ENZYME SCIENCE AND ENGINEERING

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LECTURE–13

KINETICS OF BI-SUBSTRATE ENZYME CATALYZED REACTIONS - II

So continuing from the previous lecture on kinetics of bi-substrate enzyme catalyzed reactions we will be talking about the application of King Altman method to derive rate expression for some of the complex enzyme reaction mechanisms that we often encounter in the bi-substrate reactions.

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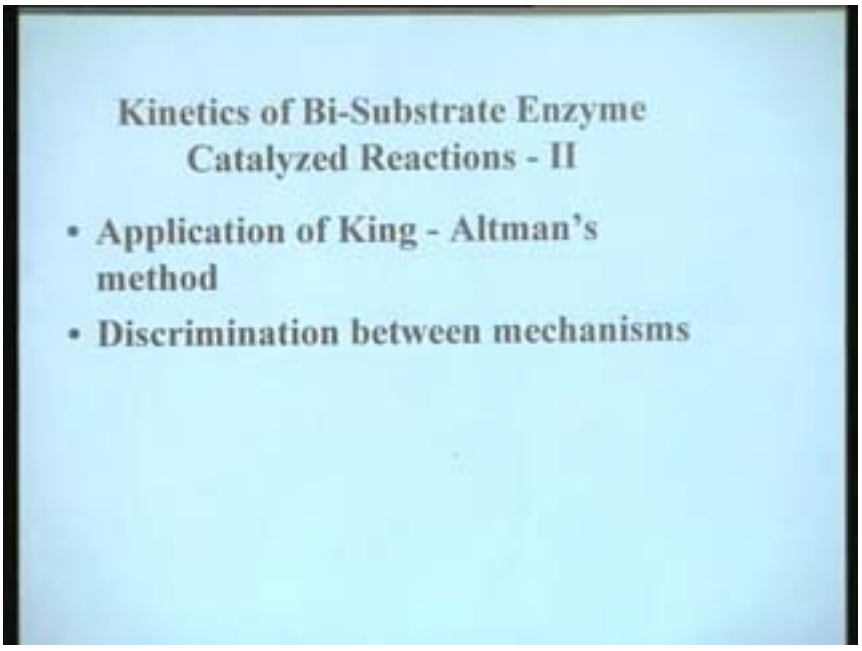

The second thing after getting the rate expression for those different mechanisms of the bi-substrate enzyme catalyzed reactions is the procedures by which one can discriminate between different mechanisms. That is for a particular enzyme catalyzed reaction how to identify the reaction mechanism it follows? We will be following general principles about that. Coming first to the application of King Altman method I will try to illustrate it with a simple bi intermediate reaction sequence. It is not bi-substrate but a single substrate.

This is just to illustrate the point so that the system does not become complex immediately and we will be able to appreciate.

Let us assume the reaction follows the type of mechanism as indicated here

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} ET \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} E + P$$

It is a totally reversible reaction and you have the rate constant k_1, k_{-1}, k_2, k_{-2}, k_3, k_{-3}.

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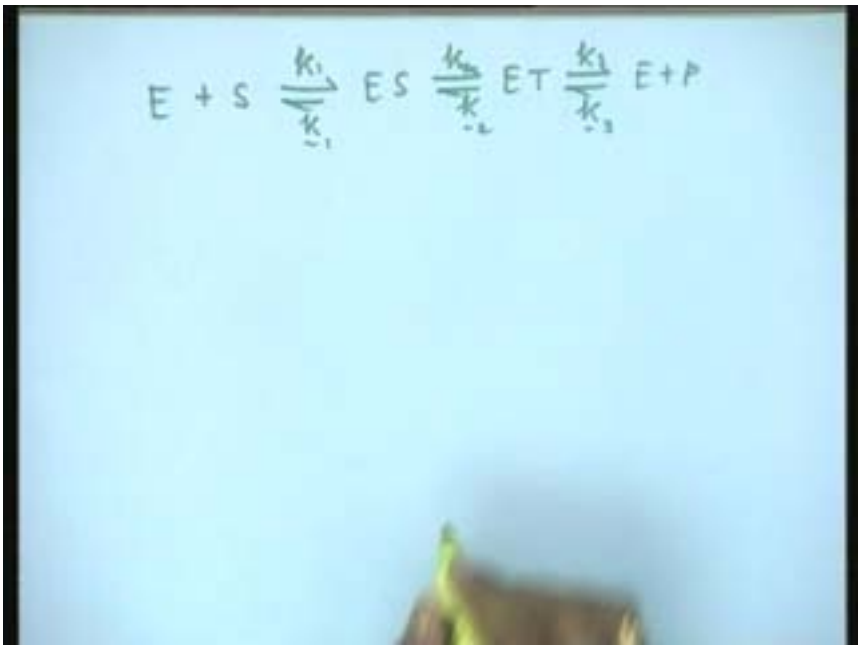

As I mentioned that the first step in any reaction mechanism would be to express a mechanism in cyclic form so that each of the enzyme species that are present, here as you see there are three enzyme species, E, ES and ET they must form the vertices of the polygon that represents the reaction mechanism. So you can just write E. It will form a triangle and E to ES, ES to ET and then ET to E. Also show the interaction between the three enzyme species that are present. Mention the rate constant in the form of Kappa values. That means Kappa value for E to ES will be k_1.S and this will be $k_{-1}$and you will notice that wherever a substrate or product species is involved you will multiply the rate constant with that. If there is no substrate or product then the rate constant will be as it is. Similarly in the case of ES it will be k_2.k_{-2}. Here you will have k_3 and k_{-3}.p.

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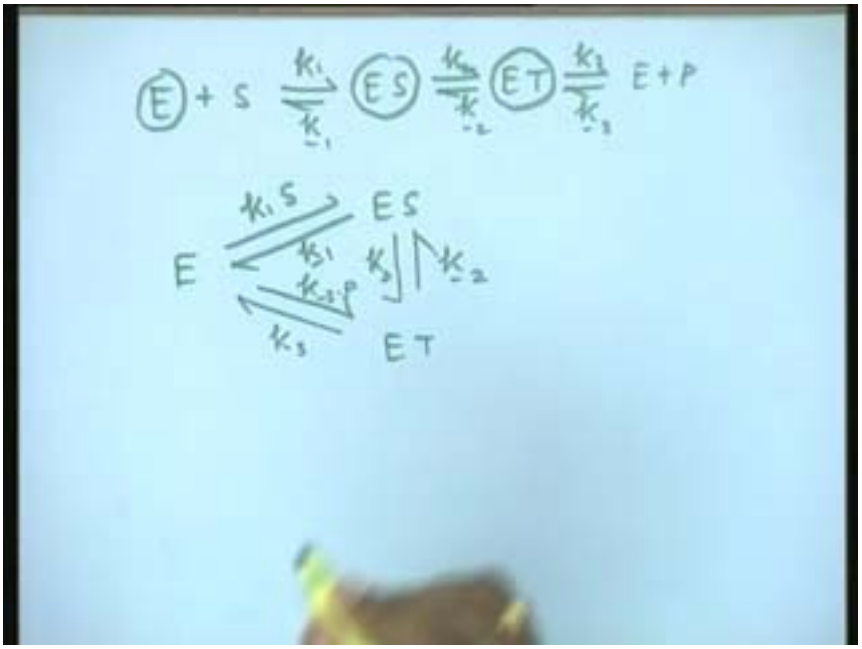

With this information that is available from the reaction mechanism then we will try to find out the fraction of each of the enzyme species of the total enzyme concentration in the form of the rate constant and the total enzyme concentration. If you consider the first enzyme species let us say E we write down all the possible patterns which contain n-1 terms where n is the number of enzyme species.

For example here the enzyme species are three so we will take all patterns that have two steps only two steps and nothing else and those patterns will be considered. Second thing is that only those patterns which end up in synthesis of that species and not the patterns that lead to the break down or which go away from the species will be considered. For example in this case since all the steps are reversible there will be three patterns.

You will notice that you have k_{-1} and k_3. You have k_{-1}, k_{-2} and also k_2, k_3. The fraction of this, the total enzyme concentration will be proportional to sum of the product of the Kappa factors. That means E upon E_0 will be equal to

$$E/E_0 = \frac{k_{-1}.k_3 + k_{-1}.k_{-2} + k_2.k_3}{\sum}$$

$\sum$ is the sum total of all the Kappa factors. That means all the Kappa factors put together for E, ES and ET. The numerator whatever we will write for ES and ET will also be added in the denominator so the sigma will be E_0.

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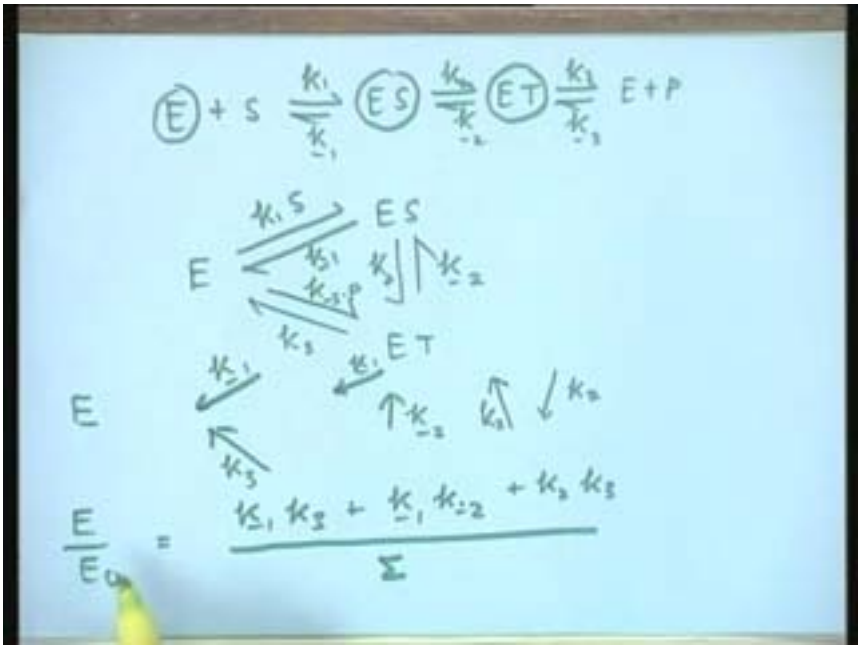

The concentration of E species will be a fraction which will be given by such an expression. Sigma is the sum total of the products of all the Kappa factors. For example we have the products of Kappa factor for E we will have for ES and also for ET. The total sum will be the sigma on the denominator. So that will represent the E_0 or you can write E will be equal to E_0 multiplied by the terms in the numerator.

Similarly you can write for ES. The ES will be given by again three different patterns. This one here it is k_1s and k_{-2}, $k_{-2}.k_{-3}p$ and k_1s and k_3. Here also you will notice that the concentration of E will be

$$E = E_0(k_1.k_{-2}s + k_{-2}.k_{-3}p + k_1.k_3s)$$

In the same fashion for ET it will come out to be $k_{-3}.p$ and k_2, $k_1s.k_2$ and $k_{-1}.\ k_{-3}.p$ and the fraction of the ET of the total enzyme concentration will be

$$E_0(k_2.k_{-3}p + k_1.k_2s + k_{-1}.k_{-3}.p)$$

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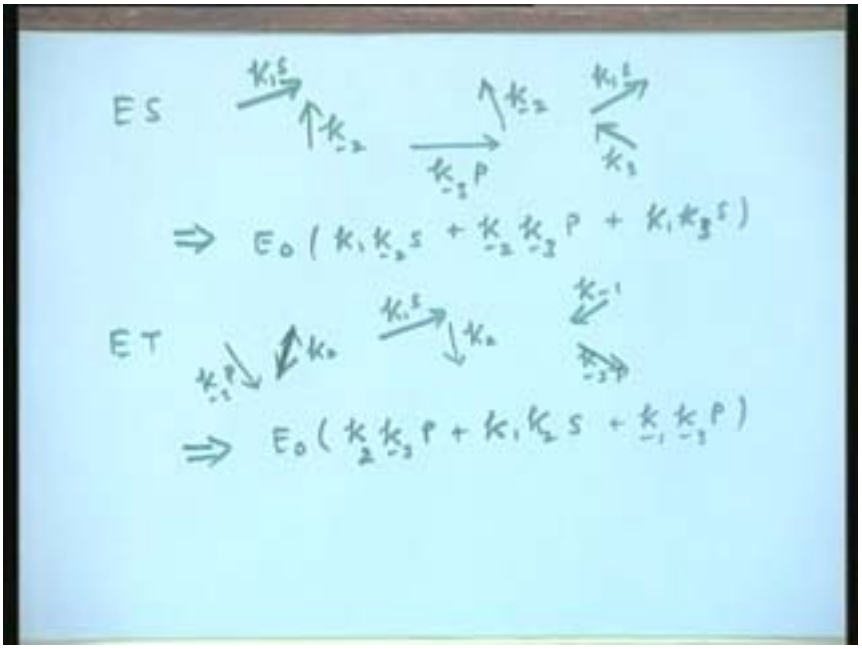

Now you can write the reaction velocity. The reaction velocity for such a reaction will be given by

$$v = k_3(ET) - k_{-3}[E].[P]$$

The rate expression for that will be this expression. You can substitute the value of ET and E in the expression and after deleting all the factors which are positive and negative you come up with

$$v = \frac{E_0(k_1k_2k_3s - k_{-1}k_{-2}k_{-3}p)}{(k_{-1}k_{-2} + k_2k_3 + k_{-1}k_3) + s(k_1k_{-2} + k_1k_3 + k_1k_2) + p(k_{-2}k_{-3} + k_2k_{-3} + k_{-1}k_{-3})}$$

This comes out to be the rate expression for such a reaction mechanism. Had this reaction not been reversible totally then the term p will become zero, irreversible reaction. You can just put p equal to zero that means this term will disappear whole of this term will disappear and the rate expression will become for a irreversible reaction step.

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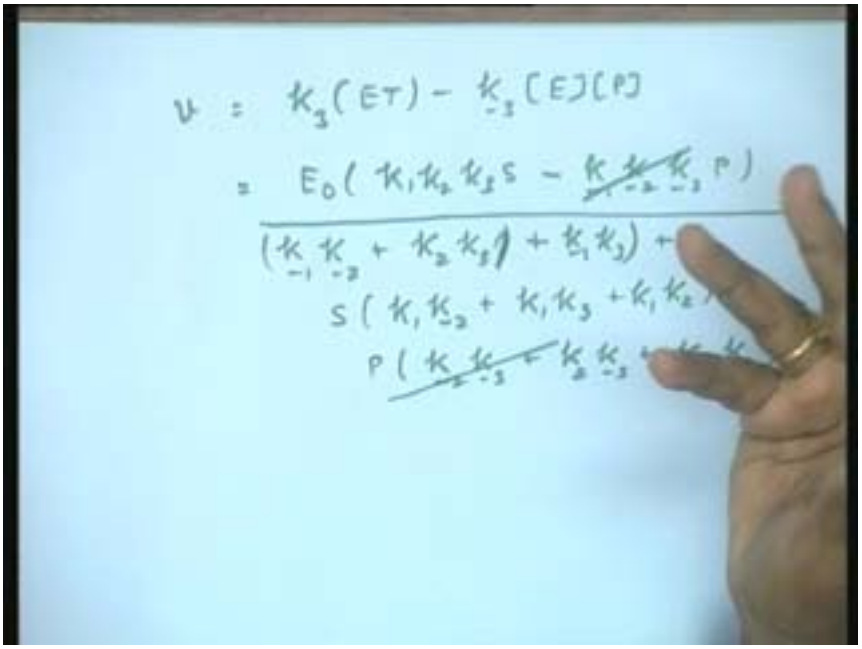

So these are the steps. Just to summarize first of all you write the reaction mechanism in a cyclic manner, write all the Kappa values at each of the inter conversion arrows of the different enzyme species express the fraction of the concentration of a particular enzyme species of the total enzyme concentration in terms of the sum of the products of the Kappa factors and then write the rate expression taking the value of the particular enzyme species from those expression. So that is what we do and one can write rate expression for a complex reaction mechanism.

The denominator is total sum of all the Kappa factors of all the three species and because here all the three enzyme species are convertible reversibly all the patterns are coming into picture. But if in a reaction mechanism some of the steps or some of the inter conversions are irreversible in nature then many of the patterns will disappear because there will be no chance of synthesizing that particular species. But we will consider only those patterns which will consist of n-1 steps.

With this background if we take a general case from a binary substrate reaction that is particularly for the ordered ternary complex formation you will recall that the reaction mechanism in this case was expressed E goes to EA then EA goes to EAB. Mind it the reaction binding of B from E is not possible in the ordered mechanism. We are considering that only A can bind to free enzyme and then this goes to EY with the release of the product X and this goes to E with the release of the product Y. Let us say this is k_1s, k_{-1}. I am just writing the Kappa values right on the arrow so that while writing the rate expression it will be easier. This is your k_2B, k_{-2}, this is k_3, and this is k_4. There are four different enzyme species E, EA, EAB and EY. The last two steps are irreversible in nature. They only lead to formation of the two products in a sequential manner.

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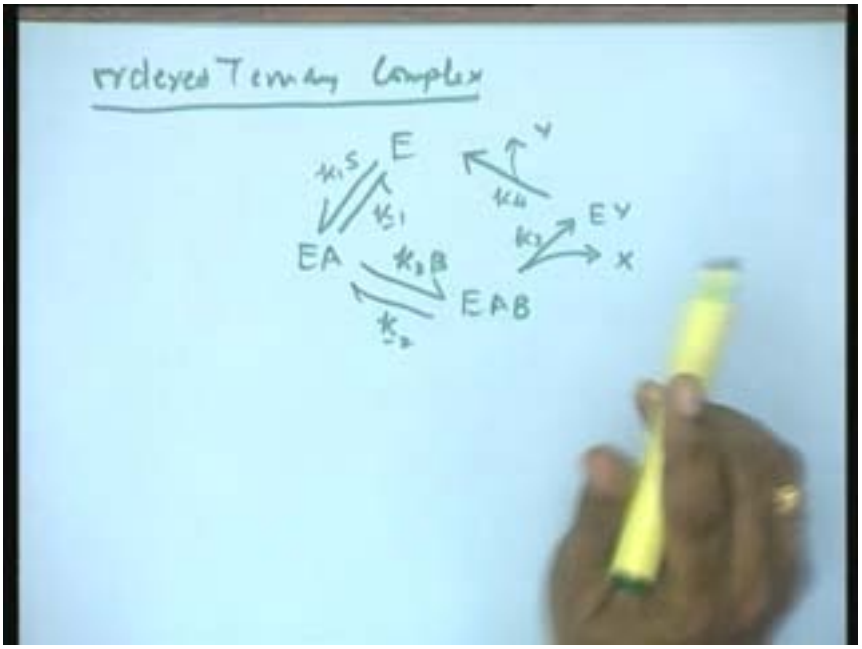

If we write for E there should be three different possibilities. One is that the E can synthesized in this route that is your k_{-1}, k_{-2} and k_4. The other is this can also be synthesized in this form k_2B, k_3 and k_4 and also k_{-1}, k_3, k_4. These are the three possible patterns which are possible and so writing for the concentration of E will be

$$E = \frac{E_0 (k_{-1} k_{-2} k_4 + k_2 k_3 k_4 B + k_{-1} k_3 k_4)}{\sum}$$

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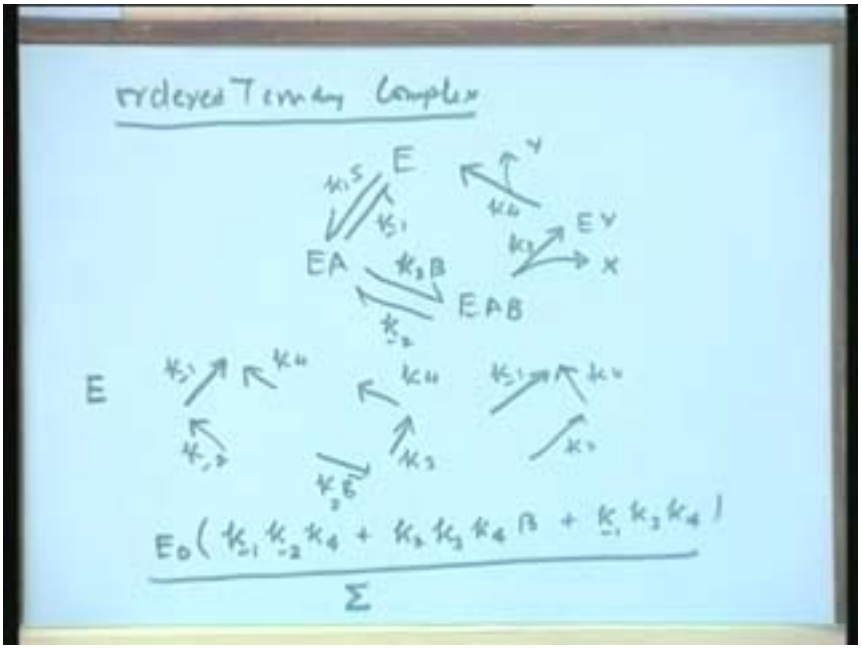

The concentration of E will be given by this factor. EA will be formed only by two patterns that is k_1A, k_{-2} and k_4 and k_1A, k_3 and k_4. These are the only two patterns which will lead to synthesis of EA and none another because in all other patterns there will be no reversible step. I again like to remind you that just as a matter of convenience we consider only those steps which lead to the synthesis of EA. All the steps which go outside from the EA I am not taking into account. The concentration of EA will be given by

$$EA = \frac{E_0 (k_1 k_{-2} k_4 A + k_1 k_3 k_4 A)}{\sum}$$

EAB will have only one pattern possible because EAB is emerging only from the E side via E and EA and it cannot come from EY. So you have only one pattern for EAB that is k_1A, k_2B and k_4. Therefore EAB will be given by

$$EAB = \frac{E_0 (k_1 k_2 k_4 A B)}{\sum}$$

Similarly EY will also have only one pattern k_1A k_2B and k_3. So this will be given by

$$EY = \frac{E_0 (k_1 k_2 k_3 A.B)}{\sum}$$

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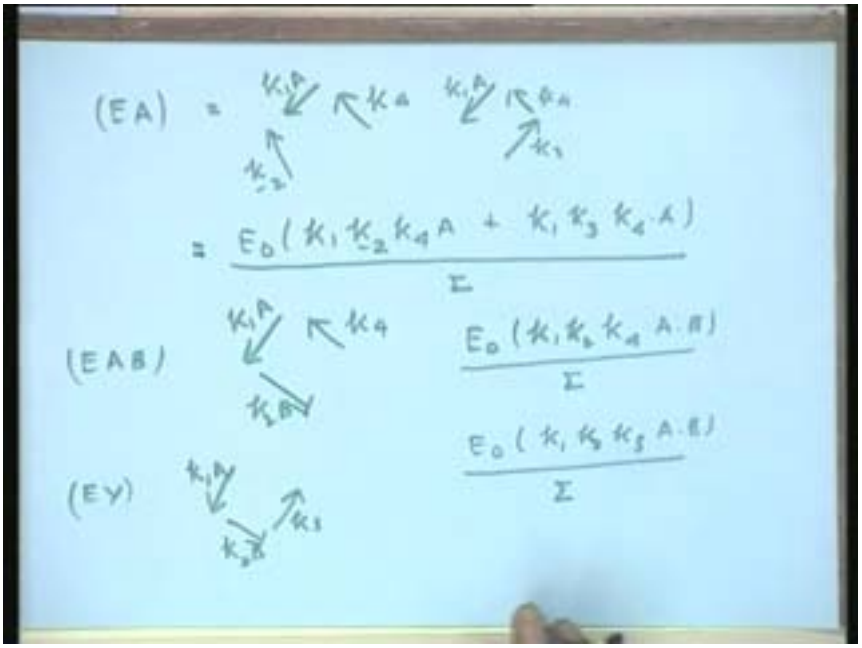

So you have now the relative concentrations or the relative fractions of each of the enzyme species that are present. You recall that in the case of allosteric enzymes also the relative concentration can be arrived from a similar analysis to have the concentration of each of the enzyme species.

From this then you can write down rate of reaction as equal to

$$v = k_3[EAB]$$

If you look at the original rate expression the product is being formed. You may have either as k_4.EY or you can have k_3.EAB. Both the reaction rates will be identical and so you can write as k_3.EAB. Right in the main reaction mechanism we are writing A plus B going to X and Y. We are considering a unimolecular reaction. If they are not unimolecular then rates will be different. But as long as we are assuming that the stoichiometric relationship is unimolecular they will be identical.

If you substitute the value of EAB you get

$$\frac{E_0\, k_1k_3k_2k_4\, A.B}{\sum}$$

$\sum$ is the total sum of all the numerators of earlier species and if you simplify that just by arithmetic manipulation you get an expression like

$$v = \frac{\{K_3k_4/k_3 + k_4\}E_0.A.B}{\frac{k_4(k_{-1}k_{-2} + k_{-1}\, k_3)}{k_1k_2\,(k_3 + k_4)} + \frac{k_4\,(k_{-2} + k_3)}{k_2(k_3 + k_4)}\, A + \frac{k_3\, k_4}{k_1(k_3 + k_4)}\, B + A.B}$$

We have rearranged it into the format of the general rate expression and in the form of individual rate constant and this term on the numerator indicate the value of V_m. This term is k'_Ak_B. This is your k_B and this is your k_A. From this again by dividing this term you can get the value of k'_A. This is identical to the generalized rate expression proposed by Alberty. You can get the individual rate constant in the form of the four parameters that we talked about V_m, k'_A, k_A and k_B which represents the kinetic parameters.

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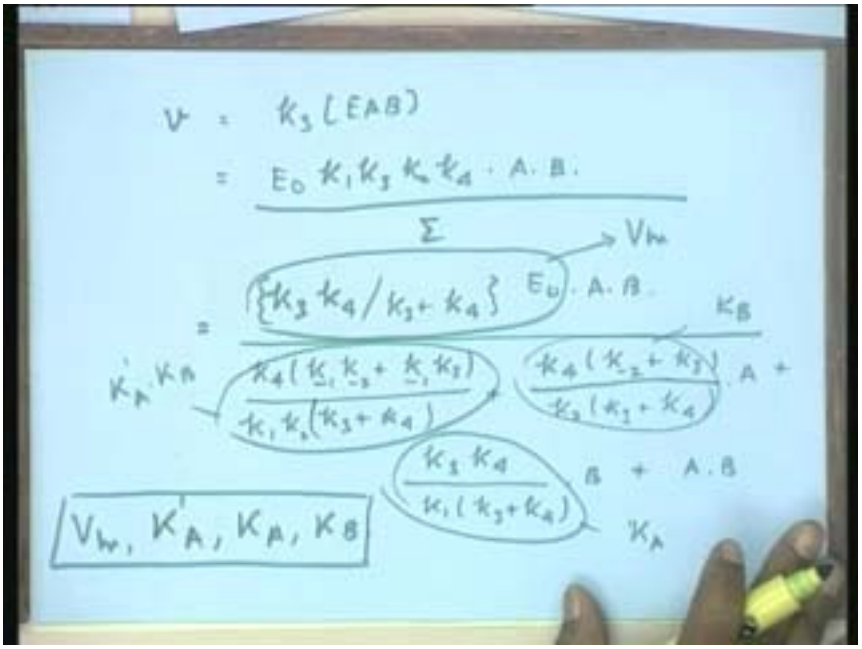

In the case of Thorell and Chance mechanism which we consider as a special case of the ordered ternary complex formation we assumed that the complex EAB was very unstable as if the EA directly goes into EY forming a product X and there is no significant concentration of EAB present.

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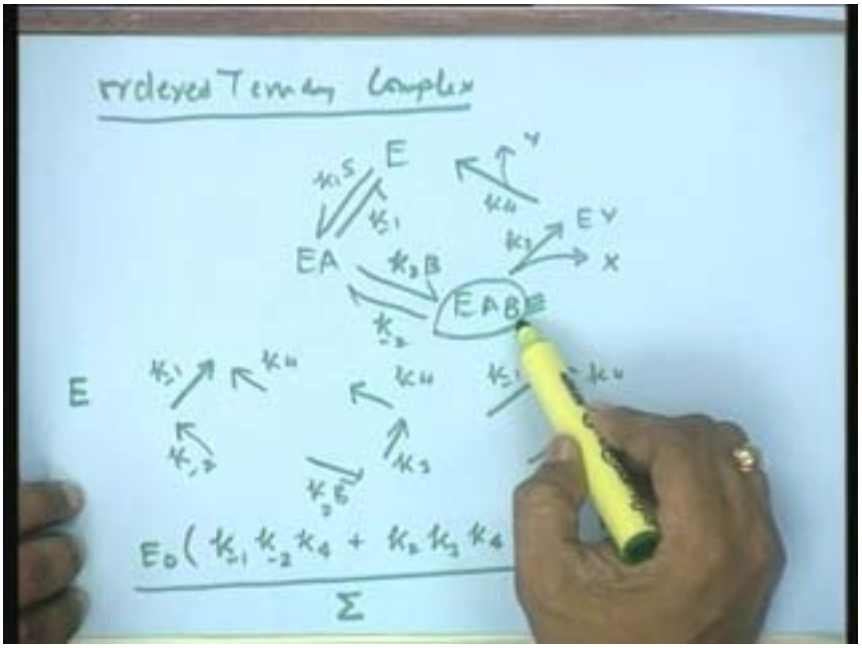

This indicates that in the case of Thorell and Chance mechanism k_3 term from the reaction mechanism is much, much greater than k_{-2}. The condition implying the non-existence of the term, in significant quantities of EAB and direct conversion of EA into

EY although it is via EAB, the complex EAB being not very stable, means that k_3 is much, much greater than k_2. When you substitute those you get a rate expression as

$$v = \frac{k_3.E_0.A.B}{k_{-1}k_3/k_1k_2 + k_3/k_1\ B + k_3/k_2\ A + A.B}$$

Again the same pattern of generalized rate expression that we noticed in the case of ternary complex formation and both the type of ternary complex formation will follow the same pattern. When we talk about the binary complex formation, in the case of ping pong mechanism the pattern will change and that will clearly indicate the existence of a different mechanism. You can further simplify this by multiplying this by k_1, k_2.

$$v = \frac{k_1k_2k_3.E_0.A.B}{k_{-1}k_3 + k_2k_3\ B + k_1k_3\ A + A.B}$$

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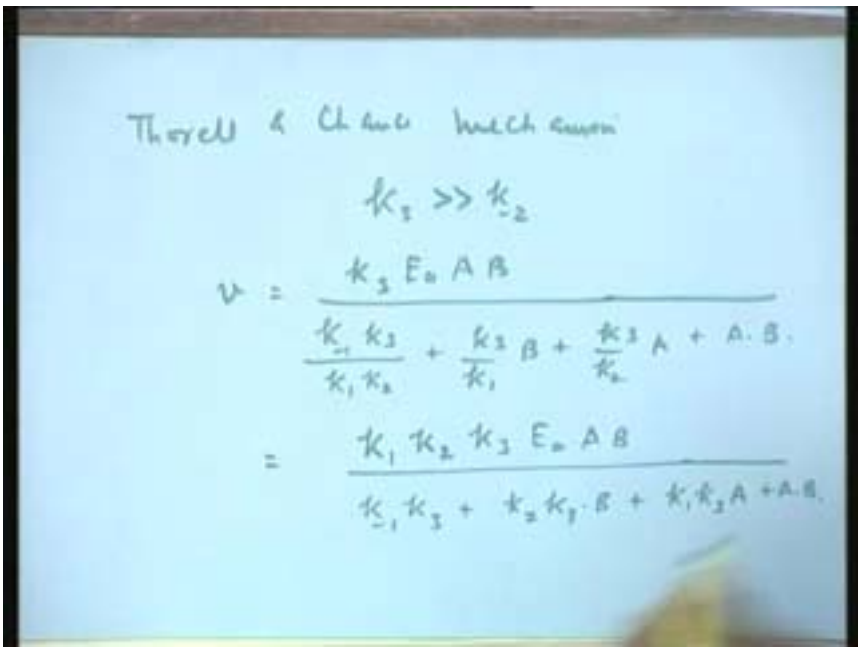

That is the final expression for Thorell and Chance mechanism and therefore one can go into such a mechanism and get rate expression.

The practical utility is two things. One is to understand the reaction mechanism that a particular reaction follows and second thing is design of reactors. You read the kinetic rate expression and the individual rate constants if you want to really design and get the final simulated values and all other simulated profiles for the reactor performance. The

significance of the kinetic studies allows you to understand the reaction mechanism. By getting their individual rate constants you can also manipulate them for the design of reactors and for simulation work. The information on the individual rate constant will also give you an understanding on how the enzymes will function at the metabolic level. A simple example is glycolytic enzymes for example hexokinase. The glucose can enter into more than one metabolic pathway. Which metabolic pathway it will enter requires understanding of the reaction mechanism and individual rate constants.

It is significant to note that most of the enzymes that are present in the living cells are bi-substrate reactions. Bi-substrate reactions can be understood and for the sake of simplicity have been analyzed keeping in view the Michaelis–Menten kinetics. But always it may not apply because the concentration of the second substrate will never be in excess in the living cell. That will always be in comparable concentration and therefore it will be always useful to have understanding of the mechanism.

The other bi-substrate reaction mechanism was a binary complex formation and the case which we talked about was Ping Pong mechanism which states that at no state ternary complex forms. As soon as the binary complex forms a product is released and the modified enzyme then interacts with the second substrate and the second product is formed. The reaction mechanism as we have expressed earlier is k_1A, k_{-1}, EA. Here at this stage a modified enzyme complex and product X will be released with k_2, k_3B and you get E'B. You again get another product form y and with the k_4 rate constant. There is only one reversible step here when the first substrate binds to enzyme rest of the steps are irreversible steps. The four different enzyme species present here E, E'_A, E' and E'_B.

For E you can write the pattern as k_{-1}, k_3B and k_4. Similarly you have another pattern k_2, k_3B and k_4 and the relative concentration of the enzyme species E will be

$$\frac{E_0(k_{-1}k_3k_4B + k_2k_3k_4B)}{\sum}$$

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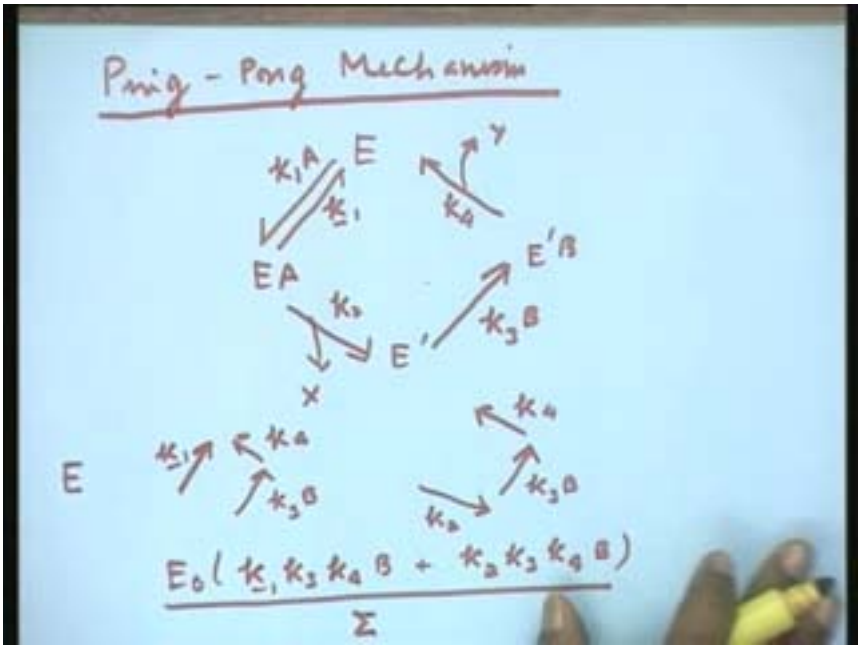

Similarly for EA you will get only one pattern which will be feasible that is you are going in the anti-clockwise fashion that is your k_3B, k_4 and $k_1.A$ and the relative concentration will be

$$\frac{E_0\,(k_1k_3k_4\,B.A)}{\sum}$$

For $E^{'}$ you again have only one pattern that is k_2, k_1A and k_4 and that will be

$$\frac{E_0\,(k_1k_2k_4\,A)}{\sum}$$

$E^{'}B$ will also have only one pattern k_1A, k_2, k_3B and you will get

$$\frac{E_0\,(k_1k_2k_3\,A.B)}{\sum}$$

So having the relative concentration of each of the enzyme species then you can always write the rate expression that is your v as equal to

$$v = k4.E^{'}B = k_2.EA$$

It can be either $k_4.E^{'}B$ or it can be $k_2.EA$. Either of that can be written.

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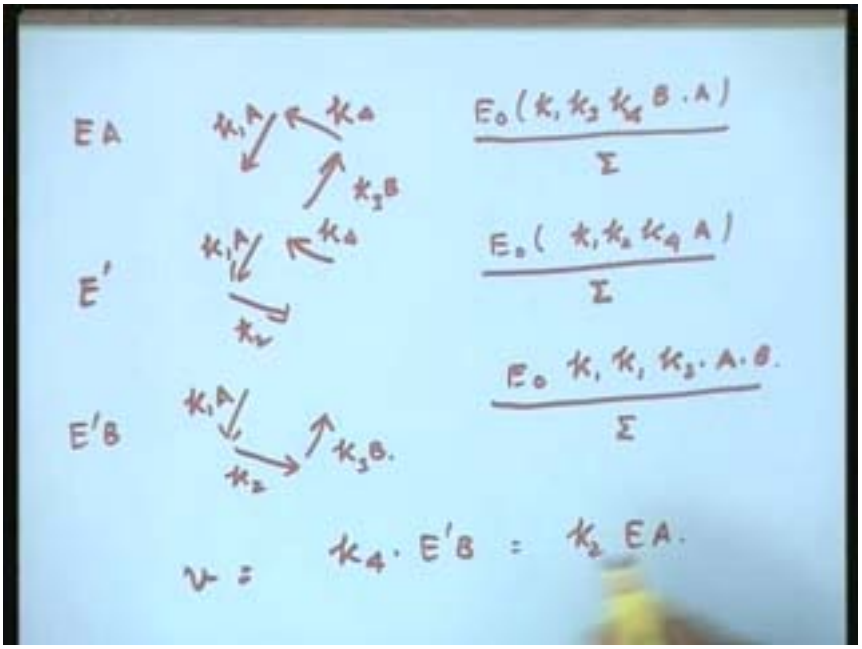

Therefore again you can substitute the values and get reaction velocity expression as

$$v = \frac{E_0 k_1 k_2 k_3 k_4 . A . B}{k_1 k_2 k_4 . A + k_3 k_4 (k_{-1} + k_2) . B + k_1 k_3 (k_2 + k_4) A . B}$$

If you try to bring it in the form of generalized rate expression you get

$$\frac{k_2 k_4 / (k_2 + k_4) . E0 . A . B}{\frac{k_2 k_4}{k_3 (k_2 + k_4)} A + \frac{k_4 (k_{-1} + k_2)}{k_1 (k_2 + k_4)} B + A . B}$$

You notice a significant difference from the generalized rate expression. You have the V_m term, you have the k_B term and you have k_A term. But the $k'_A . k_B$ term is missing which implies in the case of pong ping pong reaction mechanism, the term k'_A is zero and that makes a very big point to discriminate between the different reaction mechanisms. At least you can distinguish whether the enzyme reaction follows the binary complex mechanism or ternary complex mechanism by just evaluating the value of k'_A.

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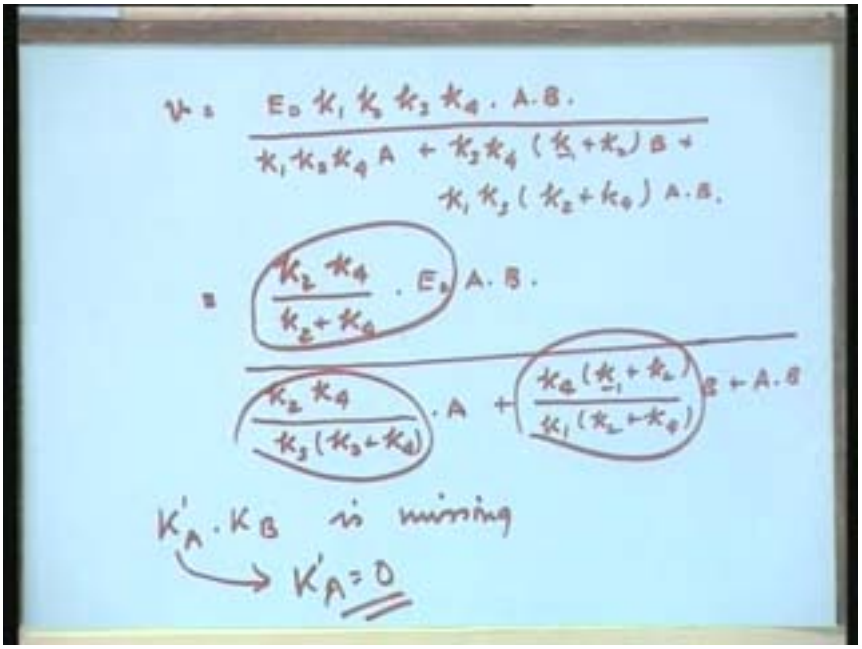

Earlier we talked about the analysis of the bi-substrate data based on the double reciprocal plot and the secondary plots of the slope and intercepts. If you plot 1/A and 1/v you get a profile like this with B varying and if you plot slope vs 1/v and similarly intercept versus 1/v you can determine the value of k_A, k_B, k'_A and V_m.

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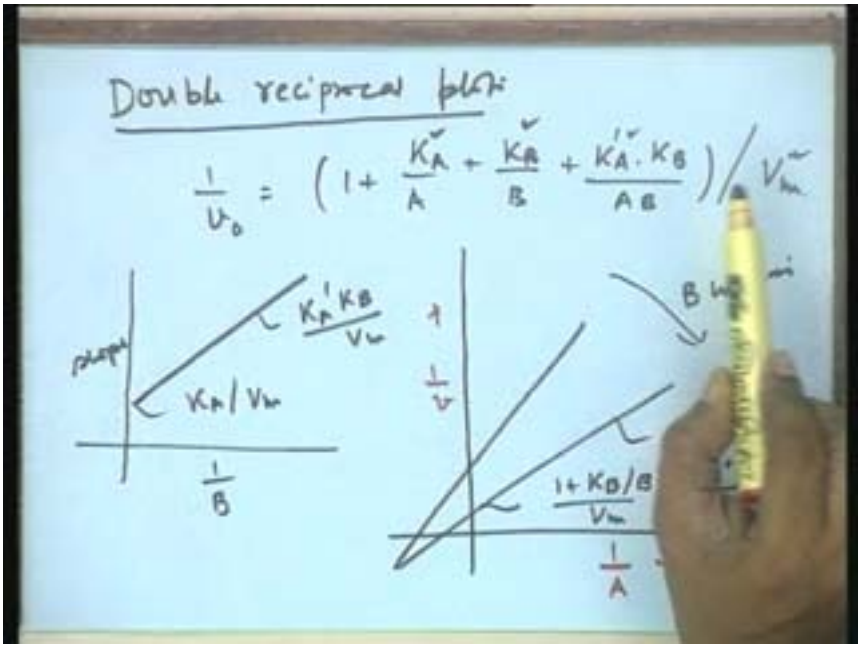

By analyzing these parameters one can distinguish if the magnitude of k'_A is zero and one can conclude that the reaction mechanism under study follows the binary reaction mechanism.

That means that the dissociation of that, that is the break down of the EA complex is very, very slow. Zero means the magnitude goes to a very small value. It becomes a very stable system.

The second part of today's lecture is to discriminate between the different reaction mechanisms. One of the tools we have got is the analysis of the reaction rate data based on double reciprocal plot. We can easily distinguish and get the magnitudes of the values of the various kinetics parameters V_m, k_A, k_B and k'_A and if the magnitude of k'_A is negligible or zero one can conclude that it is binary reaction mechanism and not ternary. Unfortunately this analysis will not yield any information about whether it is an ordered or a random ternary complex formation mechanism because in both cases the rate pattern will be identical although the magnitude of the k_A, k_B and k'_A might vary but they may have a positive value.

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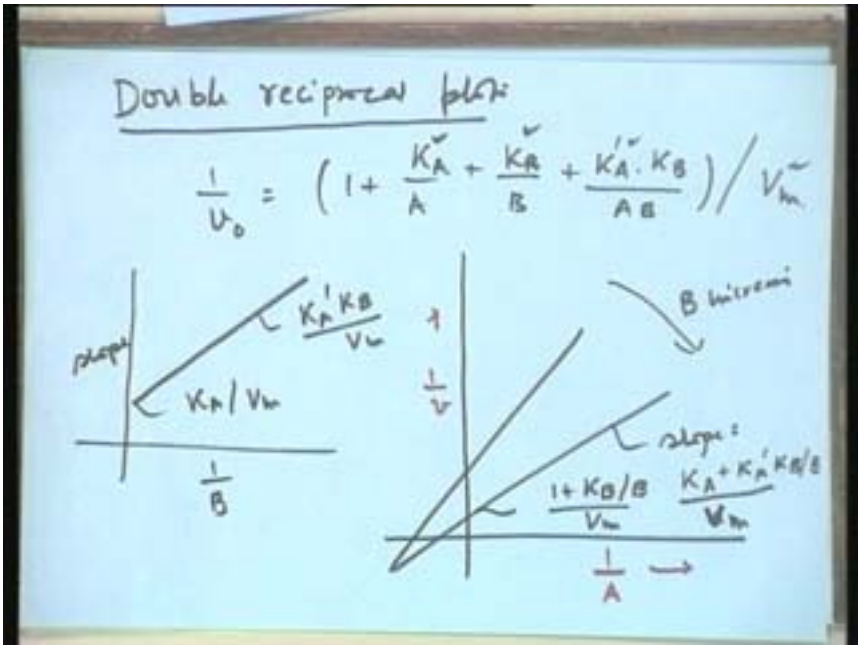

In those cases another factor which we make use often for analyzing the reaction mechanism is the enzyme inhibition data. Because if you study the use of one of the products of these bi-substrate reaction as an inhibitor and see their interaction with the effect on the rate expression you will notice that the different products X or Y when used as an inhibitor they give rise to different pattern of inhibition, which we noted earlier. That means un-competitive, non-competitive or a linear mix and the linear mix was a case of non-competitive inhibition.

I will not go into detail of analysis of those because they will only have very long expressions. But if you consider the summary of the rate equations, I summarized here the random ternary complex formation.

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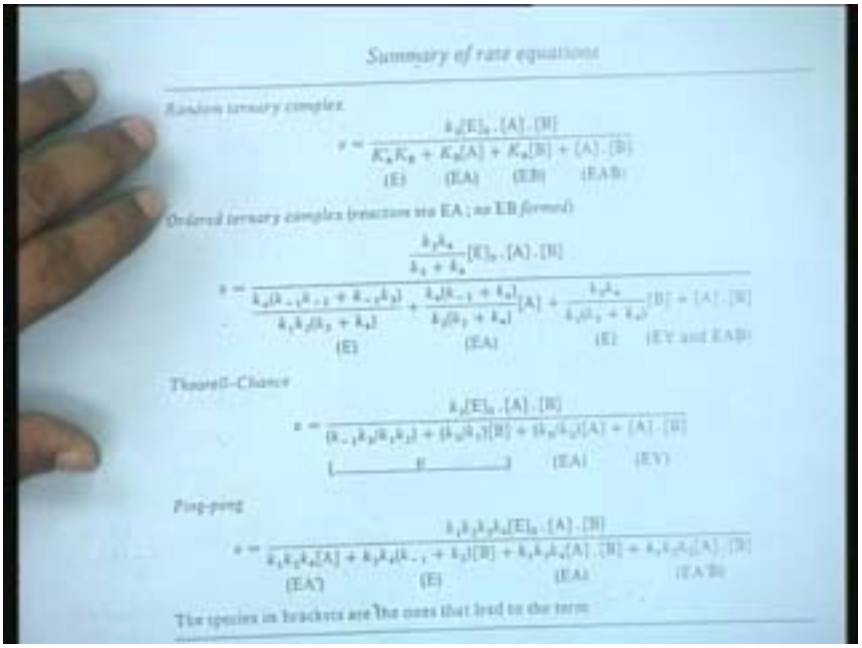

It is exactly identical to that of a generalized rate expression ordered ternary complex that we have written today and Thorell and Chance mechanism and Ping pong mechanism and the species in the brackets are the ones that correspond to that enzyme species.

For studying the inhibition pattern of each of the product used as an inhibitor let us consider a compulsory order ternary complex formation in which the substrate A binds first. Use the two products Y or X separately as inhibitors in the reaction and notice the effect of Y and X as the nature of inhibition. With respect to the varying A you get a competitive inhibition pattern. With respect to X as the inhibitor you get a mixed inhibition.

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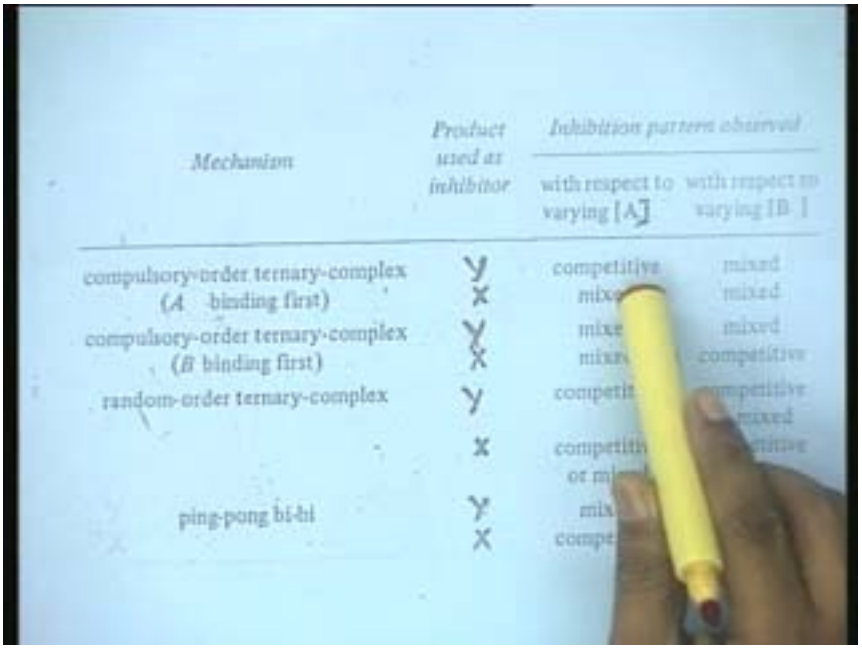

So you get a completely different pattern and as you recall that when we discussed the inhibition patterns we also mentioned very clearly that major use of the inhibition patterns is in the analyses of the bi-substrate reactions to identify their reaction mechanism. One can derive these expressions and get the different types of inhibition pattern. This is just a summary of the whole thing. When you take A binding first, you know the product and out of the two as an inhibitor you get a different pattern and with respect to varying B you get a different pattern. So by getting the inhibition data on the bi-substrate reaction kinetics either by choosing one product or the other product one can get a set of data and from which one can conclude by the ordered ternary complex formation or a random ternary complex formation

Identifying Ping-pong bi-bi or the ping pong mechanism directly from the effect of substrate concentration on the reaction rate itself can lead you to that kind of scenario. The combination of the two studies particularly the effect of substrate concentration and studying the nature of inhibition with respect to one or more of the products will give you the nature of inhibition pattern and that information can give you a conclusion about the nature of the reaction mechanism. Most of the reactions that lead to such kind of inhibition patterns have been then concluded to follow one or the other ordered or ternary complex mechanism formation.

With this we will be completing our kinetics of enzyme catalyzed reaction. Just to summarize what we have covered so far in the kinetics we have initially looked at single substrate single intermediate reactions leading to hyperbolic kinetics and the validity of this kinetic pattern to a whole range of reactions under different conditions. Thirdly we came to inhibition patterns with respect to the single substrate reactions and the effect on the inhibitor concentration on the reaction rate and also the effect of activators on the reaction rate. Then we came to the effect of pH and effect of temperature. They are used for understanding reaction thermodynamic parameters and other mechanistic aspects.

Then we came to bi-substrate reactions and the effect of substrate concentration, writing the rate expression and finally concluding with the discrimination of various reaction mechanisms that are involved in bi-substrate reactions. So that summarizes the whole of whatever we have covered in enzyme kinetics.