Fundamentals of Protein Chemistry
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Module - 07
Enzyme Kinetics and Enzyme Inhibition
Lecture - 32
Enzyme Kinetics - II

In our second lecture on enzyme kinetics, we are going to be looking at other modes of possibilities where our enzyme substrate complex is going to be formed.

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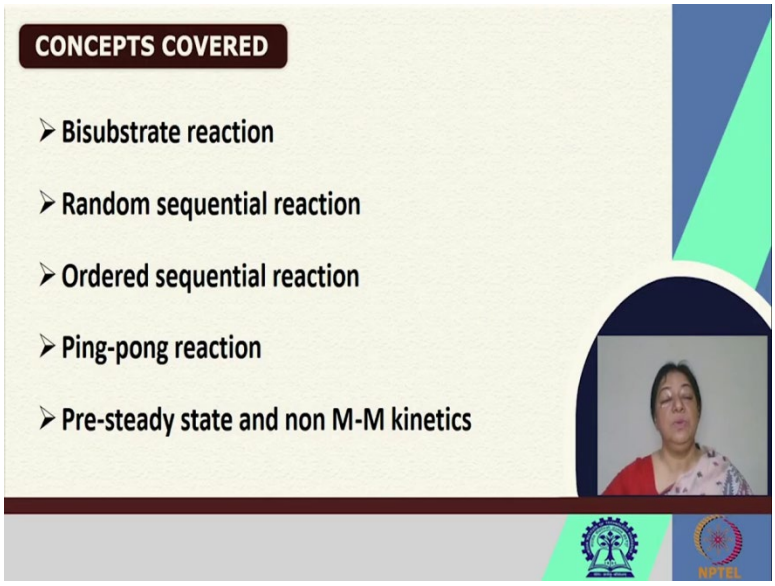

In this case what we will look at is, we will look at bisubstrate reactions, random sequential ones, ordered ones, ping-pong reactions and pre-steady state kinetics in an exemption from some of the Michaelis-Menten kinetics that we had looked at in the previous lecture.

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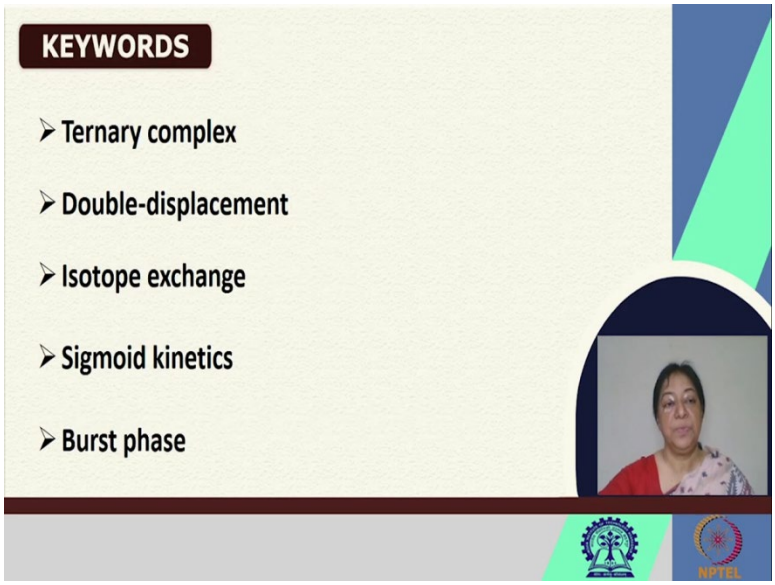

Our ternary complex tells us that we have three components in our complex formation.

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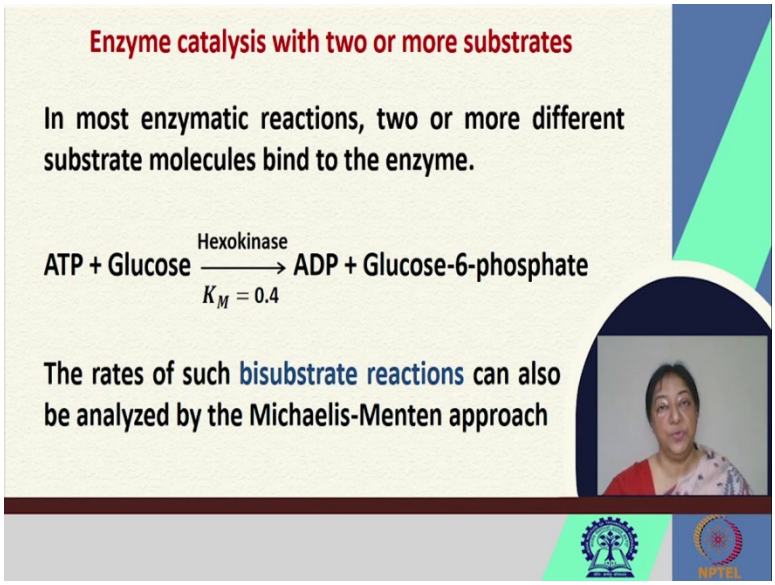

If we look at enzyme catalysis with two or more substrates, the possibilities are different. In most enzymatic reactions actually, there are at times two or more different substrate molecules that bind to the enzyme, in the specific recognition that we looked at in the previous module.

For example if we look at ATP plus glucose, we have the enzyme hexokinase that works in the transfer of the phosphate group to form glucose-6-phosphate and itself forms ADP in the process. The rates of such bisubstrate reactions can also be analyzed by a Michaelis-Menten approach. In this case, we find that there is a Michaelis constant corresponding to 0.4.

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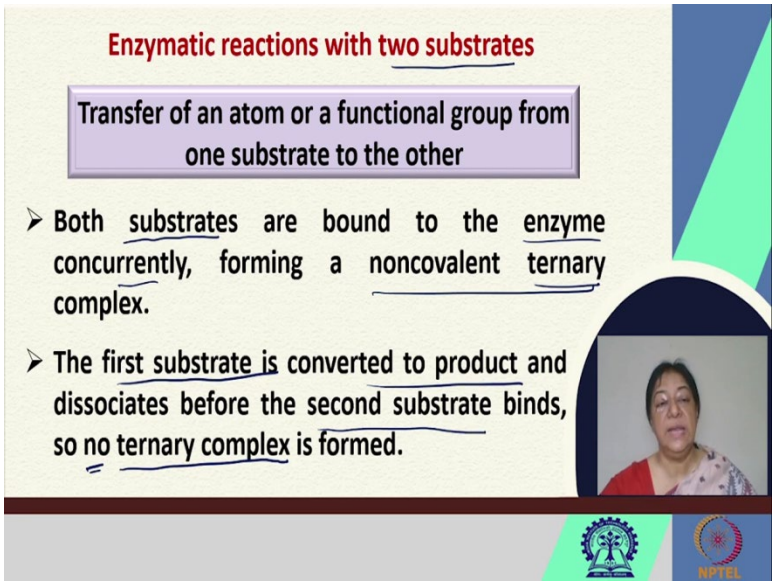

The enzymatic reactions with two substrates, involve the transfer of an atom or a functional group from one substrate to another in the formation of another compound. It may so happen that both of these substrates are bound to the enzyme concurrently, that means together forming a noncovalent ternary complex.

There are three components present in this complex that is also an enzyme substrate complex. But in this case, instead of having one substrate we have two such substrates. The first substrate is converted to the product and dissociates at times before the second substrate binds in a different case, where there is no possibility of a ternary complex form.

So, what we can have is that the possibilities with two substrates indicate that we can have both substrate bound together forming a ternary complex. It may so happen that the first substrate is converted to product before the second substrate binds, so there is no ternary complex formed. We will see specific examples of the binding of two substrates to an enzyme.

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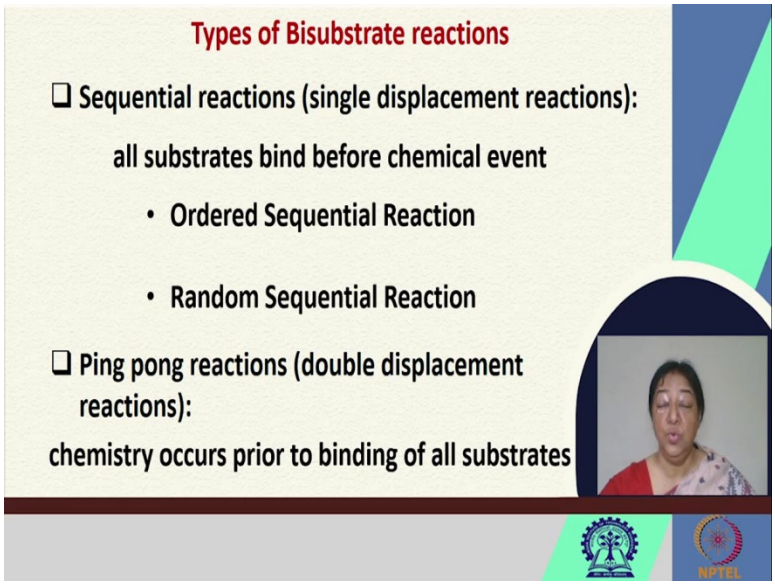

The types of bisubstrate reactions that can occur are sequential reactions, that involve single displacement reactions. In this case, all the substrates bind before the chemical event that is the catalysis, can take place. We have in this case ordered sequential reactions where the two substrates have to bind in a specific order, or we can have random sequential reactions where the substrates can bind in any order.

We can have ping-pong reactions that are known as double displacement reactions and in this case the chemistry occurs, binding to all of the substrates, so there is some catalysis that occurs before the binding of the second substrate.

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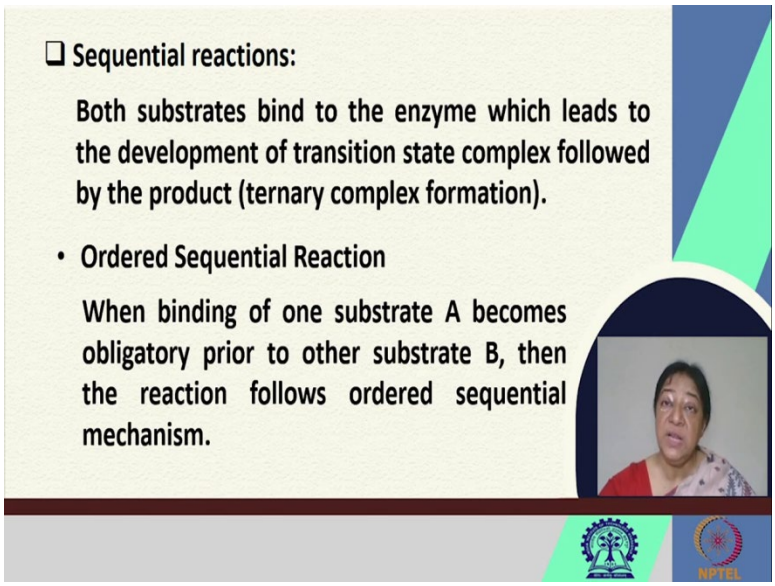

In sequential reactions both substrates bind to the enzyme which leads to the development of the transition state complex that will then result in product formation. So we have a ternary complex

form. In ordered sequential reactions, the binding of one substrate A is definitely required before the other substrate can bind to perform the specific catalytic mechanism of the enzyme.

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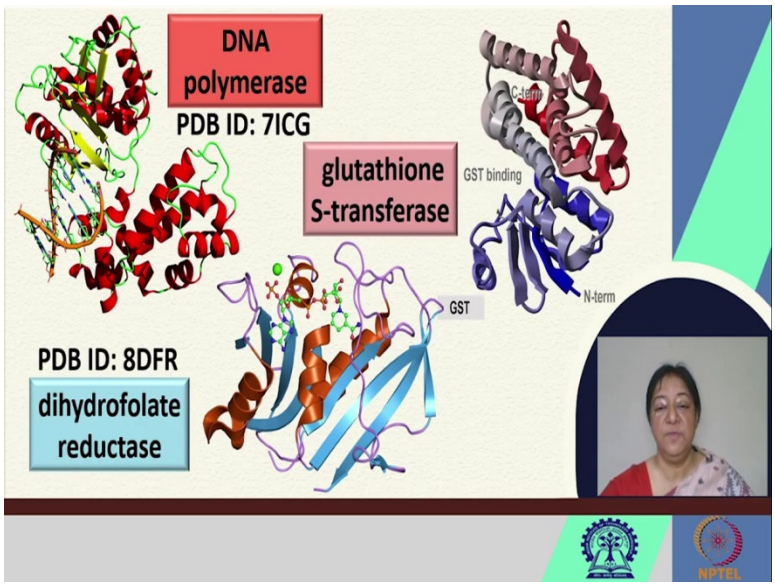

These [refer to slide] are examples of such proteins that have the binding in terms of two substrates involved.

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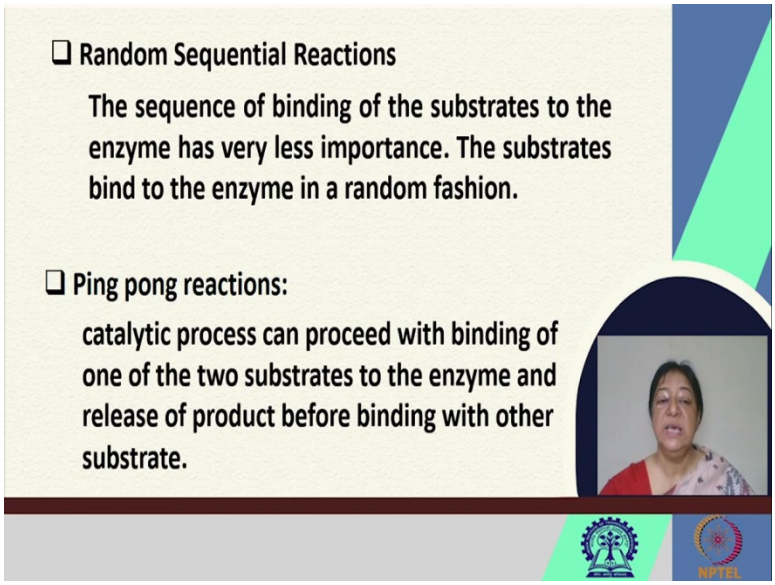

When we look at random sequential reactions, the sequence of the binding of the substrates to the enzyme is not of importance and both the substrates can bind in a random fashion. In ping pong reactions, the catalytic process as we mentioned, can proceed with binding of one of the substrates, then a release of product before the binding of the other substrate.

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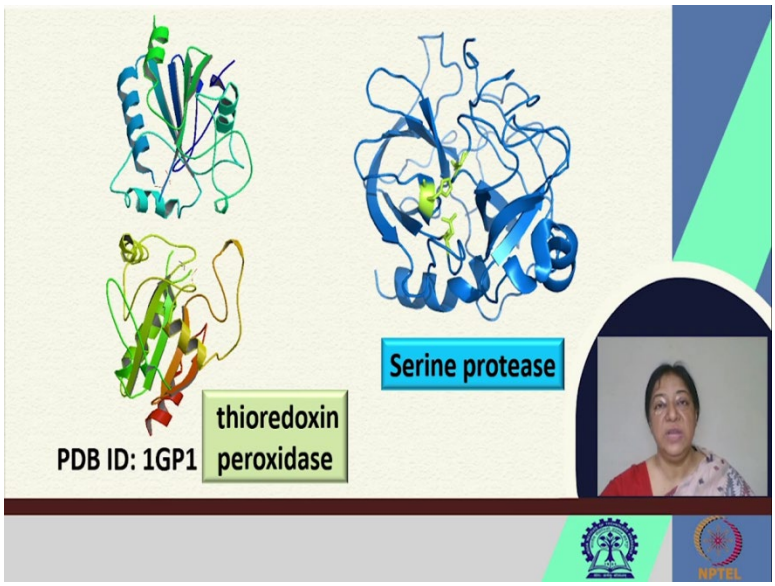

We will look at the specific schemes of binding.

These [refer to slide] are examples that would be involved in the ping pong types of reaction, some of which we have already covered in the previous module.

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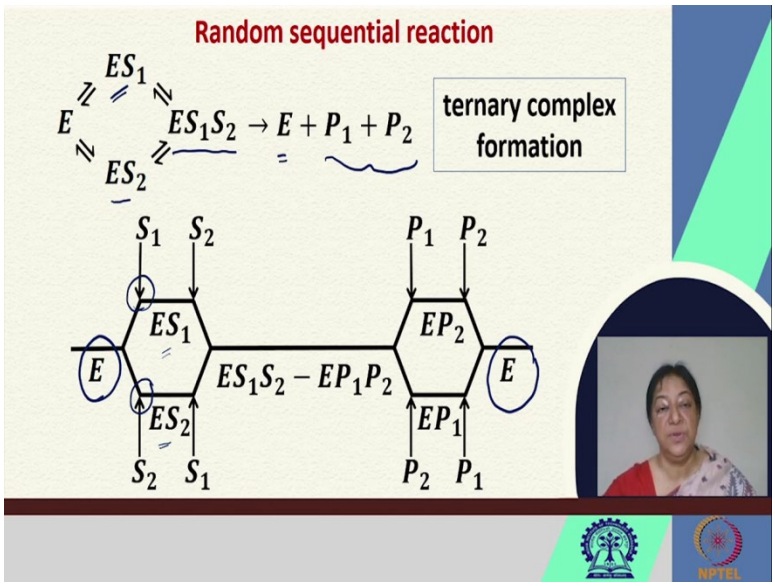

In a random sequential reaction we have our enzyme, we have the possibility of two substrates binding to the enzyme to form our enzyme substrate complex. We can have ES_1 and ES_2, these are the two substrates that have been bound to our enzyme.

We now form the ternary complex which is ES_1S_2 because we have three components present in our complex and this can result in a P_1P_2 or it may happen if we have a reaction that forms just one product. So if it is a ligase, then we would have S_1 and S_2 combine to form a single product. This indicates that the sequence of binding is not important. If we have ES_1, we can have ES_2S_2 bind to form ES_1S_2 or if ES_2 is formed first, we can have S_1 bind to form the ternary complex.

So, what happens in this case is, we can have S_1 formed first where we have S_1 arriving or recognized first, we can have S_2 where the complex formation will be ES_1 in one case and ES_2 in the other case. This would then form our ES_1ES_2, convert it to our EP_1P_2, which could then result in the release of the products that could be also one product as mentioned or we could have the release of P_1P_2 or P_2 or P_1 and there is no definite order in which the substrate binds and there is no definite order in which the product is released, but the important part is we get our enzyme back as we wanted it to be ready to bind the other substrate molecules for a further reaction.

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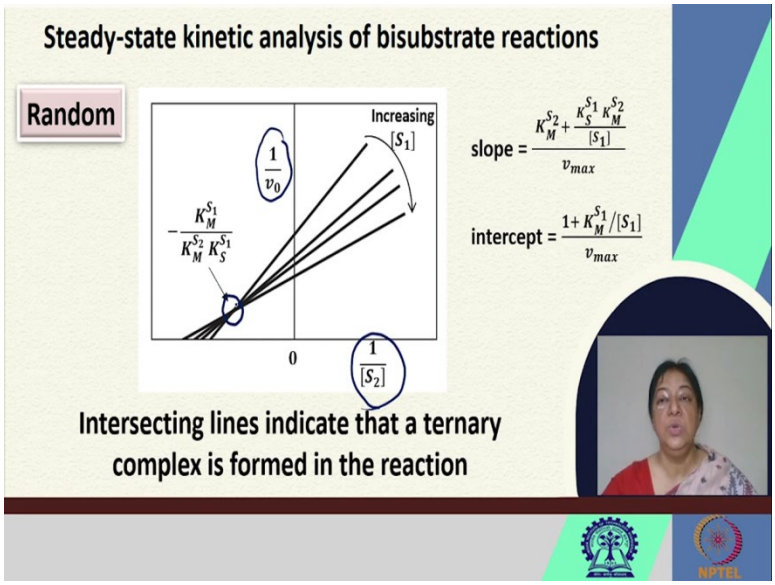

If we look [refer to slide] at the steady state kinetic analysis of the bisubstrate reactions in terms of what we had learnt in the previous classes, we have a random process if we plot $1/v_0$ versus $1/S_2$ in our double reciprocal Lineweaver-Burk plot and we have increasing concentrations of S_1 in this direction. This is a model that we get for a bisubstrate reaction and we can find out a combination of the values of the K_M for the substrate 1 and the substrate 2. We can get a slope and we can get the v_{max} of the reaction from the intercept and the slope.

These intersecting lines here indicate the formation of a ternary complex. It is much more complicated than the case of a single substrate bound that we had discussed in the previous lecture, where we looked at Michaelis-Menten kinetics corresponding to single substrate reactions.

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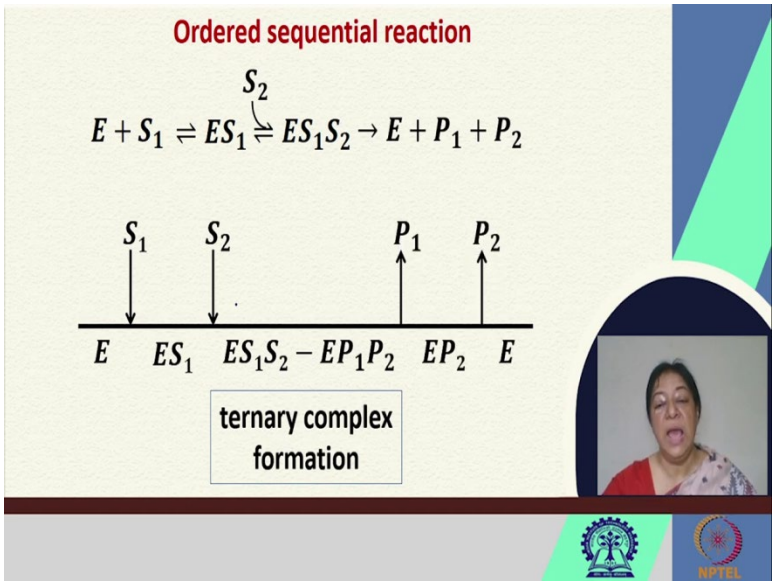

If we now look [refer to slide] at ordered sequential reactions, we have E plus S_1 form our ES_1 complex. After ES_1 is bound, it is possible for S_2 to bind in the formation of the ternary complex that will then subsequently lead to our product formation.

We have our enzyme, we have our substrate come to form our enzyme substrate 1, our ES_1 complex. This then allows the formation or the entry of S_2 as the second substrate, giving us the ES_1S_2. The specific order is important unlike our random sequential set. So again, we have the product formation and we get back our enzyme.

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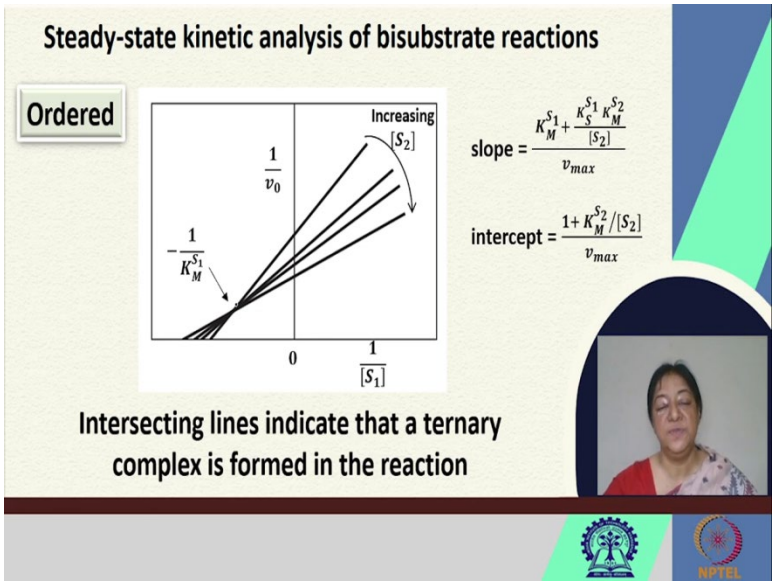

If we look [refer to slide] at the specific case of an ordered bisubstrate reaction, we have our $1/v_0$ versus $1/S_1$ in a double reciprocal plot, where we look at the increasing concentration of S_2 because only after S_1 binds, is it possible to bind S_2. And we again see the ternary complex

formation; a K_M, a slope and an intercept with expressions for K_M and v_{max} associated with them. And the intersecting lines again indicate the formation of a ternary complex.

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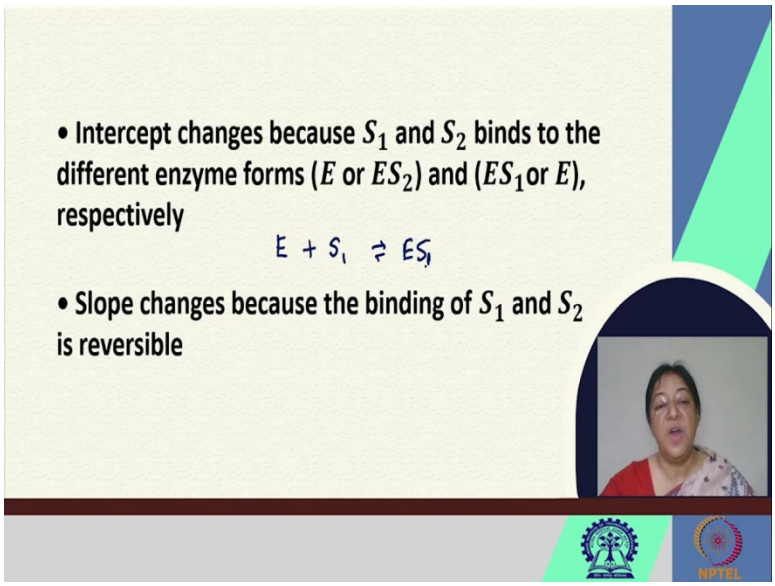

The intercept here changes because S_1 and S_2 binds to different enzyme forms. For example, when we are looking at E plus S_1, we have our ES_1 formation. The change in the intercept is because we have the different types of substrates bound. So in case of a random set, the slope will change because the binding of S_1 and S_2 is reversible.

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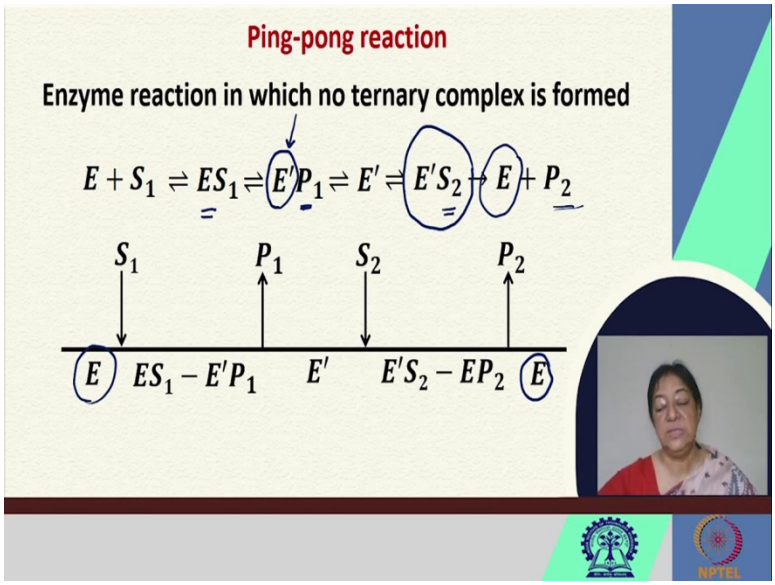

In a ping pong reaction, the case is somewhat different for a bisubstrate reaction. What happens is we have an E plus S_1 form, in the formation of the enzyme substrate complex. There is then a

conformational change in the enzyme itself where we have an E' of the enzyme and the product formation in the catalytic reaction, a transformed enzyme.

This transformed enzyme will then be able to bind to the next substrate in this bisubstrate reaction, giving us an $E'S_2$ complex; unlike the ES_1 complex which cannot bind S_2. So, S_1 binds to E in the formation of ES_1.

During the catalytic reaction, the enzyme has changed conditions related to its active site or conformational changes in its formation of the E' product complex and we have a transformed enzyme that then binds the second substrate. This then releases the other product and we get back our enzyme to where we started from.

So in this case, we do not have a ternary complex formation because at each point in time, there is one substrate bound to the enzyme. So, we have the S_1 bound to the enzyme. In the ES_1 formation, a transformation of the enzyme to E' with the first product bound to it, the release of the first product is going to have our transformed enzyme, the E' enzyme that then has to bind S_2 in the formation of another enzyme substrate complex with the second substrate that is then going to be releasing the product P_2, giving back our enzyme.

The differences in the random sequential reactions, in the ordered sequential reactions and in the ping pong reactions, are very distinct.

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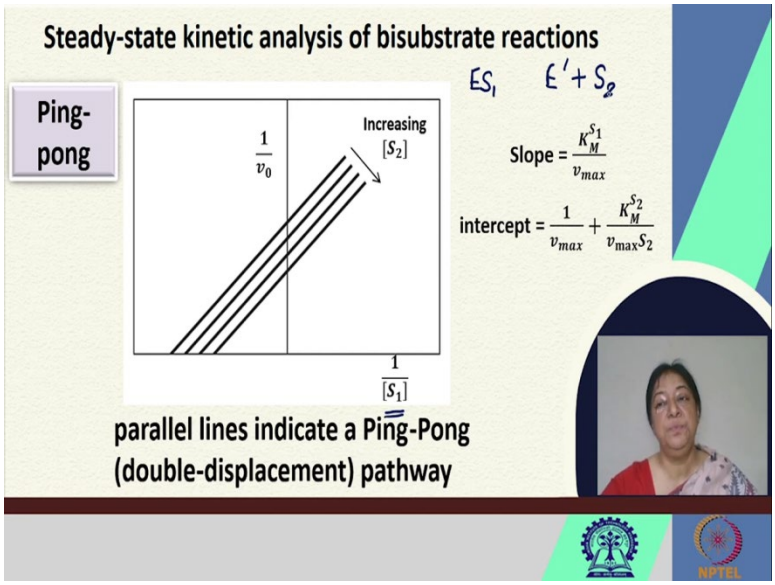

In the steady state kinetic analysis of the bisubstrate reaction in the ping pong case, what we see is when we plot the double reciprocal plot in terms of $1/v_0$ versus $1/S_1$, what we observe is increasing S_2 concentrations.

Again, in this case we have to realize that S_2 will not bind to the enzyme initially, so we have to plot $1/S_1$ because we have the formation of the ES_1 complex first, that then changes its

conformation to form our E'P_1, the release of the product that is then going to give our transformed enzyme that is only then going to be able to bind the S_2.

This [refer to slide] is the sequence of events that occurs in a ping pong bisubstrate reaction. We can get the specific slope associated with the K_M and the v_{max} values in a Michaelis-Menten type kinetics. And the parallel lines show that there is a ping pong indicating a double displacement pathway, with no formation of a ternary complex. The ternary complex as we saw in the two previous cases would result in an intersection of the lines, indicating that a ternary complex has formed.

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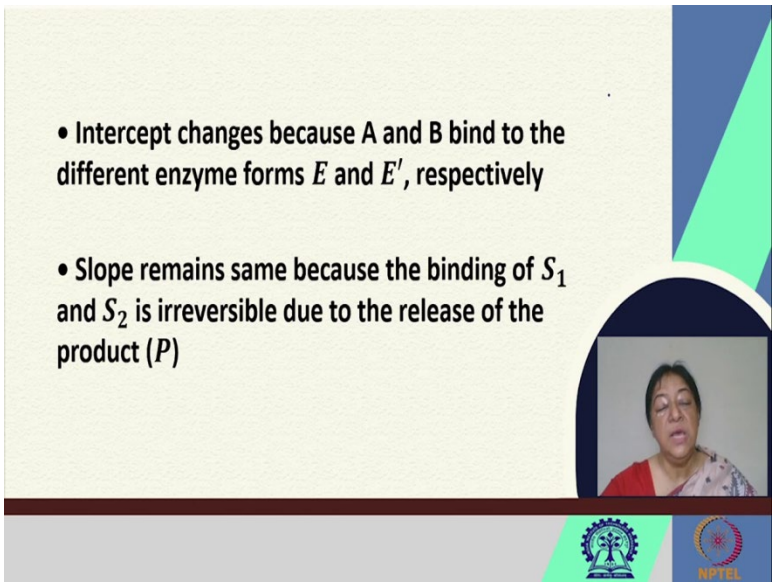

The intercept changes because the two substrates A and B bind to different enzyme forms. The different enzyme forms that we see are E and E' and the slope remains the same because the binding of S_1 and S_2 is irreversible due to the release of the product P.

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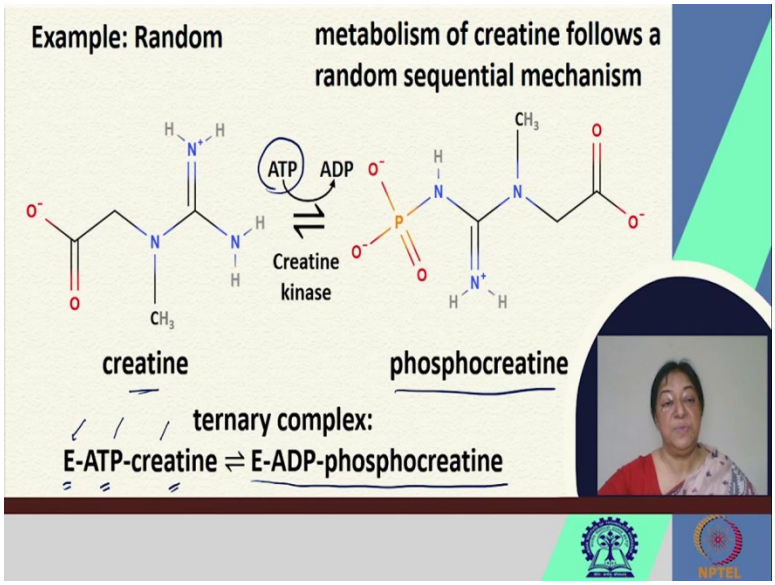

So if we look at an example now of a random case, we have the metabolism of creatine, that follows a random sequential mechanism. What do we mean by this? We mean that there are two substrates involved in the enzymatic catalytic process and the two substrates can bind in any fashion, we can have substrate 1 bind before substrate 2 and vice versa.

This [refer to slide] is our creatine, that is our starting first substrate, this is creatine kinase that is going to now transfer a phosphate group. The transfer of the phosphate group indicates that ATP also has to bind for the reaction to proceed and we would have phosphocreatine in the transfer of the phosphate group from ATP to creatine forming phosphocreatine. So in this specific reaction, we have the ternary complex that is our enzyme, our ATP and our substrate.

Now, in a random form, it would mean that the creatine could bind first to creatine kinase followed by ATP or ATP could bind first followed by creatine, but we would have the formation of the ternary complex where we would have the enzyme, our S_1 or S_2; depending upon which bound first and we would have the enzyme ADP and phosphocreatine in its specific reaction.

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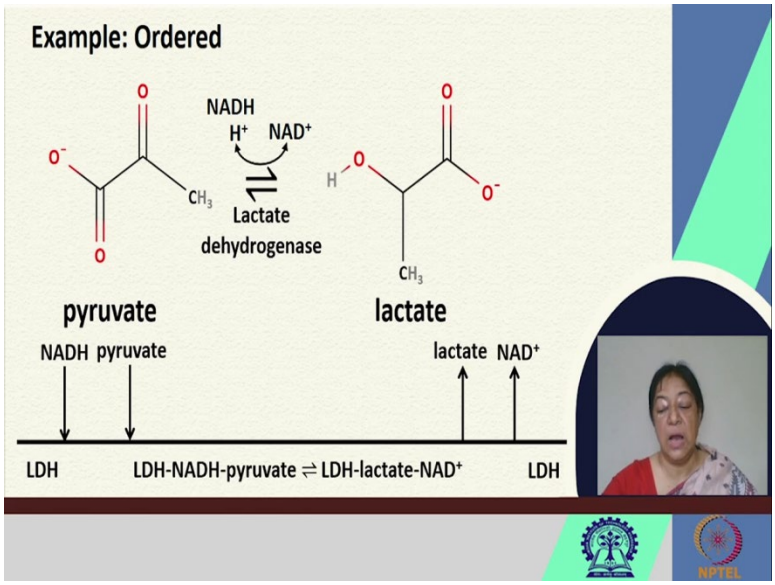

In the ordered case, there would be a specific order in which the enzymes or the enzyme would bind the substrates. In this case, we are looking at the example of pyruvate where we have the process or the enzyme lactate dehydrogenase. In this case, we have NADH going to NAD^+. What happens is we have pyruvate to lactate formation, our enzyme here is lactate dehydrogenase.

We have LDH, then we have NADH that is bound. So only after NADH binds to LDH can pyruvate bind. This signifies the ordered bisubstrate reaction, in the previous case we looked at a random set where it did not matter whether an ATP bound first or creatine bound first, but in this case for pyruvate to bind, NADH has to bind first.

This will also be followed by the ternary complex and then we will have the release of the product lactate and our NAD^+. So, there is a distinct order in which this occurs unlike our random set.

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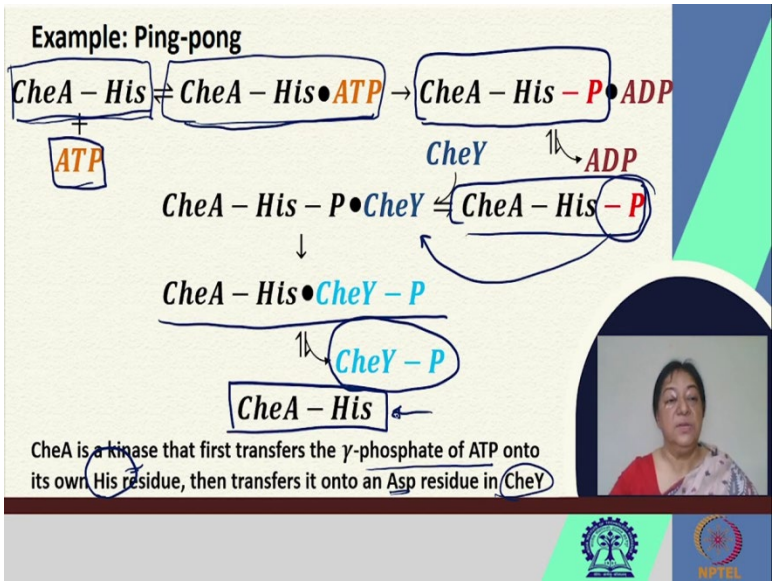

For a ping pong reaction it was a set where we had the formation of the enzyme substrate complex, the release of a product, a transformation of the enzyme that would then bind the second substrate, release the product and get back to its original form. This is one example of CheA, a protein, an enzyme that has an interesting feature in that, it is a kinase that first transfers the phosphate group of ATP onto its own histidine residue.

So, what happens is this [refer to slide] histidine residue takes up the ATP first. It then transfers it over to an aspartic acid residue of another CheY. So, this is where we have a transformation in terms of a CheA - His. What is happening is this is our enzyme, this is our first substrate, this is our ES_1, after the ES_1, what happens is we have a transformed enzyme. This transformed enzyme now has to get back to the original form.

So, now our transformed enzyme has a variation where it is going to have this phosphate transferred over to the modified enzyme and we have now the modified enzyme, the original one where we have the transfer being possible and a release of our product and the enzyme back to where it was in a ping-pong reaction.

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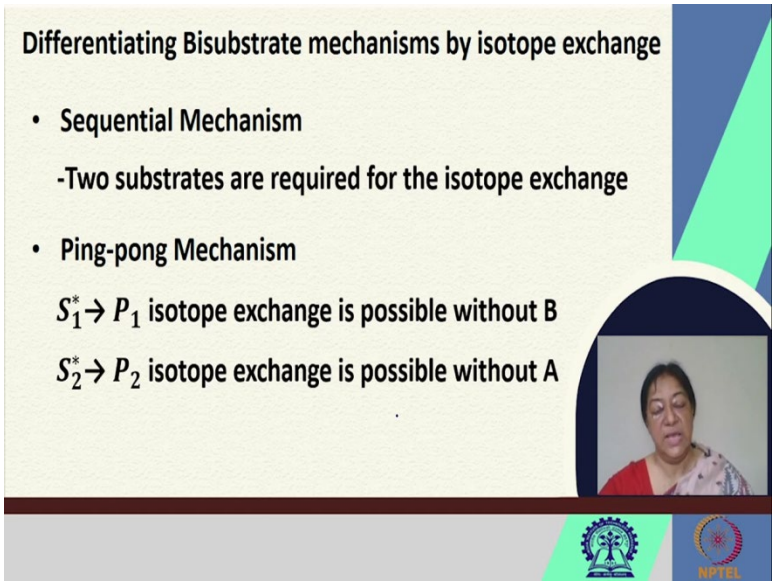

We can differentiate these bisubstrate mechanisms by isotope exchange. What do we mean by that? In a sequential mechanism, we can look at two substrates that are required for the isotope exchange. In a ping-pong mechanism, we can have the isotope exchange without S_2 and here it is possible without S_1 or without A and B being the two different substrates.

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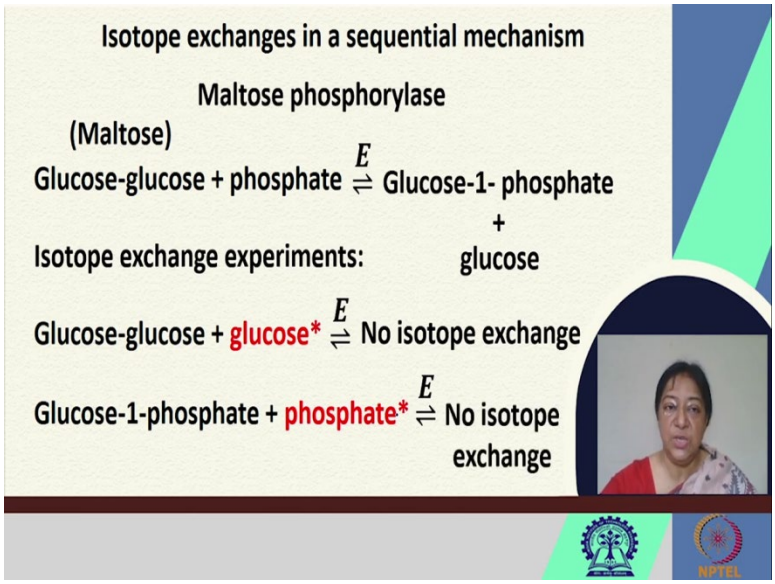

In a sequential mechanism, for example in a protein like maltose phosphorylase, we have glucose-glucose that is maltose, plus phosphate. In this case our enzyme is going to form glucose-1-phosphate plus glucose. Isotope exchange experiments indicate that we have glucose-glucose, if we add glucose* that is with the isotope to this, there is no isotope exchange; so maltose remains. If we have glucose 1 phosphate and we add a phosphate with an isotope, there is also no isotropic exchange in a sequential mechanism.

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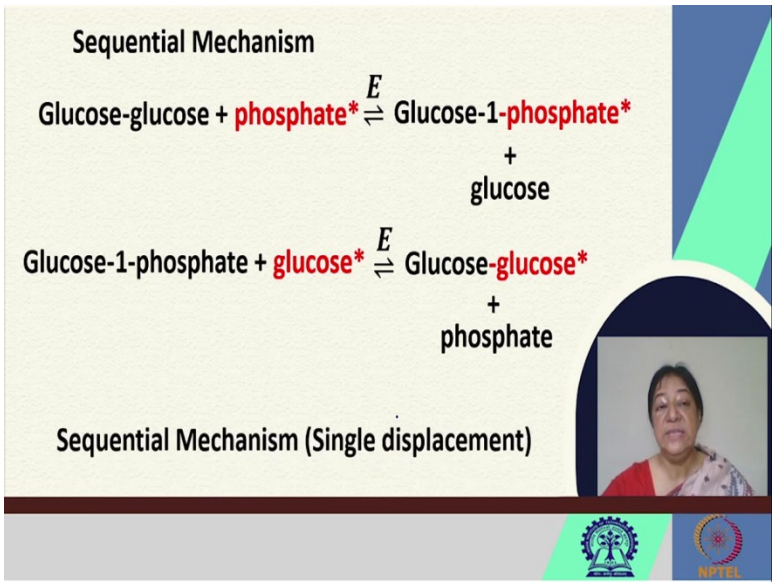

If we now look at a specific transfer where we have an isotope exchange possible, where we are looking at a glucose-glucose with the phosphate forming a glucose-1-phosphate with isotope exchange; that is also possible in this type of reaction where we have a sequential reaction indicating a single displacement.

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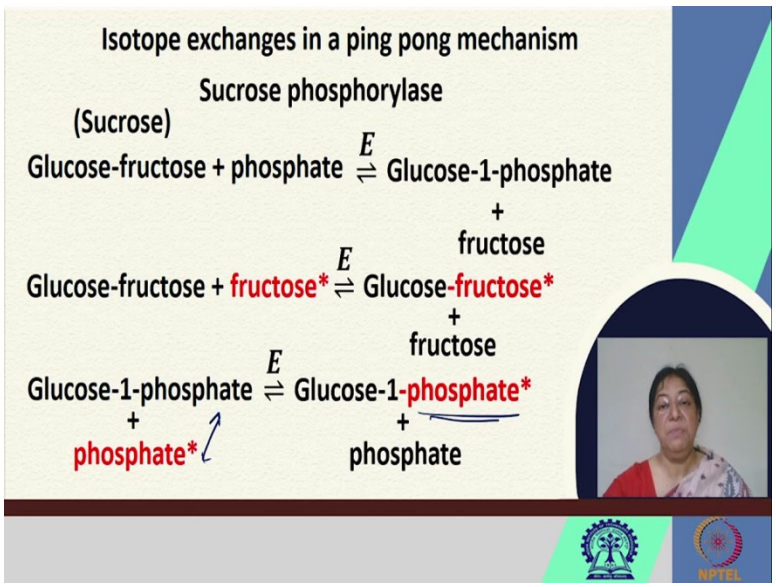

However, when we look at isotopic exchanges in a ping-pong mechanism, for example in sucrose phosphorylase where we will have glucose fructose plus the phosphate and our enzyme forming our glucose-1-phosphate plus the fructose, we can now have a glucose fructose with the fructose phosphate have the transfer of the isotope in this particular reaction, where there will be isotope exchange.

Similarly, if we look at glucose-1-phosphate plus the phosphate, here [refer to slide] an isotope exchange is possible. So in the ping pong mechanisms, we see that there is an isotope exchange possible because we have the binding in different manners in the ping pong mechanism that we look at.

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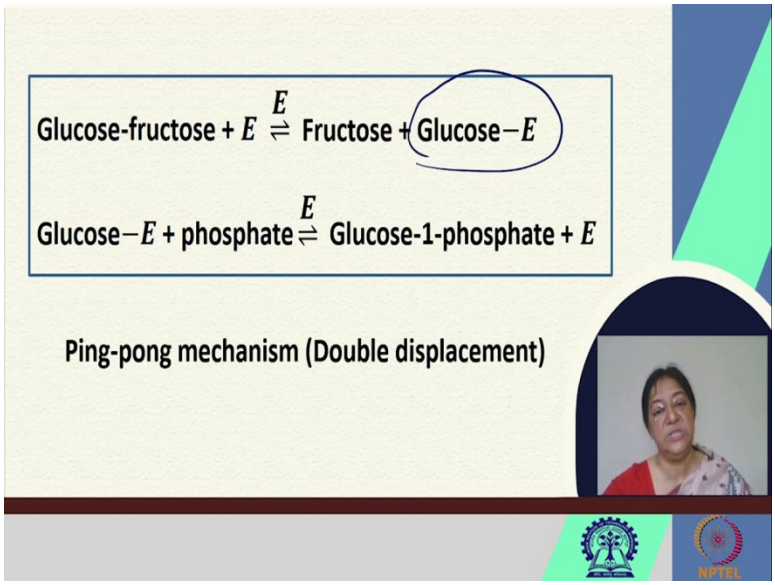

When we look at the glucose fructose plus the enzyme, we have fructose and the glucose bound to the enzyme. Then when we have the glucose enzyme complex that is now binding the phosphate, it will form glucose-1-phosphate plus the enzyme. So in this case, we see a double displacement as opposed to the single displacement that we saw in the sequential mechanism.

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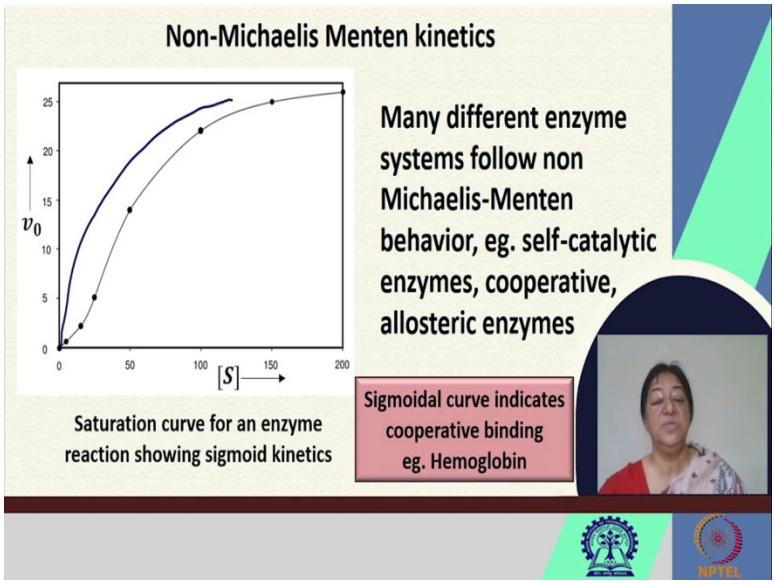

This follows non-Michaelis Menten kinetics. What we mean by that is, these different enzyme systems we realize can have their substrate bound in a manner that could change the conformation of the enzyme, change its affinity for the different substrates and so on and so forth. They could be self-catalytic enzymes, specific cooperative types, allosteric enzymes which we looked at in different mechanisms in our previous module and we will be looking at cooperativity, specifically in a protein-protein interaction or a special lecture where we look at myoglobin and hemoglobin binding.

So we have our v_0, that is the initial velocity in a typical Michaelis-Menten kinetics curve and what we observe is, we have a saturation curve for an enzyme that shows sigmoid kinetics where unlike a curve that would bind in a manner like this [refer to slide], we have a different type of binding which we observe in the case of hemoglobin.

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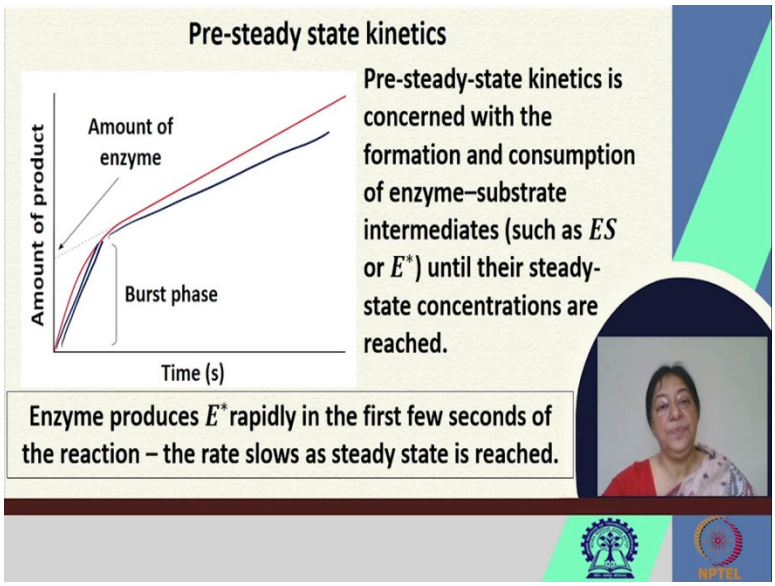

We can have pre-steady state kinetics. This is concerned with the formation and the consumption of enzyme substrate intermediates, such as the ES or the E* that is the changed enzyme, until the steady state concentrations are reached.

In this case, we observe that the amount of product is suddenly formed in a very drastic manner initially. Which means that there is a burst phase initially, in the kinetics where there is a sudden formation of the product, indicating that there is a distinct biphasic curve to our kinetics associated with this.

What we have here is the enzyme produces the transformed enzyme or the product very rapidly, in the first few seconds and then the rate slows as the steady state is reached. So we see a lot of bisubstrate kinetics, we understand how the products can form from an ordered sequential case; where we can have a random sequential case, we can have an ordered sequential case and we can have a ping pong reaction in the different types of enzyme kinetics involved.

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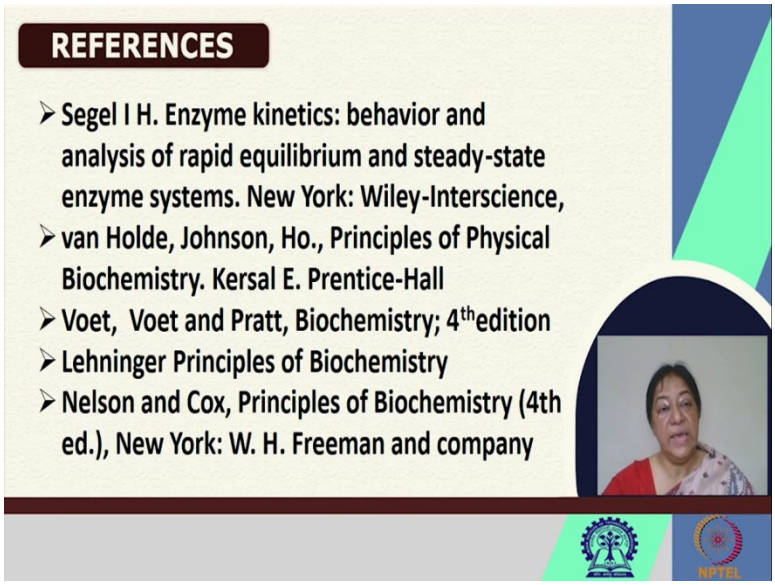

These [refer to slide] are the references.

Thank you.