Fundamentals of Protein Chemistry
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Module - 07
Enzyme Kinetics and Inhibition
Lecture - 33
Enzyme Inhibition - I

We continue our discussion with enzyme kinetics. In the subsequent two lectures, we will be looking at enzyme inhibition. In the previous lectures we looked at how substrates bind to enzymes, in what fashion they bind the formation of an enzyme substrate complex that then subsequently led to the product.

We also looked at the possibility of 2 substrates binding to the enzyme, where we could have a sequential manner or a random manner binding and also at a ping pong type of reaction. The specific methodologies where substrates could bind to enzymes, involved in most cases non covalent interactions.

But we also saw examples of covalent interactions between the enzyme and the substrate, which subsequently led to hydrolysis and other types of rearrangements that would lead us back to our original enzyme for it being ready to bind to another substrate.

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In this lecture we will be looking at the specifics of inhibition and what we mean by the different types of inhibition that enzyme inhibitors could be involved in.

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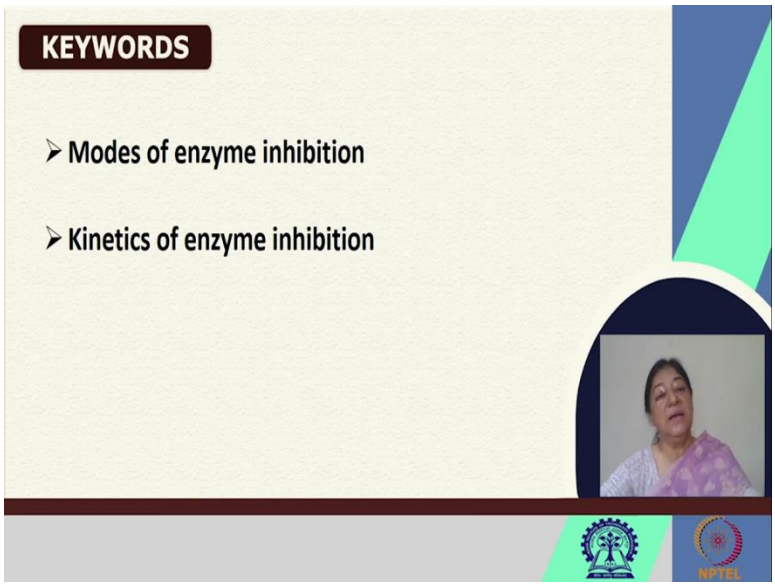

We will be looking at the different modes of inhibition and the kinetics associated with the enzyme inhibition. And in the subsequent class we will be looking at specific examples of these types of inhibition.

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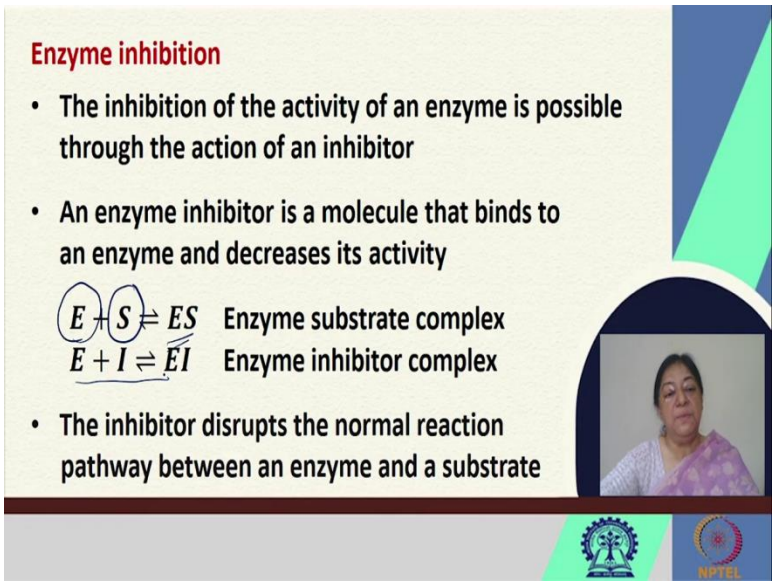

When we look at enzyme inhibition, what does this term mean? The term means that the inhibition of the activity of an enzyme, is possible through the action of an inhibitor and this process is enzyme inhibition. And we realize that the enzyme inhibitor is a molecule, that would bind to the enzyme and decrease its activity. We learnt of the enzyme substrate complex formation, when we have a single substrate bound to the enzyme in a pre equilibrium step, forming the enzyme substrate complex.

Similarly if we look at an enzyme inhibitor complex formation, this would mean that the inhibitor would disrupt the normal reaction pathway between an enzyme and a substrate because we realize that part of the enzyme has gone into binding the inhibitor. So we do not have enough of the enzyme to bind to the substrate to go through it's catalytic reaction.

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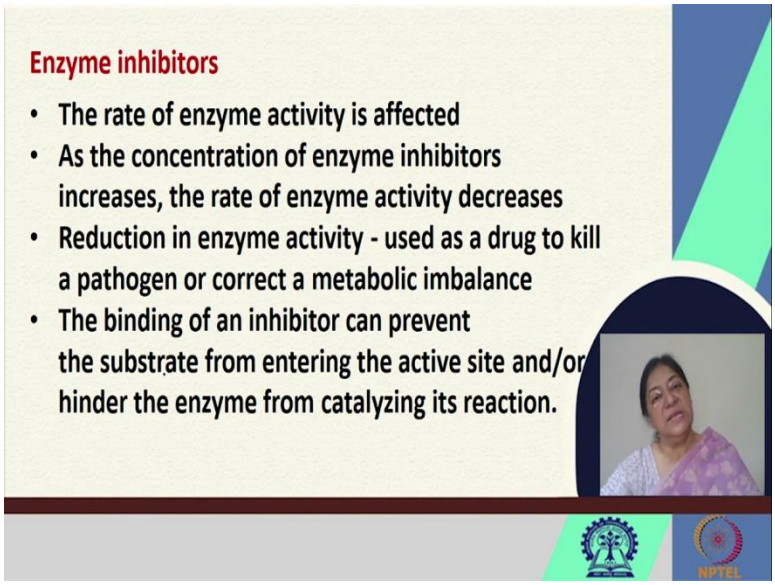

The enzyme inhibitors inhibit the rate of the activity and as the concentration of the inhibitors increases, the rate of enzyme activity would decrease because the enzyme would be inactivated by the inhibitor. This reduction in enzyme activity has beneficial properties. They may be used to kill specific or stop the activity of a specific enzyme to correct metabolic imbalance or they may be drugs that would bind to the specific enzyme molecules.

So what happens in this case, is that the binding of an inhibitor will prevent the binding from either entering this active site or will hinder the enzyme from catalyzing its specific reaction.

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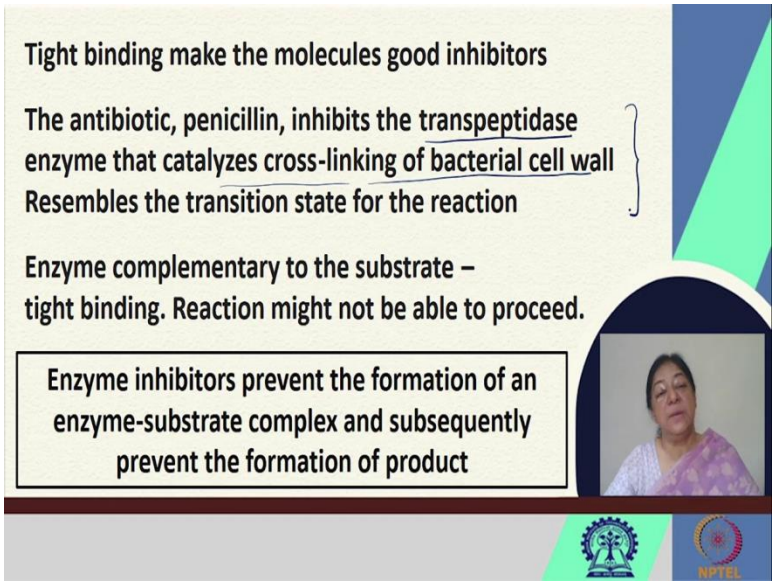

So, we are now looking for something that is going to bind tightly to the enzyme, unlike a substrate bound to the enzyme because we would like the enzyme substrate complex to be in a manner that would allow the formation of the product. But in this case where we are looking at the specific inhibition of the enzyme activity, we would be looking for tight binding.

For example, the antibiotic penicillin this inhibits the transpeptidase enzyme. As we can see from the name, the transpeptidase enzyme catalyzes the cross linking of the bacterial cell wall. In this case, as we saw in the enzyme kinetics classes that we went through, this resembles the transition state for the reaction.

We can have the enzyme complementary to the substrate in this case, that would result in tight binding, which would not allow the reaction to proceed. What happens is the enzyme substrate complex will be prevented from being formed and subsequently the product formation will be affected.

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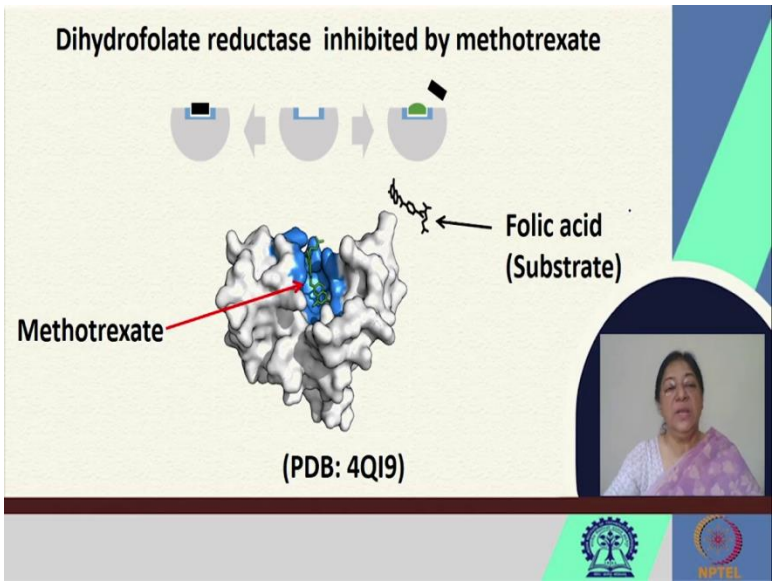

We can look [refer to slide] at this example, where we have dihydrofolate reductase, that is inhibited by methotrexate. Here Folic acid is the substrate, this marked in blue on the surface of the protein is the active site. Now methotrexate has bound to the active site, as a result of which the substrate cannot bind. So, we would need to know what kind of inhibition this is.

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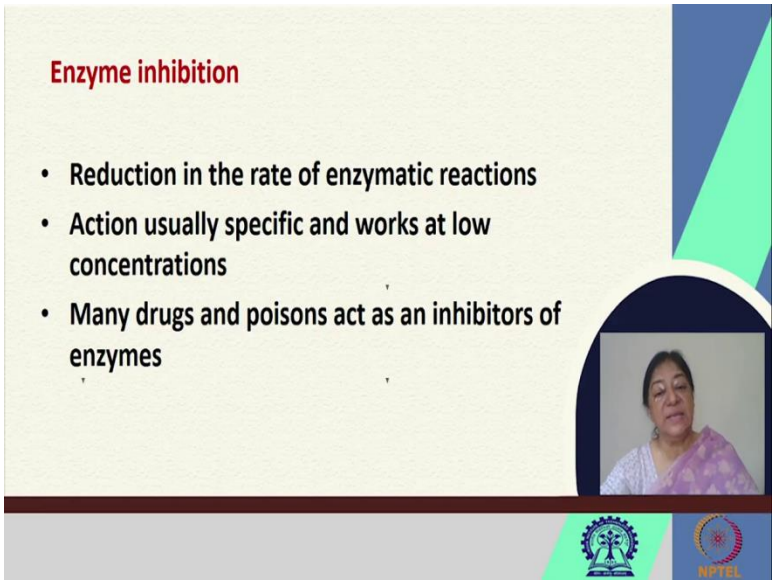

The enzyme inhibition that we considered now, indicates that there is a reduction in the rate of the enzymatic reactions. The action is specific and it can work at low concentrations, which is what we would ideally look for in a design of an enzyme. And many drugs and poisons actually act as inhibitors of enzymes.

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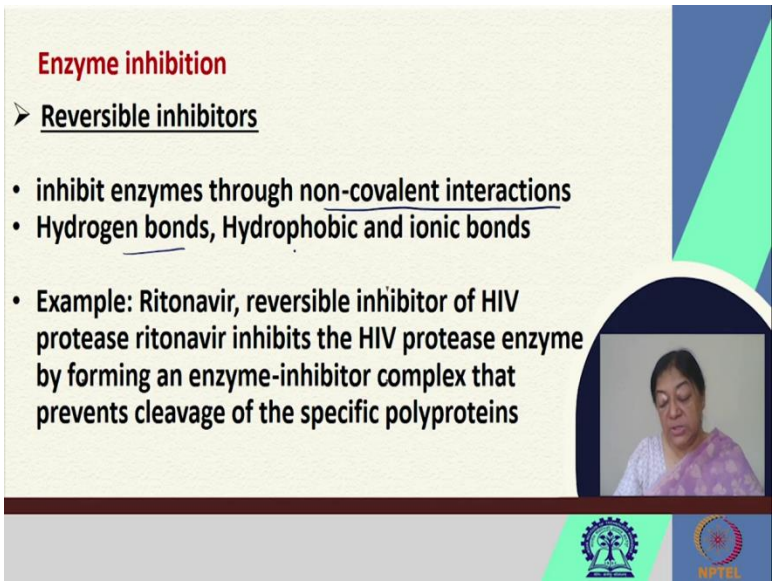

So let us look at enzyme inhibition. We have different types of enzyme inhibitors, in terms of reversible inhibitors. As the name implies, they interact through non-covalent interactions mainly hydrogen bonds, hydrophobic and ionic bonds. An example is Ritonavir, that is a reversible inhibitor of the HIV protease; the protease enzyme means that it is involved in the cleavage of specific polypeptide chains.

It creates an enzyme inhibitor complex that prevents the formation or prevents the cleavage of the specific polyproteins in HIV protease.

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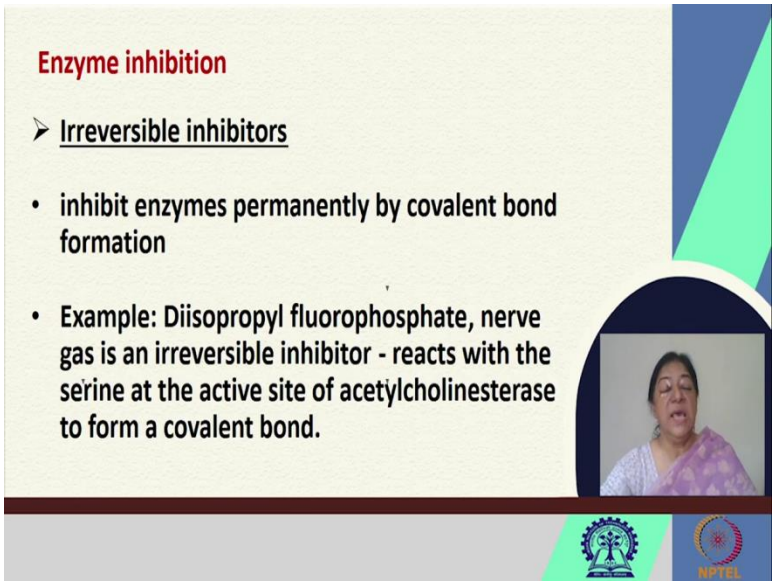

We can also have irreversible inhibitors and in irreversible inhibitors, the enzymes are permanently damaged by covalent bond formation. An example would be diisopropyl fluorophosphate, which is a nerve gas that is an irreversible inhibitor; that interacts in a covalent

fashion with the serine at the active site of acetylcholinesterase, to form a covalent bond rendering the enzyme inactive.

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We have in the case of reversible inhibitors, we have competitive inhibition, non competitive inhibition and competitive inhibition. We will see what these three types of inhibition mean in terms of the kinetics, in terms of the specific Michaelis Menten values, that we will try and observe.

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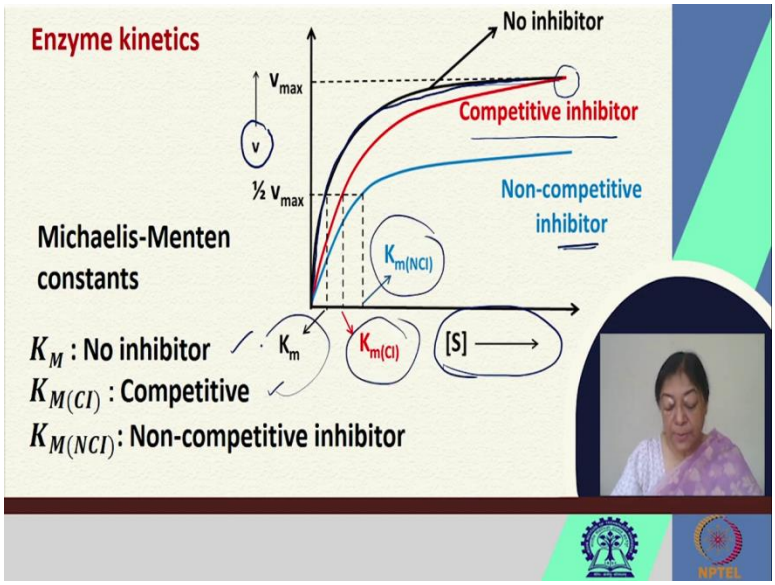

First let us look at the enzyme kinetics Michaelis-Menten, the typical velocity curves that are obtained in enzyme kinetics, where we plot the velocity of the reaction that we monitor in a

specific way versus the substrate concentration. When there is no inhibitor, we know that we have a curve where we have first order type followed by 0 order, indicating that an increase in the concentration of the substrate is not going to affect the rate of the reaction because the enzyme active sites have been saturated with the substrate. So we reach a saturation.

For a competitive inhibitor, the name implies that it competes with the substrate to bind to the enzyme. We see that it reaches the same V_{max} simply because the enzyme inhibitor complex in this case, is a reversible complex formation that, with a sufficient concentration of substrate may reach the same V_{max}.

With a non competitive inhibitor which we will also see [refer to slide], this indicates that we have an inhibition but the enzyme has been rendered ineffective, so that even with increase in substrate concentration, we do not affect or we do not reach the same V_{max} possible. We have the 3 K_m values associated with no inhibitor, competitive inhibitor and non competitive inhibitor. We know we are looking at half of V_{max} in each case, to determine the K_m values that are shown here.

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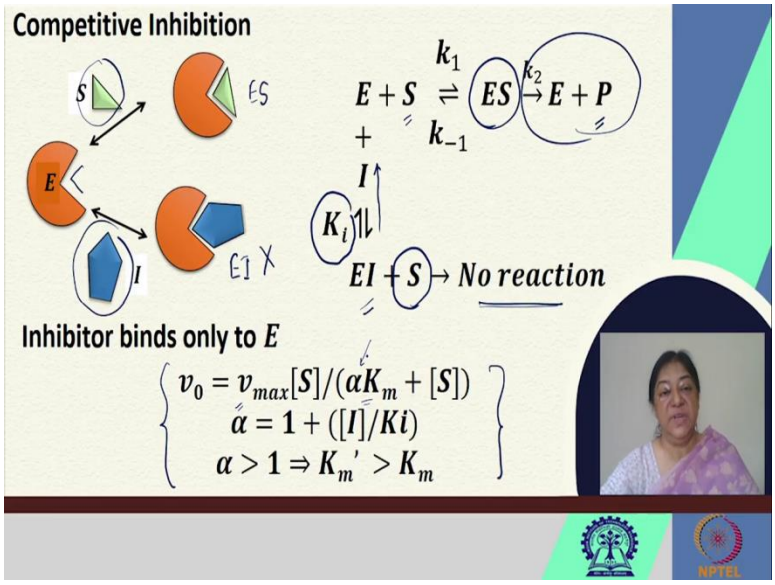

Now when we look at competitive inhibition, as the name implies our inhibitor looks [refer to slide] like the substrate as it binds to the active site. We have here an enzyme substrate complex, here we have an enzyme inhibitor complex. We have to subsequently look at the kinetics involved and we know that we would have an enzyme substrate complex to form the product.

However, in the presence of the inhibitor we are going to have an additional complex formation, where we would have an EI formed. Now, this means once the enzyme inhibitor complex is formed in a competitive inhibition, the substrate is unable to bind to the enzyme inhibitor complex, indicating that there will be no further reaction to form the product.

What has been marked here [refer to slide] is a value K_i, that is a measure of the dissociation constant that tells us how tight the inhibitor has bound to our enzyme.

The inhibitor in this case binds only to the enzyme and we have expressions related to our Michaelis-Menten kinetics, where we will see the same V_{max} attained because we realize that if we add sufficient substrate, we may have the product formation. But our K_M value is affected, so we have an α value attached to the K_m, indicating that there is a variation from the original K_m value due to the competitive inhibition.

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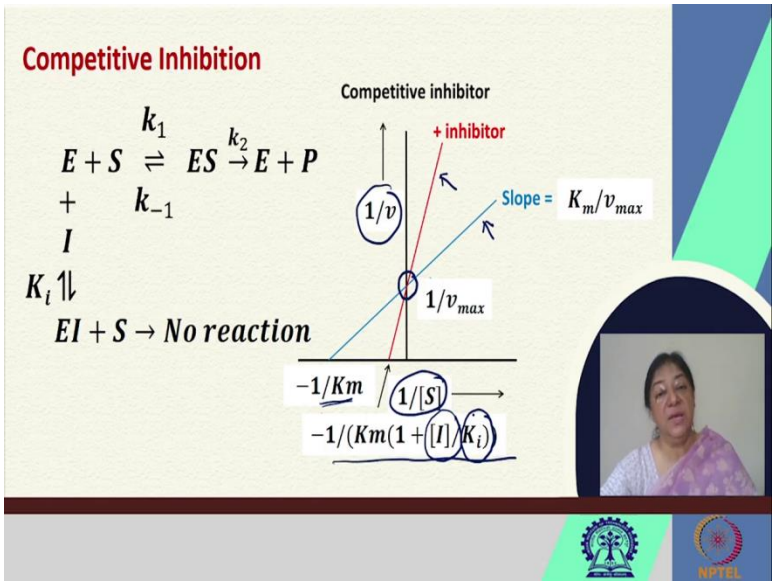

If we look [refer to slide] at the Lineweaver-Burk double reciprocal plot now, we will see that we have a specific indication where our y intercept we know is $1/V_{max}$. We see an intersection of the two lines, where the blue line indicates no inhibitor and the red line indicates the reaction or the plot with inhibitor. The 1/V versus 1/S, which is our Lineweaver-Burk plot.

In the original case we had $1/K_m$ value, now we have an inhibitor concentration and a dissociation constant the K_i value or the inhibition constant associated with the inhibitor, that is going to give us a modified Michaelis constant.

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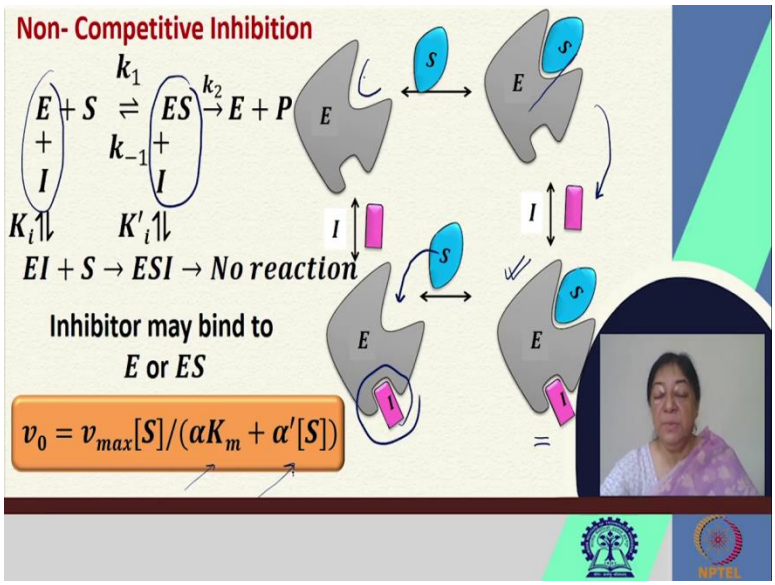

In non-competitive inhibition, what happens is the enzyme substrate complex is formed and the enzyme inhibitor complex may also be formed. Interestingly in this case, we have the formation of a ternary complex, indicating that if the enzyme substrate is bound, then if we bind the inhibitor, we will have a complex that has the substrate and the inhibitor bound.

We realize that the inhibitor does not bind to the active site of the enzyme, rather binds to it's own site that is a non-competitive inhibition. Once this ternary complex is formed, this can no longer form the product. So we have here an E + I and also an ES + I.

We will have no reaction in the formation of the ternary complex and the inhibitor in this [refer to slide] case, because it does not bind to the competitive or the active site. It may bind to the enzyme or the enzyme substrate complex because the point at which the inhibitor binds is free. So we again have a modified Michaelis Menten kinetics, where apart from the modified αK_m, we will also have an α'[S] that corresponds to the substrate concentration and the αK_m indicates the presence of the inhibitor.

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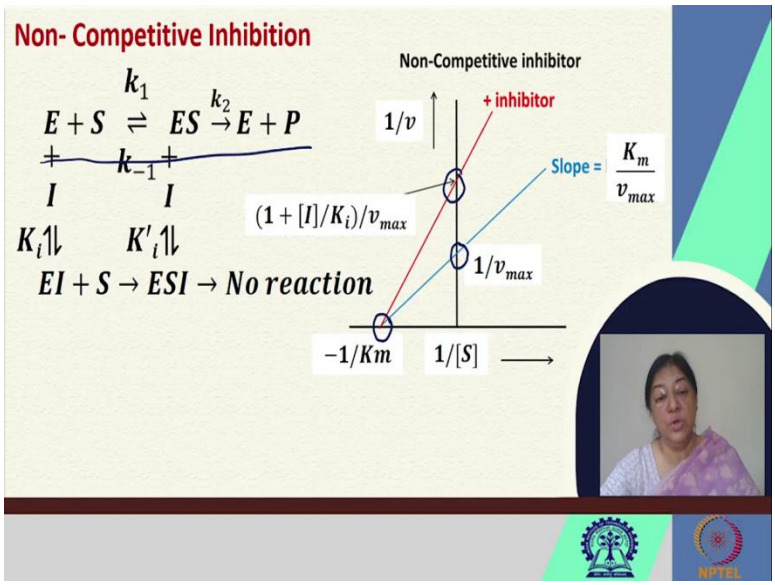

So when we look at our Lineweaver-Burk plot that gives us the specific conditions here, we will see that the V_{max} is different from the one that has no inhibitor, but the K_m value is indicating that any enzyme that is available for the reaction, would go forward to form the product.

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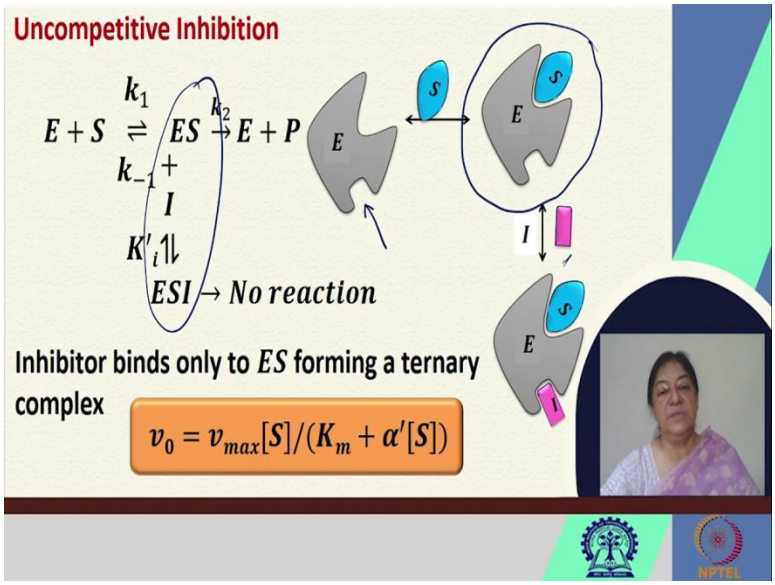

We also have uncompetitive inhibition. In uncompetitive inhibition what happens is we have the enzyme substrate complex formed and the inhibitor can only bind to the enzyme substrate complex and not the enzyme alone, but nevertheless it will inhibit the activity of the enzyme. So, we have a specific condition, where we have the ternary complex followed by the ESI formation.

But it does not bind to the enzyme alone. Only after the enzyme substrate is formed does the inhibitor bind to this to form the ternary complex. So the inhibitor binds to ES forming this ternary complex.

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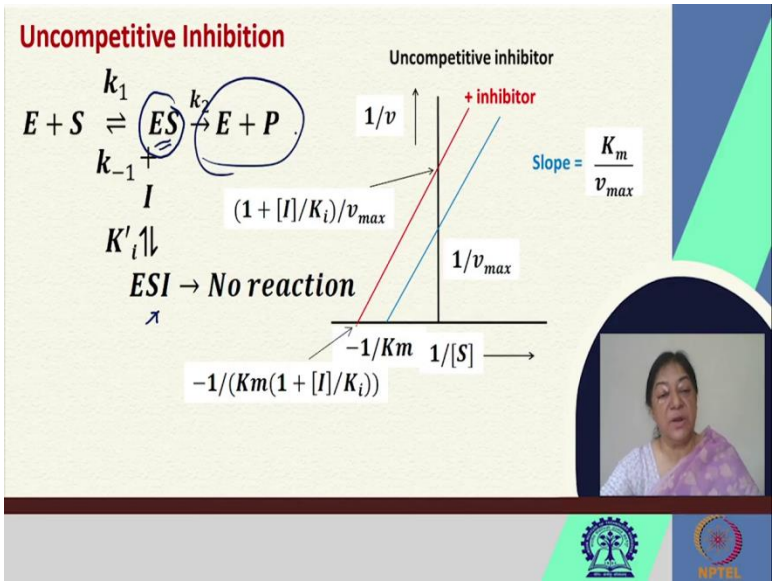

We have the modified equation for the Michaelis-Menten kinetics and when we look at our Lineweaver-Burk plot, we will see that we have parallel lines in the case of uncompetitive inhibitors because we have this binding to only the enzyme substrate complex. But we realize that if the enzyme substrate complex has an inhibitor bound to it, we cannot have our desired product formation.

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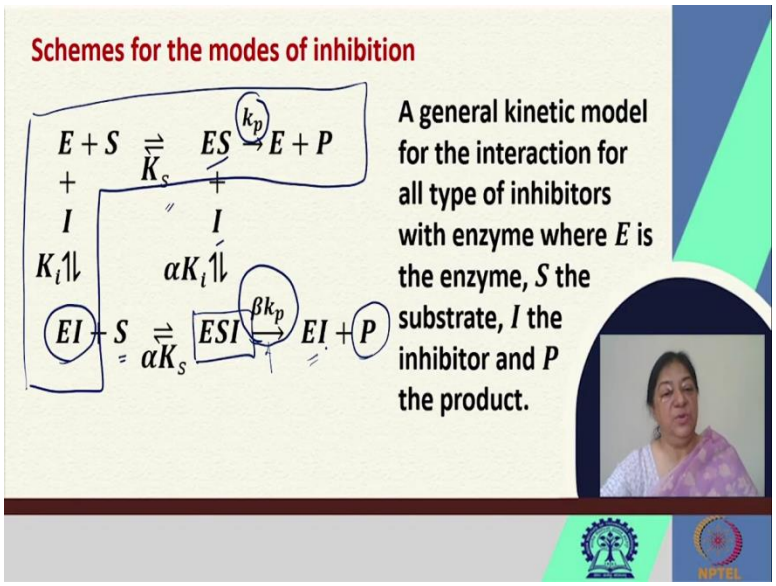

If we look at the schemes for the modes of inhibition in general and have a look at all the possibilities, we will see that we have an E + S going to an ES here [refer to slide] we have looked at the dissociation constant in the terms of K_s, going from ES back to the enzyme and substrate. And we have the small k_p indicates a specific rate constant, that is going to take the enzyme substrate complex from the product.

We now have the inhibitor addition. The inhibitor we saw in the different modes, can bind to the enzyme and can also bind to the enzyme substrate complex. So we will see a situation, where we can have an ESI. We can have an EI plus and S that would give us a ternary complex or we could have an ES plus and I, that would also give us a ternary complex. If we look at the whole scheme, it may so happen that no particular inhibition substrate analog that would look like a substrate, a competitive inhibitor, would follow say this [refer to slide] pattern only.

Then we could have a non competitive inhibitor that would follow a different scheme in the overall scheme of the kinetics that has been shown here, for a single substrate binding.

However, there may be cases where we could have a modification of this rate and still form a product with the enzyme bound to the inhibitor. There are cases that are possible. So, this is a general kinetic model for the interaction for all types of inhibitors where we have the enzyme, the substrate, the inhibitor and the product.

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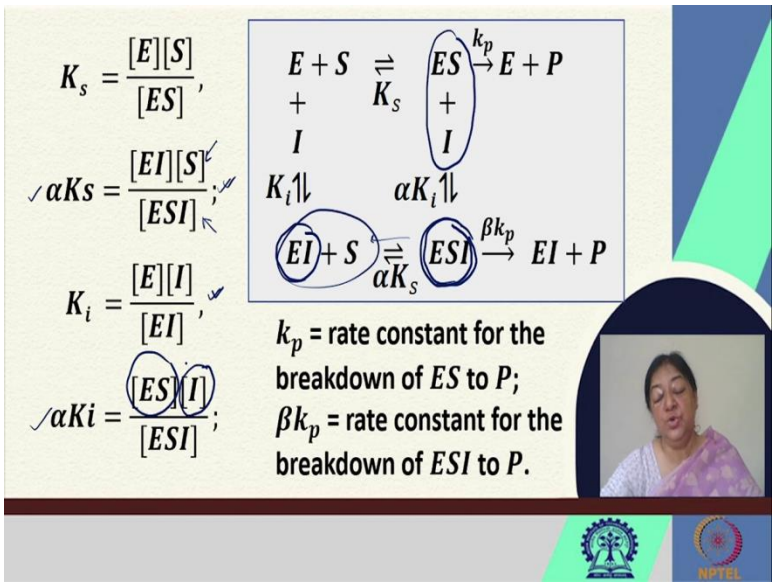

If we look at our general scheme of things like we showed, this [refer to slide] is our overall scheme. Now what is the information we can get from this scheme and how does it help us? First we can find out what the K_s value is, that is the equilibrium constant associated with the dissociation of our enzyme substrate complex. We have the αK_s value, where this is the dissociation of the enzyme substrate inhibitor complex.

We also have a K_i value that is associated with the dissociation of the enzyme inhibitor complex, which is by far the most sought after inhibition constant value that gives us an indication of how effective our inhibitor is.

We have the αK_i value, which is associated again with the enzyme substrate inhibitor ternary complex, it breaks up into ES + I. So in the initial case the αK_s, we are looking at the ternary complex breaking up into the enzyme inhibitor complex and the substrate. In the αK_i set we are looking at the enzyme substrate inhibitor ternary complex, breaking into the enzyme substrate complex, and the inhibitor.

If there is any methodology that can go to monitor the concentrations of the enzyme substrate complex or the inhibitor or the formation of the product, this will be used to determine the values of α and β in the reactions. So the k_p that we have is the rate constant for the breakdown of ES to E + P in the reaction and the βk_p is the rate constant for the breakdown of the ESI to P, that may be possible in several cases.

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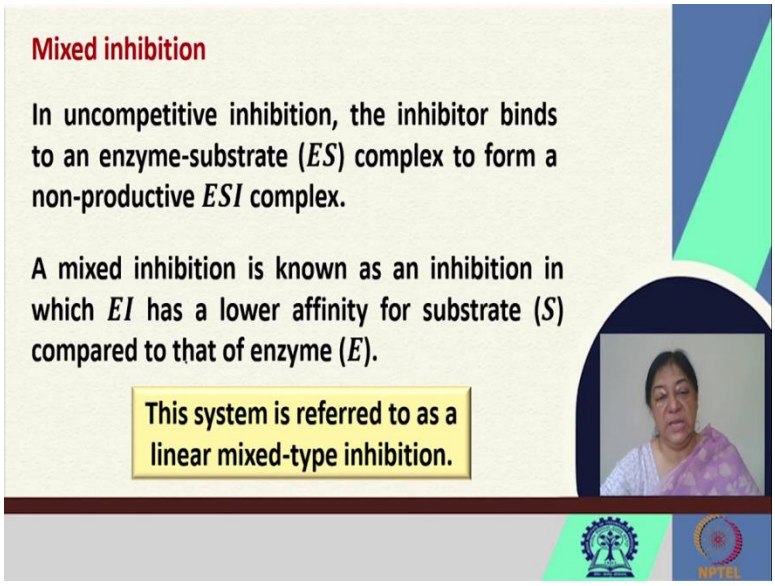

A case of mixed inhibition now indicates that sometimes in uncompetitive inhibition, where we saw that the inhibitor bound to the enzyme substrate complex, it forms a non-productive ESI complex. However, it may so happen in a mixed inhibition, where this is known as an inhibition in which the EI that is formed has a lower affinity for the substrate compared to the enzyme.

So we know that when we have the enzyme binding to the substrate, there has to be a strong affinity, a strong recognition for the substrate to bind to the enzyme. Once we have the inhibitor bound to the enzyme, then the enzyme inhibitor complex is formed. Whether this inhibitor binds to the active site of the protein in a competitive mode of inhibition or whether it is binds to a different site in a non competitive mode of inhibition. In the uncompetitive type we found that the enzyme substrate complex is formed and the inhibitor binds to this complex.

However, there may be cases for our non competitive type, where the enzyme inhibitor formed will now have less affinity for the substrate and will not be able to form the ternary complex to the extent desire. This is a linear type of mixed inhibition. We will see [refer to slide] what the graphs look like.

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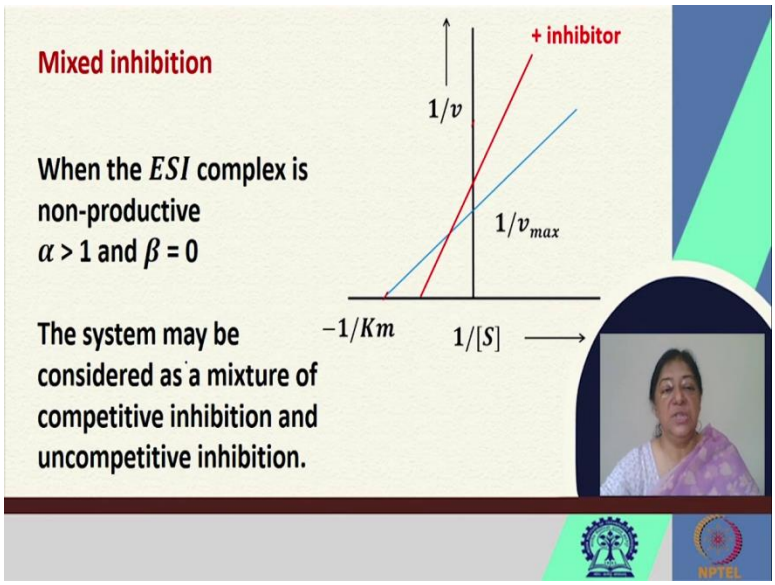

This is where we have the ESI complex that is non productive in nature and the values of the α and the β associated with the scheme of the inhibition that was described previously, gives us a set where the intersection is not on the y axis as was for competitive inhibitor, not on the x axis as was seen for a non competitive inhibitor, it is not even parallel as was seen for an uncompetitive inhibitor; but an intersection around here, indicating that we have a mixture of competitive and uncompetitive inhibition.

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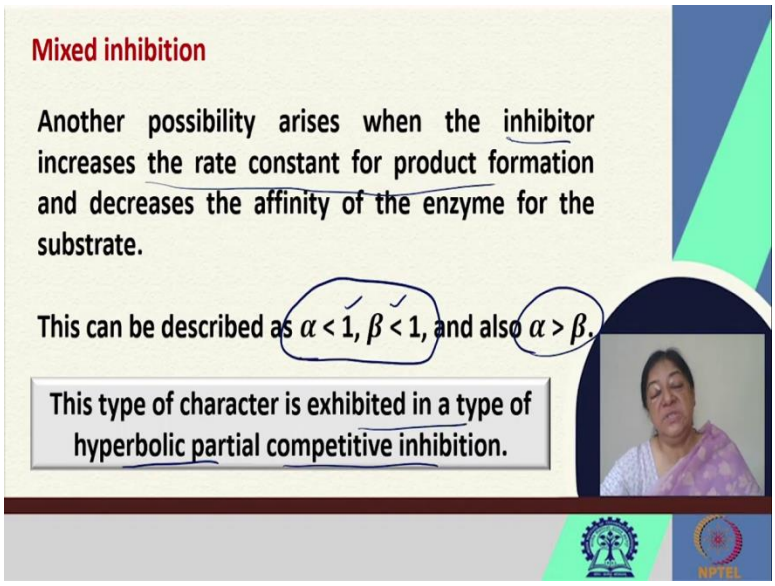

Similarly we can have another possibility, when the inhibitor increases the rate constant for the product formation and decreases the affinity of the enzyme for the substrate. Which indicates that the inhibitor is working on the enzyme in a manner, that it does not allow it to bind the substrate but nevertheless pushes it in a way, where we can consider the α less than 1, the β less than 1 and the α greater than the β, in our description in the schemes of modes of inhibition, where we looked at the α products with the different constants, the β products with the k_p value; that is the rate constant for the formation of the product. And this type of character is exhibited in a type of hyperbolic partial competitive inhibition.

Where there is partial competitive inhibition in the sense that the inhibitor is binding to the substrate. So, we realize that there may be several variations in terms of the binding of the inhibitor to the enzyme, that affects the substrate binding in different ways.

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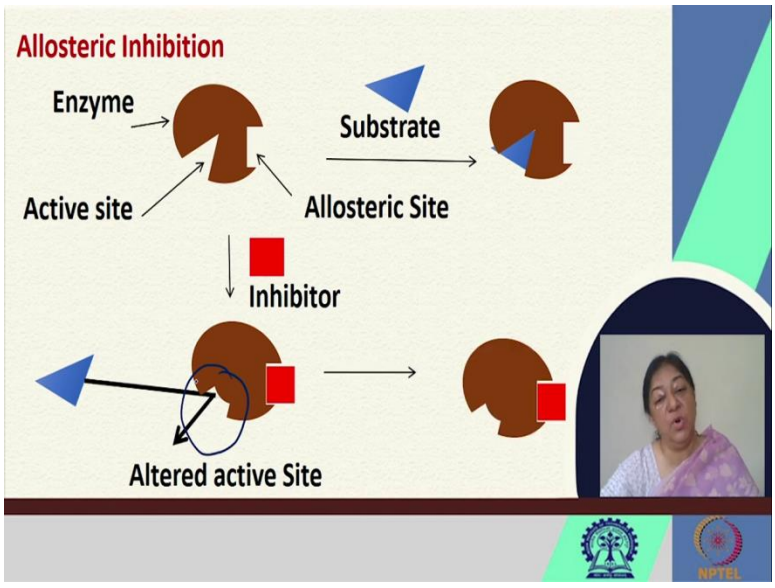

Allosteric inhibition is where we have the binding to the active site, we have an allosteric site. This is the scheme of things where we have the substrate that binds to the active site where it is supposed to go. However a binding of an inhibitor to an allosteric site, alters the active site shape complementarity and then it has an altered active side that cannot find the substrate anymore, giving us an inhibition.

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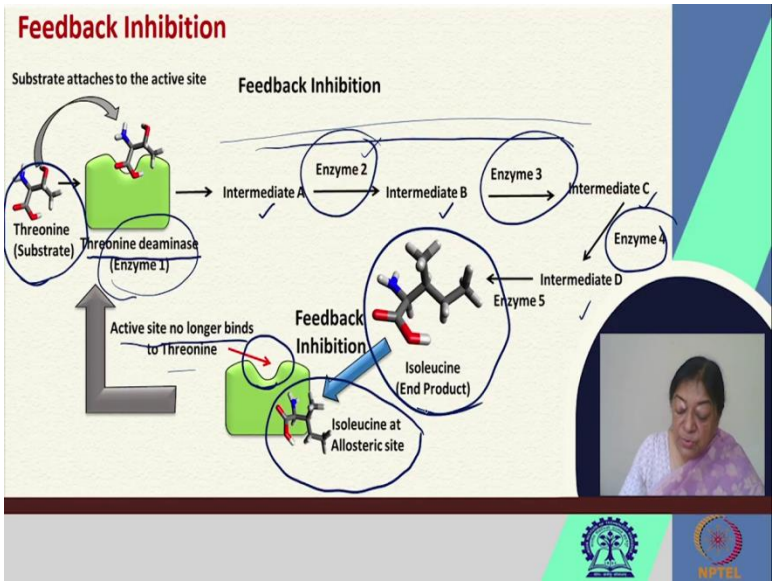

Another very interesting inhibition is feedback inhibition. In this case what happens is, there are a series of a cascade of reactions that are formed, say we have threonine as the substrate and the final product is isoleucine. In this beautiful natural way of inhibition, the substrate binds to the active site as would be essential, in an example of a threonine deaminase. We have a series of reactions, where we have different intermediates from intermediate A B C D, that involves several different other substrates.

However, isoleucine acts at an allosteric site of threonine deaminase. What happens therefore, it changes the shape of the active site and the active site can no longer bind threonine. Which means that the final end product of this series of reactions will bind to the initial enzyme 1, thus preventing it is own formation. This happens when the concentration for example, in this particular case, the enzyme would be inhibited enzyme 1.

If we go and inhibit enzyme 2, it means intermediate A would have been formed. But for that not to form, isoleucine which is the end product is an allosteric inhibitor for the first enzyme in the series of reactions. So, it prevents it's own formation when the concentration of this [refer to slide] product is high.

It is a beautiful way to control, to balance the formation of products and very smartly it goes to the first enzyme in the series. So threonine may be used elsewhere and we do not have these intermediates in the middle and enzyme 2, enzyme 3, enzyme 4 or 5 or not inhibited.

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We looked at in this lecture is competitive inhibition, non competitive inhibition, uncompetitive mix and feedback inhibition. These are all the different types of ways in which enzymes can be inhibited and these are the reversible types mostly we also have the irreversible types as we saw, where we have covalent bond formation and we will look at specific examples in the next lecture.

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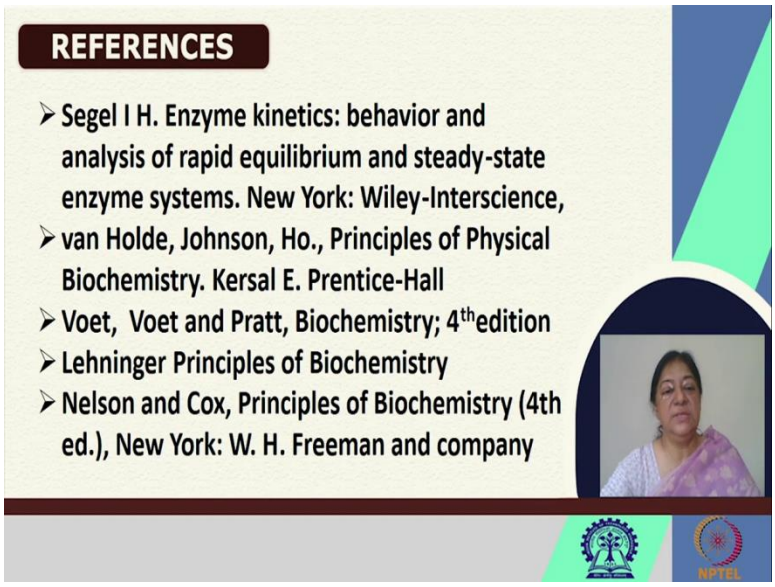

These [refer to slide] are the books that have been followed.

Thank you.