BBA 12161

THE KINETICS OF ENZYME-CATALYZED REACTIONS WITH TWO OR MORE SUBSTRATES OR PRODUCTS

I. NOMENCLATURE AND RATE EQUATIONS*

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SUMMARY

A nomenclature is proposed to facilitate discussion of possible mechanisms for enzyme-catalyzed reactions with more than one substrate or product. A shorthand notation for writing down such mechanisms is also described.

A general method for expressing the full steady-state rate equations for these mechanisms in terms of measurable kinetic constants is explained, and the resulting rate equations are given for a number of mechanisms with two or three substrates or products. The manner in which kinetic studies can be used to determine enzymic mechanisms is discussed, and examples are given to illustrate the types of information about reaction mechanisms that can be obtained.

INTRODUCTION

The steady-state kinetic theory for enzyme-catalyzed reactions having one substrate and one product is well worked out[1]. For mechanisms such as:

$$A + E \rightleftharpoons (EA) \rightleftharpoons (EP) \rightleftharpoons E + P$$

where A and P are substrate and product, E is enzyme, and there may be one, two, or any number of intermediate complexes, the steady-state rate equation for the full reversible reaction may be written in several forms. The form obtained by following the general procedure outlined in this paper is:

$$v = \frac{V_1 V_2 \left(A - \dfrac{P}{K_{\mathrm{eq}}}\right)}{K_{\mathrm{a}} V_2 + V_2 A + \dfrac{V_1 P}{K_{\mathrm{eq}}}} \tag{1}$$

where V_1 and V_2 are maximum velocities in forward and reverse directions, K_{a} and K_{p} are Michaelis constants for A and P, and K_{eq} is the equilibrium constant. Also:

$$K_{\mathrm{eq}} = \frac{V_1 K_{\mathrm{p}}}{V_2 K_{\mathrm{a}}} \tag{2}$$

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This equation, which has been called the Haldane relationship[2], relates the kinetic constants to the equilibrium constant, and can be used to eliminate K_{eq} partly or completely from Eqn. 1. For example, the equation is best written for consideration of the forward reaction as:

$$v = \frac{V_1\left(A - \frac{P}{K_{eq}}\right)}{K_a\left(1 + \frac{P}{K_p}\right) + A} \tag{3}$$

In this form both the contribution of the reverse reaction near the equilibrium point and the product inhibition caused by P are obvious. Thus only the four kinetic constants K_a, K_p, V_1, V_2 and the thermodynamic constant, K_{eq}, are necessary to describe the kinetic behavior in such a steady state system*.

For reactions with two or more substrates or products, however, the rate equations are much more complex and cannot be expressed in terms only of maximum velocities and simple Michaelis constants. Workers have usually written rate equations only for initial velocities, and when rate equations for full reversible mechanisms have been written, they have been left in terms of the individual rate constants and not expressed in terms of kinetic constants[3]. In order to express initial-velocity rate equations in terms of kinetic constants, DALZIEL has used kinetic constants from combinations of which maximum velocities and Michaelis constants may be obtained[4]. ALBERTY has used maximum velocities, Michaelis constants, and compound Michaelis constants to express initial-velocity rate equations[2]. HEARON *et al.* have recently summarized a very general approach to steady-state kinetics, but they have not written their rate equations except in terms of rate constants or groupings of rate constants[5]. When the entire rate equations for reversible reactions with two or more substrates or products are written down in any of the presently used notations they become very unwieldy and hard to work with.

BLOOMFIELD, PELLER AND ALBERTY have recently begun to write full rate equations using a notation in which a kinetic constant is defined for each term in the denominator[6]. Although well adapted for some uses, this system has the disadvantage that none of the constants correspond to Michaelis constants, and the number of constants that have to be defined can be very large. (A mechanism with sequential addition of three substrates and then release of three products with obligatory reaction order has 27 terms in the denominator.) Also the number of additional relationships between these constants is large, and these relationships are not obvious from the form of the equation.

At present there is no generally accepted nomenclature for describing different enzymic mechanisms. Thus a reaction where there are two substrates and two products, an obligatory reaction order, and a ternary complex of enzyme and substrates as well as binary complexes between enzyme and the first substrate or last product, has been called "ordered bimolecular"[3], "ternary complex mechanism"[7], or distinguished from other possibilities by being called Mechanism II (ii)

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* However, if isomerization of free enzyme is a part of the reaction sequence (that is, A and P react with different forms of E), the denominator of the rate equation also contains a term in AP, and additional kinetic constants must be defined. See below under *Iso mechanisms*.

(see ref. 4). If reactions involving two and especially three substrates or products are to be discussed, some simple way of writing down and describing the different possible mechanisms must be available. In this paper, such a shorthand nomenclature is proposed. In addition, a simple method of expressing rate equations entirely in terms of measurable kinetic constants will be described, and the uses to which such equations can be put in deciding between possible mechanisms will be discussed.

NOMENCLATURE

Substrates will be designated by the letters A, B, C, D, in the order in which they add to the enzyme, and products will be designated by P, Q, R, S, . . ., in the order in which they leave the enzyme. Enzyme forms incapable of unimolecular reaction with liberation of a substrate or product or isomerization into such a form will be called stable enzyme forms, and will be designated by E, F, G, H, . . ., with E being the least complex or "free" enzyme, if such a distinction is possible. Enzyme forms capable of unimolecular reaction with liberation of a substrate or product or isomerization into such a form will be called transitory complexes and designated by combinations of letters chosen to represent their composition, such as EA, EAB, FB.

Transitory complexes which cannot participate in bimolecular reaction steps with substrates or products, but can only undergo unimolecular degradation with release of substrates or products, or isomerize into such a form, will be called central complexes. These will be distinguished from other transitory complexes by being enclosed in parentheses: (EAB) or $(EAB\text{–}EPQ)$. Since steady-state kinetics, even when combined with other types of data, can give no evidence about the isomerization of central complexes (as opposed to the isomerization of other transitory complexes), it will normally be assumed to simplify equations that there is only one central complex in each portion of the reaction sequence, although in reality the actual catalytic reaction for most enzymes probably takes place during the conversion of one central complex of substrates and enzyme into another of products and enzyme.

The number of kinetically important substrates or products in a mechanism will be designated by the syllables Uni, Bi, Ter, Quad, and the number of reactants involved in the reaction in one direction will be the reactancy in that direction. Thus a reaction with two substrates and two products will be called Bi Bi and spoken of as being bireactant in both directions. A reaction where there is one substrate and two products will be unireactant in the forward direction and bireactant in the reverse, and will be called Uni Bi. Reactions with three substrates and two products are Ter Bi, and reactions with three substrates and three products are Ter Ter. These are both terreactant in the forward direction. Since most kinetic studies are carried out at constant pH, hydrogen ion will not normally be considered as a substrate.

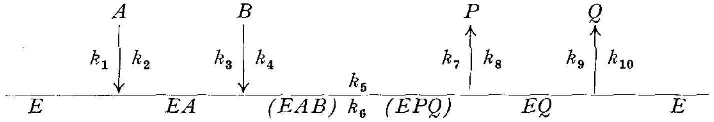
The order of addition of substrates and release of products within the reaction sequence will be described as follows: Mechanisms where all substrates must add to the enzyme before any products are released will be designated "sequential". Such mechanisms will be called "Ordered" if the substrates add in obligatory order and the products leave similarly, and "Random" if the substrates do not react in obligatory order and alternate reaction sequences exist. When one or more products are released before all substrates have added to the enzyme, the enzyme will exist in two or more stable forms between which it oscillates during the reaction. Such mechanisms will be called "Ping Pong", and if the mechanism is not obvious it will be further described by the use of Uni, Bi, Ter, to indicate the successive groups of substrate additions and product dissociations that occur.

In mechanisms described by the above definitions, isomerization of transitory enzyme forms may form a part of the reaction sequence without changing the rate equations. If isomerization of stable enzyme forms is a part of the reaction sequence, however, additional terms are present in the rate equations, and such mechanisms will be designated "Iso Ordered," "Iso Random", and "Iso Ping Pong" to indicate the stable form isomerizations. In Ping Pong mechanisms where several stable enzyme forms may occur, the mechanism can be designated "Di-Iso Ping Pong" or "Tri-Iso Ping Pong" if two or three stable enzyme forms isomerize during the reaction sequence. The syllable "Iso" can also be inserted in the complete description of a Ping Pong mechanism to indicate which enzyme form isomerizes.

For graphical presentation the reaction sequence will be written from left to right in a repeating sequence. The enzyme is represented by a horizontal line, and substrate additions and product dissociations are represented by vertical arrows. Substrates, products, and enzyme forms may be labeled for clarity and identification. Rate constants may be written next to the arrows; those on the left being for the forward reaction and those on the right for the reverse reaction. The reaction mechanism which would usually be written:

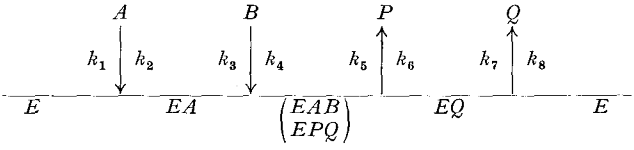
$$E + A \underset{k_2}{\overset{k_1}{\rightleftharpoons}} EA \quad EA + B \underset{k_4}{\overset{k_3}{\rightleftharpoons}} (EAB) \underset{k_6}{\overset{k_5}{\rightleftharpoons}} (EPQ) \underset{k_8}{\overset{k_7}{\rightleftharpoons}} EQ + P \quad EQ \underset{k_{10}}{\overset{k_9}{\rightleftharpoons}} E + Q \tag{4}$$

is thus Ordered Bi Bi, and can be presented graphically as:



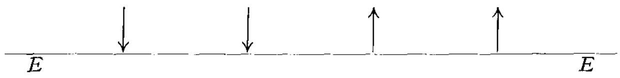
(5)

With only one central complex, the mechanism would be written:

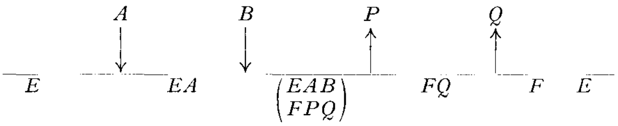


(6)

This is still Ordered Bi Bi, and both mechanisms give rate equations of identical form. "Ordered Bi Bi" is thus a kinetic description and applies to all reaction mechanisms that give the basic pattern:

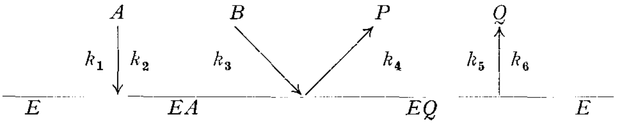


regardless of how many unimolecular isomerizations of transitory enzyme forms take place. If the free enzyme isomerizes, however, the mechanism is Iso Ordered Bi Bi and has a different rate equation:



(7)

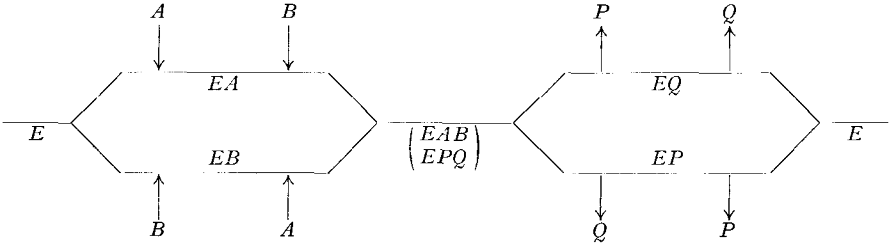
If there is no central complex formed at all, as in the mechanism proposed by THEORELL AND CHANCE for alcohol dehydrogenase[8], the mechanism is different from Ordered Bi Bi and gives a different rate equation, so that the mechanisms can be kinetically distinguished:



(8)

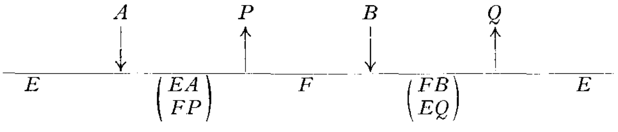
Mechanisms of this type are really limiting cases of more general sequential mechanisms in which the steady-state concentrations of the central complexes are very low. Careful kinetic studies can generally detect the central complexes, so that this type of mechanism is mainly of historical interest.

If there is not an obligatory order of addition of substrates and dissociation of products, the reaction becomes Random Bi Bi:



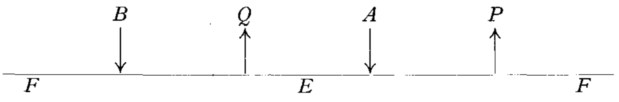
(9)

In reactions such as transaminations, the enzyme oscillates between two stable forms (*E* and *F* here) and we have Ping Pong Bi Bi:



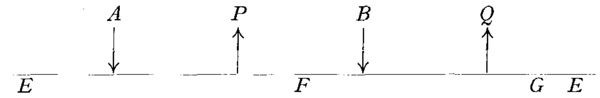
(10)

Such a sequence can just as well be interrupted at *F* and written:



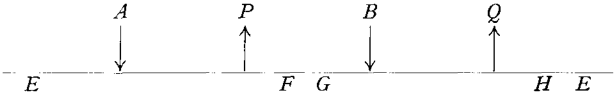
(11)

Iso Ping Pong Bi Bi with isomerization of *E* (Iso Tetra-Uni Ping Pong) would be:



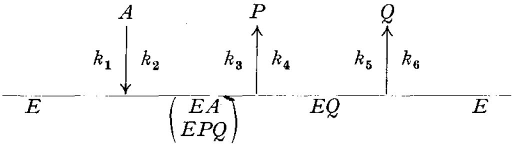
(12)

and Di-Iso Ping Pong Bi Bi would be:



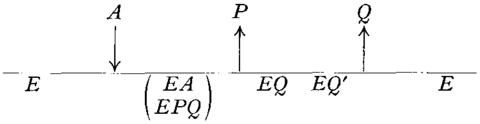
(13)

Several hydrolytic reactions, which appear to be Ping Pong Bi Bi with water as the second substrate, give apparent Ordered Uni Bi kinetics:



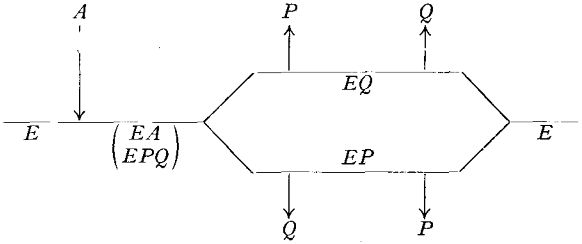
(14)

The form of the rate equation is not changed by assuming isomerization of the *EQ* complex and the mechanism is still Ordered Uni Bi:



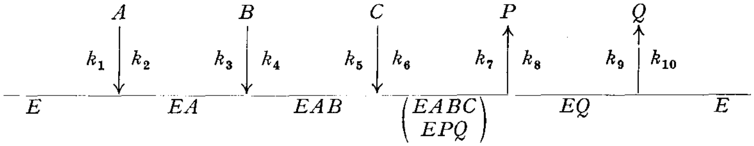
(15)

Release of products in random order gives Random Uni Bi:



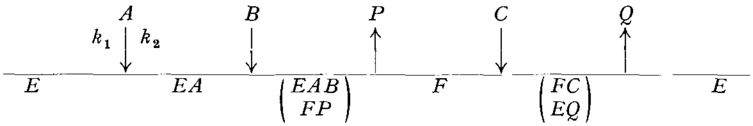
(16)

If consideration is limited to mechanisms where no alternate reaction pathways exist, stable enzyme forms do not isomerize, and central complexes are always present, there appear to be three Ter Bi mechanisms:



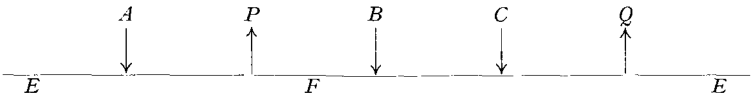
(17)

Ordered Ter Bi



(18)

Bi Uni Uni Uni Ping Pong

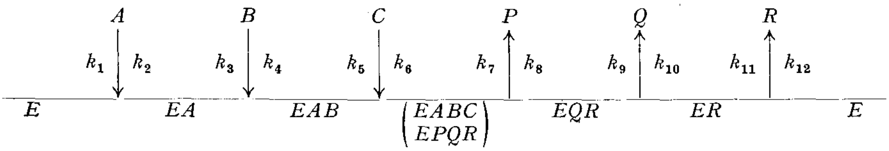


(19)

Uni Uni Bi Uni Ping Pong

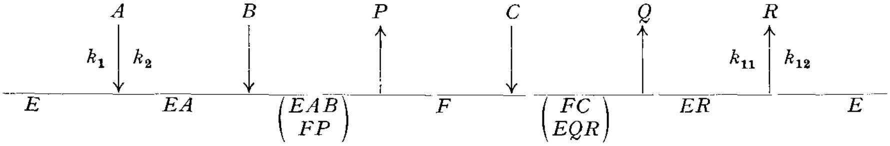
However, if the sequence of Bi Uni Uni Uni is started at F it becomes Uni Uni Bi Uni, and so these two Ping Pong mechanisms are really the same and give rate equations of identical form. The glutamic dehydrogenase reaction is Ordered Ter Bi (see ref. 9), and the enzymic synthesis of S-adenosyl methionine from ATP and methionine may have the second mechanism[10] (Uni Uni Uni Bi Ping Pong when looked at as a Bi Ter reaction).

By the same criteria there are six possible Ter Ter mechanisms:



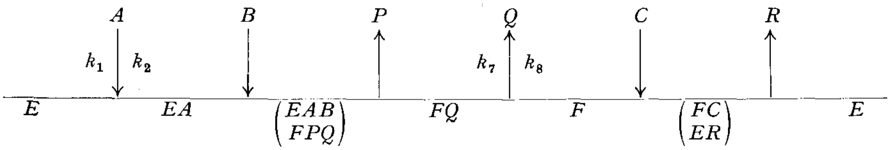
(20)

Ordered Ter Ter



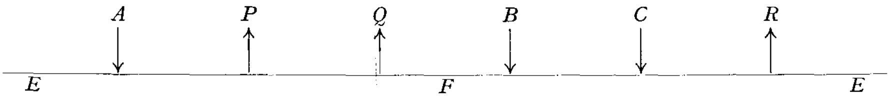
(21)

Bi Uni Uni Bi Ping Pong



(22)

Bi Bi Uni Uni Ping Pong

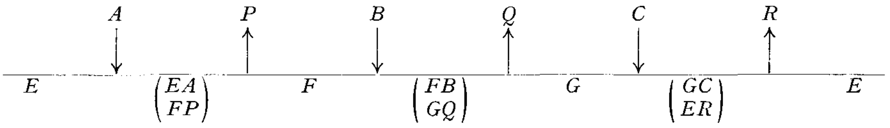


(23)

Uni Bi Bi Uni Ping Pong

(24)

Uni Uni Bi Bi Ping Pong



(25)

Hexa-Uni Ping Pong

However, Uni Uni Bi Bi is identical with the reverse reaction of Bi Bi Uni Uni and is also equivalent to it if its sequence is started in the middle. Likewise, Bi Uni Uni Bi is equivalent to Uni Bi Bi Uni if its sequence is started in the middle with form *F*. As a result there are only four really different Ter Ter mechanisms. The glutamine synthetase reaction is probably[11] Ordered Ter Ter, the activation of acetate by yeast acetate thiokinase may be[12] Bi Uni Uni Bi Ping Pong, and γ-glutamyl-cysteine synthetase is thought[13] to be Hexa-Uni Ping Pong. Propionyl CoA carboxylase has been shown[14] to resemble Bi Bi Uni Uni Ping Pong, and thus apparent examples of all four types of mechanisms are known. Similar analysis of Quad Ter mechanisms shows ten total patterns, of which only five are really different mechanisms. For Quad Quad mechanisms there are 20 total patterns, only nine of which are different. For Quad Bi there are four mechanisms, three of them different. Mevalonic pyrophosphate decarboxylase[15] is Bi Quad, the citrate-splitting enzyme[16] is Ter Quad, and carbamyl phosphate synthetase from animals is Quad Quad[17], but no detail is known as yet about these mechanisms.

RATE EQUATIONS

The simple and elegant method of King and Altman[18] allows the steady-state rate equations for mechanisms of considerable complexity to be written down in terms of the individual rate constants without going through messy algebraic expansions of large determinants. This method can easily be learned and used by anyone without a complete understanding of how or why it works. It was used to derive all of the rate equations discussed here, although some of the simpler ones were checked by the standard determinant method. In this section it will be shown how rate equations expressed in terms of rate constants can be transformed into equations expressed entirely in terms of measurable kinetic constants.

For mechanisms with more than one substrate or product, it is necessary in order to express the full rate equation in terms of kinetic constants to define more kinetic constants than just two maximum velocities and one Michaelis constant per reactant. Bloomfield, Peller and Alberty have defined a kinetic constant for each term in the denominator of the rate equation, but this requires a large number of new constants, particularly for terreactant mechanisms[6]. A simpler method is to define one "inhibition constant" for each reactant in the mechanism*. For non-random mechanisms without alternate reaction sequences this allows the complete rate equation to be expressed in terms of only two maximum velocities, one Michaelis and one inhibition constant for each reactant, and the equilibrium constant for the reaction.

For non-random mechanisms the complete rate equation will have two numerator terms: one positive term including the product of the concentrations of all reactants in the forward reaction, and one negative term including a similar product for all reactants in the reverse reaction. The denominator consists of a number of terms which are various functions of the concentrations of the reactants. For analysis it is convenient to write the rate equation in "coefficient" form, in which the various factors in each term other than the concentrations of reactants are lumped together and considered as the coefficient of the term. The coefficients of the numerator terms are designated (numerator_1) and (numerator_2) for the positive and negative terms, respectively, and the denominator term coefficients are named after the concentration factors present. Thus the equation for Ordered Uni Bi (see APPENDIX) can be written in coefficient form as:

$$v = \frac{(\text{numerator}_1)\,A - (\text{numerator}_2)\,PQ}{(\text{constant}) + (\text{coef}\,A)\,A + (\text{coef}\,P)\,P + (\text{coef}\,Q)\,Q + (\text{coef}\,PQ)\,PQ + (\text{coef}\,AP)\,AP} \quad (26)$$

Once the rate equation is written in coefficient form, the maximum velocity in either direction can be defined as the ratio of the numerator coefficient for reaction in that direction and the denominator term coefficient containing the same concentration factors (namely, the product of the concentrations of all substrates for reaction in that direction). These constants will be designated V_1 and V_2 for forward and reverse directions, respectively, and will represent velocities with all substrates saturating (at infinite concentration) and all products at zero concentration. (It should be remembered that the observed maximum velocity V for any reaction will be a function of the concentrations of the non-variable substrates and will equal V_1 or V_2 only when these substrates are all saturating.) For Ordered Uni Bi the definitions would be:

$$V_1 = \frac{(\text{numerator}_1)}{(\text{coef}\,A)} \qquad V_2 = \frac{(\text{numerator}_2)}{(\text{coef}\,PQ)}$$

V_1 and V_2 measure changes in concentration per unit time. If E_t is the concentration of the enzyme (or of independent active sites if there are several per molecule), then

$$\frac{V_1}{E_t} \text{ and } \frac{V_2}{E_t}$$

are the turnover numbers for forward and reverse reactions and have dimensions of reciprocal time.

The Michaelis and inhibition constants are defined as ratios of denominator

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[*] These constants are called "inhibition" constants because they often are identical with the actual inhibition constants for product inhibition by the reactant they are associated with, especially if all non-variable substrates are at saturating levels. They may also be identical with the dissociation constants for some of the transitory complexes (see Discussion).

term coefficients. This ratio must be such that when letters corresponding to reactants are cancelled, the reactant associated with this kinetic constant will remain in the denominator. Thus

$$\frac{(\text{constant})}{(\text{coef } A)}, \quad \frac{(\text{coef } B)}{(\text{coef } AB)}, \text{ and } \frac{(\text{coef } BP)}{(\text{coef } ABP)}$$

are possible definitions of a constant associated with A, and

$$\frac{(\text{constant})}{(\text{coef } P)}, \quad \frac{(\text{coef } B)}{(\text{coef } BP)}, \text{ and } \frac{(\text{coef } AB)}{(\text{coef } ABP)}$$

are possible definitions of a constant associated with P. Michaelis constants will be written K_{a}, K_{b}, K_{p}, and inhibition constants K_{ia}, K_{ib}, K_{ip}. Both have dimensions of concentration and are expressed in the units used for reactant concentrations.

The definition of the Michaelis constant for a reactant has the same denominator as the definition of the maximum velocity in the direction for which this reactant is a substrate. For Ordered Uni Bi the Michaelis constants are:

$$K_{\mathrm{a}} = \frac{(\text{constant})}{(\text{coef } A)} \qquad K_{\mathrm{p}} = \frac{(\text{coef } Q)}{(\text{coef } PQ)} \qquad K_{\mathrm{q}} = \frac{(\text{coef } P)}{(\text{coef } PQ)}$$

For a Ter Bi mechanism the Michaelis constants would be:

$$K_{\mathrm{a}} = \frac{(\text{coef } BC)}{(\text{coef } ABC)} \quad K_{\mathrm{b}} = \frac{(\text{coef } AC)}{(\text{coef } ABC)} \quad K_{\mathrm{c}} = \frac{(\text{coef } AB)}{(\text{coef } ABC)} \quad K_{\mathrm{p}} = \frac{(\text{coef } Q)}{(\text{coef } PQ)} \quad K_{\mathrm{q}} = \frac{(\text{coef } P)}{(\text{coef } PQ)}$$

These Michaelis constants are limiting constants. The observed Michaelis constant when A is varied will equal K_{a} only when all other substrates are saturating, and at non-saturation levels can be equal to, greater than, or less than K_{a}, depending on the mechanism.

The definitions of the inhibition constants are chosen from the remaining possible definitions so that all of the coefficients of the terms in the denominator of the rate equation can be expressed in terms of kinetic constants when the rate equation in coefficient form is multiplied by the proper factor, which is (numerator$_2$) divided by the product of the two denominator term coefficients used in defining the maximum velocities. This factor is

$$\frac{(\text{numerator}_2)}{(\text{coef } A)\ (\text{coef } PQ)}$$

for Ordered Uni Bi, for example. For ordered mechanisms that are only bireactant (Uni Bi and Bi Bi) there are two choices for some of the definitions, but if the mechanism is of Ter or greater reactancy in either direction there is only one set of definitions for the inhibition constants. The choice is simplified when some of the possible ratios are identical (in terms of rate constants), and in some cases all of the possible ratios will be identical and there is no problem of choice.

In addition to the above definitions, the thermodynamic equilibrium constant K_{eq} always equals

$$\frac{(\text{numerator}_1)}{(\text{numerator}_2)}.$$

Now when the rate equation in coefficient form is multiplied by the factor given above, all of the coefficients of terms can be expressed by using only kinetic constants and K_{eq}. The numerator has the form:

$$V_1V_2ABC\ldots - \frac{V_1V_2}{K_{eq}}PQR\ldots$$

and denominator terms contain either V_2 or V_1/K_{eq} as a factor. Division by V_2 puts the equation in the proper form for consideration of the forward reaction (division by V_1/K_{eq} does likewise for the back reaction). After this division certain denominator terms contain the factor V_1/V_2K_{eq}, which can be eliminated by substitution from the proper Haldane relationship (see below). The numerator of the equation then has the form:

$$V_1\left(ABC\ldots - \frac{PQR\ldots}{K_{eq}}\right)$$

and the denominator terms contain only Michaelis and inhibition constants and concentration factors.

The method given above is general for non-random mechanisms, regardless of reactancy. For reference, the rate equations for some of the more common multireactant mechanisms are given in the APPENDIX, together with the definitions of the inhibition constants. The definitions of K_{eq}, V_1, V_2, and the Michaelis constants are easily obtained by the rules stated above.

Haldane relationships

The various kinetic constants for a mechanism are not independent, but are related to the thermodynamic equilibrium constant by equations of the type:

$$K_{eq} = \left(\frac{V_1}{V_2}\right)^n \frac{K_{(p)}K_{(q)}K_{(r)}\ldots}{K_{(a)}K_{(b)}K_{(c)}\ldots} \tag{27}$$

where $K_{(a)}$ may be either K_a or K_{ia} and so forth. The equation includes either a Michaelis or inhibition constant for each reactant. There will always be at least one Haldane equation where $n = 1$, since this is needed for reexpressing denominator term coefficients in the rate equation for consideration of forward or reverse reactions (see above), but there may also be Haldanes where n has some other value.

Ordered mechanisms that are of Ter or greater reactancy in either direction have two Haldanes, with $n = 0$ and 1. There are two Haldanes for Ordered Uni Bi with $n = 1$, and two for Ordered Bi Bi, but the value of n in the second one depends on the definitions of the inhibition constants. For the set of definitions used in this paper (see APPENDIX) $n = 2$ for the second Haldane, and for the alternate set of definitions, n would equal 0. Ping Pong mechanisms with two stable forms have four Haldanes, one each with $n = 0$ and 2, and two with $n = 1$. For mechanisms with three stable forms, there are eight Haldanes with n equal to zero (1), one (3), two (3), and three (1). With four stable forms, there are 16 Haldanes with n equal to zero (1), one (4), two (6), three (4), and four (1). The Theorell–Chance mechanism with only six rate constants has 16 Haldanes with n equal to minus one (1), zero (4), one (6), two (4), and three (1), corresponding to 4, 3, 2, 1, and 0 inhibition constants, respectively. If the EA and EQ complexes isomerize, however, only four of these Haldanes with $n = 1$ are still valid and should be used as a test for the mechanism. This is of some interest, since the Haldane containing all Michaelis constants with $n = 3$ has been used as a test for this mechanism[2]. This situation is unique, and isomerization in mechanisms with central complexes does not influence the validity of Haldanes. In the APPENDIX Haldanes are given along with the rate equations for the mechanisms described.

Distribution equations

KING's method yields equations for E/E_t, EA/E_t, EAB/E_t, etc., which describe the distribution of the enzyme among the various possible forms. These distribution equations when multiplied by E_t give the steady-state concentrations of the various enzyme forms, and these are substituted into an equation representing the velocity of the reaction (such as $v = \mathrm{d}Q/\mathrm{d}t = k_7\,(EQ) - k_8Q(E)$) in order to obtain the overall rate equation for any given mechanism. These distribution equations have some interest by themselves, however, and are useful in deriving rate equations for reactions in the presence of dead end inhibitors[19], and equations for rates of isotopic exchange*. They all have the same denominator as the rate equation and thus if they are multiplied by the same factor (given above) used to convert the rate equation into kinetic constant form, their denominators will also be expressed in terms of kinetic constants. The terms in the numerators of these equations sometimes represent entire denominator terms, which are easily expressed in terms of kinetic constants, but some denominator terms are split between the numerators of two or more of these equations, in which case their numerators can be expressed in terms of kinetic constants only if certain rate constants can be evaluated in terms of kinetic constants (see below). In general, distribution equations can be expressed in kinetic constant form for stable enzyme forms that do not isomerize and for any non-central transitory complexes that are part of an ordered sequence of Bi or greater reactancy and do not isomerize. The amount of enzyme in the central complex or complexes can also be expressed in kinetic constant form for ordered mechanisms with no isomerizations other than of the central complex. In the APPENDIX the distribution equations that can be expressed in kinetic constant form are given for the mechanisms listed. Note that the validity of the distribution equations for transitory complexes depends on the assumption of non-isomerization, while the validity of the rate equations and Haldanes does not.

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* For instance, in Ordered Bi Bi the initial velocity with which radioactive B appears in P can easily be shown by the usual steady-state analysis to be:

$$v = \frac{V_1}{K_b} B^{*} \left(\frac{EA}{E_t}\right)$$

where B^{*} is the concentration of labeled B. Likewise the appearance of labeled A^{*} in P is given by:

$$v = \frac{V_1 A^{*} B}{K_{ia} K_b} \left(\frac{E}{E_t}\right) \left(\frac{1}{1 + \dfrac{K_a B}{K_{ia} K_b}}\right)$$

and similar equations are easily derived for A–Q and B–Q exchange. Substitution from the proper distribution equation gives the overall rate equation for the exchange with very little effort.

Rate constants

In some cases, combination of the equations resulting from definition of the kinetic constants allows calculation of some or all of the rate constants from these observed kinetic constants. Rate constants can be calculated from kinetic constants for the ordered portions of reaction sequences that are of Bi or greater reactancy and without isomerizations, except that rate constants for formation or dissociation of central complexes cannot be determined for Ping Pong mechanisms. As with the distribution equations for transitory complexes, the validity of the calculations depends on the assumption of non-isomerization of transitory complexes. The most useful applications of these calculations are in determining the degree to which some bimolecular steps are diffusion limited[20], and in detecting isomerization from failure of consistency in the calculations (see DISCUSSION). Equations for calculating rate constants from kinetic constants are given in the APPENDIX for the mechanisms listed.

Iso mechanisms

Isomerization of a transitory complex in any mechanism does not affect the form of the rate equation, but if a stable enzyme form isomerizes, additional terms occur in the denominator. Thus Iso Uni Uni has an AP term, Iso Ordered Uni Bi has AQ and APQ terms, Iso Random Uni Bi has AP^2, AQ^2, AP^2Q, and APQ^2 terms, and Iso Ordered Bi Bi has APQ, ABQ, and $ABPQ$ terms. Of the two Iso Ping Pong Bi Bi mechanisms, Iso Tetra-Uni Ping Pong (form E isomerizes) has new terms in AQ, ABQ, and APQ, while Uni Uni Iso Uni Uni Ping Pong (form F isomerizes) has BP, ABP, and BPQ terms. Di-Iso Ping Pong Bi Bi has all six of these terms. Iso Theorell–Chance has extra APQ and ABQ terms and is of special interest since its product inhibition pattern is identical with that of Ordered Bi Bi, except that B and P are the competitive pair instead of A and Q. Binding studies are necessary to distinguish between these two mechanisms.

For all non-random Iso mechanisms, the rate equations can be expressed in terms of kinetic constants by also defining, for each stable form isomerizing, two new iso-inhibition constants which carry subscripts corresponding to the reactants combining with the isomers of the stable form. The K_{ii} for a given reactant will be defined as the ratio of the denominator term coefficient used to define the maximum velocity for formation of this reactant, divided by the coefficient of the denominator term including this reactant as well. Thus for Iso Ordered Bi Bi we have the definitions:

$$K_{\mathrm{iia}} = \frac{(\text{coef } PQ)}{(\text{coef } APQ)} \quad K_{\mathrm{iiq}} = \frac{(\text{coef } AB)}{(\text{coef } ABQ)}$$

These definitions allow the ABQ and APQ terms of the rate equation to be expressed in terms of kinetic constants as:

$$\frac{V_1 APQ}{K_{\mathrm{iia}} K_{\mathrm{eq}}} \text{ and } \frac{V_2 ABQ}{K_{\mathrm{iiq}}}$$

The $ABPQ$ term can be expressed in terms of kinetic constants as:

$$\frac{K_{\mathrm{ia}} V_2 ABPQ}{K_{\mathrm{iia}} K_{\mathrm{ip}} K_{\mathrm{q}}}$$

For Iso Ordered Uni Bi, K_{iia} is the same as above, and

$$K_{\mathrm{iiq}} = \frac{(\mathrm{coef}\ A)}{(\mathrm{coef}\ AQ)}$$

For Iso Uni Uni,

$$K_{\mathrm{iia}} = \frac{(\mathrm{coef}\ P)}{(\mathrm{coef}\ AP)} \text{ and } K_{\mathrm{iip}} = \frac{(\mathrm{coef}\ A)}{(\mathrm{coef}\ AP)},$$

while for Iso Ordered Ter Ter,

$$K_{\mathrm{iia}} = \frac{(\mathrm{coef}\ PQR)}{(\mathrm{coef}\ APQR)} \text{ and } K_{\mathrm{iir}} = \frac{(\mathrm{coef}\ ABC)}{(\mathrm{coef}\ ABCR)}.$$

In each case, the iso-inhibition constants are related to the corresponding inhibition constants by an equation of the type*:

$$\frac{K_{\mathrm{iia}}}{K_{\mathrm{iiq}}} = \frac{K_{\mathrm{ia}}V_1}{K_{\mathrm{iq}}V_2} \tag{28}$$

If two stable enzyme forms isomerize, as in Di-Iso Ping Pong Bi Bi, there will be four iso-inhibition constants, and two of these equations (with subscripts containing a and q, and b and p, in this example).

Stable enzyme form isomerization does not affect the velocity in the absence of products, but product inhibition patterns are modified so that the order of addition of substrates and release of products can not be determined by kinetic experiments. Reactants can be divided into groups, however (B and P, and A and Q pairs in Iso Ordered Bi Bi, for example). Rate constants for steps involving the isomerizing stable form or any central complex are not determinable, and steady-state distributions can be calculated only for non-isomerizing stable forms and for non-central transitory forms, the rate constants for formation of which can be determined. An exception to all of the above rules is Iso Theorell–Chance (and similar mechanisms lacking central complexes) where the rate constants and enzyme form distributions can be calculated by equations analogous to those for Ordered Bi Bi, and the reaction order can be determined from the product inhibition pattern.

Random mechanisms

The rate equations for mechanisms with alternate reaction sequences contain more terms in both the numerator and denominator than do the equations for the corresponding ordered mechanisms. For Random Uni Bi, for instance, the numerator is:

$$\left(A - \frac{PQ}{K_{\mathrm{eq}}}\right)(a + bP + cQ)E_t$$

where a, b, and c are groupings of rate constants, and the denominator contains terms in AQ, APQ, P^2, Q^2, P^2Q, and PQ^2 in addition to those that would be present if the mechanism were ordered. In the forward direction the initial velocity equation

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* This equation also holds for Iso Uni Uni if K_{ia} and K_{ip} are defined properly, but for this mechanism it is more useful to know that:

$$\frac{K_{\mathrm{iia}}}{K_{\mathrm{iip}}} = \frac{K_{\mathrm{a}}}{K_{\mathrm{p}}}.$$

reduces to

$$v = \frac{V_1 A}{K_a + A} \text{ where } V_1 = \frac{a\,(E_t)}{(\text{coef } A)} \text{ and } K_a = \frac{(\text{constant})}{(\text{coef } A)},$$

in analogy with Ordered Uni Bi. Product inhibition is not the same, however, and both P and Q produce non-competitive inhibitions in which the slopes and intercepts of reciprocal plots are not linear functions of inhibitor concentration[19]. In the reverse direction, this mechanism does not give normal hyperbolic kinetics with linear reciprocal plots unless one substrate is saturating. For Random Bi Bi, the numerator of the rate equation has the form:

$$\left(AB - \frac{PQ}{K_{\text{eq}}}\right)(a + bA + cB + dP + eQ + fAP + gAQ + hBP + iBQ)E_t$$

where a, b, c, . . . i, are combinations of rate constants, and the denominator has 37 new terms in addition to those in the denominator of the Ordered Bi Bi rate equation. Again, a plot of velocity *versus* substrate concentration is not a hyperbola and reciprocal plots are not straight lines, unless one substrate is saturating. The author has written out the rate equations for these mechanisms but because of the large number of constants that would have to be defined, they have not been put into kinetic constant form.

Rapid Equilibrium Random mechanisms

If it is assumed that there are two central ternary complexes in the Random Bi Bi mechanism ((EAB) and (EPQ)) and that all steps are very rapid except the interconversion of the ternary complexes, the rate equation is greatly simplified*. This simplified equation can be derived from the full rate equation for the Random Bi Bi mechanism including two central complexes by dropping all terms including either of the two rate constants for the central complex isomerization; or more simply, by a non-steady state algebraic solution of the differential equations of the system, assuming all steps to be at equilibrium except the central complex isomerization. The rate equation is then identical in form with that of Ordered Bi Bi (see APPENDIX) except that there are no AP, BQ, ABP, and BPQ terms in the denominator. The kinetic constants are defined as for Ordered Bi Bi (see APPENDIX) except that:

$$K_{\text{ib}} = \frac{(\text{constant})}{(\text{coef } B)} \qquad K_{\text{ip}} = \frac{(\text{constant})}{(\text{coef } P)}$$

There are four Haldanes which are identical with those for the Theorell–Chance mechanism (see APPENDIX). Individual rate constants cannot be determined, other than those for the center step which are equal to V_1/E_t and V_2/E_t, but the distribution among the enzyme forms can be calculated from substrate concentrations and the various Michaelis and inhibition constants (which are all equilibrium constants for the various steps that are at equilibrium). This mechanism can be called Rapid Equilibrium Random Bi Bi.

If in such a mechanism reactants A and Q are bound at one site, and reactants B and P at an independent adjacent site, there may be inactive ternary complexes formed such as EBQ and EAP. However, if a bulky group is transferred during the reaction, the two reactants containing this group may not both be able to occupy their sites at once. In this case only one inactive ternary complex would be possible. Assuming again that the rate of formation and dissociation of such inactive complexes is much greater than the rate of isomerization between the active (*EAB*) and (*EPQ*) complexes, the effect of such inactive complexes is to add single terms to the rate equation. Formation of an *EBQ* complex adds a *BQ* term to the denominator, and formation of an *EAP* complex adds a term in *AP*. This case is of particular interest because of the recent suggestion by REYNARD *et al.*[21] that pyruvic kinase has this mechanism.

Rapid Equilibrium Random mechanisms of Ter Bi, Ter Ter, and greater reactancy are easily handled as long as the interconversion of two central complexes is the rate-limiting step. The rate equations are easily derived by the algebraic method and the denominators contain one term for each complex present during reaction. The concentration factors in each term correspond to the reactants that are released when this complex breaks down to form free enzyme along non-rate limiting pathways. For instance, an *EA* complex gives an *A* term, an *EPQ* complex a *PQ* term, and free enzyme gives a constant term. If the mechanism is of Ter or greater reactancy, more than one inhibition constant per reactant will need to be defined, unless the rates of addition and release of reactants are unaffected by the presence of the other reactants on the enzyme. The Michaelis constants are the equilibrium constants for the first dissociations of the central complexes.

If the mechanism includes two or more sets of ternary complexes, and the interconversions in each set are slow compared to other steps, but of roughly equal rate, then the rate equation becomes quite complex (although still derivable by non-steady state algebraic methods), and reciprocal plots are not likely to be linear. Such situations could occur in multireactant Ping Pong mechanisms if an enzyme-bound molecule acted as a carrier between two separate and independently reacting active sites (see DISCUSSION).

DISCUSSION

The nomenclature and notation described in this paper have proven very useful in discussing various mechanisms and in teaching enzyme kinetics to graduate students. The number of defined kinetic constants is not large and these correspond either to maximum velocities, Michaelis constants, product inhibition constants, or equilibrium constants for steps in the mechanism. Where addition of a reactant gives a non-central transitory complex, the inhibition constant for this reactant is also the dissociation constant for this complex*.

Because other notations have been used to express kinetic equations, particularly for bireactant mechanisms, a partial comparison is shown for sequential bireactant mechanisms in Table I between the system described in this paper and those most

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* The dissociation constant is for conversion of total complex of one composition to total complex of composition lacking one reactant. Thus, if $E + A \rightleftharpoons EA \rightleftharpoons EA'$, K_{ia} represents the ratio $\frac{(E)(A)}{(EA + EA')}$ at equilibrium and not just $\frac{(E)\,(A)}{(EA)}$.
The same is true if *EA'* is a dead end complex formed directly from *E* and *A*, but not participating further in the reaction.

often seen in the literature. The system described here is dimensionally simpler, since all K's and E_t have dimensions of concentration, and V's have dimensions of concentration per unit time. In the other systems the dimensions of the constants are more complex, although the system first used by ALBERTY[2] is the same as the present one except for the definitions of compound Michaelis constants instead of inhibition constants*.

TABLE I

NOMENCLATURE COMPARISON FOR SEQUENTIAL BI BI MECHANISMS

If the reaction order is obligatory, A adds first.

	DALZIEL[4]	ALBERTY[2]	*This paper*	BLOOMFIELD, PELLER AND ALBERTY[6]
Michaelis constant for A, B saturating	Φ_1/Φ_0	K_A	K_a	K_{AB}/K_B
Michaelis constant for B, A saturating	Φ_2/Φ_0	K_B	K_b	K_{AB}/K_A
Dissociation constant for EA complex	Φ_{12}/Φ_2	K_{AB}/K_B	K_{ia}	K_A
Maximum velocity, A and B saturating	e/Φ_0	V_f	V_1	V_{AB}
Turnover number	$1/\Phi_0$	$V_f/(E)_0$	V_1/E_t	$V_{AB}/(E)_0$

The various kinetic constants can all be determined from experimental data. Initial velocity analysis suffices for Michaelis constants, maximum velocities, and the inhibition constants for reactants which yield non-central transitory complexes upon combination with the enzyme, while product inhibition experiments are necessary to determine the other inhibition constants (and the order of addition of reactants in ordered sequences, if this is not known from binding studies). The usual initial velocity analysis involves varying each substrate concentration at several fixed levels of one of the others and replotting the slopes and intercepts of the reciprocal plots ($1/v$ *versus* 1/substrate) *versus* the reciprocal of the concentration of the latter substrate. This method has been described for bireactant sequential mechanisms by FLORINI AND VESTLING[22], and in such mechanisms the inhibition constant for the first substrate to combine with the enzyme is determined from the intersection point of the reciprocal plots where this substrate is the variable one, as shown by FRIEDEN[23]. In Ping Pong mechanisms, the analysis can be simplified by varying, at constant ratio, the concentrations of two or more substrates which occur in different portions of the reaction sequence[24] (that is, whose times of combination with the enzyme are separated by product release). These methods are applicable directly to terreactant mechanisms, although the analysis is somewhat more complex.

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* In order to describe complete rate equations, BLOOMFIELD, PELLER AND ALBERTY[6] have defined one kinetic constant for each term in the denominator of the rate equation. However, these K's now no longer correspond to Michaelis constants, and K_A, for instance, does not have the same meaning as K_a in either the system described here or the one used earlier by ALBERTY[2].

The full analysis mentioned above is often not necessary to identify the mechanism, which can frequently be determined from the variation of apparent Michaelis constants and maximum velocities with the concentration of the non-varied substrate. The apparent Michaelis constant is the slope of a reciprocal plot (of $1/v$ *versus* 1/substrate) divided by the vertical intercept, and the apparent maximum velocity is the reciprocal of this intercept. For sequential bireactant mechanisms such as Ordered Bi Bi, for example, the apparent Michaelis constants for A and B are:

$$K_a \frac{\left(1 + \frac{K_{ia}K_b}{K_a B}\right)}{\left(1 + \frac{K_b}{B}\right)} \quad \text{and} \quad K_b \frac{\left(1 + \frac{K_{ia}}{A}\right)}{\left(1 + \frac{K_a}{A}\right)}$$

while for bireactant Ping Pong cases they are:

$$\frac{K_a}{\left(1 + \frac{K_b}{B}\right)} \quad \text{and} \quad \frac{K_b}{\left(1 + \frac{K_a}{A}\right)}$$

For both types of mechanism the apparent V is:

$$\frac{V_1}{\left(1 + \frac{K_b}{B}\right)} \quad \text{or} \quad \frac{V_1}{\left(1 + \frac{K_a}{A}\right)}$$

when A or B, respectively, is the variable substrate.

In the Ping Pong case, the apparent Michaelis constants increase to their limiting values as the concentration of the fixed (non-varied) substrate increases, and decrease to zero as the fixed substrate concentration goes to zero. For the sequential mechanism, as the concentration of the fixed substrate rises, the Michaelis constants may increase or decrease to, or remain constant at their limiting values, depending on whether K_{ia} is less than, greater than, or equal to K_a. As the concentration of the fixed substrate decreases to zero, the K changes to K_{ia}/K_a times the limiting Michaelis constant.

Thus a mechanism where the apparent Michaelis constants decrease or remain constant with increase in the level of the non-varied substrate must be sequential. The data of BODANSKY for phosphoglucomutase[25] show that the apparent Michaelis constants do not vary when the other fixed substrate is changed through the region of its own apparent Michaelis constant, suggesting that this is a sequential rather than Ping Pong mechanism as one would be led to expect by the isolation of two stable enzyme forms*.

The ratio of apparent K to apparent V for the sequential cases is

$$\frac{K_a}{V_1}\left(1 + \frac{K_{ia}K_b}{K_a B}\right) \text{ or } \frac{K_b}{V_1}\left(1 + \frac{K_{ia}}{A}\right)$$

while for the Ping Pong case it is K_a/V_1 or K_b/V_1. Thus if the ratio of apparent Michaelis constant to apparent maximum velocity remains constant as the concentration of the fixed substrate is changed, the mechanism is Ping Pong. The data of Novick for monoamine oxidase show this relationship[27].

The advantages of having the full rate equation in hand, rather than just an initial velocity equation, are numerous. The full equation describes the steady-state kinetic behavior of the system at any time and at any point, whether near or far from equilibrium. In theory, it can be integrated and used to predict exactly the time course of a reaction as it approaches equilibrium. It easily yields simplified equations for situations where one or more reactants are at zero concentration, and thus saves the considerable time and effort necessary to derive separately a series of initial velocity and product inhibition equations. Since product inhibition experiments are often the best means of distinguishing different mechanisms, this is one of the most important uses for the full equations.

Available methods that employ steady-state kinetic data for distinguishing mechanisms include following the time course of the reaction, determining the initial velocity patterns, checking the consistency of the experimentally determined kinetic constants with the Haldane and other special relationships that are predicted between the constants[4], and determining the product inhibition patterns[9]. Following the time course of reaction is the most difficult method and has been used mainly for hydrolytic reactions that are Uni Bi. Initial velocity patterns cleanly tell sequential from Ping Pong mechanisms, but this is all. Testing for consistency with Haldane and other relationships is theoretically a good method, but in practice involves the combination of large numbers of constants, each of which may be in error to some degree[**]. As a result such comparisons have rarely enabled clear distinctions between mechanisms to be made. The greatest value of such calculations is in discovering isomerizations of transitory complexes, for which this is really the only available method (see below). Product inhibition studies will distinguish between any mechanisms whose complete rate equations have different forms, and thus is the method with greatest range of application, detecting Iso mechanisms, dead end product inhibitions[19], and the presence of central complexes in a clean and unambiguous manner. As an example, in Table II are listed the product inhibition

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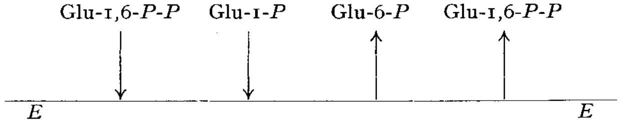
[*] If Bodansky's data are correct, this means that either the phospho- or dephospho-enzyme[26] is an artifact of preparation and not important kinetically. This author favors the mechanism:



where the dephospho-enzyme is a part of the sequence, and the phospho-enzyme might be a breakdown product of one or both of the two transitory Enzyme–Glu-1,6-*P*-*P* complexes that would be the predominate enzyme forms present in the tissue extracts from which the enzyme was prepared.

[**] Special difficulties are also encountered when the substrates activate the enzyme by combining at sites other than the catalytic site. The kinetic constants for reaction in one direction will then be determined in the presence of one substrate, while those for the reverse reaction will be determined in the presence of another substrate. If the different substrates activate to different degrees, no comparison is possible. This appears to be the case for liver alcohol dehydrogenase, where the relationships between kinetic constants predicted for both Ordered Bi Bi and Theorell–Chance mechanisms fail[28], but the product inhibition pattern clearly indicates an Ordered mechanism (C. Wratten and W. W. Cleland, unpublished experiments).

TABLE II

PRODUCT INHIBITION PATTERNS FOR BI BI MECHANISMS

Mechanism	*Inhibitory product*	*Variable substrate*			
		A		*B*	
		Unsaturated	*Saturated with B*	*Unsaturated*	*Saturated with A*
Ordered Bi Bi*	*P*	NC**	UC	NC	NC
	Q	Comp	Comp	NC	—
Theorell–Chance***	*P*	NC	—	Comp	Comp
	Q	Comp	Comp	NC	—
Iso Ordered Bi Bi***	*P*	NC	UC	NC	NC
	Q	NC	NC	NC	UC
Iso Theorell–Chance*	*P*	NC	—	Comp	Comp
	Q	NC	NC	NC	UC
Rapid Equilibrium Random Bi Bi	*P* or *Q*	Comp	—	Comp	—
Rapid Equilibrium Random Bi Bi + dead end *EBQ* complex	*P*	Comp	—	Comp	—
	Q	Comp	Comp	NC	—
Ping Pong Bi Bi	*P*	NC	—	Comp	Comp
	Q	Comp	Comp	NC	—
Iso Ping Pong Bi Bi (Isomerism of *E*)	*P*	NC	—	Comp	Comp
	Q	NC	NC	NC	NC
Di-Iso Ping Pong Bi Bi	*P* or *Q*	NC	NC	NC	NC
Random Bi Bi	*P* or *Q*	NC§	NC§§	NC§	NC§§

* These mechanisms can be distinguished only by binding studies.

** The abbreviations used are[19]: Comp, competitive; UC, uncompetitive; NC, noncompetitive; —, no inhibition.

*** The reaction order must be determined from binding studies, although AQ and BP pairs are distinguished.

§ Reciprocal plots are non-linear. Vertical intercepts are hyperbolic functions of inhibitor, while slopes are a more complex function of inhibitor[19].

§§ Both slopes and intercepts are hyperbolic functions of inhibitor[19].

patterns for all of the Bi Bi mechanisms discussed in this paper. Initial velocity studies can place these into only two groups (Ping Pong and sequential), while only two of these cannot be told apart by product inhibition analysis. In the following paper, the application of product inhibition studies in determining enzymic mechanisms is discussed in more detail[19].

Although isomerization of transitory forms does not alter the rate equation, the existence of isomerization of non-central transitory complexes can often be inferred from kinetic data alone, or from comparison of kinetic data with the equilibrium constant. A classic example of the former is the isomerization of E–DPN and E–TPN complexes in pyridine nucleotide-linked dehydrogenases. For any mechanism, the maximum velocity obtainable in either direction is limited by the magnitude of the rate constants for the unimolecular steps in the sequence, and cannot exceed any of these. If calculation of rate constants from the kinetic constants on the assumption of no isomerization gives values for unimolecular steps that are less than V/E_t for that direction, then the assumption of the simple mechanism and the calculation of at least that rate constant are not valid. The excellent kinetic data of FRIEDEN for glutamic dehydrogenase[9] allow calculation of the rate constants, assuming no isomerizations (see APPENDIX, Ordered Ter Bi mechanism). The rate constant for breakdown of the E–TPN complex, however, comes out 160/sec, while V/E_t for that direction is 1000/sec*. An identical situation occurs with the rate constant calculated for the dissociation of the E–DPN complex from the kinetic constants for lactic dehydrogenase[29] and yeast alcohol dehydrogenase[30] (but not for ribitol dehydrogenase[31]).

Since the rate equations are apparently not altered in any of these cases, there are only two possibilities which will explain the data. First, if the E–DPN (or E–TPN) complex isomerizes, the expression $\frac{K_{ia}V_1}{K_aE_t}$ which normally would equal k_2, the rate constant for dissociation of the E–DPN complex, would now equal

$$\frac{k_2k_3k_4}{(k_2 + k_3)\,(k_3 + k_4)}$$

(where k_3 and k_4 are for the isomerization step) which is necessarily less than k_2, and can easily be less than V_2/E_t (see footnote)**. An alternate explanation is that E and DPN form a dead end complex that is inactive but blocks the reaction, and has a dissociation constant less than that of the active E–DPN complex. The observed value of K_{ia} will reflect this inactive binding and be smaller than k_2/k_1. The kinetic data cannot distinguish between these two possibilities, but the former would seem the more likely.

Another example of enzyme form isomerization detected by combination of kinetic constants and K_{eq} is the analysis by HSU, CLELAND, AND ANDERSON[32] of the mechanism of potato acid phosphatase. This enzyme has an Ordered Uni Bi mechanism. Maximum values of the equilibrium constants for individual steps in the mechanism, assuming no isomerizations, were obtained from kinetic constants (k_1/k_2 from K_{ia}, k_3/k_4 from K_{ip}, k_5/k_6 from K_{iq}) and compared with K_{eq}. Since the product of these was considerably below the value of K_{eq}, it was necessary in order

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* The comparison is between k_{11} and V_f in Table II of FRIEDEN's paper[9]. The value of k_{11} is incorrectly given as 33/sec due to an arithmetic error, but the correct value can be calculated from the kinetic data in Table I of the same paper.

** An interesting point here is that if both the E–DPN and E–DPNH complexes isomerize, only one of the rate constants for dissociation of these complexes can be calculated to be less than the corresponding turnover number. This can be seen by inspecting the following relations for an Ordered Bi Bi mechanism with isomerization of EA (E–DPN) and EQ (E–DPNH):

$$\left(\frac{E_t}{V_2}\right) - \left(\frac{E_t}{V_1}\right)\frac{K_a}{K_{ia}} = \frac{1}{k_6} + \left(\frac{1}{k_{10}} - \frac{1}{k_3}\right)$$

$$\left(\frac{E_t}{V_1}\right) - \left(\frac{E_t}{V_2}\right)\frac{K_q}{K_{iq}} = \frac{1}{k_7} + \left(\frac{1}{k_3} - \frac{1}{k_{10}}\right)$$

It is obvious that only one of these relations can be negative (although both can be positive). Note that calculation of a rate constant for dissociation of E–DPN less than the turnover number (first relationship negative) depends only on whether k_3 is sufficiently smaller than the unimolecular rate constants in the reverse reaction, k_2 and k_4 excepted (these are k_6 and k_{10} here). Surprisingly, k_3 can have any value with respect to k_2 and k_4.

to explain the data to postulate isomerization of the EQ (enzyme–phosphate) complex, which introduces the equilibrium constant of the isomerization step into this product. It is probable that isomerization of enzyme forms is of common occurrence.

In discussing mechanisms in this paper, the effects of cationic activators have been ignored. This cannot be done in the laboratory, of course, since reaction rates are highly dependent on the concentrations of the activators for reactions that are metal activated. Activations have been ignored here for two reasons. First, the theory of cation activation is not well enough worked out that a complete presentation can be made at this time. But more important, it is the feeling of this author that ion concentrations should be chosen that will permit initial velocity and product inhibition experiments to be carried out without considering the kinetics of the metal activator, and then once the reaction mechanism is determined as far as the organic substrates are concerned, the effects of the metal can be analyzed. A classic example of the problems that can arise is the enzyme ATP–creatine transphosphorylase. The elegant work of KUBY *et al.*[33] restricted the mechanism to either Ordered Bi Bi or Rapid Equilibrium Random Bi Bi with a dead end E–ADP–creatine complex, but the product inhibition patterns for creatine phosphate, or for the reverse reaction, which would distinguish between the mechanisms, have not been determined. Considerable work has been done since by various authors on the effects of magnesium on this reaction, but since the mechanism is still not known, the difficulties in interpreting the Mg effects are tremendously multiplied.

A recent example of a good kinetic study where the kinetics of the activator were measured is the study of yeast hexokinase by HAMMES AND KOCHAVI[34]. These workers found that the magnesium complexes of ATP and ADP were the actual reactants, and that free nucleotides were inhibitors. For mechanisms of this type, the equations in this paper can be applied directly by using the concentrations of the active complexes, rather than of free or total nucleotide.

The mechanisms discussed in this paper all assume that there is only one active site on the enzyme that can be operative at one time*. This is probably the case for all Ordered mechanisms and for many Bi Bi Ping Pong mechanisms. It may not be true for Ping Pong mechanisms that are Ter Bi or Ter Ter, however. Here there may be two or three separate active sites combining independently with reactants and connected by an enzyme-bound group acting as a carrier between them. Since such mechanisms contain alternate reaction sequences, the rate equations become more complex, although they can still be derived for any given case by KING's method[18]. A known example of such a mechanism is the pyruvic dehydrogenase complex, which has even been separated into three separate enzymes[35]. The overall reaction catalyzed by the enzyme appears to be Hexa-Uni Ping Pong:

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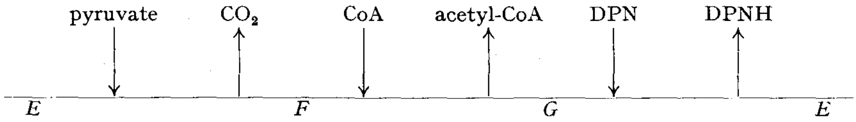
* By "enzyme" is meant a catalytic area capable of carrying out the entire reaction. There may be more than one of these areas on a given protein molecule, as long as they react independently and have identical kinetic properties. If they do not have identical properties, or the enzyme studied is a mixture of isozymes with different kinetic constants, then the observed kinetics will be that of a mixture of enzymic activities, and the simple equations given in this paper will not hold.



with isomerization of F and G, but this reaction is really the sum of three separate reactions taking place at three active centers with enzyme bound lipoic acid acting as a carrier between the sites. The biotin-containing carboxylases, which appear to be Bi Bi Uni Uni Ping Pong[14], are probably another example. In such cases, the overall kinetics will follow the rate equations given in this paper only if reactants cannot combine and react on more than one site at a time. This is probably not the case for pyruvic dehydrogenase, where pyruvate might be decarboxylated to give the hydroxyethyl-thiamine intermediate at the same time that the bound lipoic acid was reacting at one of the other two sites. It could be true for the biotin enzymes if reaction at each site were Ordered, and the biotin were the first molecule adsorbed and the last released at each site. It is also possible in such a Bi Bi Uni Uni mechanism that the Bi Bi portion of the mechanism, while sequential, is not Ordered but Random or Rapid Equilibrium Random, and the rate equation will be different. A kinetic study could give considerable evidence about the intimate mechanism of such a complex reaction. It is hoped that this paper will stimulate research on the kinetics of multireactant mechanisms and that the equations presented here can serve as a starting point for the analysis of experimental results. This author feels that it is in analyzing the most complex mechanisms that kinetic studies can give the most information and be the most useful, and that the apparent complexity of the rate equations should not be allowed to discourage work in this area.

ACKNOWLEDGEMENT

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APPENDIX

Rate equations, definitions of inhibition constants, Haldanes, distribution equations, and rate constant calculations for common multireactant mechanisms.

Ordered Uni Bi (Mechanism 14)

Rate equations. From King's method:

$$v = \frac{(k_1k_3k_5\,A - k_2k_4k_6PQ)E_t}{(k_2 + k_3)k_5 + k_1(k_3 + k_5)A + k_2k_4P + (k_2 + k_3)k_6Q + k_1k_4AP + k_4k_6PQ}$$

This transforms into:

$$v = \frac{V_1V_2\left(A - \frac{PQ}{K_{\mathrm{eq}}}\right)}{K_aV_2 + V_2A + \frac{K_qV_1P}{K_{\mathrm{eq}}} + \frac{K_pV_1Q}{K_{\mathrm{eq}}} + \frac{V_1PQ}{K_{\mathrm{eq}}} + \frac{V_2AP}{K_{\mathrm{ip}}}}$$

Inhibition constants:

$$K_{\mathrm{ia}} = \frac{(\text{coef } P)}{(\text{coef } AP)}\left(= \frac{k_2}{k_1}\right) \qquad K_{\mathrm{ip}} = \frac{(\text{coef } A)}{(\text{coef } AP)} \qquad K_{\mathrm{iq}} = \frac{(\text{constant})}{(\text{coef } Q)}\left(= \frac{k_5}{k_6}\right)$$

Haldanes:

$$K_{\mathrm{eq}} = \frac{V_1\, K_{\mathrm{ip}} K_{\mathrm{q}}}{V_2\, K_{\mathrm{ia}}} = \frac{V_1\, K_{\mathrm{p}} K_{\mathrm{iq}}}{V_2\, K_{\mathrm{a}}}$$

Distribution equations:

$$\frac{E}{E_t} = \frac{K_{\mathrm{a}} V_2 + \dfrac{K_{\mathrm{q}} V_1 P}{K_{\mathrm{eq}}}}{(\text{denominator of rate equation})}$$

$$\frac{(EA + EPQ)}{E_t} = \frac{\left(V_2 - \dfrac{K_{\mathrm{q}} V_1}{K_{\mathrm{iq}}}\right) A + \dfrac{V_2 A P}{K_{\mathrm{ip}}} + \dfrac{V_1 P Q}{K_{\mathrm{eq}}}}{(\text{denominator of rate equation})}$$

$$\frac{EQ}{E_t} = \frac{\dfrac{K_{\mathrm{p}} V_1 Q}{K_{\mathrm{eq}}} + \dfrac{K_{\mathrm{q}} V_1 A}{K_{\mathrm{iq}}}}{(\text{denominator of rate equation})}$$

Rate constants:

$$k_1 = \frac{V_2}{K_{\mathrm{ia}} E_t} \qquad k_2 = \frac{V_2}{E_t} \qquad \frac{1}{k_3} = \frac{E_t}{V_1} - \frac{1}{k_5} \qquad k_4 = \frac{k_2 + k_3}{K_{\mathrm{p}}}$$

$$k_5 = \frac{K_{\mathrm{iq}} V_2}{K_{\mathrm{q}}\, E_t} \quad k_6 = \frac{V_2}{K_{\mathrm{q}} E_t}$$

Effect of isomerizations: $(EA + EPQ)$: Calculation of k_1, k_2, k_3, k_4 invalid. *EQ:* Calculation of all rate constants invalid. Only E/E_t may be calculated.

Ordered Bi Bi (Mechanism 6)

Rate equations. from King's method:

$$v = \frac{(k_1 k_3 k_5 k_7\, AB - k_2 k_4 k_6 k_8\, PQ)\, E_t}{\begin{array}{ll} k_2(k_4 + k_5)k_7 & \\ + k_1(k_4 + k_5)k_7\, A & + k_2(k_4 + k_5)k_8\, Q \\ + k_3 k_5 k_7\, B & + k_2 k_4 k_6\, P \\ + k_1 k_3(k_5 + k_7)\, AB & + (k_2 + k_4)k_6 k_8\, PQ \\ + k_1 k_4 k_6\, AP & + k_1 k_3 k_6\, ABP \\ + k_3 k_5 k_8\, BQ & + k_3 k_6 k_8\, BPQ \end{array}}$$

This transforms into:

$$v = \frac{V_1 V_2 \left(AB - \dfrac{PQ}{K_{\mathrm{eq}}}\right)}{\begin{array}{l} K_{\mathrm{ia}} K_{\mathrm{b}} V_2 + K_{\mathrm{b}} V_2 A + K_{\mathrm{a}} V_2 B + V_2 AB + \dfrac{K_{\mathrm{q}} V_1 P}{K_{\mathrm{eq}}} + \dfrac{K_{\mathrm{p}} V_1 Q}{K_{\mathrm{eq}}} \\ + \dfrac{V_1 PQ}{K_{\mathrm{eq}}} + \dfrac{K_{\mathrm{q}} V_1 AP}{K_{\mathrm{ia}} K_{\mathrm{eq}}} + \dfrac{K_{\mathrm{a}} V_2 BQ}{K_{\mathrm{iq}}} + \dfrac{V_2 ABP}{K_{\mathrm{ip}}} + \dfrac{V_1 BPQ}{K_{\mathrm{ib}} K_{\mathrm{eq}}} \end{array}}$$

Inhibition constants:

$$K_{\mathrm{ia}} = \frac{(\text{constant})}{(\text{coef } A)} = \frac{(\text{coef } P)}{(\text{coef } AP)} \quad \left(= \frac{k_2}{k_1}\right) K_{\mathrm{ib}} = \frac{(\text{coef } PQ)}{(\text{coef } BPQ)}$$

$$K_{\mathrm{ip}} = \frac{(\text{coef } AB)}{(\text{coef } ABP)} \qquad K_{\mathrm{iq}} = \frac{(\text{constant})}{(\text{coef } Q)} = \frac{(\text{coef } B)}{(\text{coef } BQ)}\left(= \frac{k_7}{k_8}\right)$$

Haldanes:

$$K_{\mathrm{eq}} = \frac{V_1\, K_{\mathrm{p}} K_{\mathrm{iq}}}{V_2\, K_{\mathrm{ia}} K_{\mathrm{b}}} = \left(\frac{V_1}{V_2}\right)^2 \frac{K_{\mathrm{ip}} K_{\mathrm{q}}}{K_{\mathrm{a}} K_{\mathrm{ib}}}$$

Distribution equations:

$$\frac{E}{E_t} = \frac{K_{\mathrm{ia}} K_{\mathrm{b}} V_2 + \dfrac{K_{\mathrm{q}} V_1 P}{K_{\mathrm{eq}}} + K_{\mathrm{a}} V_2 B}{(\text{denominator of rate equation})}$$

$$\frac{EA}{E_t} = \frac{K_{\mathrm{b}} V_2 A + \dfrac{K_{\mathrm{q}} V_1 AP}{K_{\mathrm{ia}} K_{\mathrm{eq}}} + \dfrac{K_{\mathrm{a}} V_2 PQ}{K_{\mathrm{ia}} K_{\mathrm{eq}}}}{(\text{denominator of rate equation})}$$

$$\frac{(EAB + EPQ)}{E_t} = \frac{\left(V_2 - \dfrac{V_1 K_{\mathrm{q}}}{K_{\mathrm{iq}}}\right) AB + \left(\dfrac{V_1}{K_{\mathrm{eq}}} - \dfrac{V_2 K_{\mathrm{a}}}{K_{\mathrm{eq}} K_{\mathrm{ia}}}\right) PQ + \dfrac{V_2 ABP}{K_{\mathrm{ip}}} + \dfrac{V_1 BPQ}{K_{\mathrm{ib}} K_{\mathrm{eq}}}}{(\text{denominator of rate equation})}$$

$$\frac{EQ}{E_t} = \frac{\dfrac{K_{\mathrm{p}} V_1 Q}{K_{\mathrm{eq}}} + \dfrac{K_{\mathrm{q}} V_1 AB}{K_{\mathrm{iq}}} + \dfrac{K_{\mathrm{a}} V_2 BQ}{K_{\mathrm{iq}}}}{(\text{denominator of rate equation})}$$

Rate constants:

$$k_1 = \frac{V_1}{E_t K_{\mathrm{a}}} \qquad k_2 = \frac{V_1 K_{\mathrm{ia}}}{E_t K_{\mathrm{a}}} \qquad k_3 = \frac{V_1}{E_t K_{\mathrm{b}}}\left(1 + \frac{k_4}{k_5}\right)$$

$$\frac{1}{k_4} = \frac{E_t}{V_2} - \frac{1}{k_2} \qquad \frac{1}{k_5} = \frac{E_t}{V_1} - \frac{1}{k_7} \qquad k_6 = \frac{V_2}{E_t K_{\mathrm{p}}}\left(1 + \frac{k_5}{k_4}\right)$$

Equations for k_7 and k_8 are identical with those for k_5 and k_6 in Ordered Uni Bi.

Effect of isomerizations: $(EAB + EPQ)$: Distributions are valid; k_1, k_2, k_7, k_8 can still be calculated.

EA: E/E_t, EQ/E_t, k_7, k_8 can still be calculated.
EA and EQ: Only E/E_t can be calculated.

Theorell–Chance mechanism (Mechanism 8)

Rate equation from King's method:

$$v = \frac{(k_1 k_3 k_5 AB - k_2 k_4 k_6\, PQ)\, E_t}{k_2 k_5 + k_1 k_5 A + k_3 k_5 B + k_1 k_3 AB + k_2 k_4 P + k_2 k_6 Q + k_4 k_6 PQ + k_1 k_4 AP + k_3 k_6 BQ}$$

which transforms into the same equation as for Ordered Bi Bi except for the missing ABP and BPQ terms.

Inhibition constants:

$$K_{\mathrm{ib}} = \frac{(\text{coef } Q)}{(\text{coef } BQ)} \qquad K_{\mathrm{ip}} = \frac{(\text{coef } A)}{(\text{coef } AP)}$$

K_{ia} and K_{iq} have the same definitions as in Ordered Bi Bi.

Haldanes:

$$K_{\text{eq}} = \frac{V_1 K_{\text{ip}} K_{\text{q}}}{V_2 K_{\text{a}} K_{\text{ib}}} = \frac{V_1 K_{\text{p}} K_{\text{iq}}}{V_2 K_{\text{a}} K_{\text{ib}}} = \frac{V_1 K_{\text{p}} K_{\text{iq}}}{V_2 K_{\text{ia}} K_{\text{b}}} = \frac{V_1 K_{\text{ip}} K_{\text{q}}}{V_2 K_{\text{ia}} K_{\text{b}}}\,.$$

There are 12 more Haldanes which are true for the simple mechanism with 6 rate constants, but not valid when EA and EQ isomerize.

Distribution equations: E/E_t: Same as for Ordered Bi Bi.

EA/E_t: Same as for Ordered Bi Bi, except numerator PQ term is: V_1PQ/K_{eq}.
EQ/E_t: Same as for Ordered Bi Bi, except numerator AB term is: V_2AB.

Rate constants:

$$k_3 = \frac{V_1}{E_t K_{\text{b}}} \quad k_4 = \frac{V_2}{E_t K_{\text{p}}}$$

Equations for k_1, k_2, k_5, k_6 are the same as those for k_1, k_2, k_7, and k_8 in Ordered Bi Bi.

Effect of isomerizations: EA: k_4, k_5, k_6 may still be calculated. In addition, the rate constant for conversion of EA to EA' in the forward direction is given by:

$$\frac{1}{k} = \frac{E_t}{V_1} - \frac{1}{k_5}$$

E/E_t and EQ/E_t are given by the same equations as for Ordered Bi Bi and:

$$\frac{EA + EA'}{E_t} = \frac{K_{\text{b}} V_2 A + \dfrac{V_1 PQ}{K_{\text{eq}}} + \left(V_2 - \dfrac{V_1 K_{\text{q}}}{K_{\text{iq}}}\right) AB + \dfrac{K_{\text{q}} V_1 A P}{K_{\text{ia}} K_{\text{eq}}}}{\text{(denominator of rate equation)}}$$

EA and EQ: No rate constants can be determined. Only E/E_t can be determined.

Ping Pong Bi Bi (Mechanism 10)

Rate equation: Identical with that of Ordered Bi Bi when written in kinetic constants, except that there is no ABP, BPQ, or constant term in the denominator.

Inhibition constants: Identical with Theorell–Chance.

Haldanes:

$$K_{\text{eq}} = \frac{K_{\text{ip}} K_{\text{iq}}}{K_{\text{ia}} K_{\text{ib}}} = \frac{V_1 K_{\text{ip}} K_{\text{q}}}{V_2 K_{\text{ia}} K_{\text{b}}} = \frac{V_1 K_{\text{p}} K_{\text{iq}}}{V_2 K_{\text{a}} K_{\text{ib}}} = \left(\frac{V_1}{V_2}\right)^2 \frac{K_{\text{p}} K_{\text{q}}}{K_{\text{a}} K_{\text{b}}}$$

Distribution equations:

$$\frac{E}{E_t} = \frac{K_{\text{a}} V_2 B + \dfrac{K_{\text{q}} V_1 P}{K_{\text{eq}}}}{\text{(denominator of rate equation)}}$$

$$\frac{F}{E_t} = \frac{K_{\text{b}} V_2 A + \dfrac{K_{\text{p}} V_1 Q}{K_{\text{eq}}}}{\text{(denominator of rate equation)}}$$

The distribution between the central complexes can not be determined.

Rate constants: None can be calculated.

Effects of isomerizations: No change.

Ordered Ter Bi (Mechanism 17)

Rate equation from King's method:

$$v = \frac{(k_1k_3k_5k_7k_9ABC - k_2k_4k_6k_8k_{10}PQ)\,E_t}{\begin{array}{ll} k_2k_4(k_6 + k_7)k_9 & \\ + k_1k_4(k_6 + k_7)k_9\,A & + k_2k_4(k_6 + k_7)k_{10}\,Q \\ + k_2k_5k_7k_9\,C & + k_2k_4k_6k_8\,P \\ + k_1k_3(k_6 + k_7)k_9\,AB & + (k_2k_4 + k_4k_6 + k_2k_6)k_8k_{10}\,PQ \\ + k_1k_5k_7k_9\,AC & + k_1k_3k_6k_8\,ABP \\ + k_3k_5k_7k_9\,BC & + k_3k_5k_7k_{10}\,BCQ \\ + k_1k_3k_5(k_7 + k_9)\,ABC & + k_3k_6k_8k_{10}\,BPQ \\ + k_1k_4k_6k_8\,AP & + k_2k_5k_8k_{10}\,CPQ \\ + k_2k_5k_7k_{10}\,CQ & + k_1k_3k_5k_8\,ABCP \\ & + k_3k_5k_8k_{10}\,BCPQ \end{array}}$$

This transforms into:

$$v = \frac{V_1V_2\left(ABC - \dfrac{PQ}{K_{eq}}\right)}{\begin{array}{l} K_{ia}K_{ib}K_cV_2 + K_{ib}K_cV_2A + K_{ia}K_bV_2C + K_cV_2AB + K_bV_2AC \\[4pt] + K_aV_2BC + V_2ABC + \dfrac{K_pV_1Q}{K_{eq}} + \dfrac{K_qV_1P}{K_{eq}} + \dfrac{V_1PQ}{K_{eq}} + \dfrac{K_qV_1AP}{K_{ia}K_{eq}} \\[4pt] + \dfrac{K_{ia}K_b}{K_{iq}}\,V_2CQ + \; + \dfrac{K_qV_1ABP}{K_{ia}K_{ib}K_{eq}} + \dfrac{K_aV_2BCQ}{K_{iq}} + \dfrac{K_aK_{ic}V_2BPQ}{K_{ip}K_{iq}} \\[4pt] + \dfrac{K_{ia}K_bV_2CPQ}{K_{ip}K_{iq}} + \dfrac{K_qV_1ABCP}{K_{ia}K_{ib}K_{ic}K_{eq}} + \dfrac{K_aV_2BCPQ}{K_{ip}K_{iq}} \end{array}}$$

Inhibition constants:

$$K_{ia} = \frac{(\text{constant})}{(\text{coef } A)} = \frac{(\text{coef } P)}{(\text{coef } AP)} = \frac{(\text{coef } C)}{(\text{coef } AC)}\left(= \frac{k_2}{k_1}\right)$$

$$K_{ib} = \frac{(\text{coef } A)}{(\text{coef } AB)} = \frac{(\text{coef } AP)}{(\text{coef } ABP)}\left(= \frac{k_4}{k_3}\right)$$

$$K_{ic} = \frac{(\text{coef } BPQ)}{(\text{coef } BCPQ)} = \frac{(\text{coef } ABP)}{(\text{coef } ABCP)}\left(= \frac{k_6}{k_5}\right)$$

$$K_{ip} = \frac{(\text{coef } CQ)}{(\text{coef } CPQ)} = \frac{(\text{coef } BCQ)}{(\text{coef } BCPQ)}\left(= \frac{k_7}{k_8}\right)$$

$$K_{iq} = \frac{(\text{constant})}{(\text{coef } Q)} = \frac{(\text{coef } C)}{(\text{coef } CQ)} = \frac{(\text{coef } BC)}{(\text{coef } BCQ)}\left(= \frac{k_9}{k_{10}}\right)$$

Haldanes:

$$K_{eq} = \frac{K_{ip}K_{iq}}{K_{ia}K_{ib}K_{ic}} = \frac{V_1K_pK_{iq}}{V_2K_{ia}K_{ib}K_c}$$

Distribution equations:

$$\frac{E}{E_t} = \frac{\dfrac{K_qV_1P}{K_{eq}} + K_aV_2BC + K_{ia}K_bV_2C + K_{ia}K_{ib}K_cV_2}{(\text{denominator of rate equation})}$$

$$\frac{EA}{E_t} = \frac{\dfrac{K_q V_1 AP}{K_{ia} K_{eq}} + \dfrac{K_a V_2 PQ}{K_{ia} K_{eq}} + K_b V_2 AC + K_{ib} K_c V_2 A}{\text{(denominator of rate equation)}}$$

$$\frac{EAB}{E_t} = \frac{\dfrac{K_q V_1 ABP}{K_{ia} K_{ib} K_{eq}} + \dfrac{K_a K_{ic} V_2 BPQ}{K_{ip} K_{iq}} + \dfrac{K_b V_2 PQ}{K_{ib} K_{eq}} + K_c V_2 AB}{\text{(denominator of rate equation)}}$$

$$\frac{(EABC + EPQ)}{E_t} = \frac{\left(\begin{array}{l} \dfrac{K_q V_1 ABCP}{K_{ia} K_{ib} K_{ic} K_{eq}} + \dfrac{K_a V_2 BCPQ}{K_{ip} K_{iq}} + \dfrac{K_{ia} K_b V_2 CPQ}{K_{ip} K_{iq}} + \\ \left(\dfrac{V_1}{K_{eq}} - \left(\dfrac{K_a}{K_{ia}} + \dfrac{K_b}{K_{ib}} \right) \dfrac{V_2}{K_{eq}} \right) PQ + \left(V_2 - \dfrac{K_q V_1}{K_{iq}} \right) ABC \end{array} \right)}{\text{(denominator of rate equation)}}$$

$$\frac{EQ}{E_t} = \frac{\dfrac{K_q V_1 ABC}{K_{iq}} + \dfrac{K_a V_2 BCQ}{K_{iq}} + \dfrac{K_{ia} K_b V_2 CQ}{K_{iq}} + \dfrac{K_p V_1 Q}{K_{eq}}}{\text{(denominator of rate equation)}}$$

Rate constants:

Equations for k_1, k_2, k_9, k_{10} are the same as those for k_1, k_2, k_7, and k_8 in Ordered Bi Bi.

$$k_3 = \frac{V_1}{E_t K_b} \qquad k_4 = \frac{V_1 K_{ib}}{E_t K_b} \qquad \frac{1}{k_6} = \frac{E_t}{V_2} - \frac{1}{k_2} - \frac{1}{k_4} \qquad \frac{1}{k_7} = \frac{E_t}{V_1} - \frac{1}{k_9}$$

$$k_5 = \left(1 + \frac{k_6}{k_7}\right) \frac{V_1}{E_t K_c} \qquad k_8 = \left(1 + \frac{k_7}{k_6}\right) \frac{V_2}{E_t K_p}$$

Effect of isomerizations:

(EABC + EPQ): Calculation of k_5, k_6, k_7, k_8 invalid.
EA: E/E$_t$, EAB/E$_t$, EQ/E$_t$, k_4, k_9, and k_{10} may still be determined.
EAB: E/E$_t$, EA/E$_t$, EQ/E$_t$, k_1, k_2, k_9, k_{10} may still be determined.
EQ: E/E$_t$, EA/E$_t$, EAB/E$_t$ k_1, k_2, k_3, k_4, may still be determined.

Bi Uni Uni Uni Ping Pong (Mechanism 18)

Rate equation: Same as Ordered Ter Bi when written in kinetic constants, except no constant, *A*, *CPQ*, *ABCP* or *BCPQ* terms. The denominator also has a new *BQ* term:

$$\frac{K_a K_{ic} V_2 BQ}{K_{iq}}$$

Inhibition constants: Definitions for K_{ia}, K_{ib}, and K_{iq} are identical with those for Ordered Ter Bi, where applicable. Also:

$$K_{ic} = \frac{(\text{coef } Q)}{(\text{coef } CQ)} = \frac{(\text{coef } BQ)}{(\text{coef } BCQ)} \left(= \frac{k_8}{k_7} \right)$$

$$K_{ip} = \frac{(\text{coef } BQ)}{(\text{coef } BPQ)} = \frac{(\text{coef } AB)}{(\text{coef } ABP)} \left(= \frac{k_5}{k_6} \right)$$

Haldanes:

$$K_{\mathrm{eq}} = \frac{K_{\mathrm{ip}}K_{\mathrm{iq}}}{K_{\mathrm{ia}}K_{\mathrm{ib}}K_{\mathrm{ic}}} = \frac{V_1 K_{\mathrm{ip}}K_{\mathrm{q}}}{V_2 K_{\mathrm{ia}}K_{\mathrm{ib}}K_{\mathrm{c}}} = \frac{V_1 K_{\mathrm{p}}K_{\mathrm{iq}}}{V_2 K_{\mathrm{ia}}K_{\mathrm{b}}K_{\mathrm{ic}}} = \left(\frac{V_1}{V_2}\right)^2 \frac{K_{\mathrm{p}}K_{\mathrm{q}}}{K_{\mathrm{ia}}K_{\mathrm{b}}K_{\mathrm{c}}}$$

Distribution equations:

E/E_t: Same as Ordered Ter Bi, without numerator constant term.
EA/E_t: Same as Ordered Ter Bi, without numerator A term.
F/E_t: Sum of AB, BQ, and Q denominator terms of the rate equation, divided by the denominator of the rate equation. The distribution between the central complexes can not be determined.

Rate constants: k_1 and k_2 are determined by the same equations as for Ordered Bi Bi. The other constants can not be determined.

Effect of isomerizations:

$(EAB + FP)$ and/or $(FC + EQ)$: No change.
EA: Only E/E_t and F/E_t may be calculated.

Ordered Ter Ter (Mechanism 20)

Rate equation from King's method:

$$v = \frac{(k_1k_3k_5k_7k_9k_{11}\,ABC - k_2k_4k_6k_8k_{10}k_{12}\,PQR)\,E_t}{\begin{array}{ll}
k_2k_4(k_6 + k_7)k_9k_{11} & + k_1k_4k_6k_8k_{11}\,AP \\
+ k_1k_4(k_6 + k_7)k_9k_{11}\,A & + k_2k_5k_7k_9k_{12}\,CR \\
+ k_2k_5k_7k_9k_{11}\,C & + k_1k_3k_6k_8k_{11}\,ABP \\
+ k_1k_3(k_6 + k_7)k_9k_{11}\,AB & + k_1k_4k_6k_8k_{10}\,APQ \\
+ k_1k_5k_7k_9k_{11}\,AC & + k_3k_5k_7k_9k_{12}\,BCR \\
+ k_3k_5k_7k_9k_{11}\,BC & + k_2k_5k_7k_{10}k_{12}\,CQR \\
+ k_1k_3k_5(k_7k_9 + k_7k_{11} + k_9k_{11})\,ABC & + k_1k_3k_5k_8k_{11}\,ABCP \\
+ k_2k_4k_6k_8k_{11}\,P & + k_1k_3k_5k_7k_{10}\,ABCQ \\
+ k_2k_4(k_6 + k_7)k_9k_{12}\,R & + k_1k_3k_6k_8k_{10}\,ABPQ \\
+ k_2k_4k_6k_8k_{10}\,PQ & + k_3k_5k_7k_{10}k_{12}\,BCQR \\
+ k_2k_4k_6k_8k_{12}\,PR & + k_3k_6k_8k_{10}k_{12}\,BPQR \\
+ k_2k_4(k_6 + k_7)k_{10}k_{12}\,QR & + k_2k_5k_8k_{10}k_{12}\,CPQR \\
+ (k_2k_4 + k_2k_6 + k_4k_6)k_8k_{10}k_{12}PQR & + k_1k_3k_5k_8k_{10}\,ABCPQ \\
 & + k_3k_5k_8k_{10}k_{12}\,BCPQR
\end{array}}$$

This transforms into:

$$v = \frac{V_1V_2\left(ABC - \dfrac{PQR}{K_{\mathrm{eq}}}\right)}{\begin{array}{l}
K_{\mathrm{ia}}K_{\mathrm{ib}}K_{\mathrm{c}}V_2 + K_{\mathrm{ib}}K_{\mathrm{c}}V_2A + K_{\mathrm{ia}}K_{\mathrm{b}}V_2C + K_{\mathrm{c}}V_2AB + K_{\mathrm{b}}V_2AC + K_{\mathrm{a}}V_2BC \\
+ V_2ABC + \dfrac{K_{\mathrm{ir}}K_{\mathrm{q}}V_1P}{K_{\mathrm{eq}}} + \dfrac{K_{\mathrm{iq}}K_{\mathrm{p}}V_1R}{K_{\mathrm{eq}}} + \dfrac{K_{\mathrm{r}}V_1PQ}{K_{\mathrm{eq}}} + \dfrac{K_{\mathrm{q}}V_1PR}{K_{\mathrm{eq}}} + \dfrac{K_{\mathrm{p}}V_1QR}{K_{\mathrm{eq}}} \\
+ \dfrac{V_1PQR}{K_{\mathrm{eq}}} + \dfrac{K_{\mathrm{q}}K_{\mathrm{ir}}V_1AP}{K_{\mathrm{ia}}K_{\mathrm{eq}}} + \dfrac{K_{\mathrm{ia}}K_{\mathrm{b}}V_2CR}{K_{\mathrm{ir}}} + \dfrac{K_{\mathrm{q}}K_{\mathrm{ir}}V_1ABP}{K_{\mathrm{ia}}K_{\mathrm{ib}}K_{\mathrm{eq}}} + \dfrac{K_{\mathrm{r}}V_1APQ}{K_{\mathrm{ia}}K_{\mathrm{eq}}} \\
+ \dfrac{K_{\mathrm{a}}V_2BCR}{K_{\mathrm{ir}}} + \dfrac{K_{\mathrm{ia}}K_{\mathrm{b}}V_2CQR}{K_{\mathrm{iq}}K_{\mathrm{ir}}} + \dfrac{K_{\mathrm{ir}}K_{\mathrm{q}}V_1ABCP}{K_{\mathrm{ia}}K_{\mathrm{ib}}K_{\mathrm{ic}}K_{\mathrm{eq}}} + \dfrac{K_{\mathrm{ip}}K_{\mathrm{r}}V_1ABCQ}{K_{\mathrm{ia}}K_{\mathrm{ib}}K_{\mathrm{ic}}K_{\mathrm{eq}}}
\end{array}}$$

$$+ \frac{K_{\mathrm{r}}V_1ABPQ}{K_{\mathrm{ia}}K_{\mathrm{ib}}K_{\mathrm{eq}}} + \frac{K_{\mathrm{a}}V_2BCQR}{K_{\mathrm{iq}}K_{\mathrm{ir}}} + \frac{K_{\mathrm{a}}K_{\mathrm{ic}}V_2BPQR}{K_{\mathrm{ip}}K_{\mathrm{iq}}K_{\mathrm{ir}}} + \frac{K_{\mathrm{ia}}K_{\mathrm{b}}V_2CPQR}{K_{\mathrm{ip}}K_{\mathrm{iq}}K_{\mathrm{ir}}}$$

$$+ \frac{K_{\mathrm{r}}V_1ABCPQ}{K_{\mathrm{ia}}K_{\mathrm{ib}}K_{\mathrm{ic}}K_{\mathrm{eq}}} + \frac{K_{\mathrm{a}}V_2BCPQR}{K_{\mathrm{ip}}K_{\mathrm{iq}}K_{\mathrm{ir}}}$$

Inhibition constants:

$$K_{\mathrm{ia}} = \frac{(\mathrm{constant})}{(\mathrm{coef}\ A)} = \frac{(\mathrm{coef}\ C)}{(\mathrm{coef}\ AC)} = \frac{(\mathrm{coef}\ P)}{(\mathrm{coef}\ AP)} = \frac{(\mathrm{coef}\ PQ)}{(\mathrm{coef}\ APQ)}\left(= \frac{k_2}{k_1}\right)$$

$$K_{\mathrm{ib}} = \frac{(\mathrm{coef}\ A)}{(\mathrm{coef}\ AB)} = \frac{(\mathrm{coef}\ AP)}{(\mathrm{coef}\ ABP)} = \frac{(\mathrm{coef}\ APQ)}{(\mathrm{coef}\ ABPQ)}\left(= \frac{k_4}{k_3}\right)$$

$$K_{\mathrm{ic}} = \frac{(\mathrm{coef}\ ABP)}{(\mathrm{coef}\ ABCP)} = \frac{(\mathrm{coef}\ ABPQ)}{(\mathrm{coef}\ ABCPQ)} = \frac{(\mathrm{coef}\ BPQR)}{(\mathrm{coef}\ BCPQR)}\left(= \frac{k_6}{k_5}\right)$$

$$K_{\mathrm{ip}} = \frac{(\mathrm{coef}\ CQR)}{(\mathrm{coef}\ CPQR)} = \frac{(\mathrm{coef}\ ABCQ)}{(\mathrm{coef}\ ABCPQ)} = \frac{(\mathrm{coef}\ BCQR)}{(\mathrm{coef}\ BCPQR)}\left(= \frac{k_7}{k_8}\right)$$

$$K_{\mathrm{iq}} = \frac{(\mathrm{coef}\ R)}{(\mathrm{coef}\ QR)} = \frac{(\mathrm{coef}\ CR)}{(\mathrm{coef}\ CQR)} = \frac{(\mathrm{coef}\ BCR)}{(\mathrm{coef}\ BCQR)}\left(= \frac{k_9}{k_{10}}\right)$$

$$K_{\mathrm{ir}} = \frac{(\mathrm{constant})}{(\mathrm{coef}\ R)} = \frac{(\mathrm{coef}\ P)}{(\mathrm{coef}\ PR)} = \frac{(\mathrm{coef}\ C)}{(\mathrm{coef}\ CR)} = \frac{(\mathrm{coef}\ BC)}{(\mathrm{coef}\ BCR)}\left(= \frac{k_{11}}{k_{12}}\right)$$

Haldanes:

$$K_{\mathrm{eq}} = \frac{K_{\mathrm{ip}}K_{\mathrm{iq}}K_{\mathrm{ir}}}{K_{\mathrm{ia}}K_{\mathrm{ib}}K_{\mathrm{ic}}} = \frac{V_1K_{\mathrm{p}}K_{\mathrm{iq}}K_{\mathrm{ir}}}{V_2K_{\mathrm{ia}}K_{\mathrm{ib}}K_{\mathrm{c}}}$$

Distribution equations:

$$\frac{E}{E_t} = \frac{K_{\mathrm{a}}V_2BC + K_{\mathrm{ia}}K_{\mathrm{b}}V_2C + K_{\mathrm{ia}}K_{\mathrm{ib}}K_{\mathrm{c}}V_2 + \dfrac{K_{\mathrm{ir}}K_{\mathrm{q}}V_1P}{K_{\mathrm{eq}}} + \dfrac{K_{\mathrm{r}}V_1PQ}{K_{\mathrm{eq}}}}{(\text{denominator of rate equation})}$$

$$\frac{EA}{E_t} = \frac{\dfrac{K_{\mathrm{a}}V_2PQR}{K_{\mathrm{ia}}K_{\mathrm{eq}}} + K_{\mathrm{b}}V_2AC + K_{\mathrm{ib}}K_{\mathrm{c}}V_2A + \dfrac{K_{\mathrm{q}}K_{\mathrm{ir}}V_1AP}{K_{\mathrm{ia}}K_{\mathrm{eq}}} + \dfrac{K_{\mathrm{r}}V_1APQ}{K_{\mathrm{ia}}K_{\mathrm{eq}}}}{(\text{denominator of rate equation})}$$

$$\frac{EAB}{E_t} = \frac{\dfrac{K_{\mathrm{a}}K_{\mathrm{ic}}V_2BPQR}{K_{\mathrm{ip}}K_{\mathrm{iq}}K_{\mathrm{ir}}} + \dfrac{K_{\mathrm{b}}V_2PQR}{K_{\mathrm{ib}}K_{\mathrm{eq}}} + K_{\mathrm{c}}V_2AB + \dfrac{K_{\mathrm{q}}K_{\mathrm{ir}}V_1ABP}{K_{\mathrm{ia}}K_{\mathrm{ib}}K_{\mathrm{eq}}} + \dfrac{K_{\mathrm{r}}V_1ABPQ}{K_{\mathrm{ia}}K_{\mathrm{ib}}K_{\mathrm{eq}}}}{(\text{denominator of rate equation})}$$

$$\frac{(EABC + EPQR)}{E_t} = \frac{\left(\begin{array}{l}\dfrac{K_{\mathrm{a}}V_2BCPQR}{K_{\mathrm{ip}}K_{\mathrm{iq}}K_{\mathrm{ir}}} + \dfrac{K_{\mathrm{ia}}K_{\mathrm{b}}V_2CPQR}{K_{\mathrm{ip}}K_{\mathrm{iq}}K_{\mathrm{ir}}} + \dfrac{K_{\mathrm{q}}K_{\mathrm{ir}}V_1ABCP}{K_{\mathrm{ia}}K_{\mathrm{ib}}K_{\mathrm{ic}}K_{\mathrm{eq}}} + \dfrac{K_{\mathrm{r}}V_1ABCPQ}{K_{\mathrm{ia}}K_{\mathrm{ib}}K_{\mathrm{ic}}K_{\mathrm{eq}}} + \\ + \left(\dfrac{V_1}{K_{\mathrm{eq}}} - \dfrac{V_2}{K_{\mathrm{eq}}}\left(\dfrac{K_{\mathrm{a}}}{K_{\mathrm{ia}}} + \dfrac{K_{\mathrm{b}}}{K_{\mathrm{ib}}}\right)\right) PQR + \left(V_2 - V_1\left(\dfrac{K_{\mathrm{q}}}{K_{\mathrm{iq}}} + \dfrac{K_{\mathrm{r}}}{K_{\mathrm{ir}}}\right)\right) ABC\end{array}\right)}{(\text{denominator of rate equation})}$$

$$\frac{EQR}{E_t} = \frac{\dfrac{K_{\mathrm{a}}V_2BCQR}{K_{\mathrm{iq}}K_{\mathrm{ir}}} + \dfrac{K_{\mathrm{ia}}K_{\mathrm{b}}V_2CQR}{K_{\mathrm{iq}}K_{\mathrm{ir}}} + \dfrac{K_{\mathrm{p}}V_1QR}{K_{\mathrm{eq}}} + \dfrac{K_{\mathrm{q}}V_1ABC}{K_{\mathrm{iq}}} + \dfrac{K_{\mathrm{ip}}K_{\mathrm{r}}V_1ABCQ}{K_{\mathrm{ia}}K_{\mathrm{ib}}K_{\mathrm{ic}}K_{\mathrm{eq}}}}{(\text{denominator of rate equation})}$$

$$\frac{ER}{E_t} = \frac{\dfrac{K_aV_2BCR}{K_{ir}} + \dfrac{K_{ia}K_bV_2CR}{K_{ir}} + \dfrac{K_pK_{iq}V_1R}{K_{eq}} + \dfrac{K_qV_1PR}{K_{eq}} + \dfrac{K_rV_1ABC}{K_{ir}}}{\text{(denominator of rate equation)}}$$

Rate constants: Equations for k_1, k_2, k_3, k_4, k_5, k_6, k_8, k_9, and k_{10} are the same as for Ordered Ter Bi. Also:

$$\frac{1}{k_7} = \frac{E_t}{V_1} - \frac{1}{k_9} - \frac{1}{k_{11}} \qquad k_{11} = \frac{V_2K_{ir}}{E_tK_r} \qquad k_{12} = \frac{V_2}{E_tK_r}$$

Effect of isomerizations:

(EABC + EPQR): Distributions valid. Calculation of k_5, k_6, k_7, and k_8 invalid.

EA: Calculation of k_1, k_2, k_3 as well as k_5–k_8 invalid. Calculation of EA/E_t and $(EABC + EPQR)/E_t$ invalid.

EAB: Calculation of k_3–k_8 invalid. Calculation of EAB/E_t and $(EABC + EPQR)/E_t$ invalid.

Bi Uni Uni Bi Ping Pong (Mechanism 21)

Rate equation: Same as Ordered Ter Ter when written in kinetic constants, except no constant, A, R, $ABCP$, $CPQR$, $ABCPQ$, and $BCPQR$ terms. The denominator has two new terms:

$$\frac{K_{ip}K_rV_1ABQ}{K_{ia}K_{ib}K_{eq}} + \frac{K_aK_{ic}V_2BQR}{K_{iq}K_{ir}}$$

Inhibition constants: Definitions for K_{ia}, K_{ib}, K_{iq}, and K_{ir} (where possible) are identical with those for Ordered Ter Ter.

$$K_{ic} = \frac{(\text{coef } QR)}{(\text{coef } CQR)} = \frac{(\text{coef } ABQ)}{(\text{coef } ABCQ)} = \frac{(\text{coef } BQR)}{(\text{coef } BCQR)} \left(= \frac{k_8}{k_7}\right)$$

$$K_{ip} = \frac{(\text{coef } AB)}{(\text{coef } ABP)} = \frac{(\text{coef } ABQ)}{(\text{coef } ABPQ)} = \frac{(\text{coef } BQ)}{(\text{coef } BPQ)} \left(= \frac{k_5}{k_6}\right)$$

Haldanes:

$$K_{eq} = \frac{K_{ip}K_{iq}K_{ir}}{K_{ia}K_{ib}K_{ic}} = \frac{V_1K_{ip}K_qK_{ir}}{V_2K_{ia}K_{ib}K_c} = \frac{V_1K_pK_{iq}K_{ir}}{V_2K_{ia}K_bK_{ic}} = \left(\frac{V_1}{V_2}\right)^2 \frac{K_pK_qK_{ir}}{K_{ia}K_bK_c}$$

Distribution equations:

E/E_t, EA/E_t, ER/E_t: Same equations as for Ordered Ter Ter without, respectively, constant, A, and R numerator terms.

F/E_t: Sum of BQR, QR, AB, and ABQ denominator terms of the rate equation divided by the denominator of the rate equation. The distribution between the central complexes can not be determined.

Rate constants: k_1, k_2, k_{11}, and k_{12} are determined by the same equations as for Ordered Ter Ter. The other constants can not be determined.

Effect of isomerizations: (EAB + FP) and/or *(FC + EQR):* No change.
EA: Calculation of k_1, k_2, and EA/E_t invalid.
ER: Calculation of k_{11}, k_{12}, and ER/E_t invalid.

Bi Bi Uni Uni Ping Pong (Mechanism 22)

Rate equation: Same as Ordered Ter Ter when written in kinetic constants, except no constant, *A*, *P*, *ABP*, *CQR*, *ABCQ*, *BCQR*, *CPQR*, *AP*, *ABCPQ*, and *BCPQR* terms. The denominator has new terms in *ABQ*, *BQR*, *CP*, *BR*, *ACP*, and *CPR*. The first two have the same form that they do in Bi Uni Uni Bi, and the others are:

$$\frac{K_{\mathrm{q}}K_{\mathrm{ir}}V_1CP}{K_{\mathrm{ic}}K_{\mathrm{eq}}} + \frac{K_{\mathrm{a}}K_{\mathrm{ic}}V_2BR}{K_{\mathrm{ir}}} + \frac{K_{\mathrm{q}}K_{\mathrm{ir}}V_1ACP}{K_{\mathrm{ia}}K_{\mathrm{ic}}K_{\mathrm{eq}}} + \frac{K_{\mathrm{q}}V_1CPR}{K_{\mathrm{ic}}K_{\mathrm{eq}}}$$

Inhibition constants: Definitions for K_{ia}, K_{ib}, K_{ip}, K_{iq}, and K_{ir} (where possible) are identical with those for Ordered Ter Ter. In addition:

$$K_{\mathrm{ia}} = \frac{(\text{coef } CP)}{(\text{coef } ACP)} \quad K_{\mathrm{ib}} = \frac{(\text{coef } ACP)}{(\text{coef } ABCP)} \quad K_{\mathrm{iq}} = \frac{(\text{coef } BR)}{(\text{coef } BQR)} = \frac{(\text{coef } AB)}{(\text{coef } ABQ)}$$

$$K_{\mathrm{ic}} = \frac{(\text{coef } BR)}{(\text{coef } BCR)} = \frac{(\text{coef } PR)}{(\text{coef } CPR)} = \frac{(\text{coef } R)}{(\text{coef } CR)} \quad K_{\mathrm{ir}} = \frac{(\text{coef } CP)}{(\text{coef } CPR)}$$

Haldanes:

$$K_{\mathrm{eq}} = \frac{K_{\mathrm{ip}}K_{\mathrm{iq}}K_{\mathrm{ir}}}{K_{\mathrm{ia}}K_{\mathrm{ib}}K_{\mathrm{ic}}} = \frac{V_1K_{\mathrm{ip}}K_{\mathrm{iq}}K_{\mathrm{r}}}{V_2K_{\mathrm{ia}}K_{\mathrm{ib}}K_{\mathrm{c}}} = \frac{V_1K_{\mathrm{p}}K_{\mathrm{iq}}K_{\mathrm{ir}}}{V_2K_{\mathrm{ia}}K_{\mathrm{b}}K_{\mathrm{ic}}} = \left(\frac{V_1}{V_2}\right)^2 \frac{K_{\mathrm{p}}K_{\mathrm{iq}}K_{\mathrm{r}}}{K_{\mathrm{ia}}K_{\mathrm{b}}K_{\mathrm{c}}}$$

Distribution equations:

E/E_t: Sum of *BC*, *C*, *CP*, and *PQ* denominator terms of the rate equation, divided by the denominator of the rate equation.

EA/E_t: Sum of *AC*, *ACP*, and *APQ* denominator terms of the rate equation, plus $\frac{K_{\mathrm{a}}V_2PQR}{K_{\mathrm{ia}}K_{\mathrm{eq}}}$, divided by the denominator of the rate equation.

FQ/E_t: Sum of *QR*, *BQR*, and *ABQ* denominator terms of the rate equation, plus $\frac{K_{\mathrm{q}}V_1ABC}{K_{\mathrm{iq}}}$, divided by the denominator of the rate equation.

F/E_t: Sum of *R*, *BR*, *PR*, and *AB* denominator terms of the rate equation, divided by the denominator of the rate equation. The distribution between the central complexes can not be determined.

Rate constants: k_1, k_2, k_7, and k_8 are determined by the same equations as for Ordered Bi Bi. The other constants can not be determined.

Effect of isomerizations:
(EAB + FPQ) and/or *(FC + ER):* No change.
EA: Calculation of k_1, k_2, and EA/E_t invalid.
FQ: Calculation of k_7, k_8, and FQ/E_t invalid.

Hexa Uni Ping Pong (Mechanism 25)

Rate equation: When written in kinetic constants, the rate equation has the same numerator as the other Ter Ter mechanisms, and has denominator terms in AB, AC, BC, ABC, PQ, PR, QR, PQR, AQ, CP, BR, APQ, BCR, ABQ, BQR, ACP, and CPR. These have the same form as they do in the denominators of the other Ter Ter rate equations, and the new AQ term is:

$$\frac{K_{\mathrm{ip}}K_{\mathrm{r}}V_1AQ}{K_{\mathrm{ia}}K_{\mathrm{eq}}}$$

Inhibition constants: Definitions for K_{ia}, K_{ic}, K_{iq}, and K_{ir} (when applicable) are the same as those for the other three Ter Ter mechanisms given above. In addition:

$$K_{\mathrm{ib}} = \frac{(\text{coef } QR)}{(\text{coef } BQR)} = \frac{(\text{coef } AQ)}{(\text{coef } ABQ)} \qquad K_{\mathrm{ip}} = \frac{(\text{coef } AC)}{(\text{coef } ACP)} = \frac{(\text{coef } AQ)}{(\text{coef } APQ)}$$

Haldanes:

$$K_{\mathrm{eq}} = \frac{K_{\mathrm{ip}}K_{\mathrm{iq}}K_{\mathrm{ir}}}{K_{\mathrm{ia}}K_{\mathrm{ib}}K_{\mathrm{ic}}} = \frac{V_1K_{\mathrm{ip}}K_{\mathrm{iq}}K_{\mathrm{r}}}{V_2K_{\mathrm{ia}}K_{\mathrm{ib}}K_{\mathrm{c}}} = \frac{V_1K_{\mathrm{p}}K_{\mathrm{iq}}K_{\mathrm{ir}}}{V_2K_{\mathrm{a}}K_{\mathrm{ib}}K_{\mathrm{ic}}} = \frac{V_1K_{\mathrm{ip}}K_{\mathrm{q}}K_{\mathrm{ir}}}{V_2K_{\mathrm{ia}}K_{\mathrm{b}}K_{\mathrm{ic}}}$$

$$K_{\mathrm{eq}} = \left(\frac{V_1}{V_2}\right)^2 \frac{K_{\mathrm{p}}K_{\mathrm{q}}K_{\mathrm{ir}}}{K_{\mathrm{a}}K_{\mathrm{b}}K_{\mathrm{ic}}} = \left(\frac{V_1}{V_2}\right)^2 \frac{K_{\mathrm{p}}K_{\mathrm{iq}}K_{\mathrm{r}}}{K_{\mathrm{a}}K_{\mathrm{ib}}K_{\mathrm{c}}} = \left(\frac{V_1}{V_2}\right)^2 \frac{K_{\mathrm{ip}}K_{\mathrm{q}}K_{\mathrm{r}}}{K_{\mathrm{ia}}K_{\mathrm{b}}K_{\mathrm{c}}} = \left(\frac{V_1}{V_2}\right)^3 \frac{K_{\mathrm{p}}K_{\mathrm{q}}K_{\mathrm{r}}}{K_{\mathrm{a}}K_{\mathrm{b}}K_{\mathrm{c}}}$$

Distribution equations: E/E_t: Sum of the BC, CP, and PQ denominator terms of the rate equation, divided by the denominator of the rate equation.

F/E_t: Sum of the QR, AC, and AQ denominator terms of the rate equation, divided by the denominator of the rate equation.

G/E_t: Sum of the BR, PR, and AB denominator terms of the rate equation, divided by the denominator of the rate equation. The distribution between the central complexes can not be determined.

Rate constants: No constants can be calculated.

Effect of isomerizations: No effect.

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