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Enzyme kinetics revisited: a commentary by

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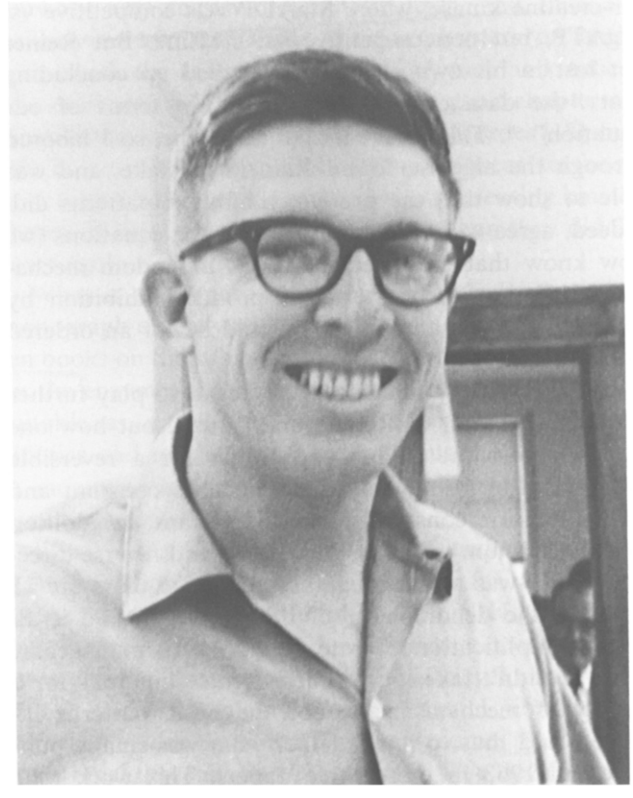
on 'The kinetics of enzyme-catalyzed reactions
with two or more substrates or products'
by W.W. Cleland
Biochim. Biophys. Acta 67 (1963) 104 ff.
with Biochim. Biophys. Acta 67 (1963) 173, 188 (summaries)

It was exactly 25 years ago that BBA published three theoretical papers on enzyme kinetics which launched such terms as 'Ping Pong Bi Bi' onto an unsuspecting scientific world [1–3]. Many advances have since been made in enzyme kinetic theory, but most of what is in these papers has stood the test of time, and is still useful today. The first of the papers has received about 1700 citations since it was published, and still gets about 50 citations per year. Thus it is not with the sorrowful mood that overcame Charles Ryder when he once again came to Brideshead that this author comtemplates the handiwork of his brash youth.

In 1959, after spending 2 years in the army and 2 years postdocing at Chicago with Eugene Kennedy, I was hired back to Wisconsin, where I had received my Ph.D. under Marv Johnson studying the intermediary metabolism of the citric acid fermentation [4,5]. The Chicago period involved study of cerebroside biosynthesis [6], and I was hired as a 'lipid biochemist'. (When I am twitted about my failure to remain in this area of research, I remind my colleagues that I have become a fat biochemist instead.) My initial research project, which was supported by NIH for 8 years, involved measurement of the substrate specificity of acylation of α-glycerophosphate by various acyl-CoA thioesters. While the basic thrust of this work was only partly successful, we did establish that acyl-CoAs form micelles [7], and that the rate of reaction is constant above the critical micelle concentration, with the extent of reaction varying with the concentration of added acyl-CoA [8]. The development of dithiothreitol as a reagent to keep CoA reduced was part of this work [9]. Acyl-CoAs are sticky molecules, and dilute solutions show weird properties, such as the formation of a monolayer at the air interface, so that the measured concentration in an aliquot of the bulk liquid is less when the same volume of a stock solution is stored in a tube with a larger diameter (and thus a greater surface/volume ratio) [10].



W. Wallace Cleland (about 1963)

Thus, after our kinetic studies were well underway, I dropped the lipid work.

As part of my duties, I was assigned to teach in the second semester of the first-year graduate level Biochemistry course in the spring of 1960. The main topic was metabolism, but thermodynamics and kinetics were also to be covered. Thermodynamics was no problem, but enzyme kinetics at that time had hardly progressed beyond the state in which J.B.S. Haldane had left it in

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1930 when he discovered the relationship between the equilibrium constant and the V/K values in forward and reverse reactions, an equation that we now call the Haldane relationship [11]. In particular, the kinetics of reactions with two or more substrates or products were not well described. The form of the initial velocity equation (that is, the description of velocity as a function of substrate concentration with no products present) was understood, but notation was awkward, and equations for velocity as a function of all reactants were very cumbersome when expressed in terms of the individual rate constants.

I found such an equation for an ordered reaction with two substrates and two products in a book by Reiner [12], along with the statement; "This complex expression is not very useful as it stands". Reiner proceeded to try to simplify the full rate equation and apply it to product inhibition data of Kuby et al. [13] for creatine kinase, where MgADP was competitive vs. MgATP, but noncompetitive vs. creatine. But Reiner got lost in his own algebra and ended up concluding that "the data cannot be analyzed in terms of our equation" *. This didn't look right to me, so I labored through the algebra, found Reiner's mistake, and was able to show that the product inhibition patterns did, indeed, agree with the predictions of the equations (we now know that creatine kinase has a random mechanism [15], but the patterns of product inhibition by MgADP are the same as they would be for an ordered mechanism).

Emboldened by this success, I began to play further with the equation of Reiner, and figured out how one could express the full rate equation for a reversible mechanism by using only one Michaelis constant and one 'inhibition constant' for each reactant, in addition to the maximum velocities in forward and reverse directions. This was a real breakthrough, since there are 11 terms in the denominator of this equation, and it allowed simplification of rate equations to manageable form. It didn't take long to derive rate equations for a variety of mechanisms, to see the basic patterns involved, and thus to put together what was finally published in 1963 in these three papers. This work took about a year, and was tried out on grad students in the spring of 1961, and again in 1962.

My interest in kinetics was kindled, and I obtained NSF support to begin experimental kinetic studies in 1960. Our first experimental paper on product inhibition in alcohol dehydrogenases came out in 1963 [16], shortly after the BBA papers, and we have continued to this day to use kinetic studies to determine enzyme mechanisms.

* The same error is repeated in the second edition of Reiner's book, published 6 years after these articles appeared [14]. So much for how well book writers read the literature!

The story of how these papers came to be published in BBA is an interesting one, since the papers were published without revision as I wrote them (a rare occurrence for any work, especially for papers of this complexity!). The papers were in preprint form by the spring of 1962, and I asked my colleagues where they thought I might publish them. Henry Lardy and David Green suggested 'Biochemistry', which had just started publication. Thus I wrote on April 23, 1962, to Hans Neurath (then, as now, the Editor), enclosing preprints and asking him to "take a look at these papers and let me know if they are the sort of thing you would like to have in 'Biochemistry'". He replied on May 14 that he would find it difficult to publish the papers both because of the limited pages available ('Biochemistry' was published only every 2 months at the time), and because the contribution was "primarily a didactical one".

I then on May 18 sent copies of the preprints and identical covering letters to three journals; J. Biol. Chem., BBA, and J. Theor. Biol. These letters said: "Could you take a preliminary look at these papers and let me know if there is any possibility of them being published in [the journal]. If so, I will put them into the proper format ... and submit them formally". I heard first from J. Theor. Biol., and Jim Danielli said: "This is the type of material we like to have for the Journal, and I shall be glad to consider the final versions of the papers if you care to send them to me". A week later I heard from J. Biol. Chem. (letter dated May 31), and John Edsall said: "I have looked over the 3 manuscripts you sent me with a great deal of interest, although I have not had time to read them in detail. However, I would like to encourage you to submit them to the Journal". When after another month I had not heard from BBA, I submitted the papers formally on July 5 to J. Biol. Chem.

It was thus with a great shock that I received two weeks later a letter dated July 12 from Dr. Westenbrink, Editor of BBA, saying: "I take pleasure to inform you that we have accepted your papers. Please send the corrections you wrote about in your letter of May 18th". Thus I had the papers submitted to one journal and accepted by another! In retrospect, I should have either written to John Edsall and withdrawn the papers immediately, or written to Dr. Westenbrink and told him that I had submitted them to J. Biol. Chem. I did neither, and it was a month later (faster reviewing in those days!) that I finally received a 5 page single spaced letter from John Edsall (probably a record for an editor's report to an author). He objected to the length of the papers, and to a good bit of the notation and nomenclature. Thus: "... we suggest the deletion of Ping Pong...". He ended with: "We recognize that the above rather drastic revisions may be disheartening after your efforts in writing 3 articles. We have taken this trouble, however, because we believe that you have

developed a system of substantial value which is well worthy of publication in the Journal of Biological Chemistry".

Under normal circumstances I would have tried to work out some compromise, but the papers would have been considerably revised before publication, and it is not all clear that I could have won the battle on notation and nomenclature. But with an ace in the hole, I wrote to John Edsall on August 27: "In view of the extensive modifications suggested, I would like at this time to withdraw these papers from consideration. I do this not because I disagree with all of the suggested changes, but because I have been teaching the system presented in these papers for three years and would prefer not to have to modify it at this late date.... I would like to thank you and the Editorial Board for the time and effort you have spent reviewing these manuscripts, and I regret that honest differences in opinion prevent publication of these articles in the Journal at this time".

On the same day I sent the final versions of the papers to BBA, and they notified me by letter dated Oct 16 that the papers had gone to the publisher. The first paper came out in January, 1963, and the other two a month later. The total bill for 400 combined reprints was only US$ 270 for a 58 page reprint! These went rather quickly, and I believe we eventually distributed over 1200 reprints.

Looking back over the last 25 years, there was certainly an element of luck in how things turned out. However, I believe the papers are better the way they are written than they would have been had I had to reach a compromise with J. Biol. Chem. It certainly says a lot for the reviewers who were willing to accept the papers as they stood; unless they were totally snowed, they truly realized that this was a workable system that would stand the test of time, and refrained from nitpicking. These papers have done wonders for my reputation, and I believe they also have enhanced the reputation of BBA. Thus Dr. Slater wrote me in 1976: "I have always considered your classical papers on enzyme kinetics as one of BBA's real scoops..."

As I said earlier, the last 25 years have been good ones, and it has been fun being a part of the field of enzyme kinetics. There has been much progress over this period, both in theory and in experimental work, and we now are able, by use of isotope effects and pH studies, to determine chemical mechanisms and get some ideas on transition state structure [17]. If structures are known from X-ray and NMR work, one can fully describe what is really happening in an enzyme active site. It is common to hear the expression that "kinetics can't prove anything; it can only disprove things", but this is not true, and because it looks at the reaction while it is taking place, a kinetic study is the ultimate arbiter of mechanism.

Why has the effort over the last 25 years been so successful? I think the major reason is that the development of theory and experiment has gone hand in hand. If either gets too far removed from the other, one goes astray. Thus, other competing kinetic theories in the 1960's did not succeed because they were not practical to apply. At the same time, the literature has always been (and probably always will be) full of data which were not properly interpreted because suitable theory was not yet available or the author was unaware of its existence.

My major disappointment over the last 25 years has been the slow pace at which modern kinetic theory has found its way into basic textbooks. Biochemistry textbooks have a major problem. First, most people, including me, are reluctant to take time away from research to write books. Second, people who do write books do not like to share their royalties (this is presumably why they write a basic text), and thus are unlikely to ask specialists to write chapters on areas with which the author is not familiar, such as kinetics. The most serious attempt to provide a good chapter on kinetics was in the book by Mahler and Cordes [18], but this has not been revised. Sadly, no present textbook does even a halfway reasonable job in presenting kinetic theory. If fact, they present little more than they did 25 years ago!

What is even more surprising is the lack of a good monograph on enzyme kinetics. Many people have written books on the subject, but most are narrowly focussed on the enzymes the author has worked on, and none is suitable as a teaching tool in a course on enzyme kinetics and mechanism. Students must go to chapters in monographs and reviews for summaries of modern enzyme kinetic theory [17].

What is the future of the field of enzyme kinetics? Has theoretical development gone as far as it can? Every 5 years when I write for another NIH grant I ask myself these questions. It has always seemed that we were nearing the end of theoretical development, although there was plenty of experimental work to do. But then suddenly something new comes along and great breakthroughs in theory occur. The development of positional isotopic exchange [19], of isotope partitioning studies [20], of the use of primary or secondary deuteration to alter heavy atom isotope effects in order to deconvolute bond-breaking steps and determine partition ratios for intermediates [21], have all snuck up on us in unpredictable fashion.

I remember trying to solve simultaneously one Sunday afternoon the three equations for a ^{13}C isotope effect with deuterated and unlabeled substrates, and the deuterium isotope effect itself when two isotope effects were on different steps in the reaction. This was a horrible algebaic mess, when suddenly things started to cancel out, and I was left with $0 = 0$! The equations

were not, independent, and thus was discovered the relationship which allows one not only to prove a stepwise mechanism, but also to tell which isotope-sensitive step comes first [21]. There is no way to predict what development of theory will come next, but I feel that there is new theory to be discovered, and that we have not yet reached the end.

Over the last 25 years, enzyme kineticists have been a rare breed, and the number of people who really understood the subject was not very great. While all of the people who went through my lab who wanted academic jobs were able to find them, most departments had a single enzymologist, and expanded in the areas of molecular and cellular biology instead. But molecular biology has been so successful that its methods have become regular lab procedures. It is now almost routine to fish out a gene, clone it, sequence it, mutate it, and express the mutant in an appropriate organism. But characterizing the mutant enzyme requires a thorough kinetic study, and thus industry is now frantically trying to hire enzymologists with a knowledge of protein chemistry and enzyme kinetics. Headhunters are even on the prowl looking for such people. It is thus an exciting time to be an enzymologist. Cloning allows one easily to produce large amounts of enzyme, and site-directed mutagenesis permits one to test one's ideas on mechanism by making specific changes in putative catalytic groups. As X-ray methods get faster and refinements more sophisticated, we also get better structural pictures to use a framework for mechanistic hypotheses. There are still things we do not understand about enzyme mechanisms, but at the rate things are going, we should by the end of the century know the detailed mechanisms of a large number of enzymes, and kinetic studies will have made a major contribution to this knowledge. It has been fun being part of this over the last 25 years, and I am looking forward to the next dozen years with great enthusiasm!

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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:

$$v = \frac{V_1 V_2 \left(A - \frac{P}{K_{eq}}\right)}{K_a V_2 + V_2 A + \frac{V_1 P}{K_{eq}}} \qquad (1)$$

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:

$$K_{eq} = \frac{V_1 K_p}{V_2 K_a} \qquad (2)$$

* Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

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)

* 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–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

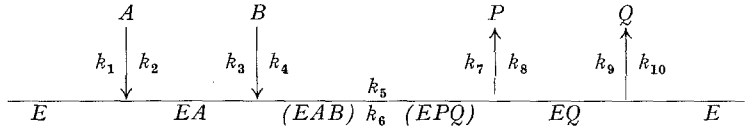
are released before all substrates have added to the enzyme, the enzyme will exist in two or more stable forms between which it oscillates during the reaction. Such mechanisms will be called "Ping Pong", and if the mechanism is not obvious it will be further described by the use of Uni, Bi, Ter, to indicate 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:

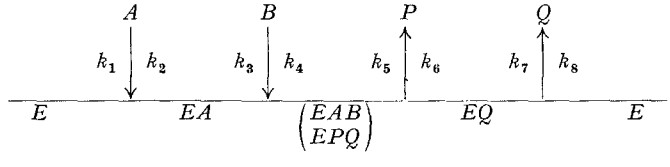
$$E + A \underset{k_2}{\overset{k_1}{\rightleftharpoons}} EA \quad EA + B \underset{k_4}{\overset{k_3}{\rightleftharpoons}} (EAB) \underset{k_6}{\overset{k_5}{\rightleftharpoons}} (EPQ) \underset{k_8}{\overset{k_7}{\rightleftharpoons}} EQ + P \quad EQ \underset{k_{10}}{\overset{k_9}{\rightleftharpoons}}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

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



(5)

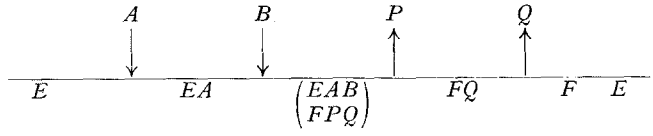
With only one central complex, the mechanism would be written:



(6)

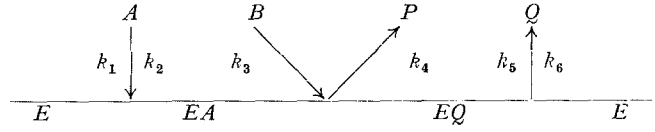
This is still Ordered Bi Bi, and both mechanisms give rate equations of identical form. "Ordered Bi Bi" is thus a kinetic description and applies to all reaction mechanisms that give the basic pattern:

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)

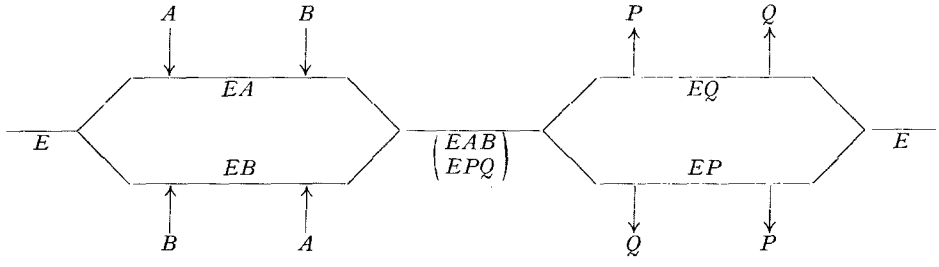
If there is no central complex formed at all, as in the mechanism proposed by THEORELL AND CHANCE for alcohol dehydrogenase[8], the mechanism is different from Ordered Bi Bi and gives a different rate equation, so that the mechanisms can be kinetically distinguished:



(8)

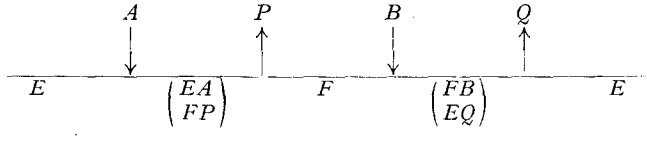
Mechanisms of this type are really limiting cases of more general sequential mechanisms in which the steady-state concentrations of the central complexes are very low. Careful kinetic studies can generally detect the central complexes, so that this type of mechanism is mainly of historical interest.

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



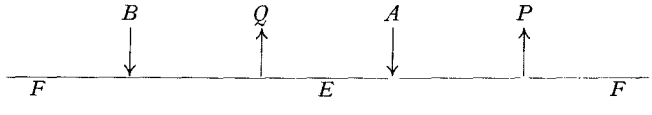
(9)

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



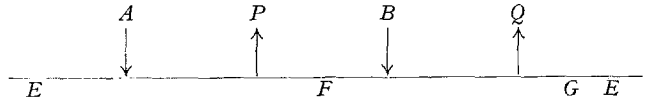
(10)

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



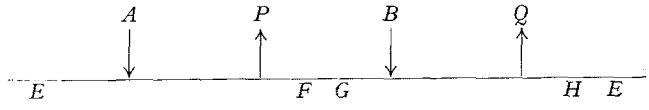
(11)

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



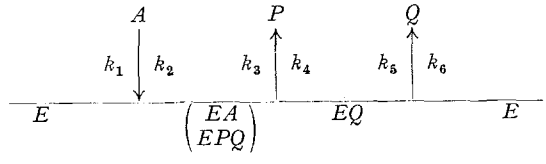
(12)

and Di-Iso Ping Pong Bi Bi would be:



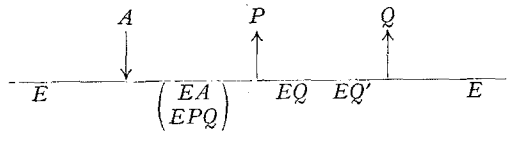
(13)

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



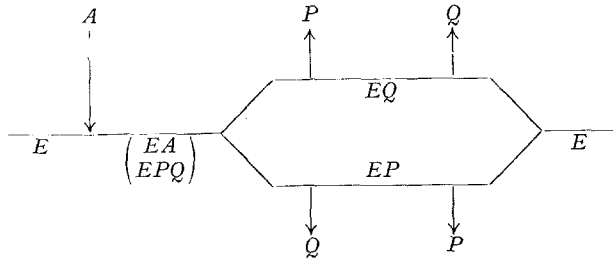
(14)

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



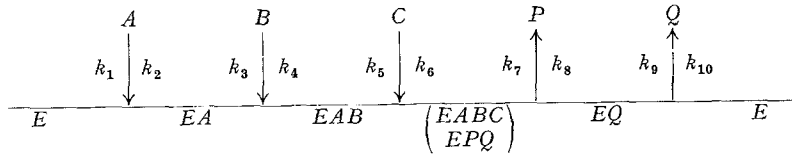
(15)

Release of products in random order gives Random Uni Bi:



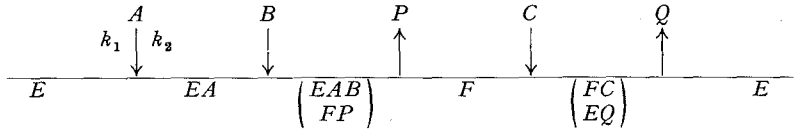
(16)

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:



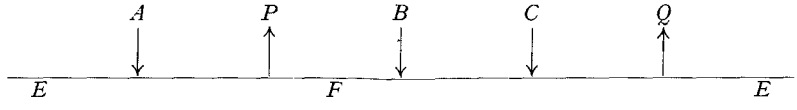
(17)

Ordered Ter Bi



(18)

Bi Uni Uni Uni Ping Pong

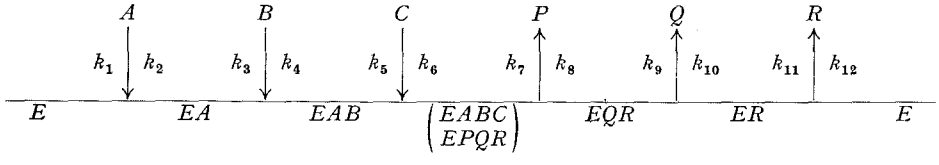


(19)

Uni Uni Bi Uni Ping Pong

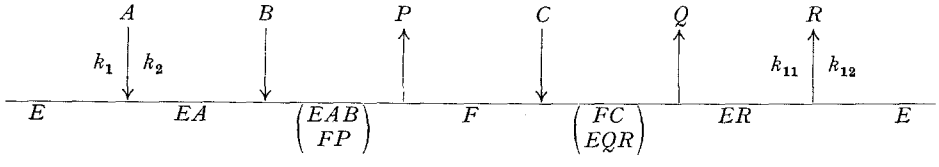
However, if the sequence of Bi Uni Uni Uni is started at F it becomes Uni Uni Bi Uni, and so these two Ping Pong mechanisms are really the same and give rate equations of identical form. The glutamic dehydrogenase reaction is Ordered Ter Bi (see ref. 9), and the enzymic synthesis of *S*-adenosyl methionine 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:



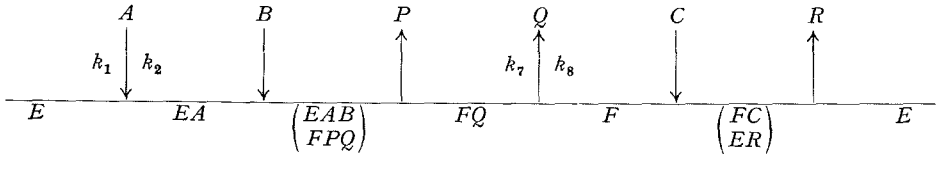
(20)

Ordered Ter Ter



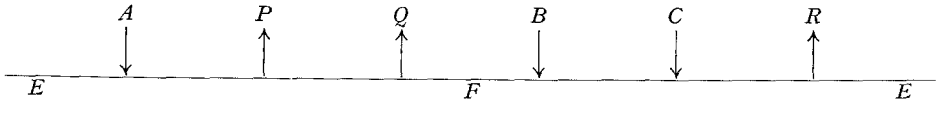
(21)

Bi Uni Uni Bi Ping Pong



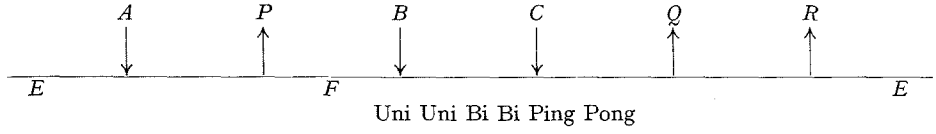
(22)

Bi Bi Uni Uni Ping Pong



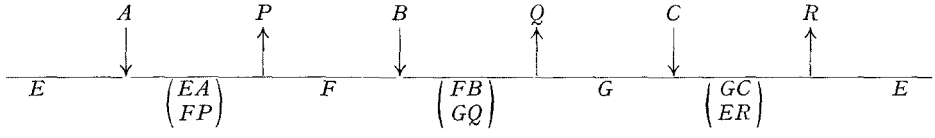
(23)

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.

BBA 12162

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

II. INHIBITION: NOMENCLATURE AND THEORY*

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(Received May 22nd, 1962)

SUMMARY

A nomenclature is proposed to describe different types of inhibitions of enzyme-catalyzed reactions, particularly for reactions with more than one substrate and product. The rate equations for such inhibitions are discussed, as are methods for distinguishing between the various types of inhibition and obtaining inhibition and kinetic constants from experimental data. Several examples are given of the type of information that can be obtained from inhibition studies.

BBA 12163

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

III. PREDICTION OF INITIAL VELOCITY AND INHIBITION PATTERNS BY INSPECTION*

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(Received May 22nd, 1962)

SUMMARY

A general method for predicting initial velocity and dead end and product inhibition patterns by inspection of the mechanism is presented. This method is applicable to any non-random mechanism without alternate reaction sequences. Several examples of the application of the method are given.