which are cited in the accompanying paper[8] bear out this assertion.

It should be pointed out that for the ordered path of reaction considered here the parameters K_A and K_R have very simple physical interpretations. From eq. 5a and 5c if follows that

$$K_A = \frac{\sum_{s=f-1}^{g-1} \frac{K_0^s}{k_{(s+1)}}}{\sum_{\alpha=1}^{f-1}\sum_{s=f-1}^{g-1} \frac{K_\alpha^s}{k_{(s+1)}}} = \frac{1}{\sum_{\alpha=1}^{f-1} K_\alpha^\circ} = \frac{(E)_{eq}(A)_{eq}}{\sum_{\alpha=1}^{f-1}(X_\alpha)_{eq}} \equiv K_{diss,A} \qquad (19a)$$

$$K_R = \frac{\sum_{s=f-1}^{g-1} \frac{K_0^s}{k_{(s+1)}}}{K_0^{n+1}\sum_{\gamma=g}^{n}\sum_{s=f-1}^{g-1} \frac{K_\gamma^s}{k_{(s+1)}}} = \frac{1}{\sum_{\gamma=g}^{n} K_\gamma^{n+1}} = \frac{(E)_{eq}(R)_{eq}}{\sum_{\gamma=1}^{n}(X_\gamma)_{eq}} = K_{diss,R} \qquad (19b)$$

These two kinetic parameters are thus to be regarded as equilibrium constants for the "dissociation" of complexes to form E and A and E and R, respectively. Agreement between K_A, for example, measured kinetically with a dissociation constant determined by some equilibrium measurement has been construed frequently as support for a reaction mechanism involving a *single* binary complex,[13] *i.e.*, $K_A = 1/K_2^\circ = k_{-1}/k_1$. From the above results it is apparent that such agreement between the two types of measurement is to be expected irrespective of the number of binary intermediates involved.

The eight independent kinetic parameters introduced above can be obtained quite readily from steady state kinetic measurements. Under the customary conditions of study, the initial steady state velocity in the forward direction, v_f, is determined with only A and B present. With (Q) = (R) = 0, eq. 3 yields the following expression for v_f

$$\frac{1}{v_f} = \frac{1}{v_{AB}} + \frac{K_{AB}}{V_{AB}K_B(A)} + \frac{K_{AB}}{V_{AB}K_A(B)} + \frac{K_{AB}}{V_{AB}(A)(B)} \qquad (20a)$$

Similarly with (A) = (B) = 0, the symmetric expression for v_r is obtained

$$\frac{1}{v_r} = \frac{1}{V_{QR}} + \frac{K_{QR}}{V_{QR}K_R(Q)} + \frac{K_{QR}}{V_{QR}K_Q(R)} + \frac{K_{QR}}{V_{QR}(Q)(R)} \qquad (20b)$$

The eight independent kinetic parameters are then easily evaluated from the usual double reciprocal plots. Equations 20a and 20b are in suitable form for such a graphical analysis, but it should be remarked that the form departs somewhat from that customarily employed, namely

$$\frac{V_{AB}}{v_f} = 1 + \frac{K_A^*}{(A)} + \frac{K_A^*}{(B)} + \frac{K_{AB}^*}{(A)(B)}$$

The relation between the quantities with asterisks and the kinetic parameters defined in eq. 20a is obvious. The representation proposed by Dalziel[14] is of a similar form. He has noted, for reaction schemes involving *specific* numbers of intermediates, some relations of the type quoted in this paper.

It is interesting to note that the minimum values of various of the rate constants are given directly by the separate terms in eq. 20a and 20b. For example, the reciprocal of the first term in eq. 20a divided by $(E)_0$ represents the minimum value for all the unimolecular rate constants for the reaction in the forward direction. The reciprocal of the coefficient of 1/(A) divided by $(E)_0$ represents the minimum value for the bimolecular rate constant for the combination of A with E. Similarly, the reciprocal of the coefficient of 1/(B) divided by $(E)_0$ is the minimum value of the rate constant for the reaction of B. A like interpretation may be given to the terms in eq. 20b with respect to the reaction in the reverse direction.

Throughout the preceding discussion we have tacitly assumed that in the ordered sequence of reaction the symbols A and B and also Q and R can be identified with the appropriate chemical spe-

(13) H. Theorell, "Ciba Foundation on Significant Trends in Medical Research," 1959, p. 18.

(14) K. Dalziel, *Acta Chem. Scand.*, **11**, 1706 (1957).

cies. There is considerable experimental evidence that for the reactions catalyzed by the dehydrogenases, it is the pyridine nucleotide which first combines with the enzyme,[13,15] *i.e.* A = DPN$^+$ and R = DPNH.

It is of some significance that this identification is in principle possible from steady state kinetic studies alone. To illustrate this possibility we can consider initial velocity measurements in the forward direction with either (a) R added or (b) Q added. From eq. 3, the following two expressions are obtained when (a) (Q) = 0 (b) (R) = 0

$$\frac{1}{v_f} = \frac{1}{V_{AB}} + \frac{K_{AB}}{V_{AB}K_B(A)} + \frac{K_{AB}}{V_{AB}K_A(B)} + \frac{K_{AB}}{V_{AB}(A)(B)} + \frac{K_{AB}(R)}{V_{AB}K_{BR}(A)} + \frac{K_{AB}(R)}{V_{AB}K_R(A)(B)} \quad (21a)$$

$$\frac{1}{v_f} = \frac{1}{V_{AB}} + \frac{K_{AB}}{V_{AB}K_B(A)} + \frac{K_{AB}}{V_{AB}K_A(B)} + \frac{K_{AB}}{V_{AB}(A)(B)} + \frac{K_{AB}(Q)}{V_{AB}K_{AQ}(B)} + \frac{K_{AB}(Q)}{V_{AB}K_Q(A)(B)} + \frac{K_{AB}(Q)}{V_{AB}K_{ABQ}} \quad (21b)$$

The first four terms in eq. 21a and 21b are of course identical to the terms in eq. 20a. Comparing these two expressions, it is immediately apparent that the effects of R and Q on v_f are not identical. More specifically, the last term in eq. 21b has no counterpart in eq. 21a involving the concentration of R. In principle then, studies of this sort should provide a means of identifying the appropriate product with Q and R. Exactly the same conclusion can be reached concerning A and B with respect to measurements of v_r. The importance of experiments of this nature has been suggested before.[16] It should be stressed, however, that kinetic measurements permitting these distinctions to be made will require high precision in the initial velocity determinations.

Mechanisms Involving Only Binary Complexes

The mechanism originally proposed to describe the kinetics of the reaction catalyzed by horse liver alcohol dehydrogenase involved only two binary complexes and no ternary complexes.[15] A generalized reaction scheme of this type with an arbitrary number of binary complexes but involving no ternary complexes can be represented

$$\begin{aligned} &E + A \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} X_1 \underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} \cdots \underset{k_{-\alpha}}{\overset{k_\alpha}{\rightleftarrows}} X_\alpha \underset{k_{-(\alpha+1)}}{\overset{k_{(\alpha+1)}}{\rightleftarrows}} \cdots \\ &\qquad \underset{k_{-(f-1)}}{\overset{k_{(f-1)}}{\rightleftarrows}} X_{f-1} \\ &\qquad B + X_{f-1} \underset{k_{-f}}{\overset{k_f}{\rightleftarrows}} X_f + Q \\ &X_f \underset{k_{-(f+1)}}{\overset{k_{(f+1)}}{\rightleftarrows}} X_{f+1} \underset{k_{-(f+2)}}{\overset{k_{(f+2)}}{\rightleftarrows}} \cdots \underset{k_{-\beta}}{\overset{k_\beta}{\rightleftarrows}} X_\beta \underset{k_{-(\beta+1)}}{\overset{k_{(\beta+1)}}{\rightleftarrows}} \\ &\qquad \cdots \underset{k_{-n}}{\overset{k_n}{\rightleftarrows}} X_n \underset{k_{-(n+1)}}{\overset{k_{(n+1)}}{\rightleftarrows}} E + R \end{aligned} \quad (22)$$

With $f = n = 2$ the above sequence is identical to that proposed by Theorell and Chance.[15]

A general equation for the steady state velocity can be readily derived in the manner previously indicated

$$v = \frac{(V_{AB}/K_{AB})(A)(B) - (V_{QR}/K_{QR})(Q)(R)}{1 + \frac{(A)}{K_A} + \frac{(B)}{K_B} + \frac{(Q)}{K_Q} + \frac{(R)}{K_R} + \frac{(A)(B)}{K_{AB}} + \frac{(Q)(R)}{K_{QR}} + \frac{(A)(Q)}{K_{AQ}} + \frac{(B)(R)}{K_{BR}}} \quad (23)$$

The kinetic parameters appearing in eq. 23 have the definitions

$$V_{AB} = (E)_0 \Big/ \left[\sum_{\alpha=1}^{f-1} \sum_{s=\alpha}^{f-2} \frac{K_\alpha^s}{k_{(s+1)}} + \sum_{\beta=f}^{n} \sum_{s=\beta}^{n} \frac{K_\beta^s}{k_{(s+1)}} \right] \quad (24a)$$

$$V_{QR} = (E)_0 \Big/ \left[\sum_{\alpha=1}^{f-1} \sum_{s=0}^{\alpha-1} \frac{K_\alpha^s}{k_{(s+1)}} + \sum_{\beta=f}^{n} \sum_{s=f}^{\beta-1} \frac{K_\beta^s}{k_{(s+1)}} \right] \quad (24b)$$

$$K_A = \frac{K_0^{f-1}/k_f}{\sum_{\alpha=1}^{f-1} \frac{K_\alpha^{f-1}}{k_f}} \quad (25a) \qquad K_B = \frac{K_0^{f-1}/k_f}{\sum_{s=0}^{f-2} \frac{K_0^s}{k_{(s+1)}}} \quad (25b)$$

$$K_R = \frac{K_0^{f-1}/k_f}{K_0^{n+1} \sum_{\beta=f}^{n} \frac{K_\beta^{f-1}}{k_f}} \quad (25c)$$

$$K_Q = \frac{K_0^{f-1}/k_f}{\sum_{s=f}^{n} \frac{K_0^s}{k_{(s+1)}}} \quad (25d)$$

$$K_{AB} = \frac{K_0^{f-1}/k_f}{\sum_{\alpha=1}^{f-1} \sum_{s=\alpha}^{f-2} \frac{K_\alpha^s}{k_{(s+1)}} + \sum_{\beta=f}^{n} \sum_{s=\beta}^{n} \frac{K_\beta^s}{k_{(s+1)}}} \quad (26a)$$

$$K_{QR} = \frac{K_0^{f-1}/k_f}{K_0^{n+1} \left[\sum_{\alpha=1}^{f-1} \sum_{s=0}^{\alpha-1} \frac{K_\alpha^s}{k_{(s+1)}} + \sum_{\beta=f}^{n} \sum_{s=f}^{\beta-1} \frac{K_\beta^s}{k_{(s+1)}} \right]} \quad (26b)$$

$$K_{AQ} = \frac{K_0^{f-1}/k_f}{\sum_{\alpha=1}^{f-1} \sum_{s=0}^{n} \frac{K_\alpha^s}{k_{(s+1)}}} \quad (26c)$$

$$K_{BR} = \frac{K_0^{f-1}/k_f}{K_0^{n+1} \sum_{\beta=f}^{n} \sum_{s=0}^{f-2} \frac{K^s\beta}{k_{(s+1)}}} \quad (26d)$$

The ten kinetic parameters are again not all independent. From eq. 25a, 25d and 26c it follows that the redundancy relation, eq. 8a, applies for this case. Similarly eq. 8b is valid for this scheme as well. Consequently, only eight parameters are again independent. Setting $v = 0$, eq. 10 is recovered.

Lower limits for the various rate constants are virtually identical to those developed above. It can be readily established that the lower limits for k_1 and $k_{-(n+1)}$ are given by 12a and 12b with the equalities applying when $f = 2$ and $f = n$, respectively. Lower limits for k_f and k_{-f} are provided by 13a and 13b with the single dif-

(15) H. Theorell and B. Chance, *Acta Chem. Scand.*, **5**, 1127 (1951).
(16) R. A. Alberty, *J. Am. Chem. Soc.*, **80**, 1777 (1958).

ference that these relations become equalities in the event that $f = 2$ and $f = n$, respectively.

Some slight modifications must be made for the lower limits on the unimolecular rate constants as given above. For $k_{(\alpha+1)}$ $(1 \leqslant \alpha \leqslant f - 2)$, 15a applies as does 15b for the symmetrically related $k_{-\beta}$ $(f + 1 \leqslant \beta \leqslant n)$. (It should be noted that rate constants with the subscript β in reaction scheme 22 are analogous to those with subscript γ in scheme 2.) However, for $k_{(\beta+1)}$ $(f \leqslant \beta \leqslant n)$, the relation 15a applies but becomes an equality if $f = n = 2$, *i.e.*, the minimal mechanism of Theorell and Chance[15] is valid. In a like manner for the reverse reaction, relation 15b holds for $k_{-\alpha}$ $(1 \leqslant \alpha \leqslant f - 1)$ but becomes an equality for the minimal mechanism.

Relations 16a and 16b are valid in this case as well for $k_{-\alpha}$ $(1 \leqslant \alpha \leqslant f - 1)$ and $k_{(\alpha+1)}$ $(1 \leqslant \alpha \leqslant f - 2)$, respectively. Similarly, 16c and 16d hold for $k_{(\beta+1)}$ $(f \leqslant \beta \leqslant n)$ and $k_{-\beta}$ $(f + 1 \leqslant \beta \leqslant n)$, respectively. Hence, identical kinetic criteria as described by 18a and 18b may be advanced for the necessity of considering binary isomers. Again K_A and K_R are dissociation constants in the same sense as described in the previous section.

Moreover, the initial steady state velocity in the forward direction with (Q) = (R) = 0 and in the reverse direction with (A) = (B) = 0 as derived from eq. 23 are identical in form to eq. 20a and 20b, respectively. Of necessity, studies of this sort provide no indication concerning the relative importance of ternary complexes. For systems in which ternary complexes are believed to be present, the adequacy of eq. 20a and 20b for describing the kinetics does *not* provide an argument that the rate constants for the interconversion of ternary complexes are much larger than the other unimolecular rate constants, contrary to a recent assertion.[17]

It is instructive to consider initial steady state velocity studies in the forward direction with only *one* of the products R or Q present. Firstly it should be noted that eq. 3 becomes identical to eq. 23 if $K_{ABQ} >> (A)(B)(Q)$ and $K_{BQR} >> (B)(Q)(R)$. This condition of large kinetic parameters, K_{ABQ} and K_{BQR}, corresponds to the steady state concentration of ternary complexes being relatively small compared to those of the binary complexes and free enzyme. Consequently for the mechanism without ternary complexes, v_f with (R) = 0 is obtained from eq. 21b by deleting the last term. When (Q) = 0, v_f is given by eq. 21a. *Steady-state kinetic data requiring the consideration of ternary complexes must then rest on the detection of the last term in eq. 21b or an analogous term proportional to (B) in the symmetric expression for* $1/v_r$. The absence of such terms also precludes the possibility of ascertaining the order of addition of substrates and products. However, it is still possible to discover the symmetrically reacting pairs A,R and B,Q by inspection of the rate law, eq. 23.

The Theorell–Chance mechanism or its generalization stated by eq. 22 is unlikely to provide a satisfactory chemical description of the mechanism of dehydrogenase action as a result of the omission of ternary complexes. The foregoing development serves to emphasize the problem of obtaining kinetic evidence for these intermediates from steady state studies.

(17) H. Theorell, *Federation Proc.*, **20**, 967 (1961).

"Transaminase" Mechanisms

The results presented above for reaction schemes with both binary and ternary complexes though of quite general validity are *not* immediately applicable to one important class of enzyme-catalyzed reactions involving two reactants and two products, *i.e.*, reactions catalyzed by the transaminases.[18] The vital characteristic distinguishing such reactions from those considered above is the dissociation of one *product* from the enzyme before the latter combines with the second *reactant*. Such a reaction can be readily resolved into two "half-reactions" each susceptible to study independently. These half-reactions might be depicted as

$$A + E \rightleftharpoons E' + Q \qquad (27a)$$

$$B + E' \rightleftharpoons E + R \qquad (27b)$$

where E′ is a chemically modified form of the enzyme E. In the best studied example of this type of system, A represents glutamic acid, Q represents α-ketoglutaric acid, B represents oxaloacetic acid and R represents aspartic acid, where the enzyme E is glutamic–aspartic transaminase.[19] The modified enzyme, E′, has been ascertained to contain a Schiff base of pyridoxal phosphate which is strongly bound to the protein moiety of the enzyme.[20] The enzyme functions to "ferry" an amino group between rather similar substrates. Such a reaction would be expected to have an equilibrium constant of the order of unity as appears to be the case.[21]

A general mechanistic scheme to which reactions of this type might be expected to conform can be represented by

$$\begin{aligned}
&A + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} X_1 \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \cdots \underset{k_{-\alpha}}{\overset{k_\alpha}{\rightleftharpoons}} X_\alpha \underset{k_{-(\alpha+1)}}{\overset{k_{(\alpha+1)}}{\rightleftharpoons}} \\
&\qquad \cdots \underset{k_{-(f-1)}}{\overset{k_{(f-1)}}{\rightleftharpoons}} X_{f-1} \underset{k_{-f}}{\overset{k_f}{\rightleftharpoons}} X_f + Q \\
&X_f \underset{k_{(f+1)}}{\overset{k_{(f+1)}}{\rightleftharpoons}} \cdots \underset{k_{-\beta}}{\overset{k_\beta}{\rightleftharpoons}} X_\beta \underset{k_{-(\beta+1)}}{\overset{k_{(\beta+1)}}{\rightleftharpoons}} \cdots \underset{k_{(g-1)}}{\overset{k_{(g-1)}}{\rightleftharpoons}} X_{g-1} \\
&B + X_{g-1} \underset{k_{-g}}{\overset{k_g}{\rightleftharpoons}} X_g \underset{k_{-(g+1)}}{\overset{k_{(g-1)}}{\rightleftharpoons}} \cdots \underset{k_{-\gamma}}{\overset{k_\gamma}{\rightleftharpoons}} X_\gamma \underset{k_{-(\gamma+1)}}{\overset{k_{(\gamma+1)}}{\rightleftharpoons}} \\
&\qquad \cdots \underset{k_{-n}}{\overset{k_n}{\rightleftharpoons}} X_n \underset{k_{-(n+1)}}{\overset{k_{-(n+1)}}{\rightleftharpoons}} E + R \qquad (28)
\end{aligned}$$

(18) For a recent discussion of such systems see A. E. Braunstein in "The Enzymes," ed. by P. D. Boyer, H. Lardy and K. Myrbäck, 2nd. ed., Vol. 2, Chapter 6, p. 113, Academic Press, Inc., New York, N. Y., 1960.

(19) A. E. Braunstein and M. G. Kritzmann, *Enzymol.*, **2**, 129 (1937).

(20) H. C. Lichstein, I. C. Gunsalus and W. W. Umbreit, *J. Biol. Chem.*, **161**, 311 (1945).

(21) S. Darling, *Acta Physiol. Scand.*, **10**, 150 (1945).

The various intermediates X_β are to be regarded as isomeric forms of the aminated enzyme if E is a transaminase. The steady state rate law for the above scheme can be written in a form analogous to those discussed previously.

$$v = \frac{(V_{AB}/K_{AB})(A)(B) - (V_{QR}/K_{QR})(Q)(R)}{\frac{(A)}{K_A} + \frac{(B)}{K_B} + \frac{(Q)}{K_Q} + \frac{(R)}{K_R} + \frac{(A)(B)}{K_{AB}} + \frac{(Q)(R)}{K_{QR}} + \frac{(A)(Q)}{K_{AQ}} + \frac{(B)(R)}{K_{BR}} + \frac{(B)(Q)}{K_{BQ}} + \frac{(A)(B)(Q)}{K_{ABQ}} + \frac{(B)(Q)(R)}{K_{BQR}}} \quad (29)$$

The kinetic parameters appearing in eq. 29 have the definitions

$$V_{AB} = \frac{(E)_0}{\sum_{\alpha=1}^{f-1}\sum_{s=\alpha}^{f-1}\frac{K_\alpha^s}{k_{(s+1)}} + \sum_{\beta=f}^{g-1}\sum_{s-\beta}^{g-2}\frac{K_\beta^s}{k_{(s+1)}} + \sum_{\gamma=g}^{n}\sum_{s=\gamma}^{n}\frac{K_\gamma^s}{k_{(s+1)}}} \quad (30a)$$

$$V_{QR} = \frac{(E)_0}{\sum_{\alpha=1}^{f-1}\sum_{s=0}^{\alpha-1}\frac{K_\alpha^s}{k_{(s+1)}} + \sum_{\beta=f}^{g-1}\sum_{s=f}^{\beta-1}\frac{K_\beta^s}{k_{(s+1)}} + \sum_{\gamma=g}^{n}\sum_{s=g-1}^{\gamma-1}\frac{K_\gamma^s}{k_{(s+1)}}} \quad (30b)$$

$$K_A = \frac{1}{\sum_{\beta=f}^{g-1}\sum_{s=g-1}^{n}\frac{K_\beta^s}{k_{(s+1)}}} \quad (31a)$$

$$K_B = \frac{1}{\sum_{s=0}^{f-1}\frac{K_0^s}{k_{(s+1)}}} \quad (31b)$$

$$K_R = \frac{1}{K_0^{n+1}\sum_{\beta-f}^{g-1}\sum_{s=0}^{f-1}\frac{K_\beta^s}{k_{(s+1)}}} \quad (31c)$$

$$K_Q = \frac{1}{\sum_{s=g-1}^{n}\frac{K_0^s}{k_{(s+1)}}} \quad (31d)$$

$$K_{AB} = \frac{1}{\sum_{\alpha=1}^{f-1}\sum_{s=\alpha}^{f-1}\frac{K_\alpha^s}{k_{(s+1)}} + \sum_{\beta=f}^{g-1}\sum_{s=\beta}^{g-2}\frac{K_\beta^s}{k_{(s+1)}} + \sum_{\gamma=g}^{n}\sum_{s=\gamma}^{n}\frac{K_\gamma^s}{k_{(s+1)}}} \quad (32a)$$

$$K_{QR} = \frac{1}{K_0^{n+1}\left[\sum_{\alpha=1}^{f-1}\sum_{s=0}^{\alpha-1}\frac{K_\alpha^s}{k_{(s+1)}} + \sum_{\beta=f}^{g-1}\sum_{s=0}^{\beta-1}\frac{K_\beta^s}{k_{(s+1)}} + \sum_{\gamma=g}^{n}\sum_{s=g-1}^{\gamma-1}\frac{K_\gamma^s}{k_{(s+1)}}\right]} \quad (32b)$$

$$K_{AQ} = \frac{1}{\sum_{\alpha=1}^{f-1}\sum_{s=g-1}^{n}\frac{K_\alpha^s}{k_{(s+1)}}} \quad (32c)$$

$$K_{BR} = \frac{1}{K_0^{n+1}\sum_{\gamma=g}^{n}\sum_{s=0}^{f-1}\frac{K_\gamma^s}{k_{(s+1)}}} \quad (32d)$$

$$K_{BQ} = \frac{1}{\sum_{s=f}^{g-2}\frac{K_0^s}{k_{(s+1)}}} \quad (32e)$$

$$K_{ABQ} = \frac{1}{\sum_{\alpha=1}^{f-1}\sum_{s,=f}^{g-2}\frac{K_\alpha^s}{k_{(s+1)}}} \quad (33a)$$

$$K_{BQR} = \frac{1}{K_0^{n+1}\sum_{\gamma,=g}^{n}\sum_{s=f}^{g-2}\frac{K_\alpha^s}{k_{(s+1)}}} \quad (33b)$$

When $v=0$, *i.e.*, the system is at equilibrium, eq. 10 is again recovered. However, from eq. 30a and 32a it follows that

$$V_{AB}/K_{AB} = (E)_0 \quad (34a)$$

From eq. 30b and 32b, we obtain

$$V_{QR}/K_{QR} = (E)_0 K_0^{n+1} \quad (34b)$$

which is of course directly deducible from eq. 10 and eq. 34a.

There are several other relations connecting the kinetic parameters defined above. From eq. 31d, 32c and 32e, it is seen that K_{ABQ} as defined by eq. 33a can be expressed as

$$K_{ABQ} = K_{AQ}K_{BQ}/K_Q \quad (35a)$$

The symmetry of the reaction scheme ensures that

$$K_{BQR} = K_{BQ}K_{BR}/K_B \quad (35b)$$

Moreover, from eq. 31a–31d it follows that

$$K_AK_B/K_QK_R = K_0^{n+1} = V_{QR}K_{AB}/V_{AB}K_{QR} \quad (36)$$

Lower limits for the various rate constants appearing in 28 can be readily obtained. From eq. 31b it follows that

$$k_1 \geqslant K_B \quad (37a)$$

with the equality holding if $f=1$, *i.e.*, no intermediates are at significant concentration levels during the steady state in the conversion of A to Q. From equation 31d, we have the result that

$$k_{-(n+1)} \geqslant K_QK_0^{n+1} = K_Q/K_{eq} \quad (37b)$$

with the equality appropriate if $g - 1 = n$ or there are no kinetically important intermediates (in the above sense) for the conversion of B to R. For the other two bimolecular rate constants, the following relations can be given

$$k_g \geqslant K_A \quad (38a)$$

$$k_{-f} \geqslant K_RK_0^{n+1} = K_R/K_{eq} \quad (38b)$$

The equality applies in (38a) if $f = g - 1 = n$. In the example cited above the latter condition is equivalent to the *mechanism* for the transfer of the amino group from the enzyme to R by reaction with B being describable by the half-reaction in eq. 27b. The equality in 38b holds if $f = g - 1 = 1$ which is equivalent to half-reaction 27a depicting the *mechanism* for A to Q interconversion.

It should be noted that relations 37a–38b are identical to 12a–13b if cognizance is taken of eq.

34a and 34b which are peculiar to this type of system. When $f = g - 1 = n = 1$, then eq. 27a and 27b can be taken to represent the mechanism of reaction. Under these circumstances all the kinetic parameters defined in eq. 30–33 are infinite[22] save K_A, K_B, K_Q and K_R. The only reactions to be considered are bimolecular, and their rate constants are given by 37a–38b. The rate law from eq. 29 becomes

$$v = \frac{(E)_0 [(A)(B) - ((Q)(R)/K_{eq})]}{\frac{(A)}{K_A} + \frac{(B)}{K_B} + \frac{(Q)}{K_Q} + \frac{(R)}{K_R}} \quad (39)$$

with the four kinetic parameters related to the equilibrium constant through eq. 36.

For more complex mechanisms with unimolecular steps for the interconversion of species, lower limits for these specific rate constants in the forward and reverse directions are given by 15a and 15b, respectively.

The steady state velocity in the forward direction (v_f) when (Q) = (R) = 0 follows from eq. 29

$$\frac{1}{v_f} = \frac{1}{V_{AB}} + \frac{1}{(E)_0 K_B (A)} + \frac{1}{(E)_0 K_A (B)} \quad (40a)$$

For the reverse direction, the symmetric expression can be written for v_r when (A) = (B) = 0

$$\frac{1}{v_r} = \frac{1}{V_{QR}} + \frac{1}{(E)_0 K_0^{n+1} K_R (Q)} + \frac{1}{(E)_0 K_0^{n+1} K_Q (R)} \quad (40b)$$

In obtaining the above expressions use has been made of eq. 34a and 34b. By comparison of these relations with similar ones derived from eq. 39, it can be seen that the mere demonstration of saturation effects (through the presence of concentration independent terms, *i.e.* $1/V_{AB}$ and $1/V_{QR}$ in the reciprocal rate laws) is sufficient to preclude a mechanism of reaction representable by eq. 27a and 27b.

The form of the reciprocal rate laws indicate that K_{AB} and K_{QR} are not determinable from steady state studies in contrast to the cases discussed previously. However, they can be calculated from the maximum velocities V_{AB} and V_{QR} by eq. 34a and 34b, and the latter are readily obtainable from the usual double reciprocal plots. In view of the foregoing relations between the kinetic parameters, eq. 34a–36, only nine of the kinetic parameters appearing in eq. 29 need be determined to fix the complete set of thirteen.

For reactions of this type questions about the order of reaction of the species do not arise. However, it should be pointed out that a parameter such as K_{ABQ} can in principle be determined from initial steady state velocity studies in the forward direction with only one product, *e.g.*, Q, present.

There is a simple distinction between the kinetics of transaminase-like reactions and the dehydrogenase systems discussed previously provided by the reciprocal steady state rate law. Comparing eq. 40a with eq. 20a, it is seen that the latter differs from the former by the presence of a term in $1/(A)\cdot(B)$ (or analogously $1/(Q)(R)$ for the reverse reaction).

(22) We adopt the convention of equating to zero all sums which run from a higher to a lower value of the stated index—a procedure which has been used implicitly elsewhere in the text.

It is of some interest that a criterion can be developed for kinetically significant isomers of an aminated enzyme. From eq. 31a–31d and 32a–32d after some algebraic manipulation the following relation can be obtained

$$K_A K_B / K_{AB} + K_Q K_R / K_{QR} \gtrless K_A K_Q / K_{AQ} + K_B K_R / K_{BR} \quad (41)$$

The equality applies if $f = g - 1$ or $X_f = X_{g-1}$ in the reaction scheme 28.

Conclusion

The preceding treatment has been directed toward the development for enzymatic reactions of general steady-state rate laws whose validity is not contingent on the presumption of the presence of a specific number of intermediates. Each of the kinetic parameters introduced is a coefficient of a term in the rate law which depends in a unique manner on the concentrations of the reactants and the products. Consequently, steady state studies alone suffice in principle to fix the magnitudes of these parameters. In practice the determination of many of them will require very high precision in the rate measurements.

Quite generally it is found that certain relations must exist between the kinetic parameters so all cannot be regarded as independent. For ordered paths of reaction with all the reactants combining with the enzyme before dissociation of any products, the total number of independent parameters appears to be given by two times the number of reactant and product species. For example, there are four independent kinetic parameters for the system involving one reactant and one product species[2] while there are eight for systems with two reactants and two products as shown above. This rule applies as well for a system involving one reactant and two products, *e.g.*, a typical hydrolytic reaction where there are six independent parameters.[23]

The lower limits for the rate constants are, as indicated above, directly determinable from the usual double reciprocal plots. The limits applying to the bimolecular rate constants are of particular interest in view of comparisons which are possible with theoretical upper limits[24] derived from the theory of diffusion-controlled reactions.[25] The magnitudes of these lower limits for a number of association reactions of substrates with dehydrogenases along with other quantitative aspects of these enzymatic reactions are taken up in the succeeding article.[3]

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(23) Further general rate laws with the appropriate definitions of kinetic parameters as well as lower limits for rate constants can be found in the doctoral dissertation of V. Bloomfield.

(24) R. A. Alberty and G. G. Hammes, *J. Phys. Chem.*, **62**, 154 (1958).

(25) For some recent discussions of the application of the theory of diffusion-controlled reactions to various areas of solution kinetics including enzyme kinetics see Chapters 5, 7, 9 by R. M. Noyes, A. Weller and L. Peller and R. A. Alberty respectively in "Progress in Reaction Kinetics," Vol. I, ed. G. Porter, Pergamon Press, Oxford, 1961.