Enzyme Science and Technology
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Module - X
Enzyme Inhibition Kinetics
Lecture - 43
Enzyme Inhibition (Part-II)

Hello everyone, this is Dr. Vishal Trivedi from Department of Biosciences and Bioengineering IIT Guwhati. And what we were discussing? We were discussing about the different properties of the enzyme in the course Enzyme Science and Technology. And so, far what we have discussed, in the previous module?

We have discussed about how you can be able to design the different types of inhibitors. So, we have discussed about the traditional approaches, we have discussed about the ligand based approach, receptor based approach and at the end we have also discussed about the computer based targeted inhibitor design.

And if you recall in the previous module, we have also designed we have also discussed about the different types the different types of tools and as well as we have also discussed in detail about how you can be able to use the docking software's to design the inhibitors.

And in the previous lecture, we have also discussed about how you can be able to study the enzyme inhibitions. So, enzyme inhibition can be of two types, it can be of reversible type or the irreversible type. In the irreversible type, the inhibitor is of two categories.

One, the irreversible inhibitors which are actually going to be block the active groups onto the active site. So, there will be actually the enzyme in a in the enzyme active site modifiers and that is how they will actually going to modify the enzyme in such a way that enzyme is no longer be active.

The other category is the societal inhibitions or the mechanism based irreversible inhibitors where you are actually going to have the inhibitor. When the enzyme is actually going to process the inhibitor, then it is actually going to modify the inhibitor to more active species or some altered species.

And because that altered species will have very high affinity for the active site, it will actually going to block the active site permanently and as a result, it is actually going to cause the societal inhibitions or it will actually enzyme itself is processing the inhibitor and it is converting it into a inactive enzyme.

So, in today's lecture, we are going to discuss about the other category which is the irreversible inhibitors. And within the reversible inhibitor, we have the three different types of inhibitors, we have the competitive inhibitors, we have non-competitive inhibitor and we also have the uncompetitive inhibitors.

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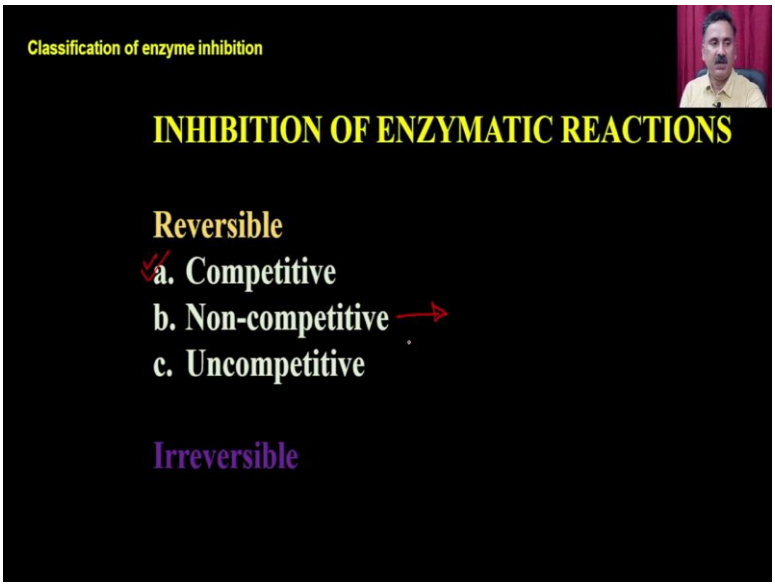

So, let us start the today's lecture. So, in the reversible inhibitors, we have the three different types of inhibitors. We have the competitive inhibitors; we have the non-competitive inhibitors and we have the uncompetitive inhibitors. So, let us start discussing first the competitive inhibitors.

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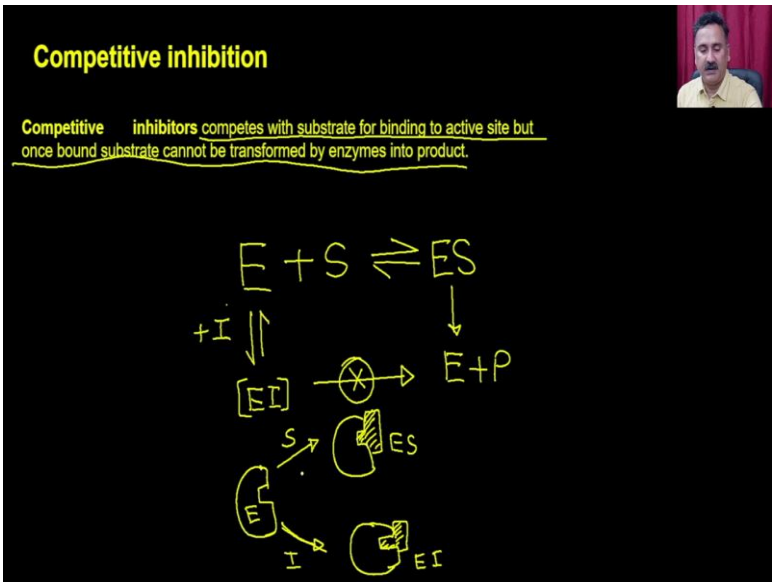

The competitive inhibitor, the competitive inhibitors, competes with the substrate for the binding to the active site, but once the bound substrate cannot be transformed by the enzyme into the product. So, what the competitive inhibitor is doing? For example, this is the enzyme ok.

So, enzyme is making a sub interaction with the substrate right and that is how the enzyme is getting converted into the enzyme substrate complex and then this enzyme substrate complex is getting converted into the enzyme plus product right. So, if the enzyme is active, it is actually going to do that. But when you add the inhibitor, for example, you add the inhibitor right. So, when you add the inhibitor, inhibitor will also have the same affinity for the active site as like the substrate right.

So, inhibitor is actually going to compete and as a result, the inhibitor is actually going to form the enzyme inhibitor complex. And because the enzyme inhibitor complex is no longer be processed because the enzyme inhibitor cannot be processed whereas, the substrate can be processed to form the product, this reaction will no going to active right. So, this is not going to happen that the inhibitor is also going to be converted into some product right.

So, this is what exactly going to happen. So, you suppose you have an enzyme right and suppose this is the active site ok. So, if this is the active site, it can actually take up the substrate on one side and it can actually take up the inhibitor on other side ok. So, if it is

taking the inhibitor right, it is forming the enzyme inhibitor complex. So, this is the inhibitor what is going to bind right and this is going to be the enzyme inhibitor complex. So, this is the enzyme ok and when it is interacting with the substrate, the substrate is also going to do the same right.

It is also going to be binding this right. So, it is also going this is the enzyme substrate complex ok. And that is how it is actually going to work. So, enzyme is actually going to (Refer Time: 06:06) inhibitor is actually competing with the substrates for the same active site. And that is why this particular type of inhibition is called as the competitive inhibitions. Let us take an examples to explain you what is the competitive inhibitor.

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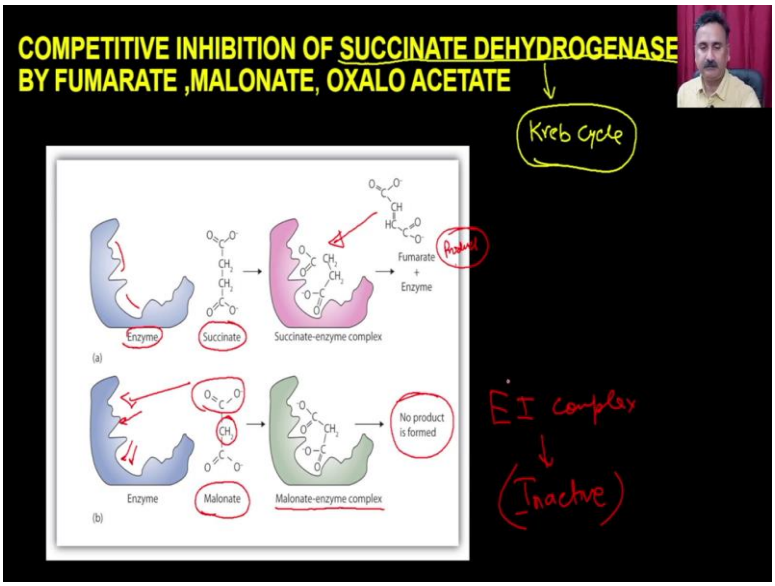

So, the competitive inhibition of the succinates dehydrogenase succinate dehydrogenase is an enzyme of the Kreb cycle. And what its job is that it is actually going to convert the succinic acid into the fumaric fumarate right.

So, this is the; this is the enzyme ok and it has some of the groups and all that. So, this is the three-dimensional structure of the enzyme, succinate dehydrogenase right. And when it is when it is say interacting with the substrate, the succinate, the succinate has the well-defined structure. So, because of that, this this region, right you see this region, this region is actually taking up the acid groups.

And this group, this this this these groups are also taking the acid group ok. And then the succinate succinic acid is getting converted into the fumarate, which is actually the product right of this reaction and then the product is going to be released. Now, the product in this case is also mimicking the same structure as the succinate.

So, in this case, what will happen is that if you add the fumarate, for example, then the fumarate is also going to block this active site and that is how the fumarate can be a competitive inhibitor. Taking this structure into the consideration, the people have developed a inhibitor, which is called as the malonate. So, malonate is exactly the same structure. You see the same structure except that you do not have 1 CH 2 ok. So, it only has 1 CH 2, here you have 2 CH 2. That is the only difference.

So, what happens is that these groups, this site and this site, these sites are crucial. And this site is where the inhibitor is going to compete so, what will happen is this acid group will go and sit here, right? And this acid group will go and sit here. This is exactly the same as for the succinate. So, as a result, what will happen is that it is actually going to form the malonate enzyme complex.

And once the malonate enzyme complex is formed, because the succinate can be get converted into the fumarate, but the malonate will not get in the product into the product. And as a result, the enzyme is actually going to be get fractionated into the inhibitor complex or the substrate complex.

So, wherever it is actually going to form the inhibitor complex that type of enzyme will no longer be active to form the product. So, as a result, ultimately all the enzyme is actually going to be get converted into the enzyme inhibitor complex.

And this enzyme inhibitor complex is going to be inactive. And that is how the enzyme is actually going to be inactive. Remember, that is all these interactions are mediated by the reversible forces, such as the Van der Waals forces, electrostatic interactions, solid bridge and all that. And that is why, this this kind of inhibition is called as a reversible inhibitions.

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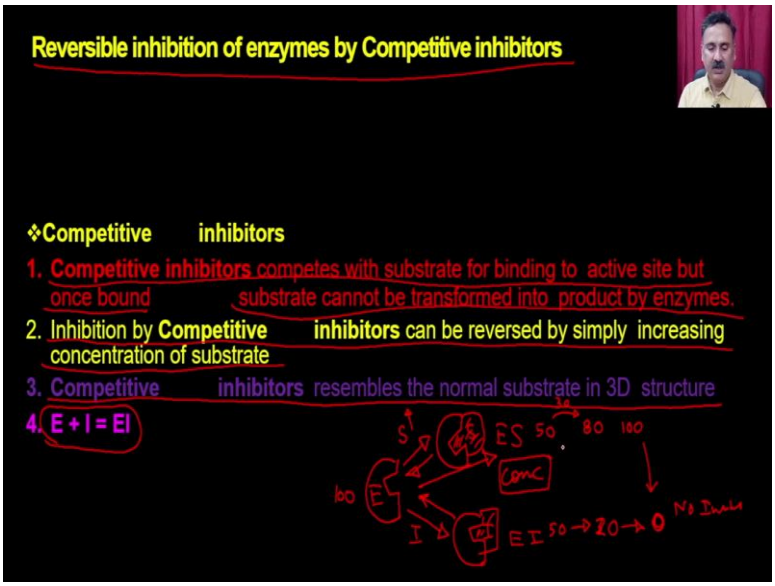

So, reversible inhibition of the enzyme by the competitive inhibitors. So, competitive inhibitors, competes with the substrate for the binding to active site. But once they bound, the substrate cannot be transformed into the product by the enzymes. Inhibition by the competitive inhibitor can be reversed by simply increasing the concentration of the substrate.

And competitive inhibitor, resembles the normal substrate in the 3 D structures. And the competitive inhibitors are making a complex with the enzyme and that is how they are forming the enzyme inhibitor complex. So, as you as you remember that when we have discussed about the enzyme, right.

So, if you have an enzyme which has the active site, this enzyme is actually going to be fractionated into 2 different types of complexes. One, it is actually going to make a complex with the substrate, right. The other complex, what it actually going to form is the inhibitor, right. So, it is also going can make the complex with the inhibitor, right. Now, these are actually going to be, you know, reversibly controlled. So, this is the, you know, the, they are in equilibrium.

So, this is the enzyme, it is forming a complex with substrate and it is forming a E S complex or E I complex. And all of these are actually the concentration driven and that is why its says that inhibition by the competitive inhibitor can be reversed by simply increasing the concentration of the substrate. So, for example, when you add the

substrate, right, it is forming the E S complex. When you add the inhibitor, it is forming the E I complex. Now, depending upon how what will be the concentration with the substrate, the amount of enzyme.

For example, if you have 100 molecules of enzyme, you can actually have the 50 molecules here and 50 molecules here. For example, because you have added the 50 molecules of inhibitor and 50 molecules of substrate. Now, imagine a situation that I will add the 80 molecules of inhibitor, a substrate, ok. So, if I add the inhibitor, the 80 molecules, which means I have added another 30 molecules, right.

Now, what will happen? For these 30 molecules, the additional 30 molecules, the substrate concentration it will go up, right. So, as a result, some of the enzyme which actually going to be free will actually go into this direction. So, this means if this will go by increase, there will be a decrease in this particular complex and that is how it is actually going to be 20, ok. So, there will be only 20 enzyme molecules which will be present in the enzyme inhibition inhibitor complex.

Whereas, the 30 molecules because it can go in the both directions, when there will be enzyme which is going to be formed, there will be a free enzyme, free substrate which is present and that is how it is going to be drive in this direction. So, if you increase the substrate concentrations, the equilibrium will go into this direction. And as a result, you will have more and more enzyme substrate complex. Imagine that if I make it 100, then the another 20 will actually going t produce.

So, this is the concentration of enzyme inhibitor complex is going to be 0. This means, all the enzyme is now present as the enzyme substrate complex and that is how at this particular stage, there will be no inhibition, right. And this is what exactly it is written here that if you increase the substrate concentration, the substrate is actually going to compete with the inhibitor and as a result, it will be keep removing the inhibitor from the enzyme complex.

And as soon as the enzyme is going to be free, the substrate will go and bind because it is all driven by the concentration gradient or concentrations. So, sub competitive inhibitor is solely been drive by the considerations. If the concentration of the inhibitor will go down, the inhibitor will again, the enzyme will again go back to the free enzyme and that

is how the enzyme is actually going to be active and it will actually going to catalyze the reactions.

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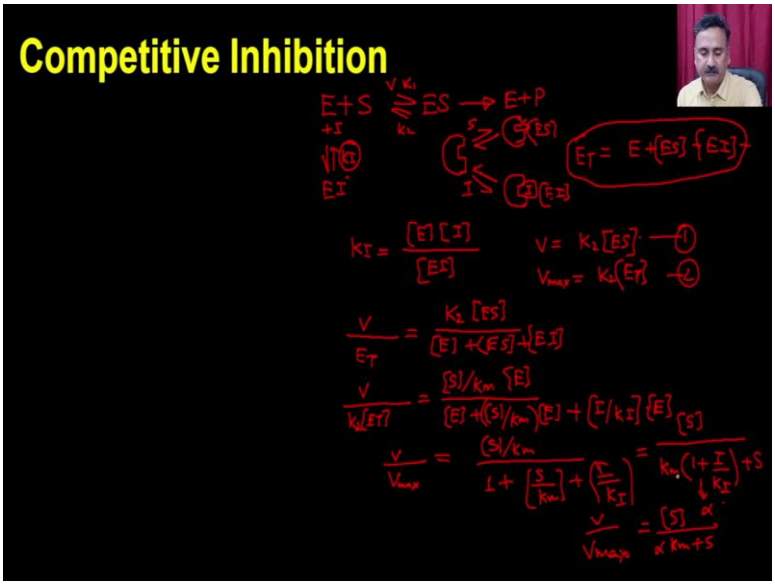

So, this is what exactly it is saying that if you have the enzyme, you can actually have the substrate, it is actually going to form the E S complex and that E S complex is actually going to form the enzyme plus product, right. If you add the inhibitor, right, then it is actually going to form the enzyme inhibitor complex, right.

And the interaction or the equilibrium constant for this is actually called as K I, whereas, in this case, it is called as the K M. So, this means you have an enzyme which is actually going to be under the equilibrium of the substrate or it is actually the under equilibrium of the inhibitor, right.

So, if you add the inhibitor, it is actually going to form the enzyme inhibitor complex. And if you add the substrate, it is actually going to form the enzyme substrate complex, which means, the for a competitive inhibitor, the enzyme is actually going to be fractionated into the two complexes, enzyme substrate complex or enzyme inhibitor complex. And this means if you have the total enzyme, the total enzyme would be the free enzyme, the enzyme substrate complex and the enzyme inhibitor complex, ok.

So, this you have to keep in concentration when you we are actually going to discuss about the kinetics. This means and remember that the equilibrium constant for the

enzyme inhibition interaction is called as K I. So, the K I is going to be called as the concentration of the enzyme, concentration of the inhibitor divided by the concentration of the enzyme inhibitor complex, ok.

Now, the velocity of the enzyme interaction is V, right. So, you remember that we when we were discussing about the Michaelis Menten constant, right. So, velocity of this is actually. So, this is called as K 1 and this is called as K 2, right. So, velocity is actually going to be called as the K 2 and it is actually going to be breakdown of E S, right.

This is a direction, right. And when you are going to have the maximum velocity, when you are actually going to have all the complexes as the, when you all enzyme is present as the enzyme substrate complex. This means the V max is actually the K 2 multiplied by the E T, correct. This is the maximum, what is possible, right.

This means if I divide the V by the total enzyme, right, it is actually going to give me the this, right. So, K 2 E S, right. This is what? This is the equation number 1, this is the equation number 2 and so, this is equal from the equation number 1, you can take this and the E T, remember this, right, this is the equation, right.

So, you can take put this value here. So, you can put that concentration of E plus concentration of E S and the concentration of E I, ok. So, these are the, this is the E T, right. So, if I put the E T from here, right, I can take this, right. So, I can take this V by K 2 E T, right. So, this is what I can put here, right.

And I can be able to do this. So, if I take this, I can put, instead of this, I can put the Michaelis Menten constant. So, you can I put the substrate divided by the Km, multiplied by the enzyme E right, and I can do the this whole right. So, I can put the enzyme concentration plus substrate divided by K m bracket right. And E plus inhibitor divided by K I, ok. And multiplied by E, ok.

So, this I have taken from here, ok. This I have taken from here, right. And if you solve this, what you are going to get is you are going to get this, V is equal divided by V max divide multi equal to S divided by K m and 1 plus. So, this I have taken the this as the, you know, outside, right.

So, 1 plus S by K m plus I by K I ok. And if you simplify this, if you simplify this, what you going to get is you are going to get this. What you are going to get? You are going to get S divided by K m bracket 1 plus I by K I plus S, ok. And this is what is very, very important equation or very, very important equation to actually be able to understand the competitive reactions, ok.

Now, if I show you the reactions. So, this this whole thing I can convert. This so, this whole thing, I can if I assume this as the alpha, right. If I, I by K I, 1 plus I by K I, if I convert, if I consider this as alpha, then it is actually going to be called as S divided by alpha K m plus S. So, it can be like this, ok. And this is very important.

So, V by V max is equal to S by alpha K m plus S. So, an alpha is equal to 1 plus I by K I, ok. So, let us discuss about how the enzyme kinetics is going to function, ok.

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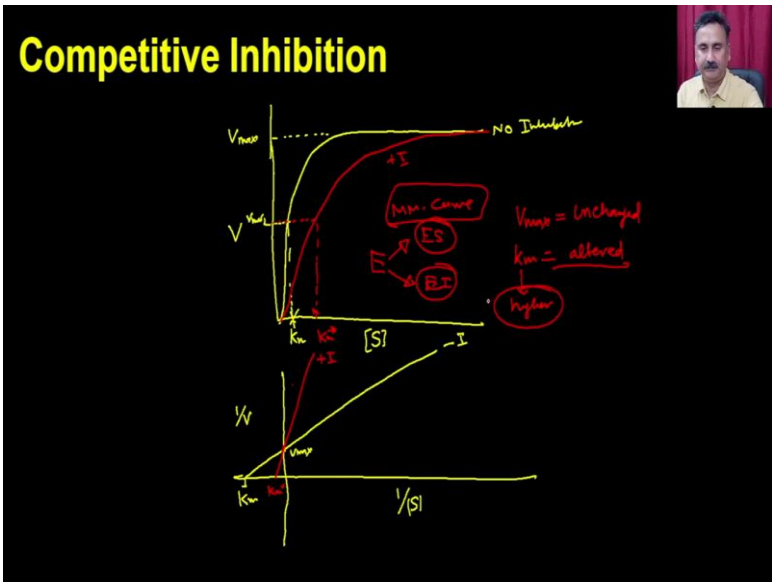

So, if I plot the substrate concentration, ok, versus the velocity, ok. So, in this case, first is we can actually be able to show you the curve, how it actually going to be. So, its going to be like this, when you have no inhibitor, ok.

So, this is the no inhibitor. And you remember that if you take this and put it like this, this is going to be the V max and this is going to be the V max by 2, ok. And if I go over like this, this is a K m, right. Remember the last time when we were talking about the Michaelis Menten curve, right. So, this is the concentration of the K V max, K m.

Now, when you add the inhibitor, ok, when you add the inhibitor, the inhibitor is actually going to take up some of the enzyme, right. So, and that is how the it will actually going to reduce the effective concentration of the enzyme. So, in that case, it is actually going to take the longer time to reach the V max.

So, that is going to be happened like this, ok. This means, it is actually going to take the longer time to reach the V max. And as a result, the velocity, the ramping of the velocity is less, right. So, this is actually in the presence of the inhibitor. This is in the absence of inhibitor.

So, when you have the inhibitor, it is actually going to take the longer time because as you are going to have one concentration of the inhibitor, it is actually reducing the concentration of the enzyme and as a result, it is actually lowering down the, it is asking the enzyme to go for more time, ok.

Now, it will still be able to reach to the V max. And as a result, the V max will remain the same. V max by 2 will remain the same. But when you calculate the K m, the K m is actually going to be different. So, this is actually going to be the revised K m or I will say the Km when you have the inhibitor. So, what you can do, what you can see is this is the Michaelis Menten curve, right.

This is the Michaelis Menten curve, ok. If I show you the same thing in the line weaver burk plot, ok. So, line weaver Burk plot is also going to give you the better idea about what is exactly happening, actually. So, this is the Michaelis Menten curve. So, 1 by V divided by 1 by S, right.

So, first is you have the no inhibitions, right. So, what will happen is that you have the no inhibition. So, this is the curve what you are going to get when you have no inhibitor. So, this is the V max and this is going to be the K m. Now, when you have the inhibitor, ok.

So, I will use the same color, right. So, when you have the inhibitor, it is actually going to be because it is taking longer period of time. So, it is actually going to be like this. This means, it is going to be this and V max will remain same. So, this is going to be in the presence of inhibitor.

So, what exactly is happening in this? Exactly its happening is that when you have the V competitive inhibition, the V max remains unchanged, but the K m is going to be altered. And what will happen to the K m? K m will be on a higher side, which means the enzyme is actually reducing enzyme is actually requiring the more amount of substrate to show you the same amount of V max.

And this is exactly what is going to happen because the enzyme is now fractionating into this right, this means, effectively the enzyme substrate concentration is getting reduced because you are some of the enzyme is getting channelized here, right. So, you require more substrate so, that if enzyme is actually be able to show you the V max. So, in a competitive inhibition, the V max is going to remain unchanged, the Km is actually going to altered.

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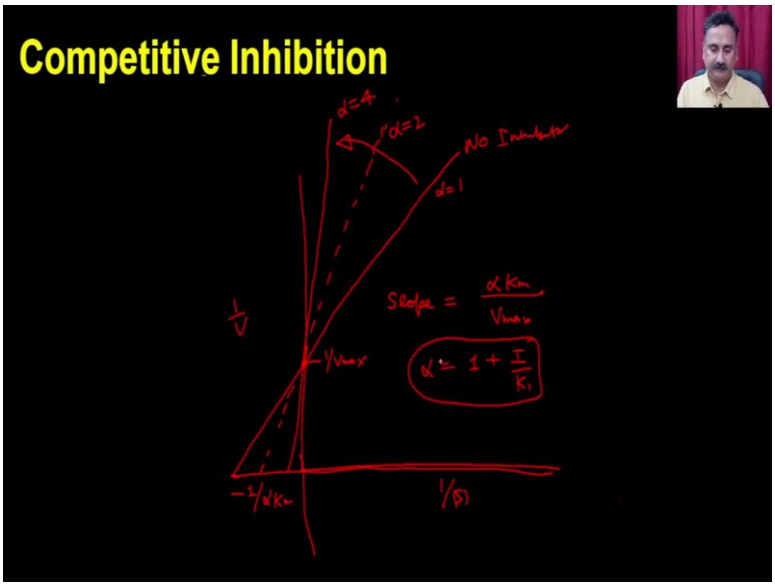

And this is what exactly happen, right, when you have the multiple inhibitors. So, when you have the multiple inhibitor and you are going to show like the line weaver plot, what will happen is that you are going to have the 1 by s versus 1 by V. So, you are going to have the.

For example, you are going to have the, this is going to be no inhibitor, right. And as you will increase the inhibitor, right. So, if you increase the inhibitor, it will go like this, it will go like this. So, here you are going to have the. So, for example, alpha is equal to 1

in this case. So, there will be no inhibitor if your alpha is equal to 2, this means you have increased the inhibitor.

So, this, if you increase the inhibitor, if you are increasing the inhibitor concentration, which means alpha is equal to 4, like that, then you are keep reducing the K m. And this is going to be the minus 1 by alpha K m, ok. So, if the alpha is 1, this is going to be the 1 by K m, if alpha is 2, then this is this, and this is that. And since this is on the minus scale, this is not a problem, ok.

So, the slope of this curve is actually going to give you the value of alpha K m divided by V max, ok. So, this is the V max. This is the 1 by V max, ok. And alpha is going to be 1 plus I by K I, ok. Remember that? So, this is actually going to be very, very useful.

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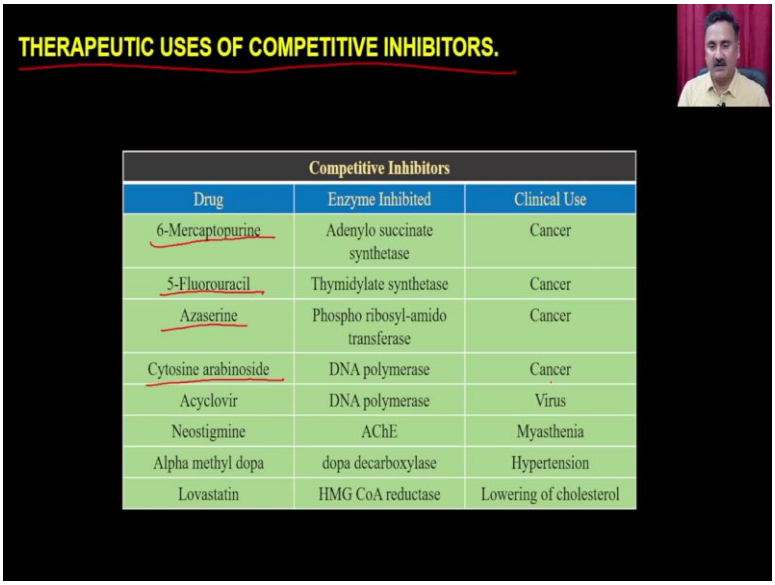

So, competitive inhibitors are easy to design because you know the size of the active side, you know the size of the inhibitor. So, you are actually going to have the many tools to develop the competitive inhibitors. And there are. So, many of these competitive inhibitors, like 6-meracaptopurine 5-fluorouracil, azaserine, cytosine, all that, ok.

And they are actually been having the very, very extensive applications in the field of cancer, virus and all that.

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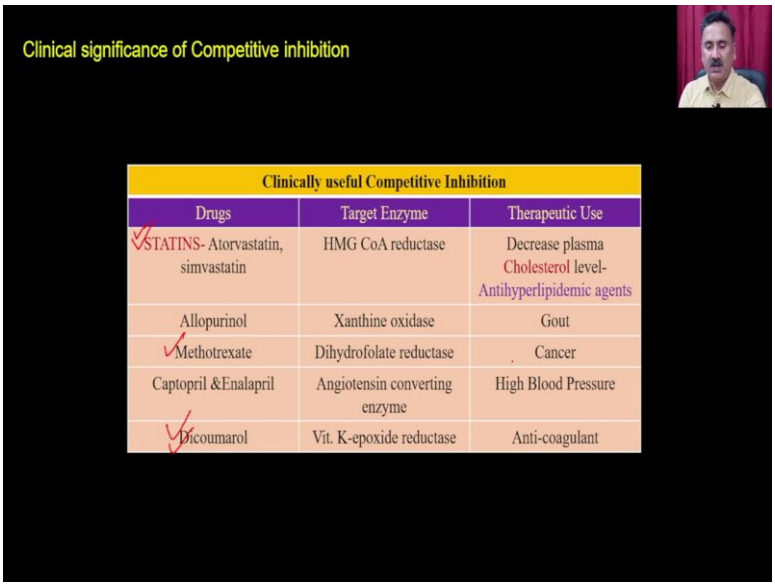

And then these are some additional inhibitors, what people have also used, for the most popular are the statins and methotrexate and dicoumarol, and all these are actually being used in different types of diseases. So, this is all about the competitive inhibitions.

Let us more discuss about. So, this is the all about the competitive inhibitions. And now we will discuss about the non-competitive inhibitions. So, in a non-competitive inhibition.

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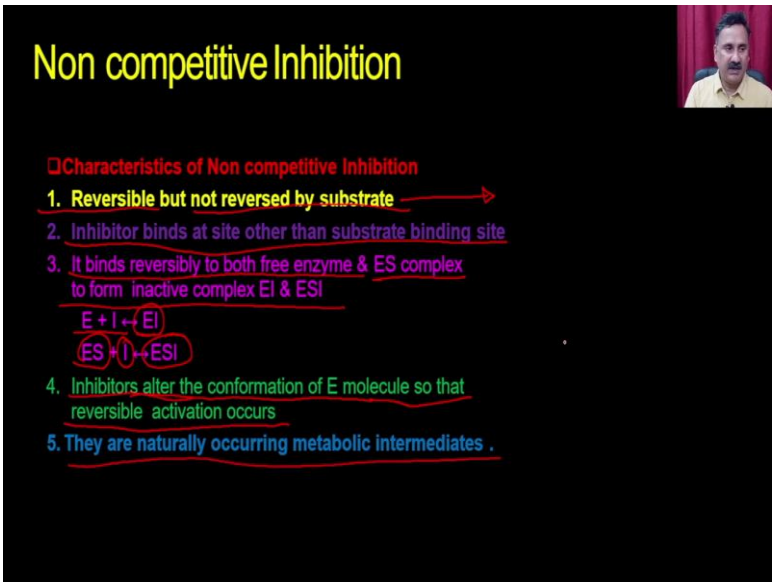

So, what are the different properties of the non-competitive inhibitor? So, non-competitive inhibition is reversible, but not reversed by the substrate. So, that is a characteristic, contrasting character, ok. Remember that in the competitive inhibitions, you can be able to reverse the competitive inhibitions by increasing the substrate concentrations. Inhibitor binds at a site other than the substrate binding site, that is another point.

It binds reversibly to both free enzyme and as well as the enzyme substrate complex to form the, to form the inactive enzyme inhibitor complex or enzyme-substrate inhibitor complex. This means, the enzyme is going to interact with the inhibitor and its going to form the enzyme inhibitor complex or enzyme-substrate complex is going to interact with the inhibitor and its going to form the enzyme inhibitor complex. The inhibitors, how it actually going to function as a inhibitor?

So, inhibitor actually altered the conformation of the enzyme molecule so, that the reversible in inactive activation occurs. They are naturally occurring metabolic, metabol intermediates.

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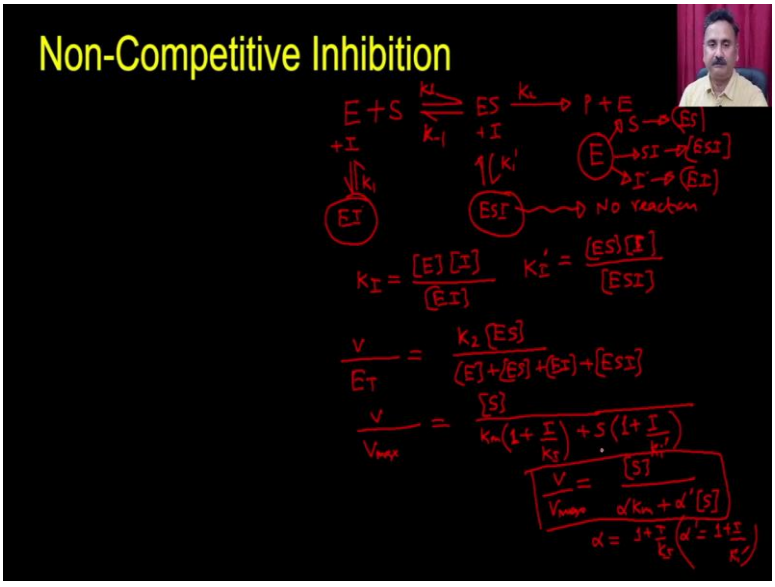

So, non-competitive inhibitions as the name suggests the enzyme, you are actually going to have the enzyme which is interacting with the substrate and it is going to form the complex, right. So, forward reaction K 1, this is K minus 1 and the enzyme-substrate

complex is going to form and then it is actually going to form the K 2 and its going to form the product and the free enzyme ok.

Now, in the non-competitive inhibition because it is not going to compete, it is actually going to bind a discrete site or allosteric site. So, it actually, because its binding to the discrete site, it actually can bind to the free enzyme in actually unbind the enzyme-substrate complex.

So, that is why its actually can bind the enzyme inhibitor ok and can form the enzyme inhibitor complex or it can actually be able to bind here and it actually can form the this enzyme-substrate inhibitor complex. Ok so, this is called as K I and this is called as K I prime ok.

And once it actually going to form the enzyme- inhibitor complex or enzyme-substrate inhibitor complex, it is actually going to lead to the no-reaction. So, remember that whenever the inhibitor binds, whether it binds to the allosteric site or whether it binds to the active site, it will never been be processed by the enzyme to form the product.

So, the K I, again we will write the reaction for the K I. So, K I is actually going to be that enzyme-inhibitor and enzyme-inhibitor complex. Remember that here again the enzyme is now fractionating into the substrate. So, its forming the enzyme-substrate complex. Enzyme is fractionating with the inhibitor.

So, it is forming the enzyme- inhibitor complex and enzyme is also binding to the this. So, its also forming the substrate inhibitor. So, its also forming this. So, its actually getting fractionated into now three individual molecular species. So, and the K I prime which is for this right is going to be the E s divide sorry I divided by concentration of E S I ok.

So, we are again going to write V by E T is going to be K 2 E S ok divided by remember this is the what is going to happen. So, this all are going to be right. So, E T E T in this case would be E ES E S I and E I ok. So, E t is going to be E plus E S from here right. Then it is going to be E I and then it is going to be E S I ok. So, if you solve this and if you it is going to be review this V by V max is equal to substrate. When will be the V max? When will the V is going to be V max? When the E is going to be E T right?

So, that is going to be K m bracket 1 plus sorry this is right 1 plus I by K i plus substrate bracket 1 plus I by K i prime ok. So, this is the substrate ok and now if you want you can actually be able to convert this into another equation and that would be like substrate divided by alpha K m plus alpha prime concentration of substrate ok. So, alpha K m again in the same way the. So, do not be get confused right. Alpha is equal to 1 plus I by K i and alpha prime is 1 plus I by K i prime ok.

So, since we do not want to make this so, complicated we have just taken the alpha as the substitute of this. So, if this is alpha this is alpha prime ok. So, alpha K m plus alpha prime substrate concentrations and so, in this V max by V by V max is equal to substrate divided by alpha K m plus alpha prime S ok and this is very important equation for non-competitive inhibitions.

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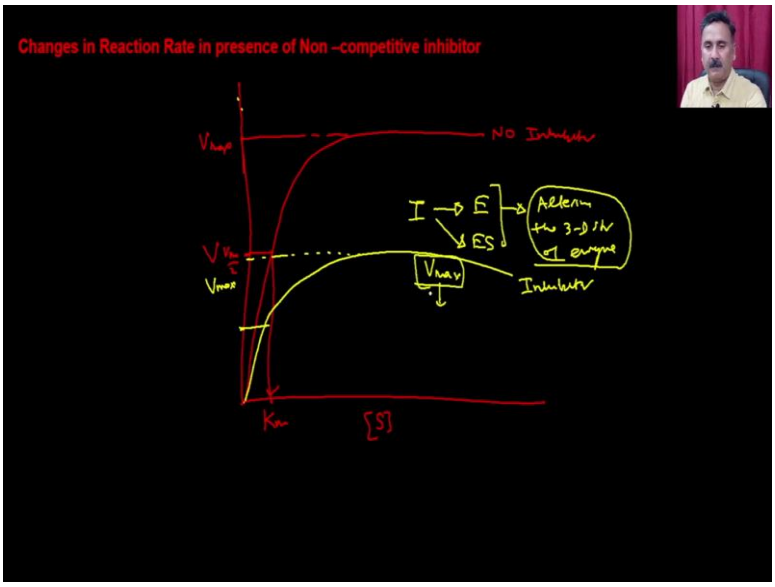

Now, we will see what will be the you know how the curve look like right. So, curve if you have the curve you have a substrate concentration right and will have the no substrate. So, first you are going to have no inhibitor. So, its going to be like this. So, it is going to be no inhibitor ok.

Now, when you have the non-competitive inhibitor ok. So, when you have the non-competitive inhibitor its going to be because you know now there is a difference right. So, sorry so, I will write all the parameters right. So, this is going to be V max this is going to be. So, this is velocity versus this and this is going to be V max by 2.

So, this is V max by 2 and I will go like this then this is going to be the K m. Now, we will show you how the non-competitive inhibitor will look like ok. So, when you have a non-competitive inhibitor ok you are actually trapping the enzyme through a alternate website alternate structure ok.

Remember that the inhibitor is actually interacting with the enzyme and inhibitor is also interacting with the enzyme substrate complex and as a mechanism it is actually changing or altering the 3D structure of the enzyme ok 3D structure of the enzyme right or I will say it is actually changing the 3D confirmation of the active site.

So, as a result it is actually going to change its affinity for the substrate or its actually going to change its interaction with the substrate. So, as a result it is actually going to affect this and so, it will not be able to attain the V max ok. So, in this case the V max is going to be like this ok.

So, this is going to be the V max when you have the inhibitor. So, this is going to be in the case of inhibitor and you will see this is the (Refer Time: 37:36) So, its actually going to alter many things. So, its going to alter V max right. So, its going to alter the V max.

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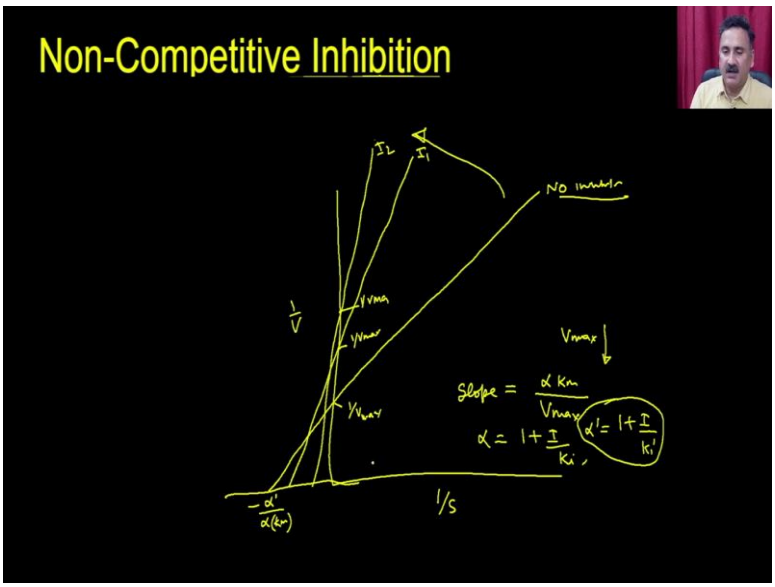

Now, will show you the line weaver Burk plot ok. So, in the line weaver Burk plot ok what you are going to do is you are going to plot 1 by S versus 1 by V ok and the slope

will be like this right. So, first you are going to have the no inhibition right. So, you are going to have no inhibition. So, this is going to be no inhibition. So, there is no inhibitor right. Now, you are going to have the as you will change the you know as you will actually going to add the inhibitors it is actually going to affect the V max.

So, its going to move like this ok. So, this is actually going to be inhibitor 1, inhibitor 2. So, the all the concentrations ok. So, all you see here is and this is actually the 1 by V max ok and this is going to be 1 by V max when you have inhibitor ok. And this is actually the minus alpha by alpha prime by alpha K m ok.

This means if alpha and alpha primes are not present then this is going to be minus 1 by K m. And you see as I am increasing the inhibitor concentrations and it is actually changing the V max. So, it is also lowering down the V max ok. So, in this case the non-competitive inhibitions you are actually going to affect V max.

So, the slope of this curve is actually going to give you the information about the alpha K m divided by V max ok. And remember that alpha is 1 plus inhibitor by K i ok; whereas, alpha prime is 1 plus inhibitor divided by K i prime ok. So, these are the two things what you have to always remember ok. So, from the slope you can be able to calculate the km and V max and this is the K V max this is the km what you are going to get.

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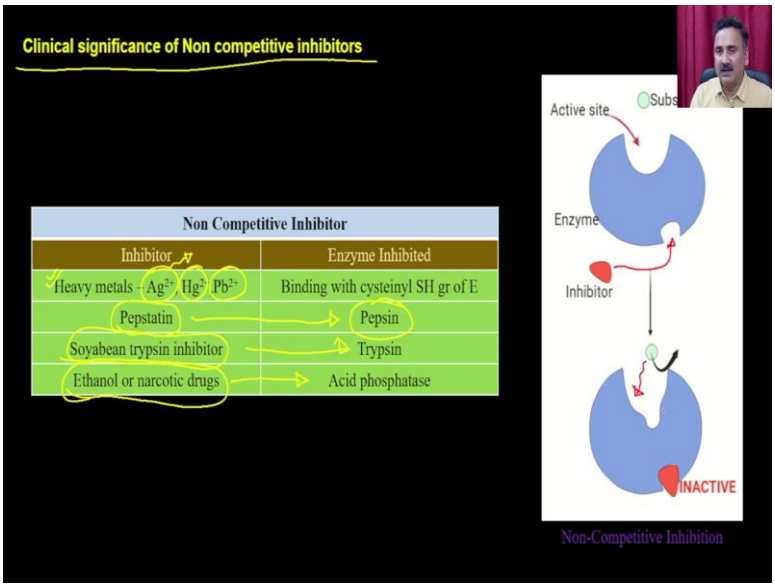

So, the clinical significance of the non-competitive inhibitions. So, non-competitive inhibitions are more potent and you know more problematic than the competitive inhibitors. So, there are so, many inhibitors what you can use you can have the inhibitors for the heavy metal toxicity.

So, for example, the heavy metals heavy metals for example, the metal which contains the silver, arginine and lead they are binding with the sulfhydryl groups of the enzyme and that is how they are actually forming the non-competitive inhibitors.

And that is why you remember that the all these heavy metals are bad for the human health because they are actually going to inactivate so, many enzymes and that is how they are actually going to force the toxicity. Then we have the pepstatin, pepstatin is the protease inhibitor for the pepsine.

Then we have the soyabean trypsin inhibitor which also have the inhibitor for the trypsin and then we also have the ethanol or the narcotic drugs. So, and that also has the inhibitor for the acid phosphatase. So, what happened in the non-competitive inhibition is that, it is actually. So, in a enzyme you have the two different sites you have the active site, you have the allosteric site.

So, substrate actually can fit into this active site right. And so, what happen is that inhibitor will go and sit here ok. So, inhibitor will go and sit in the allosteric site. It does not go and bind in the active site ok. So, that is the major difference ok from the competitive inhibitions. And once it binds it actually induces the conformational changes.

And because it change induces the conformational changes, it is actually affecting the binding of the substrate to the active sites either it will reduce the affinity or either or it may actually abolishes the substrate binding completely. So, either of these cases the inhibitor is actually going to be very very potent compared to the competitive inhibitor.

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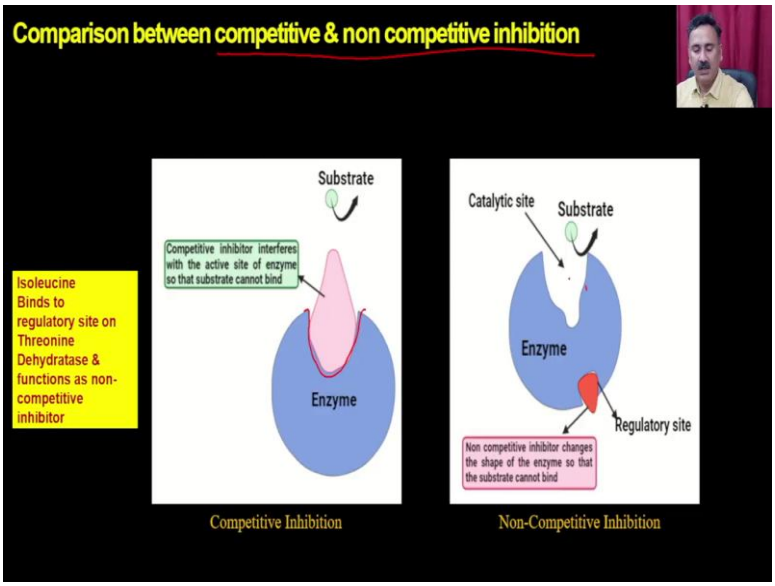

So, let us see a comparative statement how the comparison between the competitive and the non-competitive inhibitions. So, in a competitive inhibitions the substrate or the inhibitor both are competing for the active site. So, this is the active site which has the all the requisite three-dimensional structures, you have all the interactions everything available for both inhibitor as well the substrate. So, whoever will be more in concentration it will actually go and bind.

Whereas, in the case of non-competitive inhibitor, inhibitor will go and bind to a distant site. It will always been called as allosteric site and once it binds it actually brings the alterations into the active site and that is how the substrate binding is going to be compromised or the affinity is going to be reduced.

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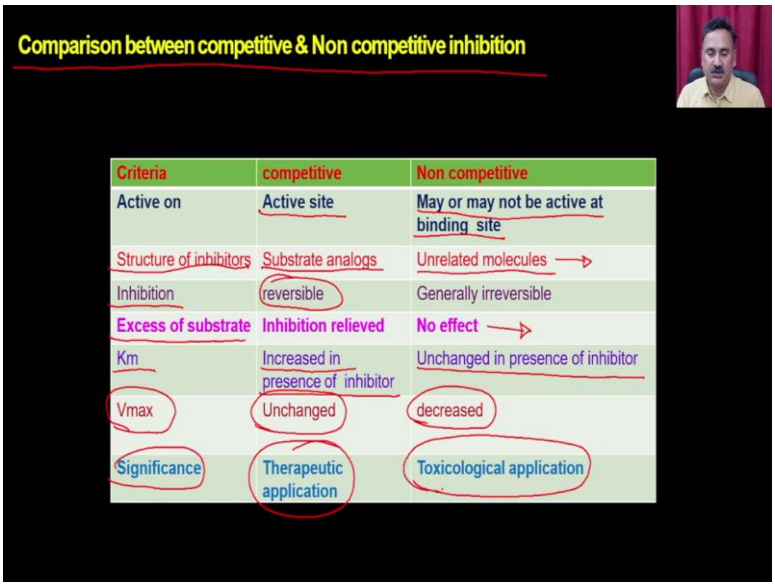

So, this is what the tabular form I have given you a comparison between the competitive and the non-competitive inhibitions. So, the competitive inhibition is active in the active site whereas, a non-competitive inhibition is may or may not be active at the binding site.

Then the structure of the inhibitor the most of the competitive inhibitors are the substrate analogs, but the non-competitive inhibitors are unrelated molecules because they are supposed to bind to the allosteric site then inhibitions more in both the cases it is reversible inhibitions.

Then if you add the excess of substrate the inhibition can be relieved in the case of competitive inhibitor, but it is having no effect on to the non-competitive inhibitor and that is how the non-competitive inhibitors are more potent compared to the competitive inhibitions. K M the K M is actually going to be increased in the presence of inhibitors.

Whereas, it is going to be unchanged in the presence of inhibitor. The V max V max is actually going to remain unaltered in the case of non-competitive inhibition whereas, it is actually going to be decreased in the case of non-competitive inhibitor. What is the significance?

Significantly is that it is actually going to have both of actually going to have the therapeutic applications whereas, the non-competitive inhibitors are mostly being used in a toxicological applications.

So, now let us move on to the third category and the third category is the uncompetitive inhibitions.

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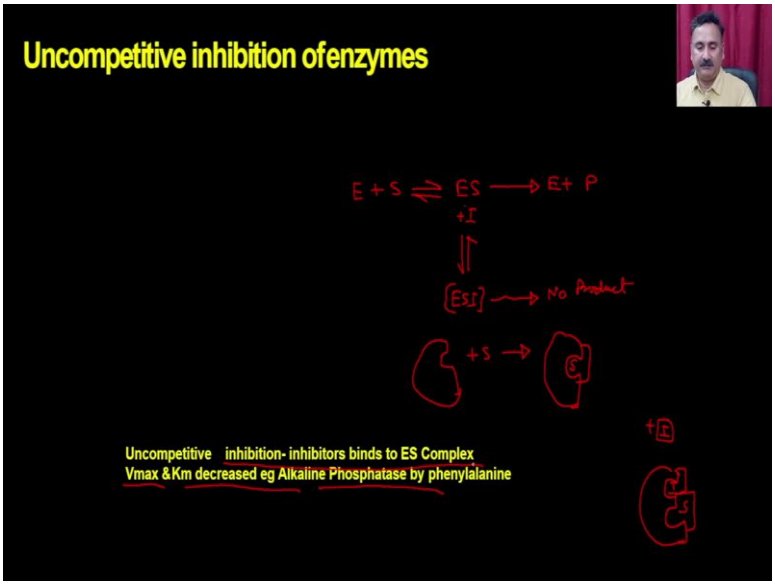

So, uncompetitive inhibitions the inhibitor is actually going to bind to the ES complex ok this means it is actually going to affect the V max and K m is actually going to be decrease. For examples, are alkaline phosphatase inhibition by the phenylalanine.

So, in this case what happens is that the enzyme is actually interacting with the substrate forming the enzyme substrate complex ok and then this enzyme substrate complex is converting into the enzyme plus product. Now, inhibitor does not have the any affinity for the enzyme, but when the substrate binds and form the enzyme substrate complex then it actually interacts with the enzyme substrate complex.

And forms the enzyme substrate inhibitor complex and this is actually not allowing the any kind of process. So, it is going to give you the no product ok. So, this is exactly what we are going to happen. So, enzyme you have an enzyme it has an active site ok. So, it is going to interact with the substrate and it is going to form the enzyme substrate that is going to form the enzyme substrate. Now, at this stage if you add the inhibitor molecule, inhibitor molecule will also going to have the additional.

So, this is going to be substrate and when you add the inhibitor it is inhibitor is actually going to have the additional active site. So, on this active site you have the substrate on

this active site you are going to have the inhibitor. So, basically when the substrate binds it actually forms inhibitor binding site. So, its actually creates the inhibitor binding site and that is how the inhibitor is actually going to bind.

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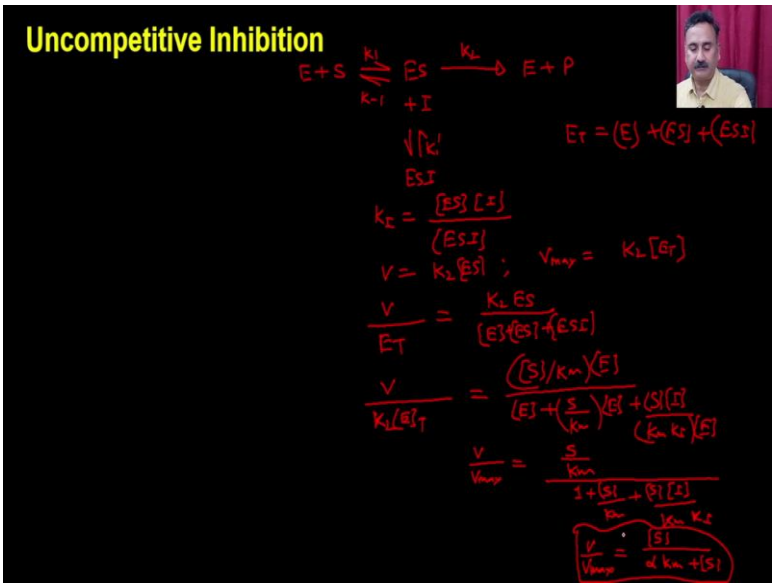

So, this is what exactly going to happen. So, the enzyme which is interacting with the substrate is giving the enzyme substrate complex. So, this is the K 1, this is a K minus 1 and then this is actually going to form K 2 is going to form the enzyme plus product and from here the inhibitor is actually going to form or then inhibitor is going to interact to give you the enzyme substrate inhibitor complex right and this will not going to process ok.

So, this is called as the K I prime because remember that we will not going to use the, we will use the same conventions. So, K I prime also we are using. When we were talking about the non-competitive inhibitors. So, K I is E S I is divided by E S I ok. Remember that the E t in this case could be E, E S and E S I ok.

So, its going to be E, E S and E S I ok. So, these are the three way in which the E t is going to divide ok. Now, V is going to be K 2 E S that is and V max this is the whole thing actually we are writing same thing right K 2 E T right. When the and then the substrate function is going to be total right.

So, V by E T is going to be K 2 E S and instead of K E T you are going to write E S sorry E plus E S plus E S I right. Now, instead of writing the E S I you can write the KI E S I and all that and because of that [FL] it is going to be and instead of E to E T you can write the K 2 E T. Then it is going to be S by K m bracket [FL] E right and divided by E plus S by Km multiplied by E plus S I.

So, S I divided by K m K I multiplied by E ok and if you simplify this ok if you simplify this you are going to get V by V max equal to because this is the V max right. So, V max V by V max is equal to S by K m divided by 1 plus S by K m plus S I divided by K m K I ok and this is what you can use you can convert this into the.

So, if you take the average of this you can actually be able to convert 1 in plus. So, you see S is also here S is also here. So, you can take the S out and you can be able to convert this. So, ultimately V by V max is going to be S divided by alpha Km plus S.

So, if you simplify all this ok S by Km S by Km all this. So, you can easily take the s by Km out from here ok and then it will actually can be used and cancelled out everything. So, then it will actually going to give you this is the final equation what you are going to get ok and this is the equation what you can use to in to understand all the parameters.

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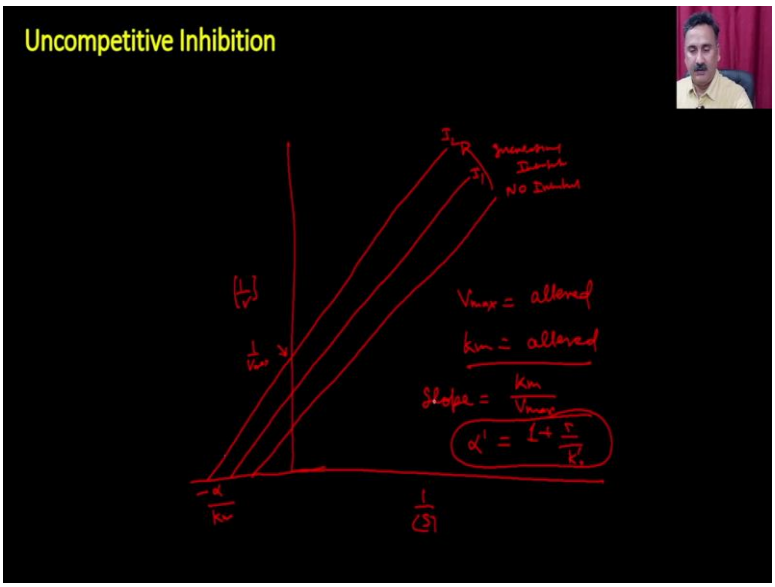

Now, we will see the line weaver Burk plot how it is actually going to work right. So, you are going to have 1 by s versus 1 by V ok and this is what exactly going to happen

ok. So, you are going to have the slopes like this like this and like this ok and this is the no inhibitor and this is the from here to here.

So, this is the inhibitor concentration 1 this is the inhibitor concentration no. So, increasing inhibitor ok and as you increase the inhibitor what you are going to do is you are. So, this is going to be minus alpha by K m ok. So, you will see that you are actually changing the K m and you are also changing the 1 by V max ok.

So, this is the 1 by V max ok. So, this is the V max and you have the inhibitor concentration 2 this is why it is so? Because in this case you are altering the when the inhibitor binds it actually induces the confirmation and that is all. So, in this case there will be a alteration in the V max.

So, it is going to be altered K m is also going to be altered ok and that is the characteristic of the uncompetitive inhibitors. The slope of this curve is actually going to be K m by V max ok and the alpha prime in this case would be 1 plus I by Ki prime ok and this is what you have to use.

So, this is all about the mechanism of the inhibition of the enzyme we have discussed about the reversible enzyme. So, we have discussed about the competitive inhibitions we have discussed about the kinetics of the competitive inhibitions and how it is actually inhibiting the enzyme and what are what we have learned that if you increase the substrate concentration. You can be able to relieve the inhibition by the competitive inhibitors. In addition to with that you also discuss about the non-competitive inhibitors.

So, non-competitive inhibitors binds to a distant site or the allosteric site and when they bind to the allosteric site they are actually inducing the conformational changes and as a result it is actually altering the binding of the substrate and then you also discuss about the uncompetitive inhibitors. So, uncompetitive inhibitor does not have any kind of binding site onto the enzyme structure.

But when the substrate binds it actually creates the binding site for the inhibitor and that is how it is actually going to bind the enzyme substrate complex rather than the free enzyme and that is how in either of these cases it is actually going to affect the K m or the V max. So, with this I would like to conclude my lecture here in our subsequent

lecture we are going to discuss some more aspects of the enzyme in the course enzyme science and technology.

Thank you.