Calculation of Steady-State Rate Equations and the Fluxes between Substrates and Products in Enzyme Reactions

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1. Two methods are described for deriving the steady-state velocity of an enzyme reaction from a consideration of fluxes between enzyme intermediates. The equivalent-reaction technique, in which enzyme intermediates are systematically eliminated and replaced by equivalent reactions, appears the most generally useful. The methods are applicable to all enzyme mechanisms, including three-substrate and random Bi Bi Ping Pong mechanisms. Solutions are obtained in algebraic form and these are presented for the common random Bi Bi mechanisms. The steady-state quantities of the enzyme intermediates may also be calculated. Additional steps may be introduced into enzyme mechanisms for which the steady-state velocity equation is already known. 2. The calculation of fluxes between substrates and products in three-substrate and random Bi Bi Ping Pong mechanisms is described. 3. It is concluded that the new methods may offer advantages in ease of calculation and in the analysis of the effects of individual steps on the overall reaction. The methods are used to show that an ordered addition of two substrates to an enzyme which is activated by another ligand will not necessarily give hyperbolic steady-state-velocity kinetics or the flux ratios characteristic of an ordered addition, if the dissociation of the ligand from the enzyme is random.

The conventional method of deriving steady-state rate equations involves setting up simultaneous equations describing the concentration of each enzyme species in terms of the concentrations of its nearest neighbours. As is well known, the algebraic complexity in solving these equations becomes very great for all but the simplest systems. King & Altman (1956) introduced an elegant schematic method which has been further developed by a number of authors [see Kinderlerer & Ainsworth (1976) and Indge & Childs (1976)]. However, even this technique becomes unwieldy with complex mechanisms and it generates solutions in the form of coefficients in a polynomial which may not necessarily be the most convenient form of presentation, particularly if the influence of a particular rate constant on the overall rate is to be examined.

In the present paper alternative procedures of deriving the steady-state rate equations are described based on the flux concept. The most general of these is one in which the reaction is progressively simplified by replacement of reaction sequences with simpler equivalent reactions. This leads directly to an algebraic solution for the rate equations and solutions are given for the common random Bi Bi mechanisms in the presence of all substrates and products. The calculation of fluxes between substrates in mechanisms with triple branch points is also described.

Terminology

In the following sections on fluxes and the equivalent reaction, the interconversion of enzyme species E_1 , E_2 , E_3 etc. will be considered (Figs. 1a and 1b). These steps may represent isomerizations, or the addition and dissociation of ligands. In the latter case the concentrations of the ligands are omitted from the rate expressions. Thus k_{+1} , k_{-1} etc. are equivalent to the 'kappas' of King & Altman (1956).

The term 'flux'

The present method is based on the flux concept (Britton, 1966) in which the term flux is used to indicate the actual number of individual molecules transformed per unit time from one form to another. For example, considering two adjacent enzyme species E_1 and E_2 (Fig. 1a), the flux of E_1 to E_2 is given by $k_{+2}E_1$ (where E_1 indicates the total quantity of E present) and the flux of E_2 to E_1 is given by $k_{-2}E_2$. The net rate of chemical transformation of E_1 to E_2 is the difference between the two fluxes.

Calculation of Fluxes

The following summarizes the methods described in Britton (1966). They are applicable to all enzyme systems where the steps can be arranged in series or

Fig. 1. The equivalent reaction

(a) Scheme I represents three enzyme species E_1 , E_2 and E_3 and their interconversions. The rate constants are indicated beside the arrows. E_1 , E_2 and E_3 may represent different isomeric forms of the enzyme, or the steps may indicate the addition or dissociation of ligands. In the latter case the concentrations of the ligands are omitted, for simplicity. In Scheme II, E_2 has been omitted, but the fluxes between E_1 and E_3 are the same as in Scheme I provided that the concentrations of E_1 and E_3 are maintained. Scheme II differs from Scheme I in that the total amount of enzyme involved is less by the amount represented by E_2 . Scheme III represents an 'equivalent reaction' to Scheme I and cannot be distinguished from Scheme I in the steady state. The fluxes between E'_1 and E'_3 are the same as in Scheme I and the total amount of enzyme involved is the same. E'_1 and E'_3 are marked with primes to emphasize that they are apparent values. (b) Scheme IV represents Scheme III (Fig. 1a) with additional steps and rate constants. Scheme V is an equivalent reaction, with E_4 omitted. The primes indicate apparent values.

in parallel. In certain cases, in systems with triple branch points this is not possible. The treatment of such cases is discussed in the section on the star-delta transformation.

Steps in parallel

Consider the conversion of E_1 into E_2 (Fig. 1a) and suppose that there are two different molecular pathways by which this conversion can occur. Let the rate constants for the two pathways be denoted by k_{+2} and k'_{+2} . The total flux of E_1 to E_2 will be given by the sum $(k_{+2}E_1 + k'_{+2}E_1) = (k_{+2} + k'_{+2})E_1$. Clearly therefore the flux of E_1 to E_2 may be calculated by multiplying the sum of the rate constants by E_1 . This also applies when k_{+2} and k'_{+2} are apparent values (see below).

Steps in series

Consider the conversion of E_1 into E_3 (Scheme I, Fig. 1a), which occurs via an intermediate form E_2 . Following the probability approach of Britton (1964, 1966), in the steady state:

$$\text{Flux of } E_1 \text{ to } E_3 = E_1 k_{+2} k_{+3} / (k_{-2} + k_{+3}) = E_1 r_{12} k_{+3} \quad (1)$$

$$\text{where } r_{12} = k_{+2} / (k_{-2} + k_{+3}) \quad (2)$$

Similarly

$$\text{Flux of } E_3 \text{ to } E_1 = E_3 k_{-2} k_{-3} / (k_{-2} + k_{+3}) = E_3 r_{32} k_{-2} \quad (3)$$

$$\text{where } r_{32} = k_{-3} / (k_{-2} + k_{+3}) \quad (4)$$

Thus to calculate the flux of E_1 to E_3 , E_1 should be multiplied by the 'apparent' rate constant $r_{12}k_{+3}$, and conversely the 'apparent' rate constant for the flux of E_3 to E_1 is $r_{32}k_{-2}$. This procedure may be repeated to calculate fluxes between more distantly related species. Thus, for example, to calculate the fluxes between E_1 and E_4 , E_2 may be eliminated with the insertion of the 'apparent' rate constants between E_1 and E_3 , and the process may then be repeated to eliminate E_3 .

The Equivalent Reaction

Compare Schemes I and II (Fig. 1a). The rate constants in Scheme II have been calculated so that the fluxes of E_1 to E_3 and E_3 to E_1 in Scheme II will be the same as in Scheme I provided that the quantities of E_1 and E_3 are the same. In this respect the Schemes are not distinguishable, but they are not identical, because the total amount of enzyme involved in Scheme I is greater by an amount represented by E_2 . In Scheme I, in the steady state

$$\begin{aligned} E_1 + E_2 + E_3 &= E_1[1 + k_{+2}/(k_{-2} + k_{+3})] + E_3[1 + k_{-3}/(k_{-2} + k_{+3})] \\ &= E_1(1 + r_{12}) + E_3(1 + r_{32}) \end{aligned} \quad (5)$$

where r_{12} and r_{32} are defined by eqns. (2) and (4). In Scheme III (Fig. 1a), therefore the outgoing rate constants from E_1 have been divided by $(1 + r_{12})$ and the outgoing rate constants from E_3 divided by $(1 + r_{32})$. In this Scheme the steady-state quantities of E'_1 and E'_3 , (see below), are given by the equations:

$$E'_1 = E_1(1 + r_{12}) \quad (6)$$

$$E'_3 = E_3(1 + r_{32}) \quad (7)$$

From eqn. (5) therefore

$$E_1 + E_2 + E_3 = E'_1 + E'_3 \quad (8)$$

Thus the total amount of enzyme involved in Scheme III is the same as in Scheme I. Further, the fluxes between E'_1 and E'_3 are the same as between E_1 and E_3 . In addition, the fluxes of E'_1 to E_0 and E'_3 to E_4 are the same as the fluxes of E_1 to E_0 and E_3 to E_4 . Because all of the fluxes are identical, eqns. (6) and (7) represent the steady-state values of E'_1 and E'_3 , and in the steady-state, Scheme III is an exact equivalent of Scheme I. Scheme III may therefore be described as an equivalent reaction.

By the above procedure, the enzyme reaction represented by Scheme I (Fig. 1a) may be simplified by deletion of E_2 and replacement by the equivalent reaction $E'_1 \rightleftharpoons E'_3$ (Scheme III). More than one step can be deleted in relation to a given enzyme species. E_2 has been omitted in Scheme IV (Fig. 1b) and replaced by an equivalent reaction as in Scheme III.

E_4 may also be omitted to give the equivalent reaction (Scheme V, Fig. 1b). In Scheme V

$$r_{34} = k_{+4}/(k_{-4} + k_{+5}) \quad (9)$$

and

$$r_{54} = k_{-5}/(k_{-4} + k_{+5}) \quad (10)$$

Calculation of the Rate Constants of Equivalent Reactions

The above arguments may be generalized to give the following rules. They are applicable to most enzyme systems apart from some mechanisms with triple branch points, where it may be necessary to carry out star-delta transformations as discussed below. Thus to derive an equivalent reaction between E_1 and E_3 , thereby deleting E_2 (Fig. 1a), the following procedure is used.

(1) Calculate r_{12} by dividing the rate constant for the step $E_1 \rightarrow E_2$ by the sum of the rate constants for the steps directed from E_2 to E_1 and E_3 . Similarly calculate r_{32} by dividing the rate constant for the step $E_3 \rightarrow E_2$ by the same sum.

(2) Delete E_2 and write an apparent rate constant for the step E_1 to E_3 which is given by the product of r_{12} and the rate constant for the step $E_2 \rightarrow E_3$. Similarly the rate constant for the step $E_3 \rightarrow E_1$ is given by the product of r_{32} and the rate constant for the step $E_2 \rightarrow E_1$.

(3) Divide all outgoing rate constants from E_1 by $(1 + r_{12})$ and all outgoing rate constants from E_3 by $(1 + r_{32})$.

(4) Several steps may be deleted simultaneously in relation to a given enzyme species. Steps (1) and (2) are followed, but in step (3) division should be by $(1 + \Sigma r)$, where Σr refers to the sum of the appropriate r values for the groups deleted (see, for example, Fig. 1b).

(5) The process of deletion may be repeated so that apparent values are deleted. The same procedures are followed, but the apparent values for the rate constants are used.

(6) If steps are in parallel, or if steps appear in parallel after deletion of intermediates (see, for example, Fig. 3), the rate constants (real or apparent) should be added together provided that the reactions contribute in the same direction (i.e. forward or backward) to the overall reaction. This last proviso is a necessary requirement for the calculation of reaction velocity (see below), although it is not necessary for calculating the quantities of enzyme intermediates.

Calculation of Quantities of Enzyme Intermediates

By successively deleting steps in an enzyme reaction it should be possible to simplify an enzyme reaction

Fig. 2. Derivation of steady-state rate equation from the equivalent reaction

In Scheme VI the enzyme reaction has been reduced to an equivalent reaction between E' , the free enzyme, and ES' , the enzyme-substrate complex. k_{+s} and k_{+p} represent the rate constants for the reaction proceeding in a forward direction. k_{-s} and k_{-p} represent the constants for the reaction proceeding in the reverse direction. Scheme VII is the equivalent reaction from which ES' has been deleted. Since only one form of the enzyme is now considered, $E' = E_T$, where E_T represents the total quantity of enzyme.

sufficiently for the quantities of the intermediates to be calculated by conventional methods (see, for example, Scheme VI; Fig. 3). The values obtained, however, will be apparent values, and to obtain the true value the apparent value must be divided by the appropriate r terms. Thus, for example, for the random Bi Bi mechanism shown in Fig. 3, EX' should be divided by $(1 + r_{32} + r_{32}' + r_{34} + r_{34}')$ to obtain EAB, and E' should be divided by $(1 + r_{12} + r_{12}' + r_{14} + r_{14}')$ to obtain E.

Calculation of the Steady-State Velocity Equation

As just discussed, it should be possible to simplify an enzyme reaction sufficiently for the quantities of enzyme intermediates to be calculated by conventional methods and the velocity of the steady-state reaction can be calculated from these quantities. Thus in Scheme VI (Fig. 2) the velocity will be given by $(k_p ES' - k_{-p} E')$. Alternatively the equivalent-reaction technique can be used to derive the initial-velocity equation directly. Thus consider Scheme VI (Fig. 2): by applying the rules described above, Scheme VI can be simplified to Scheme VII by deleting ES' and:

$$k'_{+s} = r_1 k_{+p} / (1 + r_1 + r_2) \quad (11)$$

and

$$k'_{-p} = r_2 k_{-s} / (1 + r_1 + r_2) \quad (12)$$

where

$$r_1 = k_{+s} / (k_{-s} + k_{+p}) \quad (13)$$

and

$$r_2 = k_{-p} / (k_{-s} + k_{+p}) \quad (14)$$

The two r values arise because there are two pathways to ES' . In Scheme VII there is only one form of the enzyme E' and this may therefore be equated with E_T the total amount of enzyme. Thus the steady-state velocity (v) of the enzyme reaction will be given by:

$$v = k'_{+s} E_T - k'_{-p} E_T \quad (15)$$

Substituting eqns. (11)–(14) in eqn. (15)

$$v = \frac{k_{+s} k_{+p} E_T}{k_{+s} + k_{-s} + k_{+p} + k_{-p}} - \frac{k_{-p} k_{-s} E_T}{k_{+s} + k_{-s} + k_{+p} + k_{-p}} \quad (16)$$

The coefficients of E_T in eqn. (15) give the number of complete catalytic cycles of the enzyme per unit time in each direction.

Solutions for the Steady-State Rate Equation for Random Bi Bi Reactions

The application of the equivalent-reaction technique may be illustrated by the calculation of the steady-state velocity equations for two random Bi Bi mechanisms (Schemes VIII and IX, Figs. 3 and 4). In Scheme VIII (Fig. 3), EA, EB, EP and EQ may be deleted to yield Scheme VI where ES' represents the ternary enzyme-substrate complex EAB' (Fig. 2). Four groups are deleted in relation to both E and EAB, and there will be four r values in relation to both E' and ES' in Scheme VI. There are two pathways for the formation of EAB from E by the forward reaction (i.e. by the addition of substrates), that via EA and that via EB. When EA and EB are deleted, the equivalent reactions appear in parallel, and therefore the corresponding rate constants are added together. Similar considerations apply to the dissociation of the products. The apparent rate constants in Scheme VI are therefore given by the expressions:

$$k_{+s} = \frac{r_{12} k_{+2} [B] + r_{12}' k_{+2}' [A]}{1 + r_{12} + r_{12}' + r_{14} + r_{14}'} \quad (17)$$

$$k_{-s} = \frac{r_{32} k_{-1} + r_{32}' k_{-1}'}{1 + r_{32} + r_{32}' + r_{34} + r_{34}'} \quad (18)$$

$$k_{+p} = \frac{r_{34} k_{+4} + r_{34}' k_{+4}'}{1 + r_{32} + r_{32}' + r_{34} + r_{34}'} \quad (19)$$

$$k_{-p} = \frac{r_{14} k_{-3} + r_{14}' k_{-3}'}{1 + r_{12} + r_{12}' + r_{14} + r_{14}'} \quad (20)$$

where

$$r_{12} = k_{+1}[A]/(k_{-1} + k_{+2}[B]) \quad (21)$$

$$r_{12'} = k'_{+1}[B]/(k'_{-1} + k'_{+2}[A]) \quad (22)$$

$$r_{14} = k_{-4}[Q]/(k_{+4} + k_{-3}[P]) \quad (23)$$

$$r_{14'} = k'_{-4}[P]/(k'_{+4} + k'_{-3}[Q]) \quad (24)$$

$$r_{32} = k_{-2}/(k_{-1} + k_{+2}[B]) \quad (25)$$

$$r_{32'} = k'_{-2}/(k'_{-1} + k'_{+2}[A]) \quad (26)$$

$$r_{34} = k_{+3}/(k_{+4} + k_{-3}[P]) \quad (27)$$

$$r_{34'} = k'_{+3}/(k'_{+4} + k'_{-3}[Q]) \quad (28)$$

If these values of k_s , k_{-s} , k_p and k_{-p} are substituted into eqn. (16) the steady-state rate equation for Scheme VIII is obtained. This is the general equation and it applies when all substrates and products are present. The expression is considerably simplified if P or Q = 0. Under these conditions k_{-p} and the second term in eqn. (16) are zero. There are then no negative terms in the rate equation and the number of terms in the denominator is decreased to three. Further, either r_{14} or $r_{14'}$ will be zero and either r_{34} or $r_{34'}$ become a ratio between two rate constants.

Scheme IX (Fig. 4) illustrates a random Bi Bi mechanism in which there is an isomerization of the central ternary complex. When EA, EB, EP and EQ are deleted the equivalent reaction, Scheme X, is obtained. In Scheme X, k_{+s} and k_{-p} are as defined for Scheme VIII and

$$k'_{+x} = \frac{k_{+x}}{1 + r_{32} + r_{32'}} \quad (29)$$

$$k_{+p} = \frac{r_{34}k_{+4} + r_{34'}k'_{+4}}{1 + r_{54} + r_{54'}} \quad (30)$$

$$k'_{-x} = \frac{k_{-x}}{1 + r_{54} + r_{54'}} \quad (31)$$

and

$$k_{-s} = \frac{r_{32}k_{-1} + r_{32'}k'_{-1}}{1 + r_{32} + r_{32'}} \quad (32)$$

r_{12} , $r_{12'}$, etc. are as defined in relation to Scheme VIII.

The general steady-state velocity (v) for Scheme X is given by the expression

$$v = \frac{(k_{+s}k'_{+x}k_{+p} - k_{-s}k'_{-x}k_{-p})E_T}{\left(k_{-p} + \frac{k_{+s}k'_{+x}}{k_{-s} + k'_{+x}}\right)(k_{-s} + k'_{+x} + k'_{-x}) + \left(k_{+p} + \frac{k_{-s}k'_{-x}}{k_{-s} + k'_{+x}}\right)(k_{+s} + k_{-s} + k'_{-x})} \quad (33)$$

As discussed in relation to Scheme VIII, k_{-p} and the negative term in eqn. (33) disappear if one or other product is absent. One of the r terms also becomes zero.

Scheme VIII

Fig. 3. Random Bi Bi reaction with a single species of the free enzyme (E) and a single species of the ternary complex (EAB)

The numbers in parentheses adjacent to the enzyme species are given to enable the r values to be identified.

The same technique may be used to calculate the steady-state velocity equations when the enzyme isomerizes after release of the products before combining with the substrates. Indeed, the random Bi Bi case, where there is a single ternary enzyme-substrate complex (EAB), but in which the products dissociate to give one form of the free enzyme E_2 and the substrates combine with a form E_1 may be obtained directly from Schemes IX and X. In Scheme IX, E may be identified with EAB, EPQ with E_1 and EAB with E_2 . With appropriate changes in the significance of the rate constants the steady-state velocity will then be given by eqn. (33).

Isomerization of Enzyme-Substrate Complexes

The reaction of a substrate (A) with an enzyme to form an enzyme-substrate complex (EA) may be a slow process. This may be due to steric factors which render the majority of collisions ineffective, or it may

be because the binding of the substrate occurs in stages. In the latter case the reaction will be more accurately represented by Scheme A (Fig. 5) rather than as a simple step. Parallel pathways are shown,

Fig. 4. *Random Bi Bi mechanism with isomerization of the ternary complex, and the equivalent reaction*
 Scheme IX represents a random Bi Bi reaction with an isomerization of the ternary complex ($EAB \rightleftharpoons EPQ$). The labelling of the rate constants for the other steps in the reaction is as in Scheme VIII (Fig. 3). Scheme X is the equivalent reaction to Scheme IX with the species EA, EB, EP and EQ deleted. The numbers in parentheses adjacent to the enzyme species enable the r values to be identified.

Fig. 5. *Schemes involving isomerization of an enzyme-substrate complex*

E represents the enzyme and EA_{Ia} , EA_{Ib} etc. enzyme-substrate complexes. In Scheme B the step $E \rightleftharpoons EA_I$ is rapid. In Scheme C, EA_I represents an abortive complex. Scheme D represents the equivalent reaction.

since the formation of bonds between the enzyme and substrate may not always occur in the same sequence. Additional possibilities include Scheme B (Fig. 5), where the initial addition of substrate is rapid, and Scheme C (Fig. 5), where an abortive complex is formed. All of these Schemes give the same equivalent reaction (Scheme D, Fig. 5) in which:

$$k'_{-1} = \alpha / \left(1 + \frac{[A]}{K_A} \right) \quad (34)$$

$$k'_{+2} = \beta [A] / K_A \left(1 + \frac{[A]}{K_A} \right) \quad (35)$$

where α , β and K_A represent various combinations of

rate constants and in which k'_{-2} and k'_{+3} are independent of A.

Scheme C has been discussed by Dalziel (1963) and shown to give the same steady-state velocity equation as an isomerization of the binary complex in an ordered reaction. The present approach generalizes this finding to all mechanisms and shows also that the different Schemes cannot be distinguished by flux measurements with isotopes.

To obtain the general rate equation for a random Bi Bi mechanism in which there is isomerization of the binary enzyme-substrate complexes, the values for the rate constants of the equivalent reactions (Scheme D) may be calculated and inserted into the appropriate equations.

Fig. 6. Diagram to illustrate the star-delta transformation

E_1 , E_2 , E_3 and E_4 are different enzyme species. Scheme XI represents a star configuration with a triple branch point at E_4 and Scheme XII is the corresponding star-delta transformation with the deletion of E_4 . With the values of k'_{12} , k'_{-1} etc. given in the text, the fluxes between E_1 , E_2 and E_3 and the total amount of enzyme involved in Scheme XII will be the same as in Scheme XI. Scheme XII is thus an equivalent reaction and cannot be distinguished from Scheme XI in the steady state.

Alternative Application of the Flux Approach to the Calculation of the Amounts of Enzyme Intermediates and the Steady-State Rate Equation

In the steady state, the flux from one enzyme species, E_n , to another, E_m , must be equal to the flux in the opposite direction. If the fluxes are equated, the quantity of one intermediate can be expressed directly in terms of the other without the quantities of any other intermediates being involved. For example, in Scheme X (Fig. 4) the flux of E to EPQ will be made up of two components, that via the direct reaction involving k_{-p} and that via EAB. Therefore:

$$\text{Flux of E to EPQ} = E \left(k_{-p} + \frac{k_{+s}k'_{+x}}{k_{-s} + k'_{+x}} \right) \quad (36)$$

Similarly

$$\text{Flux of EPQ to E} = \text{EPQ} \left(k_{+p} + \frac{k_{-s}k'_{-x}}{k_{-s} + k'_{+x}} \right) \quad (37)$$

Since the two fluxes are equal

$$\text{EPQ} = E \left(k_{-p} + \frac{k_{+s}k'_{+x}}{k_{-s} + k'_{+x}} \right) / \left(k_{+p} + \frac{k_{-s}k'_{-x}}{k_{-s} + k'_{+x}} \right) \quad (38)$$

All of the enzyme species can be expressed in this way in terms of E, and a solution for E may be obtained by equating the sum of all of the species with the total amount of enzyme E_T . This approach may therefore be used to calculate the amounts of the enzyme intermediates and thus indirectly the reaction velocity.

Vol. 161

The Star-Delta Transformation

The methods described so far enable the majority of enzyme mechanisms to be analysed, since in these mechanisms the steps are in series or parallel. With the random Bi Bi Ping Pong mechanism considered by Wong & Hanes (1962) (Fig. 7, below) or the random addition of three substrates, these methods cannot be applied, since the presence of triple branch points (for example, at EA and EQ, Fig. 7) prevent the steps being arranged in this way. Such mechanisms may be converted into equivalent mechanisms containing only parallel and series paths by a procedure which may be termed a star-delta transformation because of the appearance of the equivalent reaction and by analogy with the corresponding electrical transformation (Fig. 6). In Scheme XI (Fig. 6) the rate of entry of E_1 molecules into the pool of molecules represented by E_4 is $k_{+4}E_1$, and the probability that such a molecule will be transferred to E_2 is given by the ratio $k_{-5}/(k_{-4} + k_{-5} + k_{-6})$ (Britton, 1964, 1966). Thus:

$$\text{Flux of } E_1 \text{ to } E_2 = E_1 k_{+4} k_{-5} / (k_{-4} + k_{-5} + k_{-6}) \quad (39)$$

Corresponding expressions apply to the fluxes between E_1 , E_2 and E_3 . Further:

$$E_4 = (E_1 k_{+4} + E_2 k_{+5} + E_3 k_{+6}) / (k_{-4} + k_{-5} + k_{-6}) \quad (40)$$

By analogy with Scheme III (Fig. 1a), therefore

$$r_{14} = k_{+4} / (k_{-4} + k_{-5} + k_{-6}) \quad (41)$$

Scheme XIII

Scheme XIV

Fig. 7. Diagram to illustrate the derivation of the steady-state rate equation for the combined Bi Bi Ping Pong mechanism. Scheme XIII represents the combined and Bi Bi Ping Pong mechanisms of Wong & Hanes (1962) for the reaction $RX + R' = R'X + R$. $RX = A$, $R' = B$, $R'X = Q$ and $R = P$. Step (1) consists of a star-delta transformation about EA. In step (2) a star-delta transformation about EQ has been followed by the addition of parallel rate constants between EX and EAB. Similarly in step (3) a star-delta transformation about EX has been followed by an addition of rate constants between E and EAB to give Scheme XIV. Scheme XIV can be further simplified to Scheme VII (Fig. 2).

If Scheme XII represents a reaction which is equivalent to Scheme XI then

$$k'_{-1} = k_{-1}/(1 + r_{14}) \quad (42)$$

$$k'_{12} = k_{-5}r_{14}/(1 + r_{14}) \quad (43)$$

Corresponding expressions may be written for r_{24} and r_{34} and for the rate constants k'_{-2} , k'_{-3} , k'_{21} , k'_{13} , k'_{31} , k'_{23} and k'_{32} .

The application of the star-delta transformation to the Wong & Hanes (1962) mechanism is shown diagrammatically in Fig. 7 with a description of the steps in the legend. A similar procedure may be used when three substrates add in random order to an enzyme. In this case four star-delta transformations are required.

Calculation of Fluxes from Substrates to Products

The flux of a particular substrate to a product may be defined as the number of individual molecules of this substrate converted into the product per unit time. Britton (1964, 1966) described a method of calculating such fluxes when the steps in the enzyme reaction are in series or parallel and it can be applied when there are triple branch points by making use of

the star-delta transformation. This is illustrated in Fig. 8, where the calculation of the flux of A to Q for the Wong & Hanes (1962) mechanism is set out schematically. The flux A to P may be similarly calculated with a single star-delta transformation. The calculation of such fluxes is simpler than the derivation of the equivalent reaction (Fig. 7), since only the fluxes between the enzyme species have to be considered and division of rate constants by terms of the type $(1 + r)$ is not required (compare Schemes II and III, Fig. 1a). A similar procedure may be used when three substrates add in random order to an enzyme and/or there is a random dissociation of three products. For the addition of three substrates, two star-delta transformations are required.

Discussion

The systematic elimination of steps in an enzyme reaction and their replacement by equivalent reactions to obtain the steady-state velocity equation has a number of advantages. A solution is obtained as an explicit algebraic expression which, in the random Bi Bi mechanism (Figs. 3 and 4), for example, lends itself readily to computation. If certain steps are known at the outset to be rapid, the r terms and the overall calculation will be further simplified. Should

Scheme XV

Fig. 8. Diagram to illustrate the calculation of the flux of A to Q in Scheme XIII (Fig. 7).

Scheme XV represents the routes of conversion of A into Q. Step (1) consists of deleting EX and replacing the individual rate constants with the expressions for the fluxes of EA to EQ and EQ to EA (compare Schemes I and II, Fig. 1a). Step (2) consists of a star-delta transformation with deletion of EA and replacement of the rate constants with the flux expressions for the fluxes of A to EX etc. Fluxes which appear in parallel are added together. Step (3) consists of a similar deletion of EQ. In step (4), after deletion of EAB the parallel fluxes are added together. Note that the expressions for the fluxes are used, and division by terms of the type $(1+r)$ to obtain equivalent reactions (compare Schemes II and III, Fig. 1a) is not necessary. Further, only the fluxes from the enzyme intermediates to Q need be considered.

the polynomial describing the velocity in terms of a given substrate or product be required, the appropriate terms need only be expanded, leading to relatively simple expressions. The particular algebraic form of the solution enables the influence of the rate constants of a given step on the overall rate to be seen relatively readily and the stepwise nature of the process enables, in effect, different parts of the reaction to be examined in isolation. If required, the equivalent reaction procedure may be combined with other techniques. For example, a mechanism might by the use of equivalent reactions be simplified sufficiently to yield a manageable solution by the King & Altman (1956) technique. This has some similarities to the procedure of Cha (1968), but the Cha technique is limited to rapid steps that are at chemical equilibrium.

The equivalent-reaction technique may be used to insert additional steps in a reaction for which the steady-state velocity equation is already known. This has been discussed in relation to the isomerization of

binary complexes, but clearly the same procedure may be applied to other steps in the enzyme reaction. For example, if the steady-state velocity equation is known for a reaction in which the enzyme-substrate complex breaks down in a single step, into products and free enzyme, the effects of various modes of dissociation of the products on the equation can be investigated. In this last case, if the concentration of one of the products is to be set to zero, this simplification should be introduced at the outset to decrease the amount of calculation.

The isomerization of binary complexes (Fig. 5) also illustrates how the equivalent-reaction technique may be used to test whether variants of a particular part of an enzyme reaction can be distinguished by steady-state kinetics without necessarily examining the kinetics of the whole system.

The alternative procedure described for calculating the rate equation depends on the fact that the ratio of the amounts of any two enzyme species may be obtained in terms of the rate constants if the fluxes

between the two species are equated. It is a less systematic procedure than the equivalent-reaction technique, but it can be useful in simpler cases where it may lead very directly to the rate equation. It may also be used to calculate the quantities of enzyme intermediates once the quantity of one or more of the intermediates is known.

The star-delta transformation enables fluxes between enzyme intermediates to be calculated in reactions where there is a random addition of three substrates and in the random Bi Bi Ping Pong mechanism (Wong & Hanes, 1962). Steady-state velocities can therefore be calculated and the fluxes between the substrates and products can also be derived. The method of calculating fluxes has similarities to that described by Flossdorf & Kula (1972), but the latter workers used a procedure that restricted them to chemical equilibrium. An unexpected finding from the present analysis is that in a triple-substrate reaction the ratio (flux of Q to B)/(flux of Q to A) will not necessarily be a linear function of [B] if the addition of A and B to the enzyme is ordered, if C can dissociate randomly

from the enzyme-substrate complexes. Similarly hyperbolic steady-state-velocity kinetics will not necessarily be obeyed with A and B as varied substrates. However, the characteristics of an ordered reaction will be approached at high concentrations of C. Since C may represent an activating metal, such circumstances may not be uncommon.

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