and obtain 7 μM. Their experiments also indicate that at 25° the value of D_{red} is greater than at 4°. Calculations such as described above show that if DPNH is the only substrate appreciably bound such a value for D_{red} is too large to account for the increase in K_{app}/K_{eq} which is observed. A value of D_{red} of 0.8 μM would be required if this were the case. As pointed out by Neilands[6] the low value of the Michaelis constant for pyruvate suggests that this substance is also strongly bound. If it is assumed that the dissociation constant for pyruvate is the same as that for DPNH (that is, 7 μM) the values of K_{app}/K_{eq} calculated with equations (15)–(17) are in agreement with the experimental values. If the binding of DPN is negligible the value of K_{app}/K_{eq} would be expected to be proportional to the enzyme concentration squared at higher enzyme concentrations.

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The Relationship between Michaelis Constants, Maximum Velocities and the Equilibrium Constant for an Enzyme-catalyzed Reaction

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In the case of reversible reactions which are catalyzed by enzymes the Michaelis constants and maximum velocities may be determined for both the forward and reverse reactions. The steady state treatment of a number of commonly used mechanisms has been used to obtain the equations for the initial rates of the forward and reverse reactions. It is possible to show that for these mechanisms there is a relationship between the various kinetic constants and the equilibrium constant for the over-all reaction. These relations offer a means of testing the suitability of a particular mechanism for an enzyme-catalyzed reaction. These equations also call attention to important new kinetic constants.

In the case of enzyme-catalyzed reactions for which the equilibrium is not displaced strongly in favor of reactants or products it is possible to determine the maximum velocities and Michaelis constants for both the forward and reverse reactions. The question then arises as to whether the values of these constants are independent of the equilibrium constant for the over-all reaction under the same conditions. Haldane[1] was the first to show that such relations exist. As shown in the preceding paper[2] the Haldane relationship is satisfied by the fumarase reaction. The purpose of the present article is to discuss such relationships for more complicated reactions and mechanisms.

The objective of completely describing the kinetics of a reaction requires the determination of the mechanism and the individual rate constants. In the absence of means for directly studying the intermediate complexes in an enzymatic reaction this objective is not generally attained and the maximum amount of information must be obtained from a study of the kinetics of the over-all reaction; that is, the rate of disappearance of substrate or appearance of product when the reaction is in a nearly steady state. The problem is then to interpret the constants obtainable for the over-all reaction in terms of the individual rate constants of the appropriate mechanism. The system of differential equations for a particular mechanism may be solved in a perfectly general way,[3] but frequently one of two approximations is satisfactory. The first is that made by Michaelis and Menten[4] who assumed that the enzyme–substrate complex is in rapid equilibrium with free substrate and enzyme. A less restrictive assumption is that introduced to enzyme kinetics by Briggs and Haldane[5] who assumed that the enzyme–substrate complex may be considered to be in a steady state; that is, the rate of formation of the complex is equal to its rate of decomposition. It should be pointed out that the steady state assumption used in enzyme kinetics is somewhat different from the assumption of unstable intermediates frequently used in kinetics[6] since the usual corollary of the latter assumption is that an appreciable fraction of *none* of the reactants is in the form of the intermediate. In the case of enzymatic mechanisms it is generally assumed that an appreciable fraction of the enzyme, but of no other reactant, may be in the form of an intermediate complex. A number of mechanisms for reversible enzymatic reactions may not be treated by the rapid equilibrium method since contradictory assumptions would be involved in treating the forward and reverse reactions. Therefore, in the following discussion only the more general steady state method[7] will be used except in

(1) J. B. S. Haldane, "Enzymes," Longman's, Green and Co., London, 1930, pp. 80–82.

(2) R. M. Bock and R. A. Alberty, THIS JOURNAL, **75**, 1921 (1953).

(3) B. Chance, *J. Biol. Chem.*, **151**, 553 (1943); B. Chance, D. S. Greenstein, J. Higgins and C. C. Yang, *Arch. Biochem. Biophys.*, **37**, 332 (1952).

(4) L. Michaelis and M. L. Menten, *Biochem. Z.*, **49**, 333 (1913).

(5) G. E. Briggs and J. B. S. Haldane, *Biochem. J.*, **19**, 338 (1925).

(6) L. P. Hammett, "Physical Organic Chemistry," McGraw-Hill Book Co., New York, N. Y., 1940, p. 105.

(7) In order to avoid details in subsequent discussions the following description of the steady state method is given: The rate equations for the n enzyme–substrate complexes involved in the mechanism are written and the rates of change for the concentrations of these complexes are set equal to zero. The concentration of free enzyme is eliminated by use of $[E] = [E]_0 - \sum_{i=1}^{i=n} [ES_i]$, where $[E]_0$ is the total concentration of the enzyme. The resulting n equations in n un-

the case of the most complicated mechanisms.

The relationship between the various kinetic constants for a mechanism and the equilibrium constant for the over-all reaction presents a means of testing the suitability of a particular mechanism. The following list of mechanisms is far from complete but is suggestive of the types of relations which are encountered.

Mechanisms for Enzymatic Reactions Involving Two Reactants and Two Products.—A large number of enzymatic reactions involve a coenzyme or an acceptor so that the over-all reaction which is catalyzed may be represented by

$$A + B \rightleftarrows C + D \qquad (I)$$

where B and D, for example, may be oxidized and reduced coenzyme. A quite general mechanism for the enzymatic catalysis of such a reaction is

1. $E + A \rightleftarrows EA$
2. $E + B \rightleftarrows EB$
3. $EA + B \rightleftarrows EAB$
4. $EB + A \rightleftarrows EAB$
5. $EAB \underset{k_2}{\overset{k_1}{\rightleftarrows}} ECD$
6. $ECD \rightleftarrows EC + D$ (I,1)
7. $ECD \rightleftarrows ED + C$
8. $EC \rightleftarrows E + C$
9. $ED \rightleftarrows E + D$

The equilibrium constants for the successive steps, expressed as dissociation constants, will be referred to as K_1, K_2, ..., K_9. These dissociation constants are not completely independent since

$$K_1K_3 = K_2K_4 \qquad (1)$$
$$K_6K_8 = K_7K_9$$

The steady state treatment of this mechanism does not yield a linear relationship between the reciprocal initial velocity and the reciprocal initial concentrations of substrates. This conclusion is in agreement with that of Segal, Kachmar and Boyer[8] who have given the steady state treatments for a number of mechanisms involving coenzymes or activators.

Simple relationships are obtained, however, in the special case that all the equilibria are adjusted rapidly except for the fifth step. In this special case the initial rate of the forward reaction is given by

$$-\left(\frac{d(A)}{dt}\right)_0 = \frac{V_f}{1 + (K_A/[A]) + (K_B/[B]) + (K_{AB}/[A][B])} \qquad (2)$$

The kinetic constants V_f, K_A, K_B and K_{AB} may be calculated from measurements of the initial reaction rate at various concentrations of A and B. The maximum velocity of the forward reaction which is approached when the concentrations of A and B are both increased is V_f which is equal to $k_1[E]_0$. The Michaelis constant for A, K_A, is equal to the concentration of A at which the initial velocity of the forward reaction is $V_f/2$ when the concentration of B is sufficiently high so that further increasing it will not affect the rate. The Michaelis constant for B, K_B, may be determined in a similar way, and in the present case $K_A = K_4$ and $K_B = K_3$. Equation (2) is the most general rate equation which will appear in this article and all subsequent rate equations may be considered to be special cases.

The fourth kinetic constant, K_{AB}, is of a type which has not been previously described and is equal to K_1K_3 in the present case. This constant may be calculated by several different methods from kinetic data at concentrations of A and B insufficient to saturate the enzyme. For example, if the concentration of B is held constant at a value $[B]_0$ which is of the order of magnitude of K_B while the concentration of A is varied, equation (2) may be written

$$-\left(\frac{d(A)}{dt}\right)_0 = \frac{V_f'}{1 + K_A'/[A]} \qquad (3)$$

where V_f', the apparent maximum velocity, is $V_f/(1 + K_B/[B]_0)$ and K_A', the apparent Michaelis constant for A, is $(K_A[B]_0 + K_{AB})/(K_B + [B]_0)$ which may be obtained as the ratio of slope to intercept of a plot according to the method of Lineweaver and Burk.[9] Solving for K_{AB} yields

$$K_{AB} = K_A'K_B + [B]_0(K_A' - K_A) \qquad (4)$$

An alternative procedure would be to determine the slopes of Lineweaver–Burk plots for two different initial concentrations of B. Since the slope is equal to $(K_A + K_{AB}/[B]_0)/V_f$ two simultaneous equations may be solved for K_A and K_{AB}.

The values of K_1 ... K_4 may be calculated from the values of K_A, K_B, K_{AB} and equation (1). The rate equation for the reverse reaction is analogous to equation (2) and $V_r = k_2[E]_0$, $K_C = K_7$, $K_D = K_6$, and $K_{CD} = K_6K_8$ so that K_6 ... K_9 may also be obtained.

If V_f and V_r are determined at the same total enzyme concentration,[10] it may be shown that certain of the kinetic constants are related to the equilibrium constant for the over-all reaction, K_{eq}

$$K_{eq} = V_fK_{CD}/V_rK_{AB} \qquad (5)$$

This equation may be readily verified by substituting the values of the kinetic constants in terms of dissociation constants and individual rate constants for mechanism (I,1).

A further simplification of equation (2) is possible in the special case that the affinity of the enzyme for A is uninfluenced by the presence of B, etc. In this case $K_1 = K_4 = K_A$, $K_2 = K_3 = K_B$. $K_7 = K_9 = K_C$ and $K_6 = K_9 = K_D$ so that for the forward reaction

$$-\left(\frac{d(A)}{dt}\right)_0 = \frac{V_f}{(1 + (K_A/[A]))(1 + (K_B/[B]))} \qquad (6)$$

This equation is identical with the equation obtained by Laidler and Socquet[11] by considering

known complex concentrations are then solved simultaneously. The expressions for the concentrations of the various complexes are then substituted into the rate expression for *any* reactant or product since as a consequence of the steady state approximation the rate of disappearance of any reactant is equal to the rate of appearance of any product. It is assumed that the enzyme concentration is very small so that the amount of substrate in enzyme–substrate complexes may be neglected. The expression for the *initial* reaction velocity is obtained by setting all concentrations of products equal to zero in the rate expression.

(8) H. L. Segal, J. F. Kachmar and P. D. Boyer, *Enzymologia*, **15**, 187 (1952).

(9) H. Lineweaver and D. Burk, THIS JOURNAL, **56**, 658 (1934).

(10) This will also be assumed in all subsequent discussions.

(11) K. L. Laidler and I. M. Socquet, *J. Phys. Colloid Chem.*, **54**, 519 (1950).

adsorption of the two substrates on two types of sites on the enzyme for the case that there is no competition between the substrates and the enzyme concentration is negligibly small. Schwert and Hakala[12] have discussed the determination of K_A and K_B for reactions for which equation (6) applies. An analogous equation holds for the reverse reaction. In this case the various kinetic constants are related to the equilibrium constant for the over-all reaction by

$$K_{eq} = V_f K_C K_D / V_r K_A K_B \qquad (7)$$

Mechanism Involving Four Complexes.—If the various substrates combine and dissociate in a certain order, mechanism (I,1) may be simplified to

$$\begin{gathered} E + A \underset{k_2}{\overset{k_1}{\rightleftharpoons}} EA \qquad (I,2) \\ EA + B \underset{k_4}{\overset{k_3}{\rightleftharpoons}} EAB \underset{k_6}{\overset{k_5}{\rightleftharpoons}} ECD \underset{k_8}{\overset{k_7}{\rightleftharpoons}} ED + C \\ ED \underset{k_{10}}{\overset{k_9}{\rightleftharpoons}} E + D \end{gathered}$$

The steady state treatment of this mechanism yields equation (2) for the initial velocity of the forward reaction in which $V_f = k_7[E]_0/F$, $K_A = k_4k_7/k_1k_5F$, $K_B = G/k_3F$, $K_{AB} = k_2G/k_1k_3k_5F$, $F = k_3(k_6 + k_7)/k_1k_5 + k_7/k_9 + 1$, and $G = k_4k_6 + k_4k_7 + k_5k_7$. Similarly for the reverse reaction $V_r = k_4[E]_0/H$, $K_C = G/k_6k_8H$, $K_D = k_4k_7/k_{10}H$, $K_{CD} = k_9G/k_6k_8k_{10}H$ and $H = k_8(k_4 + k_5)/k_6k_{10} + k_4/k_2 + 1$. Four of these kinetic constants are related to the equilibrium constant for the over-all reaction by equation (5). Thus it is seen that the over-all kinetics for this mechanism are indistinguishable from those for the preceding case.

Mechanism Involving Three Complexes.—If there is but a single ternary complex mechanism (I,2) may be simplified to

$$\begin{gathered} E + A \underset{k_2}{\overset{k_1}{\rightleftharpoons}} EA \qquad (I,3) \\ EA + B \underset{k_4}{\overset{k_3}{\rightleftharpoons}} EXY \underset{k_6}{\overset{k_5}{\rightleftharpoons}} EC + D \\ EC \underset{k_8}{\overset{k_7}{\rightleftharpoons}} E + C \end{gathered}$$

where the ternary complex has been represented by the indefinite EXY since the nature of this complex is not important from a kinetic standpoint. Although the same is true of EA and EC it is convenient to write them as they are to avoid confusion. The expressions for the initial rates of the forward and reverse reactions are of the type indicated in equation (2), and the over-all kinetic constants are given by

$$\begin{aligned} V_f &= k_5k_7[E]_0/(k_5 + k_7) \\ K_A &= k_5k_7/k_1(k_5 + k_7) \\ K_B &= k_7(k_4 + k_5)/k_3(k_5 + k_7) \\ K_{AB} &= k_2k_7(k_4 + k_5)/k_1k_3(k_5 + k_7) \qquad (8) \\ V_r &= k_2k_4[E]_0/(k_5 + k_7) \\ K_C &= k_2k_4/k_8(k_2 + k_4) \\ K_D &= k_2(k_4 + k_5)/k_6(k_2 + k_4) \\ K_{CD} &= k_2k_7(k_4 + k_5)/k_6k_8(k_2 + k_4) \end{aligned}$$

If EXY dissociates directly into products rather than in two successive steps the relations for the various kinetic constants may be obtained by putting $k_7 \gg k_5$ for the forward reaction or as $k_2 \gg k_4$ for the reverse reaction.

Four of these kinetic constants are related by equation (5). Since mechanisms (I,1) (rapid equilibrium case), (I,2) and (I,3) yield the same rate equation and four of the kinetic constants are related in the same way to the equilibrium constant for the over-all reaction, it is not possible to distinguish between them with only measurements of over-all kinetics, and so the simplest of the three mechanisms should be used.

Mechanism Involving Two Complexes.—If in the preceding mechanism $k_5 \gg k_7$ and $k_4 \gg k_2$ the same relations are obtained as if

$$\begin{gathered} E + A \underset{k_2}{\overset{k_1}{\rightleftharpoons}} EA \qquad (I,4) \\ EA + B \underset{k_4}{\overset{k_3}{\rightleftharpoons}} EC + D \\ EC \underset{k_6}{\overset{k_5}{\rightleftharpoons}} E + C \end{gathered}$$

Theorell and Chance[13] have applied this mechanism in the case of the reaction catalyzed by liver alcohol dehydrogenase and have given the steady state treatment. The initial velocity of the forward reaction is expressed by equation (2) where $V_f = k_5[E]_0$, $K_A = k_5/k_1$, $K_B = k_5/k_3$, and $K_{AB} = k_2k_5/k_1k_3$. Similarly for the reverse reaction $V_r = k_2[E]_0$, $K_C = k_2/k_6$, $K_D = k_2/k_4$ and $K_{CD} = k_2k_5/k_4k_6$. As pointed out by Theorell and Chance,[13] the Michaelis constant for A determined in the presence of a high concentration of B depends upon the rate of dissociation of C from EC, and not at all upon the rate of dissociation of A from EA. The equilibrium constant for the over-all reaction is related to K_{AB} and K_{CD} by equation (5), but in the case of the present mechanism there is another relation not satisfied by other mechanisms which are discussed, namely

$$K_{eq} = V_f^3 K_C K_D / V_r^3 K_A K_B \qquad (9)$$

Mechanism Involving a Single Complex.—The simplest, but probably least likely, mechanism for reaction (I) is

$$E + A + B \underset{k_2}{\overset{k_1}{\rightleftharpoons}} EXY \underset{k_4}{\overset{k_3}{\rightleftharpoons}} E + C + D \qquad (I,5)$$

The steady state treatment for this mechanism yields the following equation for the initial velocity of the forward reaction.

$$-\left(\frac{d(A)}{dt}\right)_0 = \frac{V_f}{1 + (K_{AB}/[A][B])} \qquad (10)$$

where $V_f = k_3[E]_0$ and $K_{AB} = (k_2 + k_3)/k_1$. Thus the apparent Michaelis constant for A obtained by holding the concentration of B constant for a series of experiments in which the concentration of A is varied will be inversely proportional to the

(12) G. W. Schwert and M. T. Hakala, *Arch. Biochem. Biophys.*, **38**, 57 (1952).

(13) H. Theorell and B. Chance, *Acta Chem. Scand.*, **5**, 1127 (1951).

TABLE I

COMPARISON OF EQUILIBRIUM CONSTANTS FOR THE ALCOHOL DEHYDROGENASE REACTION AT 20° WITH THOSE CALCULATED FROM KINETIC CONSTANTS

pH	K_{eq},[a] Exptl.	K_{eq}, calcd. Eq. (7)	Eq. (14)	Eq. (9)	V_f/V_r
7.0	1.11×10^{-4}	39×10^{-4}	1.07×10^{-4}	0.030×10^{-4}	0.029
8.0	0.71×10^{-3}	5.5×10^{-3}	0.49×10^{-3}	$.040 \times 10^{-3}$	0.088
9.0	1.06×10^{-2}	21×10^{-2}	4.0×10^{-2}	$.75 \times 10^{-2}$	0.19
10.0	0.90×10^{-1}	1.2×10^{-1}	1.5×10^{-1}	1.8×10^{-1}	1.2

[a] The average value for the lowest enzyme concentration is given.

concentration of B. An equation similar to (10) applies for the reverse reaction where $V_r = k_2[E]_0$ and $K_{CD} = (k_2 + k_3)/k_4$. These kinetic constants are related to the equilibrium constant by equation (5). The same relation would be obtained if there were more intermediate complexes of the same composition as EXY.

Mechanisms in which One Substrate Can React with the Enzyme to Form a Product.—In the following mechanism the enzyme might, for example, be alternately oxidized or reduced.

$$E + A \underset{k_2}{\overset{k_1}{\rightleftharpoons}} E' + C \qquad (I.6)$$

$$E' + B \underset{k_4}{\overset{k_3}{\rightleftharpoons}} E + D$$

The initial rate of the forward reaction is given by

$$-\left(\frac{d(A)}{dt}\right)_0 = \frac{V_f}{(K_A/[A]) + (K_B/[B])} \qquad (11)$$

where $V_f = k_1k_3[E]_0$, $K_A = k_3$ and $K_B = k_1$. In this case V_f is not the velocity which is approached as the concentrations of A and B are increased. If the initial velocity is measured at concentrations of A at which $K_A/[A] \gg K_B/[B]$, equation (11) becomes $-(d[A]/dt)_0 = V_f[A]/K_A$ so that V_f/K_A may be determined. The quantities V_f/K_B, V_r/K_C and V_r/K_D may also be determined since a similar expression applies to the initial velocity of the reverse reaction for which $V_r = k_2k_4[E]_0$, $K_C = k_4$ and $K_D = k_2$. These kinetic constants are related to the equilibrium constant for the over-all reaction by

$$K_{eq} = V_f/V_r = K_AK_B/K_CK_D \qquad (12)$$

An interesting feature of this mechanism is that since $V_f/K_A = k_1[E]_0$, $V_f/K_B = k_3[E]_0$, $V_r/K_C = k_2[E]_0$ and $V_r/K_D = k_4[E]_0$, the individual rate constants may be determined if the molar concentration of the enzyme is known. The usual Michaelis–Menten behavior will be shown by this mechanism if, for example, the initial concentration of B is held constant for a series of experiments in which the concentration of A is varied over a wide range. In this case equation (3) applies with $V_f' = V_f[B]_0/K_B$ and $K_A' = K_A[B]_0/K_B$.

If there is an enzyme–substrate intermediate in both steps of the preceding mechanism, it becomes

$$E + A \underset{k_2}{\overset{k_1}{\rightleftharpoons}} EA \underset{k_4}{\overset{k_3}{\rightleftharpoons}} E' + C \qquad (I,7)[14]$$

$$E' + B \underset{k_6}{\overset{k_5}{\rightleftharpoons}} ED \underset{k_8}{\overset{k_7}{\rightleftharpoons}} E + D$$

(14) The author is indebted to Dr. G. W. Schwert for suggesting this mechanism.

The steady state treatment yields the following relation for the initial velocity of the forward reaction.

$$-\left(\frac{d(A)}{dt}\right)_0 = \frac{V_f}{1 + (K_A/[A]) + (K_B/[B])} \qquad (13)$$

where $V_f = k_3k_7[E]_0/(k_3 + k_7)$, $K_A = k_7(k_2 + k_3)/k_1(k_3 + k_7)$, and $K_B = k_3(k_6 + k_7)/k_5(k_3 + k_7)$. A similar relation is obtained for the reverse reaction with $V_r = k_2k_6[E]_0/(k_2 + k_6)$, $K_D = k_2(k_6 + k_7)/k_8(k_2 + k_6)$ and $K_C = k_6(k_2 + k_3)/k_4(k_2 + k_6)$. It is easily verified that these kinetic constants are related to the equilibrium constant for the over-all reaction by the equation

$$K_{eq} = V_f^2K_CK_D/V_r^2K_AK_B \qquad (14)$$

Application to the Alcohol Dehydrogenase Reaction.—The only reaction of type (I) for which all four Michaelis constants as well as the forward and reverse maximum velocities have been determined appears to be the alcohol dehydrogenase reaction studied by Theorell and Bonnichsen[15] and Theorell and Chance.[13] Their best values of the kinetic constants at *p*H 7, 8, 9 and 10 have been used to calculate equilibrium constants using equations (7), (14) and (9). For this purpose the equilibrium constant is defined by

$$K_{eq} = [\text{aldehyde}][\text{DPNH}]/[\text{alcohol}][\text{DPN}] \qquad (15)$$

These calculated values may be compared with the actual values in Table I.

Since equations (7), (14) and (9) differ only in the power of the V_f/V_r ratio the differences between the results for the various equations will be greatest under conditions where this ratio is considerably different from unity. At *p*H 10 this ratio is sufficiently close to unity so that it is not possible to make a choice between the three equations without more accurate values for the kinetic constants. At *p*H 7 and 8 equation (14) gives the value which agrees best with the directly determined equilibrium constant, while at *p*H 9 equation (9) gives the best agreement. Whether there is actually a change in mechanism with *p*H and whether (I, 7) is the mechanism at *p*H 7 and 8 will have to be determined by further experiments.

Mechanisms for Enzymatic Reactions Involving One Reactant and Two Products.—Many enzyme-catalyzed reactions are of the type

$$A \rightleftharpoons C + D \qquad (II)$$

For example, the hydrolysis of esters or peptides in aqueous solutions could be expressed in this way since the concentration of water is high and very nearly constant. Since the treatments of

(15) H. Theorell and R. Bonnichsen, *Acta Chem. Scand.*, **5**, 1105 (1951).

possible mechanisms for this reaction follow closely those for reaction (I) the rate expressions obtained from the steady-state treatment are simply summarized in Table II. In all these cases the initial rate of the forward reaction is given by

$$-\left(\frac{d(A)}{dt}\right)_0 = \frac{V_f}{1 + (K_A/[A])} \qquad (16)$$

Mechanism (II, 1) has been treated only for the case that the conversion to EA to ECD is rate-determining. The kinetic constants for this case are related to the equilibrium constant by

$$K_{eq} = V_f K_{CD}/V_r K_A \qquad (17)$$

TABLE II

RATE EXPRESSIONS FOR THE INITIAL RATES OF THE REVERSE REACTION FOR REACTION (II)

Mechanism	$-(d[C]dt)_0$
(II,1) $E + A \rightleftarrows EA \rightleftarrows ECD \rightleftarrows EC + D$, $ECD \rightleftarrows ED + C$, $ED \rightleftarrows E + D$, $EC \rightleftarrows E + C$	$\frac{V_r{}^a}{1 + \frac{K_C}{[C]} + \frac{K_D}{[D]} + \frac{K_{CD}}{[C][D]}}$
(II,2) $E + A \rightleftarrows EX \rightleftarrows EC + D$, $EC \rightleftarrows E + C$	$\frac{V_r}{1 + \frac{K_C}{[C]} + \frac{K_D}{[D]} + \frac{K_{CD}}{[C][D]}}$
(II,3) $E + A \rightleftarrows EX \rightleftarrows E + C + D$	$\frac{V_r}{1 + (K_{CD}/[C][D])}$
(II,4) $E + A \rightleftarrows EC + D$, $EC \rightleftarrows E + C$	$\frac{V_r}{\frac{K_C}{[C]} + \frac{K_D}{[D]} + \frac{K_{CD}}{[C][D]}}$

[a] This expression is obtained only for the case that the conversion of EA to ECD is rate-determining.

If, in addition, the binding of C is not affected by the binding of D, and *vice versa*

$$K_{eq} = V_f K_C K_D/V_r K_A \qquad (18)$$

This relation is given by Haldane.[16] In the case of the other three mechanisms, equation (17) is also found to apply.

Mechanisms for Enzymatic Reactions Involving One Reactant and One Product.—The simplest type of enzyme-catalyzed reaction may be represented by

$$A \rightleftarrows C \qquad (III)$$

(16) Reference 1, p. 51.

The steady state treatments for the following two mechanisms have been given by Haldane.[1]

$$E + A \underset{k_2}{\overset{k_1}{\rightleftarrows}} EX \underset{k_4}{\overset{k_3}{\rightleftarrows}} E + C \qquad (III,1)$$

$$E + \underset{k_2}{\overset{k_1}{\rightleftarrows}} EX \underset{k_4}{\overset{k_3}{\rightleftarrows}} EY \underset{k_6}{\overset{k_5}{\rightleftarrows}} E + C \qquad (III,2)$$

The over-all kinetic behaviors for these mechanisms are indistinguishable, and the initial rates of the forward and reverse reactions are given by equations of the type of (16). The expressions for the over-all kinetic constants in terms of the individual rate constants are given in the preceding paper.[2] Haldane pointed out that

$$K_{eq} = V_f K_C/V_r K_A \qquad (19)$$

This relation is borne out by the data of the β-glucoside reaction[1] and the fumarase reaction.[2]

The following mechanism involves the reaction of a second molecule of substrate with the enzyme–substrate complex.

$$E + A \underset{k_2}{\overset{k_1}{\rightleftarrows}} EA;\ EA + A \underset{k_4}{\overset{k_3}{\rightleftarrows}} EC + C;\ EC \underset{k_6}{\overset{k_5}{\rightleftarrows}} E + C \qquad (III,3)$$

The initial rate of the forward reaction is given by

$$-\left(\frac{d(A)}{dt}\right)_0 = \frac{V_f}{1 + (K_A/[A]) + (K_A{}^2/[A]^2)} \qquad (20)$$

where $V_f = 2k_5[E]_0$, $K_A = k_5(k_1 + k_3)/k_1k_3$ and $K_{A^2} = k_2k_5/k_1k_3$. A similar expression holds for the reverse reaction in which $V_r = 2k_2[E]_0$, $K_C = k_2(k_4 + k_6)/k_4k_6$ and $K_{C^2} = k_2k_5/k_4k_6$. Thus it can be seen that the equilibrium constant for the over-all reaction is related to these kinetic constants by

$$K_{eq} = [C]/[A] = \sqrt{V_f K_C{}^2/V_r K_A{}^2} \qquad (21)$$

The number of mechanisms which may be invoked in the study of enzymatic reactions is practically limitless but the above mechanisms are suggestive of some general types and the way in which the relation between kinetic constants and equilibrium constants may be used in selecting a suitable mechanism.

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