THE KINETICS OF TERNARY ENZYME COMPLEXES

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Summary

Equations have been derived for the kinetics of transferring enzymes on the assumption that the intermediate complex is ternary (enzyme–donor–acceptor) rather than binary (enzyme–donor). Deductions have been made from these equations which can be compared with the consequences of the most probable form of the binary-complex hypothesis. The two hypotheses lead to the expectation of quite different results in experiments using competing acceptors.

I. Introduction

The classical theory of enzyme action, as developed by Michaelis and Menten (1913), envisages the reaction sequence

enzyme+substrate $\rightleftharpoons$ enzyme–substrate complex $\rightarrow$ products

or, in short notation,

$$E+S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \overset{k_3}{\rightarrow} P.$$

From the rate equation for the steady state of the system it follows that†

$$v=\frac{VS}{S+(k_2+k_3)/k_1}$$
$$=\frac{VS}{S+K_m},$$

where v is the velocity of the enzyme reaction, K_m the Michaelis constant $[=(k_2+k_3)/k_1]$, and V the theoretical maximum velocity when all the enzyme is bound into the intermediate complex $(=k_3E)$. To Lineweaver and Burk (1934) is usually assigned the credit of developing graphical methods for determining V and K_m from kinetic data. The most used method is to invert the Michaelis–Menten equation into the form

$$\frac{1}{v}=\frac{1}{V}+\frac{K_m}{V}.\frac{1}{S}.$$

If the plot of $1/v$ against $1/S$ is linear, then V and K_m can be determined from the graph.

There exists, however, whole classes of enzymes for which it is doubtful whether the simple Michaelis–Menten treatment can be applied. These include the transferring enzymes where an acceptor (A) as well as the substrate (S), otherwise the donor

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† In equations throughout this paper E, S, X, etc. will be used to denote the concentration of the species E, S, X, etc.

(D), is involved. A good deal of evidence, summarized in the following paper (Jermyn 1962), suggests that in this case the reaction sequence passes through a ternary complex, EAD, acceptor and donor then being co-substrates.

Woolf (1929) was the first to investigate such "two-substrate" systems and our present knowledge of them has been summarized by Segal (1959). However, such mathematical treatments as those of Alberty (1953) for what is here called the sequential case and of Ingraham and Makower (1954) for what is here called the reciprocal case have not, in general, been cast in a form readily applicable to such enzymes as the transferring glycosidases. The present paper is an attempt to derive expressions containing quantities easily measured in experimental work with such enzymes in a form that can be used as the basis of empirical tests of mechanisms.

II. Kinetics of Binary Complexes

(a) *Single Acceptor*

The usual picture of the action of transferring enzymes in terms of binary complexes may be illustrated for the specific case of glycosidases, which split the glycosides, GlyOR. A variety of steric and other considerations (Koshland 1953) lead to the belief that the reaction passes through a glycosyl–enzyme intermediate (E–Gly) which then reacts with the acceptor (AOH) to give GlyOA and regenerate the enzyme. This can be cast in a general form, applicable to all transferases, as

$$E+\alpha\beta \underset{k_2}{\overset{k_1}{\rightleftharpoons}} E.\alpha\beta \underset{k_4}{\overset{k_3}{\rightleftharpoons}} \underset{\alpha}{\overset{}{E\beta \; \underset{}{+}}} + \gamma \underset{k_6}{\overset{k_5}{\rightleftharpoons}} E.\beta\gamma \underset{k_8}{\overset{k_7}{\rightleftharpoons}} E+\beta\gamma,$$

where $\alpha\beta$ is the donor and γ the acceptor, and leading to the equation*

$$v = E(k_1k_3k_5k_7.\alpha\beta.\gamma - k_2k_4k_6k_8.\alpha.\beta\gamma)/Z, \tag{1}$$

where

$$\begin{aligned} Z = {} & k_1k_3(k_6+k_7).\alpha\beta + k_2k_4(k_6+k_7)\alpha + k_5k_7(k_2+k_3)\gamma \\ & + k_6k_8(k_2+k_3).\beta\gamma + k_1k_4(k_6+k_7).\alpha\beta.\alpha + k_1k_5(k_3+k_7).\alpha\beta.\gamma \\ & + k_4k_8(k_2+k_6)\alpha.\beta\gamma + k_5k_8(k_2+k_3)\gamma.\beta\gamma. \end{aligned}$$

This equation is that of a reversible reaction and not easily applied to the analysis of data; if certain simplifying assumptions are made, i.e. that the measurements are made under initial conditions where the concentration of the species α and $\beta\gamma$ is zero and that the reaction is irreversible ($k_4 = k_6 = 0$), equation (1) reduces to

$$v = \frac{k_1k_3k_5ADV}{k_1k_3k_7D + k_5k_7(k_2+k_3)A + k_1k_5(k_3+k_7)AD}, \tag{2}$$

where species $\alpha\beta$ is now written as D (donor) and γ as A (acceptor). The condition of irreversibility is fulfilled by the β-glucosidase system that is examined in the following

* For reasons of space, the detailed derivation of the equations in this paper will not be given, but those which do not already occur in the literature in guises that are only formally different (references in Segal 1958) may be obtained from the author.

paper. Under initial conditions of a reversible reaction, i.e. where the only assumption made is that the concentration of the species α and $\beta\gamma$ is zero, the simplified form of equation (1) is the same as equation (2), except that the coefficient of D in the denominator is $k_1k_3(k_6+k_7)$. The following treatment still applies with a little modification. Inverting equation (2)

$$\frac{1}{v}=\frac{1}{V}\left[\frac{k_7(k_2+k_3)}{k_1k_3}\cdot\frac{1}{D}+\frac{k_3+k_7}{k_3}+\frac{k_7}{k_5}\cdot\frac{1}{A}\right]. \tag{3}$$

If A is taken as constant, which is the case, for instance, for hydrolysis in aqueous solution, equation (3) may be written

$$\frac{1}{v}=\frac{1}{V}\left\{\frac{k_3+k_7}{k_3}+\frac{k_7}{k_5A}\right\}+\left\{\frac{k_7(k_2+k_3)}{Vk_1k_3}\right\}\frac{1}{D}. \tag{4}$$

The kinetics are thus formally the same as those of a simple Michaelis complex, with the derived values of V and K_m depending on a different set of constants. The apparent value of the limiting velocity will be equal to

$$\frac{k_3k_5AV}{\{k_3k_7+k_5A(k_3+k_7)\}},$$

where V is defined as the limiting velocity at saturating (infinite) concentrations of both donor and acceptor. The attempt to find the Michaelis constant of the enzyme–donor complex by dividing the value of the intercept of the Lineweaver–Burk line on the axis of $1/v$ into the value of its slope will give a K_m equal to

$$k_3k_5k_7A(k_2+k_3)/\{(k_3+k_7)k_1k_5A+k_1k_3k_7\}.$$

If A is a species other than the solvent, the derived values of V and K_m will thus depend on acceptor concentration, both rising with increase in this concentration.

Equation (3) may also be rewritten as

$$\frac{1}{v}=\frac{1}{V}\left\{\frac{k_3+k_7}{k_3}+\frac{k_7(k_2+k_3)}{k_1k_3D}\right\}+\frac{k_7}{k_5V}\cdot\frac{1}{A}, \tag{5}$$

showing that Michaelis–Menten kinetics also apply to the case of fixed donor concentration and varying acceptor concentration.

If equation (3) and its derivatives are to apply to the irreversible case under conditions other than the initial ones, the further assumption must also be made that $k_8=0$, i.e. that the product ($\beta\gamma$ or P) is not a competitive inhibitor of the enzyme. If this assumption cannot be made then a term

$$\frac{k_8(k_2+k_3)}{k_1k_3}\cdot\frac{P}{D}$$

must be added to the right-hand side of equation (5).

So long as $P \ll D$, this will not lead to serious departures from Michaelis–Menten kinetics but, as in less complex cases of inhibition of enzymes by their products, when $P \doteqdot D$, such kinetics will no longer hold even approximately.

(b) Two Acceptors (irreversible case)

An irreversible statement of the binary-complex hypothesis with competing acceptors may be written

$$E+\alpha\beta \underset{k_2}{\overset{k_1}{\rightleftharpoons}} E.\alpha\beta \xrightarrow{k_3} E.\beta + \alpha \begin{cases} +\gamma_1 \xrightarrow{k_5} E.\beta\gamma_1 \xrightarrow{k_6} E+\beta\gamma_1 \\ +\gamma_2 \xrightarrow{k_7} E.\beta\gamma_2 \xrightarrow{k_8} E+\beta\gamma_2 \end{cases}.$$

The ratio of the velocities of formation of the two products $\beta\gamma_1(P_1)$ and $\beta\gamma_2(P_2)$ is given by

$$\frac{v_1}{v_2} = \frac{k_6.E.\beta\gamma_1}{k_8.E.\beta\gamma_2} = \frac{k_5.E\beta.\gamma_1}{k_7.E\beta.\gamma_2} = \frac{k_5}{k_7}.\frac{\gamma_1}{\gamma_2},$$

so that if the two acceptors are present in concentrations substantially unchanged during the course of the reaction, the ratio of products is given by

$$\frac{P_1}{P_2} = \frac{k_5}{k_7}.\frac{A_1}{A_2} = KA_1/A_2. \tag{6}$$

The ratio of products is thus proportional to the ratio of the acceptors. If one acceptor (A_2) is fixed, as when a competing acceptor is added to an enzymic hydrolysis in aqueous solution, the ratio of products will be proportional to A_1. Since the values of neither k_5 nor k_7 depend on the donor, the ratio of the products is thus independent of both the nature and the concentration of the donor.

The overall velocity is given by

$$v = v_1+v_2$$
$$= \frac{k_1k_3(k_5k_8A_1V_1+k_6k_7A_2V_2)D}{k_1k_5k_8(k_3+k_6)A_1D+k_1k_6k_7(k_3+k_8)A_2D+k_1k_3k_6k_8D+k_5k_6k_8(k_2+k_3)A_1+k_6k_7k_8(k_2+k_3)A_2}, \tag{7}$$

where V_1, V_2 are defined as the maximum velocities in the presence of A_1, A_2 alone. Equation (7) reduces to equation (2) if A_2 is equated to zero. If A_1, A_2 are held constant, equation (7) reduces to an equation of form

$$1/v = K_1+(K_2/D)$$

on inversion so that ordinary Michaelis–Menten kinetics will hold for variations in the concentration of the donor.

The more interesting case is that in which A_2 and D are held constant and A_1 is varied, e.g. the case in which an alternative acceptor is added to a hydrolytic reaction proceeding in aqueous solution. Equation (7) reduces to the form

$$v = (pA_1+q)/(rA_1+S),$$

and the limits of v lie between

$$\frac{k_1k_3k_6k_7A_2V_2D}{k_7k_8(k_2+k_3)A_2+k_1k_3k_8D+k_1k_7(k_3+k_8)A_2D}$$

when $A_1 = 0$, and

$$\frac{k_1 k_3 V_1 D}{k_1 D(k_3+k_5)+k_5(k_2+k_3)}$$

as $A_1 \to \infty$. According to the value of the constants involved, the overall reaction velocity (as measured by the disappearance of donor) can therefore increase or decrease with increasing concentration of the acceptor; also v varies monotonically with A_1 and there are no maxima or minima. Furthermore, since both limits involve the value of D, the amount and direction of the trend will depend on donor concentration.

(c) *Two Acceptors* (*reversible case*)

A formulation of the binary-complex hypothesis including a completely reversible reaction sequence and two acceptors may be written

$$E+P_1 \underset{k_2}{\overset{k_1}{\rightleftharpoons}} EP_1 \underset{k_4}{\overset{k_3}{\rightleftharpoons}} A_1+E_D \begin{cases} +A_2 \underset{k_6}{\overset{k_5}{\rightleftharpoons}} EP_2 \underset{k_8}{\overset{k_7}{\rightleftharpoons}} E+P_2 \\ +A_3 \underset{k_{10}}{\overset{k_9}{\rightleftharpoons}} EP_3 \underset{k_{12}}{\overset{k_{11}}{\rightleftharpoons}} E+P_3 \end{cases},$$

where the donor (D) is now considered as a reaction product (P_1) exactly parallel to the products P_2 and P_3 produced by transfer to the two acceptors A_2 and A_3.

It may be deduced that

$$\frac{v_2}{v_3} = \frac{k_1k_3k_5k_7(k_{10}+k_{11})P_1A_2+k_5k_7k_{10}k_{12}(k_2+k_3)P_3A_2-k_2k_4k_6k_8(k_{10}+k_{11})P_2A_1-k_6k_8k_9k_{11}(k_2+k_3)P_2A_3}{k_1k_3k_9k_{11}(k_6+k_7)P_1A_3+k_6k_8k_9k_{11}(k_2+k_3)P_2A_3-k_2k_4k_{10}k_{12}(k_6+k_7)P_3A_1-k_5k_7k_{10}k_{12}(k_2+k_3)P_3A_2}. \tag{8}$$

If either of the pairs of velocity constants k_8 and k_{12} or k_6 and k_{10} are equated to zero, i.e. P_2 and P_3 have no affinity for the enzyme (first case) or are competitive inhibitors only (second case), equation (8) reduces to the form $v_2/v_3 = KA_2/A_3$ as in the irreversible case, and the reversibility of the rest of the system does not preclude the application of the same conclusions as drawn for that case. The deduction that

$$K = k_5k_7(k_{10}+k_{11})/k_9k_{11}(k_6+k_7),$$

in particular, shows that P_2/P_3 is independent of the *nature* and *concentration* of the donor. For initial conditions, where P_2 and $P_3 = 0$, equation (8) reduces to the same form.

Finally, where the only restriction is that A_2, $A_3 \gg A_1$, i.e. the acceptors are present in excess, the reduced form is

$$\frac{v_2}{v_3} = \frac{(aP_1+bP_3)A_2-cP_2A_3}{-dP_3A_2+(eP_1+fP_2)A_3},$$

and the ratio of the products *will change as the reaction proceeds. The ratio P_2/P_3 is dependent* on both the *nature* and *concentration* of the donor.

The equation for overall reaction velocity (disappearance of P_1) is too long to set out in full but takes the form

$$v = E.\frac{aP_1A_2+bP_1A_3-cP_2A_1-dP_3A_1}{eA_1+fA_2+gA_3+hP_1+iP_2+jP_3+kP_1A_1+lP_1A_2+mP_1A_3+nP_2A_1+oP_2A_2+pP_2A_3+qP_3A_1+rP_3A_2+sP_3A_3}. \tag{9}$$

When A_2 and A_3 are fixed, the following form is obtained for the dependence of v on P_1:

$$v = \frac{aP_1 - A_1(bP_2+cP_3)}{d+eA_1+P_1(f+gA_1)+P_2(h+iA_1)+P_3(k+lA_1)}.$$

For A_3 and P_1 fixed, the form for the dependence of v on A_2 is

$$v = \frac{a+bA_2-A_1(cP_2+dP_3)}{(e+fP_2+gP_3)A_1+(h+iP_2+jP_3)A_2+kP_2+lP_3}.$$

Under initial conditions (A_1, P_2, $P_3 \doteqdot 0$) these reduce to the standard forms

$$v = P_1/(a+bP_1),$$

and

$$v = (a+bA_2)/(c+dA_2).$$

Hence, only by considering initial velocities is it possible to obtain an analysable account of the effect of changing one of the variables on the overall enzymic reaction.

III. Kinetics of Ternary Complexes

(a) *Linear Sequence*

The simplest hypothesis about enzyme action through ternary complex formation is that the formation of the complex must take place in a fixed sequence; i.e. the alternative pictures are

$$E+D \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ED+A \underset{k_4}{\overset{k_3}{\rightleftharpoons}} EDA \overset{k_5}{\rightarrow} P,$$

or

$$E+A \underset{k_2}{\overset{k_1}{\rightleftharpoons}} EA+D \underset{k_4}{\overset{k_3}{\rightleftharpoons}} EAD \overset{k_5}{\rightarrow} P,$$

leading to the equations

$$v = \frac{k_1k_3VAD}{(k_2k_4+k_2k_5)+(k_1k_4+k_1k_5)D+k_3k_5A+k_1k_3AD}, \tag{10a}$$

or

$$v = \frac{k_1k_3VAD}{(k_2k_4+k_2k_5)+(k_1k_4+k_1k_5)A+k_3k_5D+k_2k_3AD}. \tag{10b}$$

Inversion of equation (10a) gives

$$\frac{1}{v} = \frac{1}{V} + \frac{k_5}{k_1V}\cdot\frac{1}{D} + \frac{k_4+k_5}{k_3V}\cdot\frac{1}{A} + \frac{k_2(k_4+k_5)}{k_1k_3V}\cdot\frac{1}{AD}. \tag{11a}$$

As $A \rightarrow \infty$, the situation that occurs for simple hydrolysis in aqueous solution, equation (11a) reduces to

$$v = \frac{1}{V} + \frac{k_5}{k_1V}\cdot\frac{1}{D},$$

which is the form for simple Michaelis–Menten kinetics, except that the apparent K_m is now equal to k_5/k_1 instead of the value $(k_5+k_2)/k_1$ expected when a ternary complex is not assumed.

For finite values of A we have

$$\frac{1}{v}=\frac{1}{V}\cdot\frac{k_3A+k_4+k_5}{k_3A}+\frac{Ak_3k_5+k_2k_4+k_2k_5}{VAk_1k_3}\cdot\frac{1}{D}.$$

The derived values of V and K_m will vary as A varies, apparent V increasing monotonically with increasing A, and

$$K_m=\frac{k_3k_5A+k_2(k_4+k_5)}{k_1(k_3A+k_4+k_5)},$$

varying between the limits k_5/k_1 and k_2/k_1. It is apparent also from equation (11a) that there will be a rectilinear relationship between $1/v$ and $1/A$ when acceptor concentration is varied at a fixed donor concentration, similar to the Michaelis–Menten relationship usually considered only as applying to donors.

Inversion of equation (10b) gives

$$\frac{1}{v}=\frac{1}{V}+\frac{k_4+k_5}{k_3V}\cdot\frac{1}{D}+\frac{k_5}{k_1V}\cdot\frac{1}{A}+\frac{k_2(k_4+k_5)}{k_1k_3V}\cdot\frac{1}{AD}. \tag{11b}$$

As $A\to\infty$ donor $K_m\to(k_4+k_5)/k_3$, a form identical with the original Michaelis–Menten formulation. At finite values of A,

$$K_m=(k_4+k_5)(k_2+k_1A)/k_3(k_1A+k_5),$$

and at $A=0$,

$$K_m=k_2(k_4+k_5)/k_3k_5.$$

Not only the relation between $1/v$ and $1/D$ at fixed A but also the relationship between $1/v$ and $1/A$ at fixed D is once again rectilinear.

For the case where $A=D$, i.e. the donor is its own acceptor, both equations (11a) and (11b) reduce to

$$\frac{1}{v}=\frac{1}{V}+\frac{k_1k_4+k_1k_5+k_3k_5}{k_1k_3V}\cdot\frac{1}{D}+\frac{k_2k_4+k_2k_5}{k_1k_3V}\cdot\frac{1}{D^2},$$

and the Lineweaver–Burk plot of $1/v$ against $1/D$ will be approximately linear for high D and parabolic for low D.

(*b*) *Competing Donors or Acceptors* (*first case*)

The two schemes

$$E+D\underset{k_2}{\overset{k_1}{\rightleftharpoons}}ED\begin{cases}+A_1\underset{k_4}{\overset{k_3}{\rightleftharpoons}}EDA_1\overset{k_7}{\rightarrow}P_1\\[2ex]+A_2\underset{k_6}{\overset{k_5}{\rightleftharpoons}}EDA_2\overset{k_8}{\rightarrow}P_2\end{cases},$$

and

$$E+A\underset{k_2}{\overset{k_1}{\rightleftharpoons}}EA\begin{cases}+D_1\underset{k_4}{\overset{k_3}{\rightleftharpoons}}EAD_1\overset{k_7}{\rightarrow}P_1\\[2ex]+D_2\underset{k_6}{\overset{k_5}{\rightleftharpoons}}EAD_2\overset{k_8}{\rightarrow}P_2\end{cases},$$

are formally the same, leading to equation (12) for competing acceptors or its equivalent with D and A interchanged for competing donors:

$$v = v_1+v_2$$

$$= \frac{k_1k_3(k_6+k_8)V_1A_1D+k_1k_5(k_4+k_7)V_2A_2D}{k_3k_7(k_6+k_8)A_1+k_5k_8(k_4+k_7)A_2+k_1k_3(k_6+k_8)A_1D+k_1k_5(k_4+k_7)A_2D+k_1(k_4+k_7)(k_6+k_8)D+k_2(k_4+k_7)(k_6+k_8)}, \quad (12)$$

where V_1 and V_2 are the maximum velocities in the absence of other competitors. Certain corollaries emerge from equation (12) or during its derivation:

(i)
$$\frac{v_1}{v_2} = \frac{k_3(k_6+k_8)V_1}{k_5(k_4+k_7)V_2}.\frac{A_1}{A_2}$$
$$= KA_1/A_2$$

($= P_1/P_2$ under reaction conditions where the concentration of the acceptors is not substantially changed since this ratio will be that of the reaction velocities so long as these remain constant). Hence the ratio of the products is that of the acceptors. This ratio is independent of the *concentration* of the donor but not of its *nature*, since the values of all the constants k_3, k_4, k_5, k_6, k_7, and k_8 will depend on the nature of the donor.

(ii) Where one acceptor or one donor of a pair acts only as a competitive inhibitor, $k_8 = 0$ and consequently $V_2 = 0$, and we have (for the donor case)

$$\frac{1}{v} = \frac{1}{V}+\frac{k_4+k_7}{k_3V}.\frac{1}{D_1}+\frac{k_5(k_4+k_7)}{k_3k_6V}.\frac{D_2}{D_1}+\frac{k_7}{k_1V}.\frac{1}{A}+\frac{k_2(k_4+k_7)}{k_1k_3V}.\frac{1}{AD_1}, \quad (13)$$

where D_1 = donor and D_2 = competitive inhibitor. The usual procedure for the determination of the inhibitor constant involves determining the relationship of $1/v$ and $1/D_1$ at $D_2 = 0$ and at some other fixed value.

Equation (13) may be rewritten

$$\frac{1}{v} = \frac{1}{V}\left(1+\frac{k_7}{k_1A}\right)+\frac{1}{D_1}\left\{\frac{k_4+k_7}{k_3V}+\frac{k_5(k_4+k_7)D_2}{k_3k_6V}+\frac{k_2(k_4+k_7)}{k_1k_3AV}\right\}, \quad (14)$$

the coefficient of $1/D_1$ in equation (14) reducing to

$$\frac{k_4+k_7}{k_3V}+\frac{k_2(k_4+k_7)}{k_1k_3AV}$$

for $D_2 = 0$. The Michaelis–Menten treatment leads to the presumption that the ratio of the Michaelis constants in the absence and presence of a known concentration of competitive inhibitor equals $1+K_iD_2$. For the present case, however, this ratio is $1+\{k_5k_1A/k_6(k_1A+k_2)\}$ and K_i only extrapolates to the true K_i ($= k_5/k_6$) as $A \to \infty$. This non-equivalence of the true and derived values of K_i will also be true for the more complex cases to be discussed.

(iii) Equation (14) may be rewritten for the acceptor case

$$\frac{1}{v} = \frac{1}{V}\left\{1+\frac{k_7}{k_1D}+\frac{k_4+k_7}{k_3A_1}+\frac{k_2(k_4+k_7)}{k_1k_3D}\right\}+\frac{k_5(k_4+k_7)}{k_3k_6A_1V}.A_2. \quad (15)$$

The effect of adding a competitive inhibitor of acceptance to the enzymic system with A_1 and D fixed therefore takes the form

$$1/v = p+qA_2,$$

and a plot of $1/v$ against A_2 should be linear.

(iv) If both acceptors are active (i.e. $k_8 \neq 0$), equation (12) reduces to

$$v = (p+qA_2)/(r+sA_2)$$

when A_1 and D are fixed and, depending on the values of the nonce-constants, continued addition of A_2 to the system will increase or decrease the reaction velocity monotonically. If a partial reaction velocity is measured by the rate of appearance of one of the products, rather than the overall reaction velocity by the disappearance of the donors, the analysis of the next corollary will apply.

(v) The case of two competing donors is not readily amenable to analysis unless it is possible to measure v_1 or v_2, the partial velocities of decomposition of D_1 and D_2, respectively. In this case

$$\frac{1}{v_1} = \frac{1}{V_1} + \frac{k_7}{V_1k_1}\cdot\frac{1}{A} + \frac{k_5k_8(k_4+k_7)}{V_1k_1k_3(k_6+k_8)}\cdot\frac{D_2}{AD_1} + \frac{k_5(k_4+k_7)}{V_1k_3(k_6+k_8)}\cdot\frac{D_2}{D_1} + \frac{k_4+k_7}{V_1k_3D_1} + \frac{k_2(k_4+k_7)}{V_1k_1k_3}\cdot\frac{1}{AD_1}. \quad (16)$$

Normal experimental technique is to hold A and D_2 constant and to obtain an apparent Michaelis constant from the plot of $1/v_1$ against $1/D$. Now equation (16) can be arranged to give

$$\frac{1}{v_1} = \frac{1}{V_1}\left(1+\frac{k_7}{Ak_1}\right) + \left\{\frac{k_5k_8(k_4+k_7)D_2}{AV_1k_1k_3(k_6+k_8)} + \frac{k_5(k_4+k_7)D_2}{V_1k_3(k_6+k_8)} + \frac{k_4+k_7}{V_1k_3} + \frac{k_2(k_4+k_7)}{AV_1k_1k_3}\right\}\cdot\frac{1}{D_1} \quad (17)$$

and from equation (17) it can be deduced that the ratio of the apparent Michaelis constants in the presence and absence of D_2 is

$$1 + \frac{k_5(k_1A+k_8)}{(k_6+k_8)(k_1A+k_2)}\cdot D_2.$$

The value of K_i thus varies between $k_5k_8/k_2(k_6+k_8)$ for $A = 0$ and $k_5/(k_6+k_8)$ as $A \to \infty$.

(c) *Competing Donors or Acceptors (second case)*

The scheme

$$\begin{array}{llll}
E+A_1 \underset{k_2}{\overset{k_1}{\rightleftharpoons}} EA_1 & & & EA_1D \xrightarrow{k_9} P_1 \\
 & & k_5 \nearrow\!\!\swarrow k_6 & \\
 & +D & & \\
 & & k_7 \searrow\!\!\nwarrow k_8 & \\
E+A_2 \underset{k_4}{\overset{k_3}{\rightleftharpoons}} EA_2 & & & EA_2D \xrightarrow{k_{10}} P_2
\end{array},$$

and the corresponding one for two donors, present an alternative picture of competition in the sequential reaction scheme. It leads to the formula

$$v = [k_1k_5A_1V_1\{k_4(k_8+k_{10})+k_7k_{10}D\} + k_3k_7A_2V_2\{k_2(k_6+k_9)+k_5k_9D\}]D/Y \quad (18)$$

where

$$\begin{aligned}
Y = {} & k_5k_7D^2(k_9k_{10}+k_1k_{10}A_1+k_3k_9A_2)+D[\{k_1k_4k_5(k_8+k_{10})+k_1k_7k_{10}(k_6+k_9)\}A_1 \\
& +\{k_2k_3k_7(k_6+k_9)+k_3k_5k_9(k_8+k_{10})A_2+k_4k_5k_9(k_8+k_{10})+k_2k_7k_{10}(k_6+k_9)\}] \\
& +A_1k_1k_4(k_6+k_9)(k_8+k_{10})+A_2k_2k_3(k_6+k_9)(k_8+k_{10})+k_2k_4(k_6+k_9)(k_8+k_{10}).
\end{aligned}$$

Corollaries to equation (18) follow:

(i)
$$\frac{v_1}{v_2}=\frac{A_1}{A_2}\cdot\frac{k_1k_5k_9\{k_4(k_8+k_{10})+k_7k_{10}D\}}{k_3k_7k_{10}\{k_2(k_6+k_9)+k_5k_9D\}}.$$

The ratio of the velocities of formation of the products P_1 and P_2 is here dependent not only on the *nature* of the donor but also its *concentration*. When A_1 and A_2 are fixed, this ratio reduces to the form

$$v_1/v_2=(p+qD)/(r+sD),$$

with the ratio rising or falling monotonically with increasing D. When A_1 and D are fixed, the form is

$$v_1/v_2=K/A_2,$$

and the ratio is inversely proportional to acceptor concentration. For any case where the concentration of the donor changes appreciably throughout the reaction, the ratio P_1/P_2 must be appreciably different at different stages of the reaction.

(ii) Competitive inhibition of either donor or acceptor types results when $k_7=0$. For the donor case

$$v=\frac{k_1k_4k_5VAD_1}{k_1k_4k_5AD_1+k_3k_5k_9AD_2+k_4k_5k_9A+k_1k_4(k_6+k_9)D_1+k_2k_3(k_6+k_9)D_2+k_2k_4(k_6+k_9)}. \quad (19)$$

Inversion of equation (19) and deduction of the values of the apparent K_m in the presence and absence of D_2 leads to the result that the value of their ratio is $1+(k_3/k_4)D_2$. The value of K_i is therefore k_3/k_4, independent of A as would be expected, so that the normal Lineweaver–Burk analysis gives the correct value when applied to this case.

(iii) Rewriting equation (19) for the acceptor case and inverting leads to

$$\frac{1}{v}=\frac{k_5}{k_2V}+\frac{k_3k_5k_9}{k_1k_2k_4V}\cdot\frac{A_2}{A_1}+\frac{k_5k_9}{k_1k_2V}\cdot\frac{1}{A_1}+\frac{k_6+k_9}{k_2V}\cdot\frac{1}{D}+\frac{k_3(k_6+k_9)}{k_1k_4V}\cdot\frac{A_2}{A_1D}+\frac{k_6+k_9}{k_1}\cdot\frac{1}{A_1D}. \quad (20)$$

Hence, for fixed A_1 and D, the reduced form of equation (20) shows that the effect of adding a competitive inhibitor of acceptance once more takes the form

$$1/v=p+qA_2.$$

(iv) If both acceptors are active we have as before the two reduced equations

$$v=(p+qA_2)/(r+sA_2),$$

and

$$1/v_1=p+qA_2,$$

for the effect of adding A_2 to a system where A_1 and D are fixed.

(v) The system where there are two acceptors and one donor obviously defies Lineweaver–Burk analysis in terms of $1/D$ and either $1/v$ or $1/v_1$. For the case of two donors and one acceptor, the reduced form of equation (18) is

$$v=(aD_1+b)/(cD_1+d),$$

when A and D_2 are fixed, and the Lineweaver–Burk analysis also fails; on the other hand

$$v_1 = aD_1/(bD_1+c),$$

and here some analysis is possible. When A and D_1 are fixed, therefore, and the reaction velocity measured is v_1, the rate of disappearance of D_1, it can be shown that the ratio of the apparent values of K_m in the presence and absence of D_2 is

$$\left(1+\frac{aA^2+bA+c}{dA^2+eA+f}.D_2\right).$$

The inhibitor constant K_i is thus a complex function of the concentration of acceptor.

(d) *Reciprocal Complex Formation*

When the same ternary complex can be formed along two different paths we have the scheme

$$\begin{array}{lll} E+D \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ED+A & & \\ & k_5 \searrow\nwarrow k_6 & \\ & & EAD \overset{k_9}{\rightarrow} P, \\ & k_7 \nearrow\swarrow k_8 & \\ E+A \underset{k_4}{\overset{k_3}{\rightleftharpoons}} EA+D & & \end{array}$$

leading to the equation

$$v = ADV(k_3k_5k_7A+k_1k_5k_7D+k_1k_4k_5+k_2k_3k_7)/W, \tag{21}$$

where

$$\begin{aligned} W = {} & k_3k_5(k_8+k_9)A^2+k_1k_7(k_6+k_9)D^2+k_3k_5k_7A^2D+k_1k_5k_7AD^2 \\ & +(k_5k_7k_9+k_1k_4k_5+k_1k_5k_8+k_2k_3k_7+k_3k_6k_7)AD \\ & +(k_1k_4k_6+k_1k_4k_8+k_1k_4k_9+k_2k_6k_7+k_2k_7k_9)D \\ & +(k_2k_3k_6+k_2k_3k_8+k_2k_3k_9+k_4k_5k_8+k_4k_5k_9)A \\ & +k_2k_4(k_6+k_8+k_9). \end{aligned}$$

On inverting, equation (21) takes the form

$$1/v = 1/V+(1/V)\{1/(aD+bA+c)\}(d+eD/A+fA/D+g/A+h/D+i/AD),$$

and, if A is taken as fixed, this further reduces to

$$1/v = 1/V+(1/V)\{1/(D+p)\}(qD+r+s/D).$$

Enzyme experiments are generally performed in concentration ranges where the order of magnitude of the quantities is $1/D \gg A \gg 1/A \gg D$, so that the above equation is of the form

$$\frac{1}{v} = \frac{1}{V}+\frac{1}{V}\cdot\frac{1}{Q_{(\text{small})}}\left(Q_{(\text{very small})}+Q_{(\text{small})}+Q_{(\text{large})}\cdot\frac{1}{D}\right).$$

Hence the kinetics of such a system will be approximately Michaelis–Menten when A is large and D small, and depart measurably from this scheme as $D \to A$. In fact when $A \to \infty$, equation (21) reduces to

$$v = \frac{k_7 DV}{(k_8+k_9)+k_7 D},$$

a typical Michaelis–Menten form.

If we equate both k_7 and k_8 to 0 in equation (21) we have the important practical case where the acceptor is also a competitive inhibitor of the donor. This leads to equation (22)

$$v = \frac{k_1k_4k_5ADV}{k_3k_5k_9A^2+k_1k_4k_5AD+k_1k_4(k_6+k_9)D+(k_2k_3k_6+k_2k_3k_9+k_4k_5k_9)A+k_2k_4(k_6+k_9)}. \tag{22}$$

This equation is of such a form that for a given value of D, v must pass through a maximum with increasing A, the maximum occurring at

$$A = \{k_4(k_6+k_9)(k_1D+k_2)/k_3k_5k_9\}^{\frac{1}{2}}.$$

Analysis also shows that apparent $V = V\{1+(k_6+k_9)/k_5A\}$ and apparent K_m for the donor equals

$$\frac{k_2k_4(k_6+k_9)+(k_2k_3k_6+k_2k_3k_9+k_4k_5k_9)A+k_3k_5k_9A_2}{k_1k_4(k_5A+k_6+k_9)};$$

apparent V thus falls and K_m rises as A increases.

(e) Reciprocal Complex Formation with Competing Donors or Acceptors

The scheme here is given by

$$\begin{array}{cccccccc}
E+A_1 & \underset{k_2}{\overset{k_1}{\rightleftharpoons}} & EA_1+D & \underset{k_8}{\overset{k_7}{\rightleftharpoons}} & EA_1D & \overset{k_{15}}{\rightarrow} & P_1 \\
 & & & k_9 \swarrow\!\!\nearrow k_{10} & & & \\
E+D & \underset{k_4}{\overset{k_3}{\rightleftharpoons}} & ED+A_1 & & & & \\
 & & +A_2 & \underset{k_{12}}{\overset{k_{11}}{\rightleftharpoons}} & EA_2D & \overset{k_{16}}{\rightarrow} & P_2 \\
 & & & k_{13} \swarrow\!\!\nearrow k_{14} & & & \\
E+A_2 & \underset{k_6}{\overset{k_5}{\rightleftharpoons}} & EA_2+D & & & &
\end{array}$$

This formulation leads to an equation which has been worked out but is far too complex to be given in full, even in reduced form. But the reduced form for the ratio of the two partial velocities is rather shorter; it is

$$v_1/v_2 = M/N, \tag{23}$$

where

$$M = \alpha A_1+\beta A_1^2+\gamma A_1^3+\delta A_1A_2+\epsilon A_1^2A_2+\zeta A_1A_2^2+\eta A_1D+\theta A_1^2D+\iota A_1^3D+\kappa A_1^2A_2D$$
$$+\lambda A_1A_2^2D+\mu A_1A_2D+\nu A_1D^2+\xi A_1^2D^2+oA_1A_2D^2,$$

and

$$N = aA_2+bA_2^2+cA_2^3+dA_1A_2+eA_1A_2^2+fA_1^2A_2+gA_2D+hA_2^2D+iA_2^3D+jA_1A_2^2D$$
$$+kA_1^2A_2D+lA_1A_2D+mA_2D^2+nA_2^2D^2+oA_1A_2D^2.$$

If A_1 and A_2 are fixed, equation (23) reduces to

$$v_1/v_2 = (pD^2+qD+r)/(xD^2+yD+z),$$

whence, as $0 \leftarrow D \rightarrow \infty$, $r/z \leftarrow v_1/v_2 \rightarrow p/x$. Hence, for experiments in which A_1, A_2 are not sensibly altered, these are also the limits for the ratio P_1/P_2, which will obviously depend in a complex way on the *concentration* of the donor as well as its *nature*.

If A_1 and D are fixed,

$$v_1/v_2 = (pA_2^2+qA_2+r)/(wA_2^3+xA_2^2+yA_2+z),$$

whence as $0 \leftarrow A_2 \rightarrow \infty$, $r/z \leftarrow v_1/v_2 \rightarrow 0$, and the same remarks as before apply to the ratio P_1/P_2.

(f) *Donor as One of the Competing Acceptors*

Equations (12), (18), and (23) can all be adapted to the case where the donor can also act as one of the competing acceptors. This is a case of frequent practical importance.

(i) Equation (12): putting $A_2 = D$, equation (12) becomes

$$v = v_1+v_2$$

$$= \frac{k_1k_3(k_6+k_8)V_1AD+k_1k_5(k_4+k_7)V_2D^2}{k_3k_7(k_6+k_8)A+k_1k_3(k_6+k_8)AD+k_1k_5(k_4+k_7)D^2+\{k_5k_8(k_4+k_7)+k_1(k_4+k_7)(k_6+k_8)\}D+k_2(k_4+k_7)(k_6+k_8)}. \quad (24)$$

For $k_8 = 0$, i.e., where the donor acts as a competitive inhibitor of the acceptor only, equation (24) becomes

$$v = \frac{k_1k_3k_6VAD}{k_3k_6k_7A+k_1k_3k_6AD+k_1k_5(k_4+k_7)D^2+k_1k_6(k_4+k_7)D+k_2k_6(k_4+k_7)}, \quad (25)$$

and v passes through a maximum at any given value of A when

$$D = [\{k_2k_6(k_4+k_7)+k_3k_6k_7A\}/k_1k_5(k_4+k_7)]^{\frac{1}{2}}.$$

For a given value of D, v increases monotonically with increasing A. Furthermore, for a fixed value of A

$$\frac{1}{v} = \frac{1}{V}\left(1+\frac{k_4+k_7}{k_3A}\right)+\frac{k_3k_7A+k_2(k_4+k_7)}{k_1k_3AV}\cdot\frac{1}{D}+\frac{k_5(k_4+k_7)}{k_3k_6AV}\cdot D, \quad (26)$$

and the system will approximate to Michaelis–Menten kinetics at low values of D and depart increasingly as D increases. When $k_8 \neq 0$, it can be deduced from the partial forms of equation (24) that, for a fixed value of A, v_1 has a maximum at

$$D = [\{(k_6+k_8)(k_2k_4+k_2k_7+k_3k_7A)\}/k_1k_5(k_4+k_7)]^{\frac{1}{2}}.$$

Also

$$1/v = (1/V)(a+b/D+cD),$$

and the system will depart from Michaelis–Menten kinetics at high values of D; and

$$1/v_2 = (1/V_2)(p+q/D+r/D^2),$$

and the system will depart from Michaelis–Menten kinetics at low values of D.

The ratio

$$v_1/v_2 = \{k_3(k_6+k_8)V_1/k_5(k_4+k_7)V_2\}(A/D) \\ = KA/D,$$

so that increasing A at fixed D, or D at fixed A, merely leads to proportionate increase or decrease in this ratio.

(ii) Equation (18): for $A_2 = D$, equation (18) reduces to the form

$$v = v_1+v_2 \\ = (aD^3+bD^2+cAD^2+dAD)/(aD^3+3D^2+fD+gAD^2+hAD+iA+j). \qquad (27)$$

For the competitive inhibitor case, i.e. k_7, $k_8 = 0$, this form may be written in full

$$v = \frac{k_1k_4k_5k_{10}VAD}{k_3k_5k_9k_{10}D^2+k_1k_4k_5k_{10}AD+\{k_4k_5k_9k_{10}+k_2k_3k_{10}(k_6+k_9)\}D+k_1k_4k_{10}(k_6+k_9)A+k_2k_4k_{10}(k_6+k_9)}, \qquad (28)$$

with a maximum for v at a fixed value of A when

$$D = [\{k_4(k_6+k_9)(k_1A+k_2)\}/k_3k_5k_9]^{\frac{1}{2}},$$

and no maximum with increasing A at fixed D. At a fixed value of A, also

$$\frac{1}{v} = \frac{1}{V}\left\{1+\frac{k_9}{k_1A}+\frac{k_2k_3(k_6+k_9)}{k_1k_4k_5A}\right\}+\left\{\frac{k_6+k_9}{k_5V}+\frac{k_2(k_6+k_9)}{k_1k_5AV}\right\}\frac{1}{D}+\frac{k_3k_9D}{k_1k_4AV}, \qquad (29)$$

showing departures from Michaelis–Menten kinetics only at high values of D.

For k_7, $k_8 \neq 0$, the partial equations for v_1 and v_2 are not readily analysable, although it can be shown that neither v_1 nor v_2 shows a maximum when A is increased at a fixed value of D. Attempts to define the course of v_1 and v_2 when D is increased at a fixed value of A lead to quartic equations.

The ratio

$$v_1/v_2 = (cAD^2+dAD)/aD^3+bD^2 \\ = (cAD+dA)/(aD^2+bD).$$

For A increasing at a fixed value of D, the ratio is proportional to A; for D increasing at fixed A there is a formal minimum at

$$D = -(b/a)+\{(b^2/a^2)-(bd/ac)\}^{\frac{1}{2}},$$

which cannot, however, represent any meaningful positive concentration of the donor, and the ratio will in fact decrease monotonically with increasing D.

(iii) Equation (23): when $A_2 = D$, equation (23) reduces to the form

$$\frac{v_1}{v_2} = \frac{\alpha A+\beta A^2+\gamma A^3+\delta AD+\epsilon A^2D+\zeta A^3D+\eta AD^2+\theta A^2D^2+\iota AD^3}{aD+bD^2+cD^3+dD^4+eAD+fAD^2+gAD^3+hA^2D^2}. \qquad (30)$$

For fixed D, equation (30) further reduces to the form

$$v_1/v_2 = (aA^3+bA^2+cA)/(pA^2+qA+r),$$

and, for fixed A, to

$$v_1/v_2 = (aD^3+bD^2+cD+d)/(pD^4+qD^3+rD^2+sD+t).$$

Thus in either case the value of the ratio will depend in a very complex way on the value of the variable.

IV. Conclusions

The conclusions to be drawn from the equations presented in Sections II and III must depend on the purposes to which they are to be applied. Thus, the following paper (Jermyn 1962) describes a series of experiments directed to determining the ratio of the products formed from two competing acceptors by a single enzyme using a variety of donors. From Sections II(*b*) and II(*c*), the only possible case, on the binary-complex hypothesis, in which the nature and concentration of the donor can have any influence on the ratio of the products, is that of a completely reversible system. Even then the concentrations of the donor and products must be of the same order of magnitude for the effect to be sensible. Section III(*b*) outlines a form of the ternary-complex hypothesis where the ratio is influenced by the nature of the donor but not its concentration. The form discussed in Section III(*c*) leads to the result that the ratio depends on both the nature and the concentration of the donor, and the same result emerges from Section III(*e*). But the effect on the ratio of the products of holding the concentration of the donor and one acceptor constant and varying the concentration of the second acceptor is quite different in the last two cases.

By experiments suited to a given enzyme it should at least be possible to eliminate some of the sub-hypotheses as not giving an adequate account of the observed data.

V. References

Alberty, R. A. (1953).—*J. Amer. Chem. Soc.* **75**: 928.

Ingraham, L. L., and Makower, B. C. (1954).—*J. Phys. Chem.* **58**: 266.

Jermyn, M. A. (1962).—*Aust. J. Biol. Sci.* **15**: 248.

Koshland, D. E. (1953).—*Biol. Rev.* **28**: 416.

Lineweaver, H., and Burk, D. (1934).—*J. Amer. Chem. Soc.* **56**: 658.

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

Segal, H. L. (1959).—In "The Enzymes". 2nd Ed. (Ed. P. D. Boyer, H. Lardy, and K. Myrback.) Vol. 1. p. 35. (Academic Press, Inc.: New York.)

Woolf, B. (1929).—*Biochem. J.* **23**: 472.