Biochemistry - I

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Lecture –9
Enzymes –III
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Welcome, in the last class we spoke about Enzyme kinetics and how we have the specific active site of the enzyme and how we found the enzyme-substrate complex.

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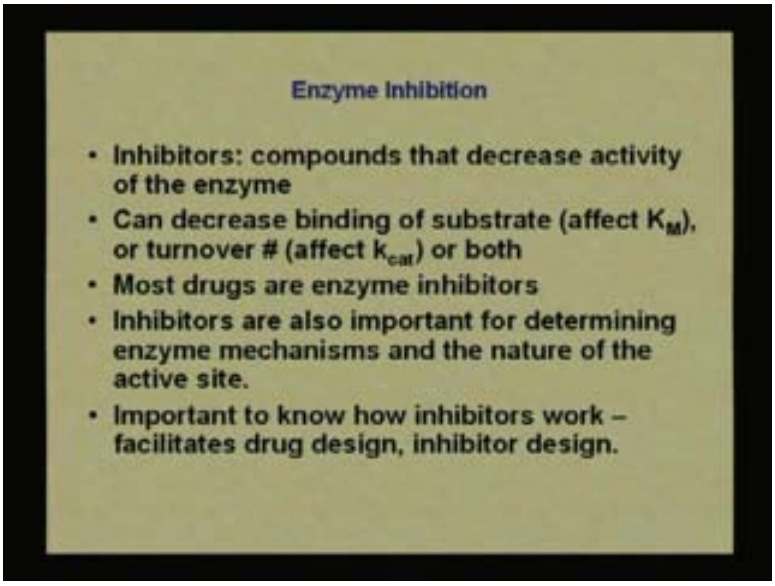

What we are going to speak about in this class is enzyme inhibition because enzyme inhibition is a very important factor in determining drugs or specific compounds that are going to inhibit the action of definite or specific enzymes. So apart from knowing the structure and once we know the function there are certain designs to the drugs that can be made to determine how we can design a inhibitor of particular enzyme.

Now what we want to do is we want to stop the particular reaction that the enzyme is catalyzing. So these inhibitors are compounds that decrease the activity of the enzyme. How can they decrease the activity of the enzyme? They have to affect the efficiency of the enzyme. They decrease the efficiency of the enzyme by affecting the K_M value or the K_{cat} value or both of the values. (Refer Slide Time 01:50 min). So we have to decrease the binding of the substrate if an inhibitor of an enzyme has to act. We want to stop the enzyme from forming the enzyme-substrate complex as simple as that.

So if I want to form or I want to deter the formation of the enzyme-substrate complex
I have to have the inhibitor bind to the substrate some how or bind to the enzyme some how.

Most drugs are enzyme inhibitors because there is certain bodily function that goes wrong that is why you have a specific activity of an enzyme in the wrong fashion. So what would you want? You want some drug that is going to inhibit the enzyme so that it does not act in a fashion that it is acting as right now. For example if you have Antibiotics, what does it do? It inhibits the enzymatic reactions of the bacteria that have formed the infection. So inhibitors are also used to determine enzyme mechanisms and also for the nature of the active site. And of course it is extremely important to know how these inhibitors work because this can facilitate drug design and inhibitor design.

For example what are the effects of the enzyme inhibition? The antibiotics, what do they do? They inhibit the enzymes by effecting bacterial metabolism. So they act on bacterial enzymes and you need to have inhibition of those enzymes. So they do not form infection for you.

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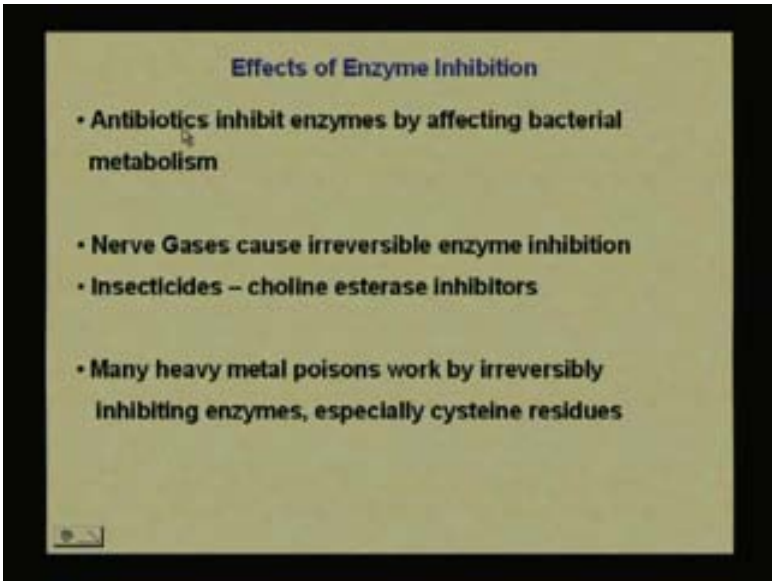

For example, if you look at Nerve gases, Nerve gases actually cause irreversible enzyme inhibition. Obviously you know they are extremely dangerous. This stop of particular in fact called as choline esterase only that transports your nerve signals. So that is lost the activity of the enzyme is completely lost. For insecticides we have choline esterase inhibitors, again from the name you can see that it acts on an ester. (Refer Slide Time 4:05 min)

Then many heavy metal poisons work irreversibly inhibiting enzymes, especially cysteine residues. So how do we study enzyme inhibition or what are the different types of enzyme inhibitions? Actually there are two different types. We have a reversible inhibition. In reversible inhibition as the name implies the inhibitor can bind and dissociate from the enzyme. And usually in most cases the activity of the enzyme is

recovered on removal of the inhibitor. And the association is usually non covalent in nature as we studied earlier. So you just have a lose association of the enzyme and the inhibitor just like you have the enzyme and the substrate. (Refer Slide Time 5:10 min)

The reversible inhibition can be of three types. You can have Competitive inhibition, Noncompetitive inhibition and Uncompetitive inhibition. We will see how these different types are going to change the overall activity of the enzyme and how actually they occur.

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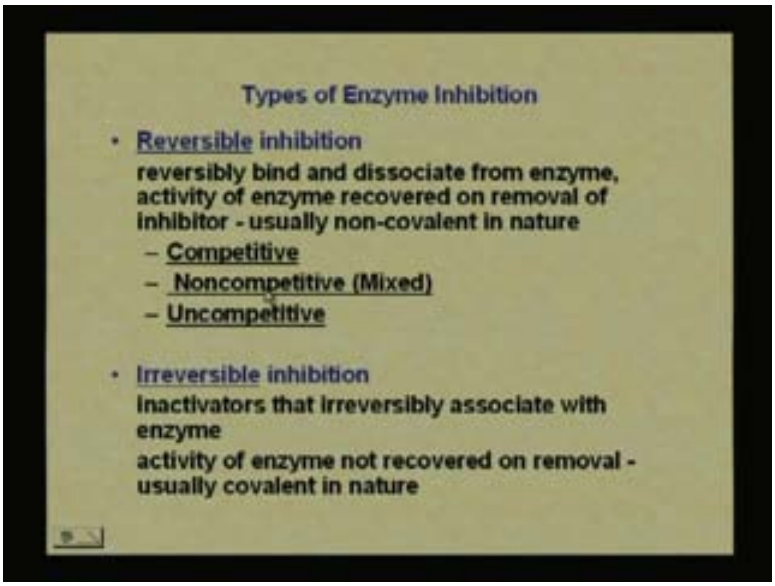

So these are three different types of reversible inhibition. Again the features of reversible inhibition are there is a reversible binding and dissociation from the enzyme and removal of an inhibitor, the activity of the enzyme is recovered and the association is non covalent in nature. Then we can have Irreversible inhibition. In this case what you are going to have is the inactivators of the inhibitors irreversibly associate with the enzyme by usually forming covalent bonds. You know it is extremely difficult to break a covalent bond once a covalent bond is formed. So there will be irreversible inhibition of the enzyme and the enzyme will be rendered in active that is not the case with reversible inhibition.

Because of the loose association once the inhibitor is removed the activity of the enzyme is recovered. But in the case of irreversible inhibition you have inactivators that will irreversibly associate with the enzyme and the activity of the enzyme is not recovered on removal, it is usually covalent in nature. So these are the two main different types that are reversible and irreversible inhibitions. Under reversible inhibition we have Competitive, Noncompetitive and Uncompetitive. What is competitive inhibition? Competitive inhibition is basically competition. We have a competition where the substrate or rather the inhibitor looks like the substrate. So it is called a substrate mimic or a substrate analogue.

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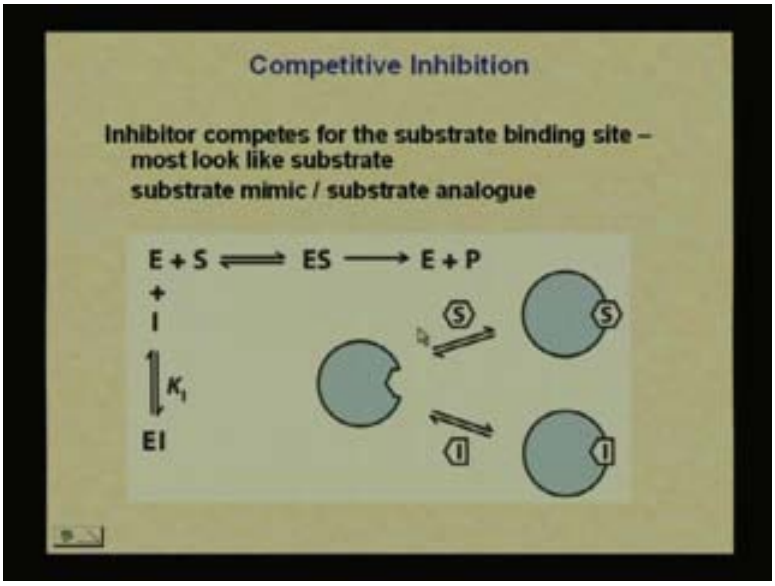

So the inhibitor competes for the substrate binding site. Now why does it do that? You have to remember what we discussed about active sites of enzymes they have a particular shape. What is this particular shape come from? This particular cleft means it has particular residues in that. These specific residues are there for a specific reason. So it is going to bind to a particular region of the substrate. (Refer Slide Time 07:56 min)

Now the inhibitor has the same types of moieties here that are going to bind to that enzyme active site. So what you essentially have is instead of having just an enzyme-substrate complex in addition you can also have an enzyme inhibitor complex. So the inhibitor competes for this site, it competes for the substrate binding site and obviously therefore it must also look like the substrate. if it has to compete form the same site since the active site is so specific it has to look like the substrate which is why it is also called a the substrate mimic or a substrate analogue. So if you want to design an inhibitor that is going to act on this particular active site of this enzyme you have to design something that looks like the substrate.

so that what you do is you have the enzyme fool the enzyme basically into believing that it is actually binding the substrate but it is binding the inhibitor. And what are you doing is you are decreasing the activity in a sense of the enzyme. So what do we have in our enzyme kinetic set is we have E + S go to the ES which goes to the E + P. So this is our normal reaction. Now here we have a normal reaction but I have an inhibitor. (Refer Slide Time 09:22 min)

What sort of an inhibitor? It is a competitive inhibitor. So the competitive inhibitor is going to interact with the enzyme in an equilibrium that is an inhibition constant that I get here forming an enzyme inhibitor complex. Since the enzyme inhibitor complex is in equilibrium with the enzyme and the inhibitor and I know it is a loose association. So I have an enzyme plus the inhibitor form an enzyme inhibitor complex.

Now what is going to happen in my normal kinetic set is this is what I get. If I have the inhibitor then how is this going to change? It is going to change in a fashion that since here I have an equilibrium the enzyme inhibitor complex also dissociates to the enzyme and the inhibitor. So again once the enzyme is available the substrate can form the complex with enzyme substrate. (Refer Slide Time 10:54 min)

This is my V_{max}, if I increase the substrate concentration to a large enough value then the enzyme-substrate complex can form because the enzyme and inhibiter actually has in equilibrium. This is a loose association so I can have free enzyme dissociated from the inhibitor. So I add a large amount of substrate I can have the enzyme actually reach the same value of V_{max}. What is my V_{max}? It is my k_2 total enzyme concentration. My total enzyme concentration is fixed. If my total enzyme concentration is fixed then this enzyme part of it may have formed an enzyme inhibitor complex but that enzyme inhibitor complex can also dissociate to form back E.

Now the total enzyme is actually the free enzyme plus enzyme-substrate complex plus enzyme inhibitor complex. So that is my total enzyme but since here we have an equilibrium and what can happen is I will not start of with the same velocity when I had no inhibitor so this is minus inhibitor. (Refer Slide Time 12:58 min)

If I had an inhibitor then what will happen to the velocity of the reaction? Part of the enzyme is going for the enzyme inhibitor so this is going to be flatter because the velocity is going to be less, it is decreased. So once I have a decrease then it is going to be this but it is eventually going to reach V_{max} why because the total enzyme is the same. If the total enzyme is same then this V_{max} at some point in time all the enzyme will actually be able to fall first. The V_{max} is the same because the total enzyme available is the same. (Refer Slide Time 13:43 min) That is extremely important. Something that you have to understand to know what is happening to V_{max}.

so in competitive inhibition, when my inhibitor is competing for the same site that the substrate is then the initial velocity with the inhibitor is going to be less than the initial velocity without the inhibitor. My V_{max} is going to be the exactly the same because the available total enzyme is the same. Then what is going to happen to K_M? What is my K_M? Now I go to half of V_{max} which is approximately this point here.

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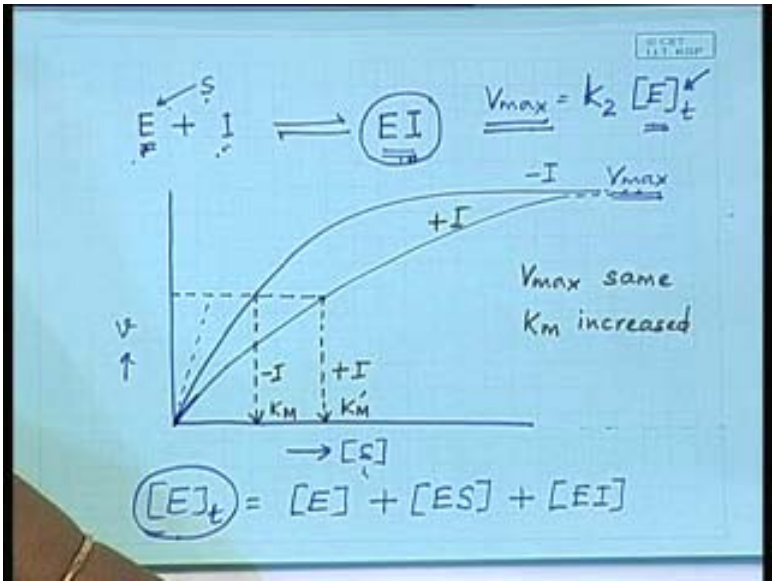

So this is my K_M value without the inhibitor, this is my K_M value, –I this is +I. So what is happened to my K_M? It has increased. So I put K'_M. So my V_{max} is same, K_M increased for competitive inhibition of the enzyme. Now we go back to the slides. For example, this is a very specific example that I have here. This is an example of succinate dehydrogenase. What does dehydrogenase do? The name implies that it is it is going to dehydrogenate succinate. So here is succinate and here are the hydrogens then these are going to form double bonds. If I tell you that that there is a specific enzyme called succinate dehydrogenase and obviously it will works on only succinate and here it removes the hydrogens to form a double bond. It does not work for malonate. What is the difference is just this CH_2. So this succinate dehydrogenase active site is just enough to fit this. It does not fit this it is that specific, each enzyme is this specific. (Refer Slide Time 16:34 min)

So you could have Competitive inhibition when we speak about it. So this could act as an inhibitor for succinate dehydrogenase. It is not going to be any reaction it is just going to sit their property.

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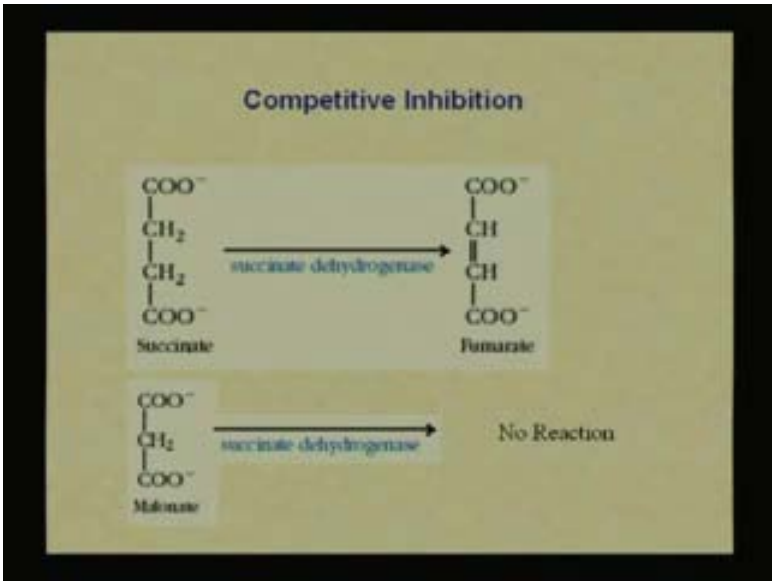

Then I am getting back to kinetics. This is what I was talking about. There is no reaction with the enzyme inhibitor complex why is that in competitive inhibition because it is already occupied the site that it is sitting in the same site.

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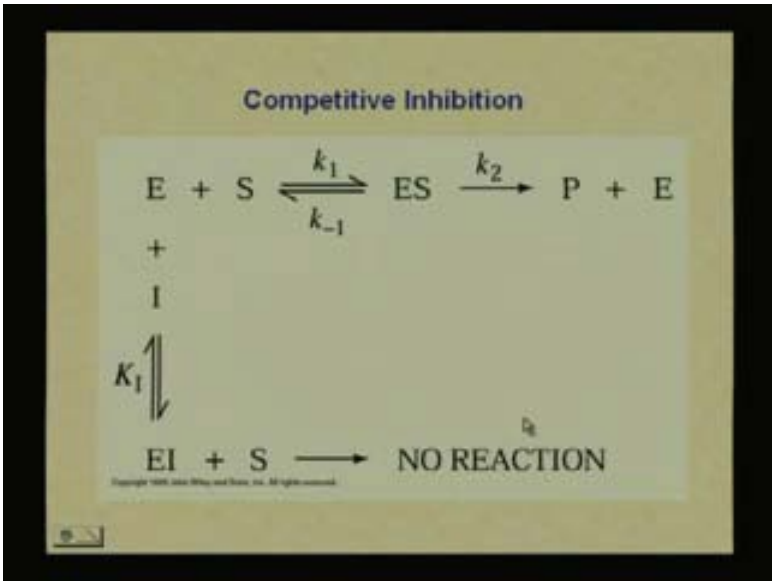

So for competitive inhibition if the enzyme or the inhibitory sitting in the same site that the substrate is sitting. For example, you are sitting in a chair then some body else cannot occupy the same chair as simple as that. So the inhibitor is sitting in the pocket where substrate is supposed to be. So that is exactly why it is acting as an inhibitor.

We have our overall kinetics with the k_1, k_{-1} and k_2 values that actually looks like this. So you can also work out the kinetics or Michaelis-Menten kinetics for this expression because here we have considered an additional K_I and that is going to change the whole expression for the kinetics. (Refer Slide Time 17:52 min). So what are the features of competitive inhibition? There is reversible binding of I to E. what is the K_I? It is the [E][I] to the [EI] complex.

So what complex I am talking about, It is this an association or a dissociation? I am saying that K_I = [E][I] / [EI], the dissociation. So we have [EI] ↔ [E] + [I]. So what is my K_I? It is product of this and this. the unit for K_I a molar unit. (Refer Slide Time 18:54 min). What are the features do we have? The amount of enzyme available for binding to substrate is less because the inhibitor has taken the some of the enzyme but effectively the total enzyme is same.

Now what we have to look at is the expression for V. how does that change? The K_M value has α associated with them. The K_M value is not K_M anymore it is alpha αK_M because the K_M is being modified and we saw how it is being modified. The V_{max} is same. The V_{max} does not change because the total enzyme is same. this α is given by this expression it is one plus the inhibitor concentration divided by K_I, the α is always greater than one which means the modified K_M in the presence of the inhibitor is always greater than the K_M value for uninhibited enzyme. So we have an enzyme reaction with no inhibitor and an enzyme reaction with inhibitor. We have a modified K_M value that is larger for competitive inhibition.

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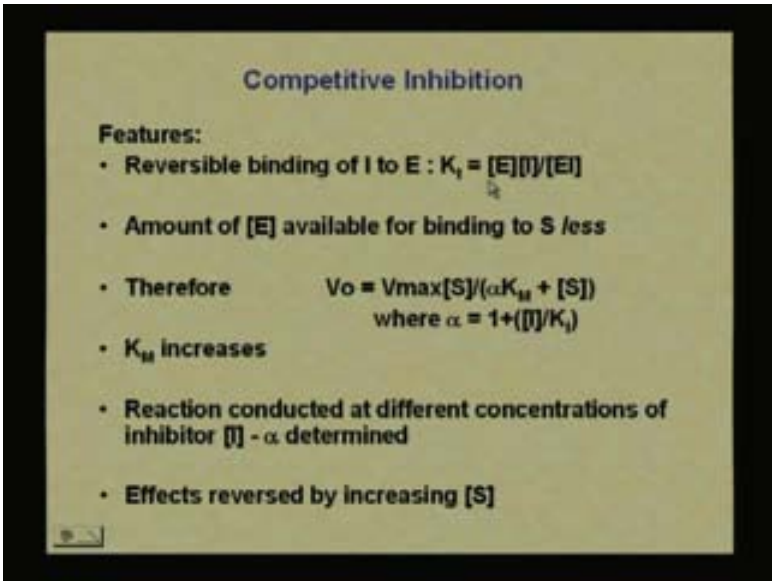

So we have an increase in the K_M. The reactions conducted at different concentrations of inhibitor and I can find out α and we will see how we can do that.

So these are the specific features. I have less amount of the enzyme available because some of the inhibitor as bound to the enzyme. I have a modified Michaelis constant because of the presence of the inhibitor which is going to keep the V_{max} same but my K_M is going to change. And I can determine the value of α. So how do I have to determine α? All I have to do is do the reaction. How did we do the original Michaelis-Menten? We just conducted a reaction at different substrate concentrations. Now I have to conduct my experiment at different inhibitor concentrations. (Refer Slide Time 21:42 min)

The effects are reversed by increasing the substrate amount. As soon as this equilibrium is going to release some amount of the enzyme. if I increase the substrate concentration to a larger value then the free enzyme can react with the substrate to form the normal products in the normal way it work. So this is what we have. This is the case the green line is with out the inhibitor, the normal Michaelis-Menten constant that you would see which eventually gets to V_{max}. With the inhibitor we have the slow reaction because some of the enzyme has been taken up by the inhibitor. And we know that if you want to calculate km I have to consider half of V_{max}.

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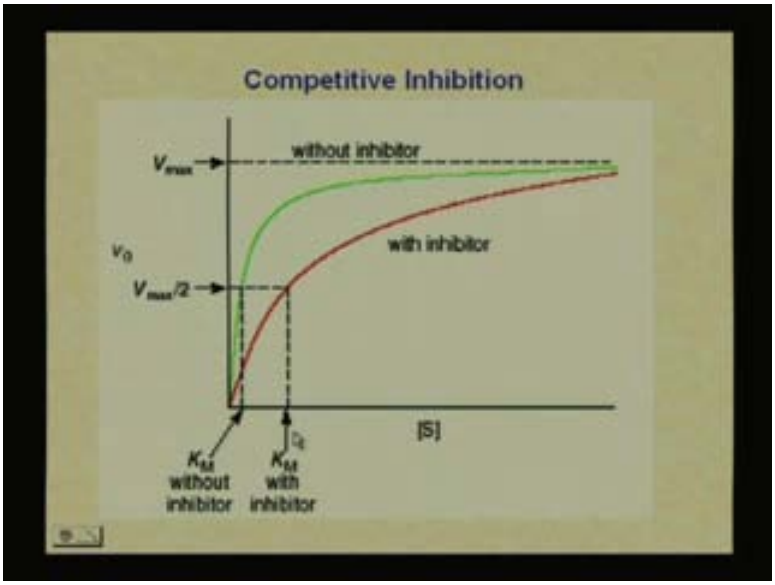

So this K_M corresponds to without the inhibitor and this K_M corresponds to with the inhibitor. Now how does this change our Lineweaver-Burke plot? What is the Lineweaver-Burke plot? We have a $1/V_0$ versus a 1/[S]. Now let us work on that. So now I have 1/V versus a 1/[S]. Here is 1/V and here is 1/[S]. What is this? No inhibitor. My y intercept is $1/V_{max}$. This is $-1/K_M$ now the slope increases but the V_{max} is the same. Now I have inhibitor, I have the same V_{max} $-1/K'_M$.

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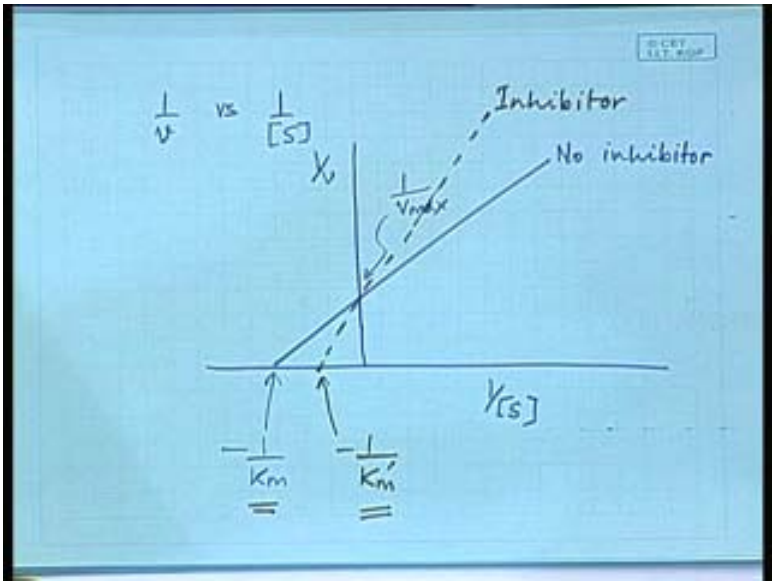

So I have no inhibitor in one case and in the next case I can determine my K_M values for the uninhibited enzymes and the K'_M value for the presence of the inhibitor and I can also find out what V_{max} is. So let us get back here, which is exactly what we have. So we have the uninhibited enzyme plus the competitive inhibitor.

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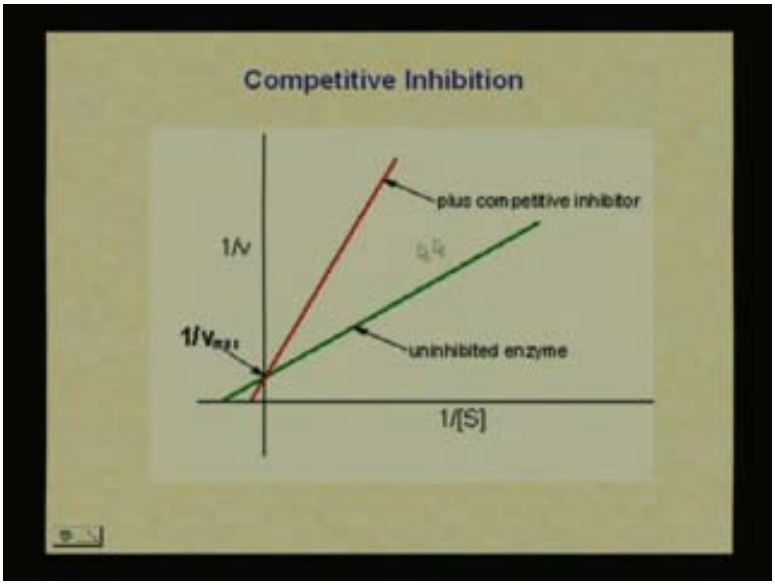

This is $1/V_{max}$ and we know that what these two values are. They are the Michaelis constants for the different sets of reactions that we have. So that is competitive inhibition.

Remember that this α that we spoke about was 1 + [I]/K_I. So when α is one it means the concentration of I is zero.

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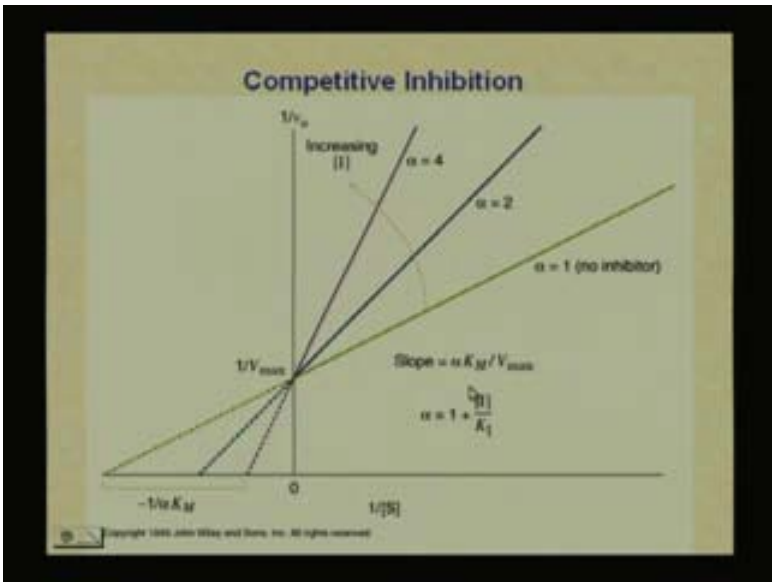

The concentration of I is zero means that I have an uninhibited reaction. So my α is one which essentially means that my K_M is what I would have in a normal Lineweaver-Burke plot. So here I have no inhibitor, this is my original -1/ K_M. We increase the slope with inhibitor.

Now if I add more of the inhibitor the slope is going to increase even further because my K_M is going increase even more. We did this just for one set but if you do this for a series of sets then you have to remember that your V_{max} remains the same. So the intersection or the intercept on the y-axis is going to be same but you are going to get increasing slopes with increasing inhibitor concentrations. And if you know the inhibitor concentrations then you can actually work out what α is because you know the modified αK_M. Then from the inhibitor concentration you can find out the value of K_I. (Refer Slide Time 27:05 min)

If I know what the inhibitor concentration is in each case then I can find out what α is. Then from the α I can find out what K_I is. So usually this experiment is done for increasing or different concentrations of inhibitor. And you get steeper and steeper curves for competitive inhibition. So as soon as you look at graph like this which is the Lineweaver-Burke plot for competitive inhibition.

Now we are going to look at a different type of inhibitions. So what are the features of competitive inhibition? The features of competitive inhibition are that the enzyme is or the substrate and enzyme complex is formed the inhibition is competing for the same site. In the event of this the V_{max} is the same the maximal velocity can be same because the total enzyme concentration is same. (Refer Slide Time 28:12 min)

What about the Lineweaver-Burke plot for the inhibitor? It is going to have a steeper slope because we are going to have increased K_M. So that completes competitive inhibition. This is a type of reversible inhibition. Now we do noncompetitive inhibition. Here the difference is the inhibitor not only binds the enzyme but also binds to the enzyme substrate complex. So it is not competing with the same site. It is competing or rather it is binding to a different site.

So let us look at this picture here we have the enzyme and we have the substrate. Then we form the enzyme substrate complex.

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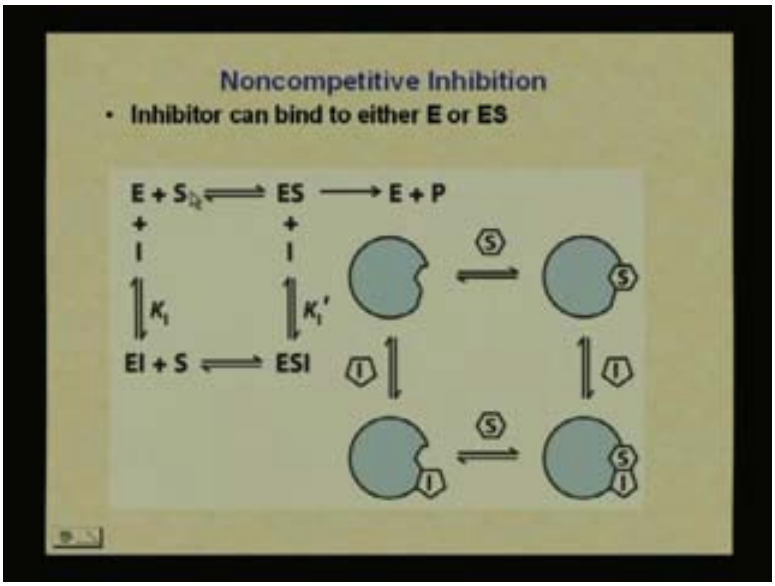

This enzyme substrate complex can bind the inhibitor and so can in the enzyme itself. In competitive inhibition we did not have this extra part here. So we do not have a ternary complex in competitive inhibition.

But in noncompetitive inhibition what does this do? It makes, basically what it is doing is it effects the formation of the product. The inhibition of a reaction is going to effect the formation of the product. So it is going to effect how the enzyme or how the substrate is eventually going to the product because you should have the enzyme substrate complex that is going to dissociate to form the product. So neither will the EI complex will give you the product nor will ESI complex will give you the product. It is only the ES going to give you the product. (Refer Slide Time 30:04 min)

We only have to have this complex that is going to give us the product. This is not going to give us the product and this is definitely not going to give us the product because the inhibitor sits in such a way or acts in active site in such a manner that the catalytic reaction cannot take place.

The catalytic reaction takes place only if the active site of the enzyme is bound to the substrate in this manner only. The inhibitor sits here so basically it prevents the substrate or it prevents product formation. So the feature of noncompetitive inhibition is that the inhibitor can bind to either the enzyme or the enzyme substrate and it is only enzyme-substrate complex that eventually will form the product. This is what we have. We have EI, again we have this and this ESI is also not going to give any reaction.

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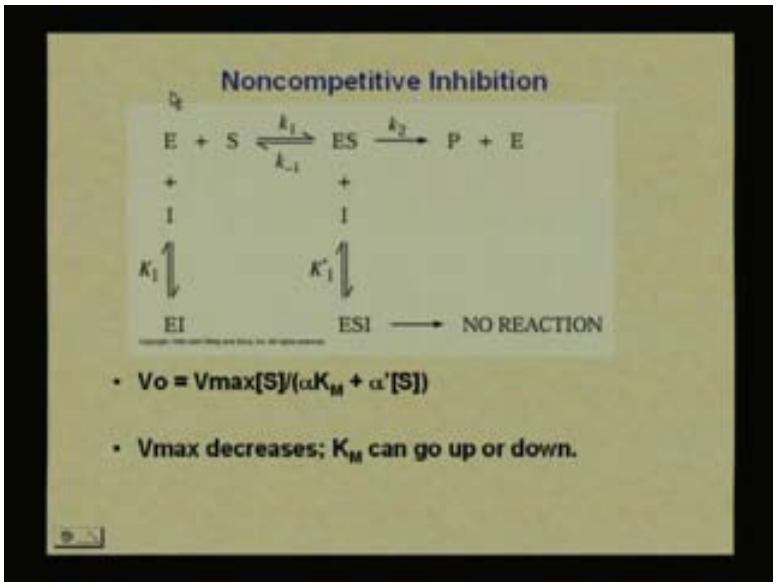

It is only the ES that is capable of giving us the reaction. Now what we have is we have a decrease in the V_{max}. Why do we have a decrease in the V_{max}? Because what is happening is all of the enzyme is not available for product formation. Initially for the competitive reaction what happened was the total enzyme was available. If the total enzyme was available at some point I can form the product but if I have inactivated the enzyme in a fashion that it is not available for formation then I will have a V_{max} decreased. What does that look like? This is what is going to look like. The K_M usually is the same. What we have is we have an uninhibited enzyme and we have a noncompetitive inhibitor.

What we are talking is in terms of our substrate concentration and our velocity. In a normal reaction we have this so this is our V_{max} and we can find our initial velocity. When I have an inhibitor the start is always going to be slower, obviously you have an inhibition for the reaction so the start of the reaction going to be slower. In the competitive case what happen is it reaches the V_{max} because the total enzyme was available. in this case what actually happens is if this ternary complex formed some how then all the enzyme is never available for the total amount of reaction to go so you never reach this V_{max}.

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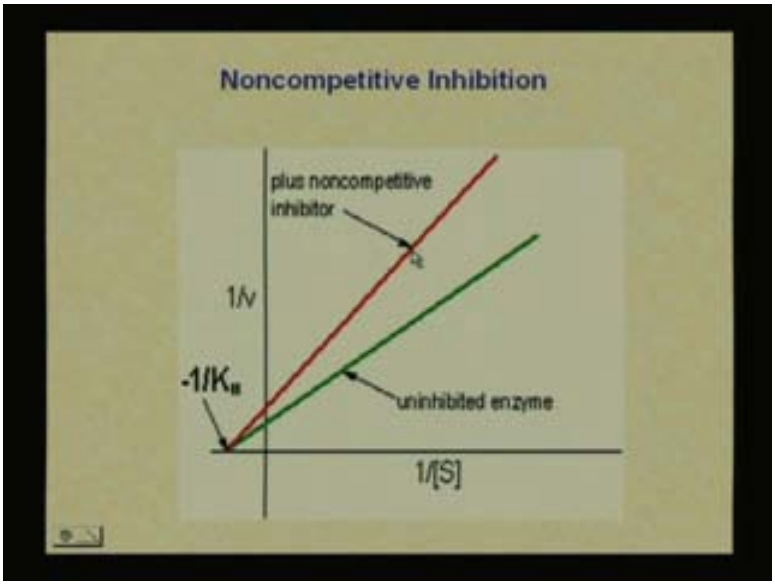

You reach a lower value here. So this is the V_{max} reached with the inhibitor. Now what happens is this V_{max} and this V_{max} and half of this V_{max} is going to be some where here, half of this V_{max} is going to be some where here. So in this case what happens is that the total of the V_{max} that could attained for uninhibited enzyme is not reached but the K_M value which is half of the V_{max} and half of this V_{max}. So this is where we have the inhibitor, this is where we have no inhibitor. So this is usually the same. (Refer Slide Time 34:57 min)

Now if I look at the Lineweaver-Burke plot what I am going to have is I have a uninhibited enzyme -I. I have an inhibited added with the same K_M. here again my intercept is $1/V_{max}$, here my intercept is -1/ K_M. My initial velocity is always going to increase because I have an inhibitor so my line is always going to be above the uninhibited enzyme.

Now my V_{max} has decreased. So what happens to $1/V_{max}$? It increases say some where here. And my K_M is same plus non competitive inhibitor. So I have a case with the V_{max} is same, the K_M is different for the competitive inhibition. I have K_M same the Vmax is different for noncompetitive inhibition. Here I have my noncompetitive inhibition why are the lines of inhibitor always above the uninhibited enzyme because the velocity or reaction velocity is always slower in the presence of an inhibitor. When I am plotting 1/V then it has to be high. (Refer Slide Time 37:07 min)

Now we will consider uncompetitive inhibition. What is uncompetitive inhibition? Uncompetitive inhibition does not react with the enzyme at all. There is no complex between the enzyme and this inhibitor. This inhibitor only reacts with the enzyme substrate complex so after the enzyme substrate complex is formed will this inhibitor act.

So you understand the distinction between each of the reversible inhibitors. In competitive inhibition the reaction was only with E, in noncompetitive inhibition the reaction is with E or ES and in uncompetitive inhibition it is only with ES. That is the difference between these.

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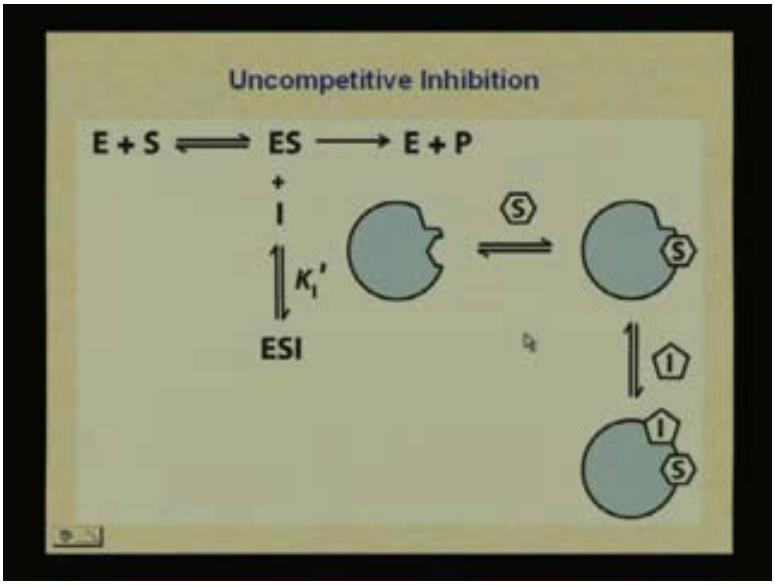

So we have E + S going to the ES complex which can add the inhibitor to it forming this ternary complex again and the ES dissociated to the E + P. so this is our enzyme, this is our substrate which sits here and we have the enzyme substrate inhibitor ternary complex look like this. But again it is the enzyme substrate that is going to form the product. So this is the basic difference between these.

So what happens is again this ternary complex or this ESI not going to give us any reaction. The active site is distorted after binding of the substrate, this usually occurs in the multisubstrate enzymes where you have more than one substrate actually formed or like the bisubstrate reaction that I showed you where you can act inhibitors there.

You have an active site that is distorted after binding of the substrate and it decreases both the K_M and the K_{cat} values. If the K_{cat} is decreased is then the V_{max} going to decrease and the K_M also effected and because the available enzyme again is decreased. What happens is even if you add more substrate nothing is going to happen. So what do our kinetic curves look like for uncompetitive inhibition?

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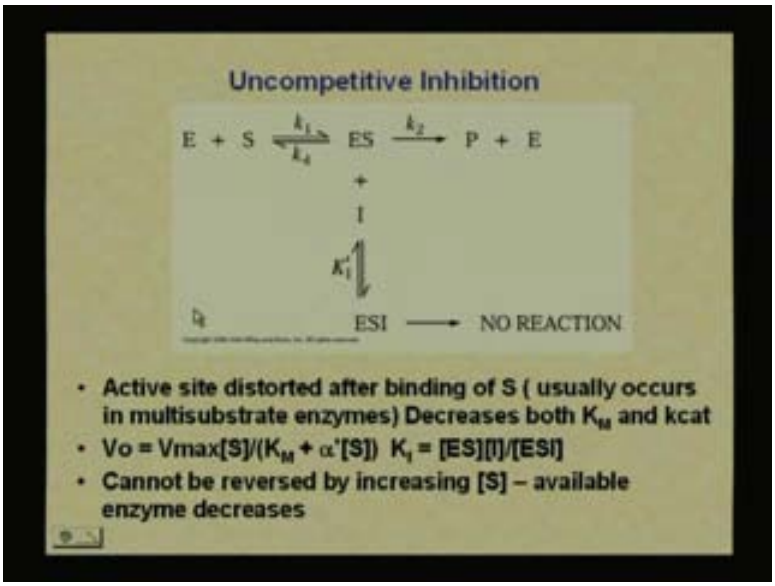

It is going to be a parallel line. First of all you know that this red line that is the line with the inhibitor is always above the uninhibited enzyme because now the reaction is slower.

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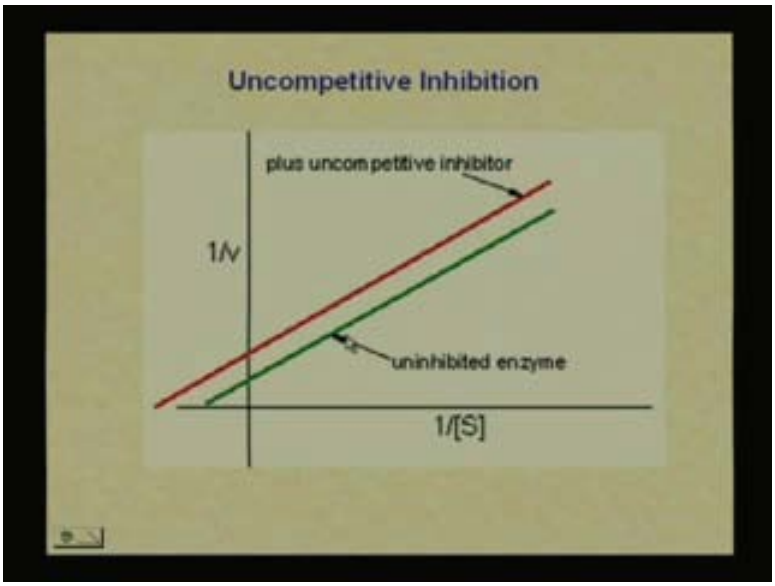

But what happens to our K_M? Let us go back to Michaelis-Menten, this is minus inhibitor, this is my initial velocity. This is my velocity, this is substrate concentration. what I am saying is this V_{max} will not be attained because the total enzyme is not available and the K_{cat} also decreases, the K_M also is effective but here the difference is that E + I does not take place, here there is no E + I, there is only ES + I to form ESI. Let us go back here, E + I does not happen here that happens only for non competitive inhibition.

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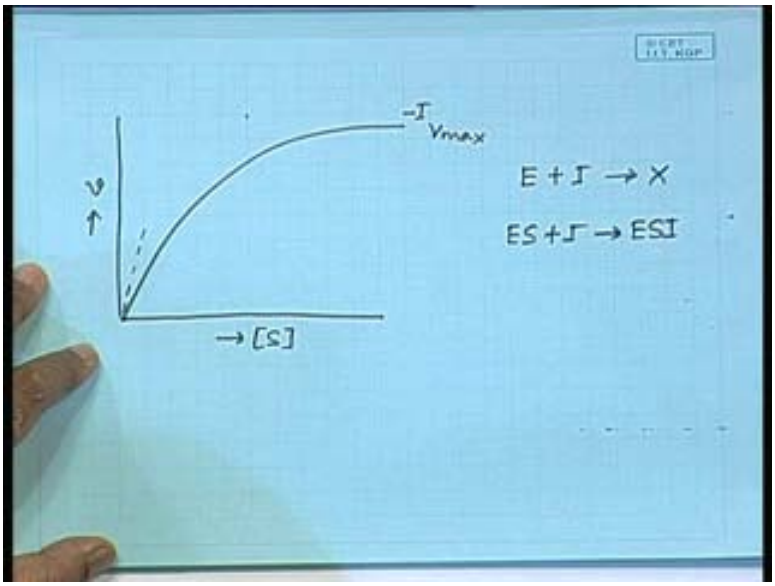

When we have ES here ES + I will give you the ternary complex ESI and there is no reaction. It usually has an effect on the K_{cat} value where your V_{max} is decreased. The decrease in the V_{max} is because the available enzyme actually is decreased. So what you have is you have an increase in the initial velocity due to the presence of the inhibitor and a parallel line for the uncompetitive inhibitor.

What happens for the competitive inhibitor? You have the same V_{max} so you had same intersection on the y-axis. For the non-competitive set so you had an intersection on the x-axis because the K_M was the same. In this case for the uncompetitive inhibition there is no such intersection at all you have only a parallel line with the uninhibited enzyme.

so when you increase the inhibitor concentration the first line corresponds to no inhibitor and each of the additional lines that you have here correspond to increasing concentrations of I. why do we have increasing concentrations of I here? Because this V_0 is going to be more for an inhibitor compared to one without inhibition. If you add more of the inhibitor then that is going to decrease the initial velocity even more. So you will have an even larger value for the V_0. If you get parallel lines for your inhibition then you know that the inhibitor is an uncompetitive inhibitor of the enzyme. If you get lines that have an intersection on the y-axis then it is competitive inhibitor.

If you have an intersection on the x-axis then it is noncompetitive inhibitor. (Refer Slide Time 44:04 min). Now if we go back to the problem that we were doing in the last class then I gave you a set of V_0 theV values and [S] values.

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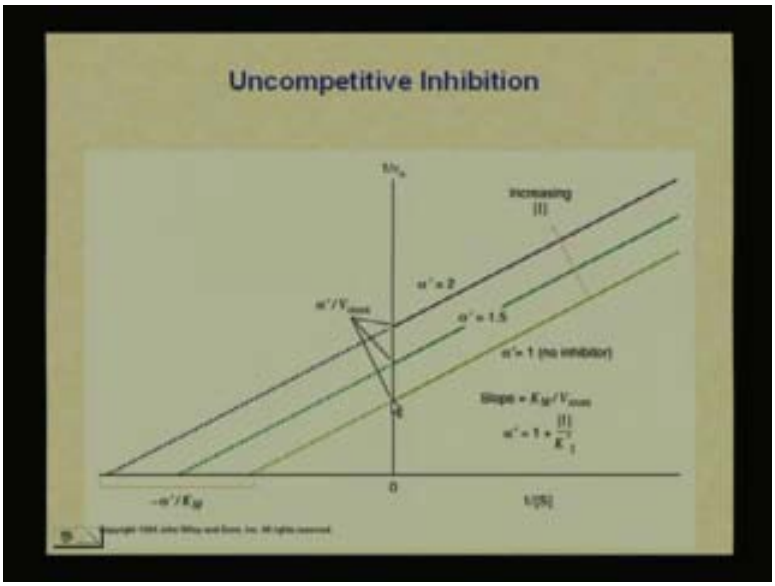

So we got a series of [S] values and a series of V values.

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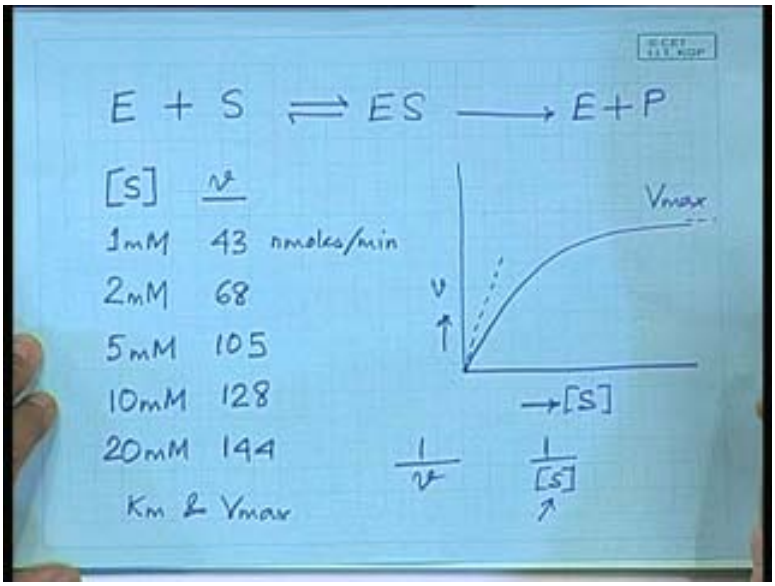

Now, suppose here we introduce an inhibitor so we have [S] values, we have V values for an uninhibited enzyme. If I put in an inhibitor [A] and an inhibitor [B] then I need the velocities for the presence of A and the velocities for the presence of B for the same

substrate concentrations. So say we had a value 1 2 5 10 20, the velocity for no inhibitor 43 68 105 128 144, with the inhibitor A we have 30 50 86 113 134 and for the inhibitor B we have 26 41 64 77 and 88. You can see how the initial velocities have decreased than the uninhibited enzyme. (Refer Slide Time 46:20 min)

Now what we are going to do is we are going to plot the Lineweaver-Burke plot for this case. So if I have these S concentrations then what information do I require to plot my Lineweaver-Burke plot is 1/[S], 1/V, $1/V_A$ and $1/V_B$. From this plot we can determine what type of inhibition we can have. So I am going get a plot like this for 1/[S].

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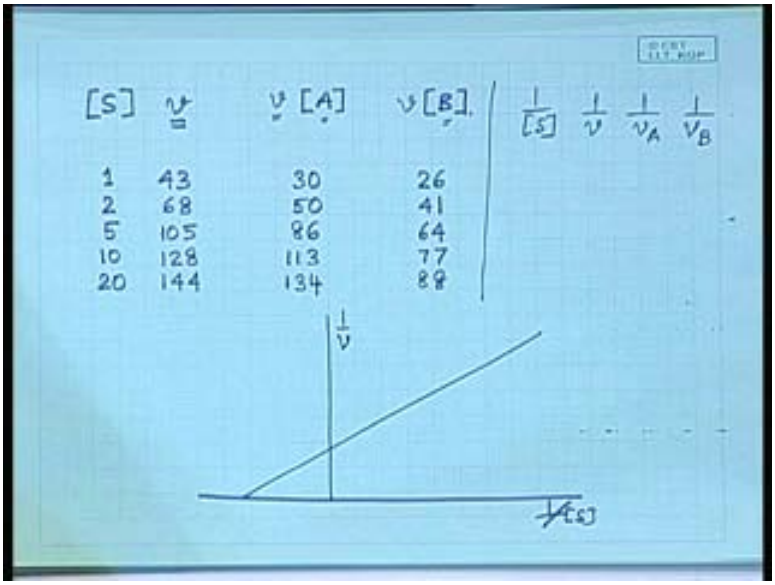

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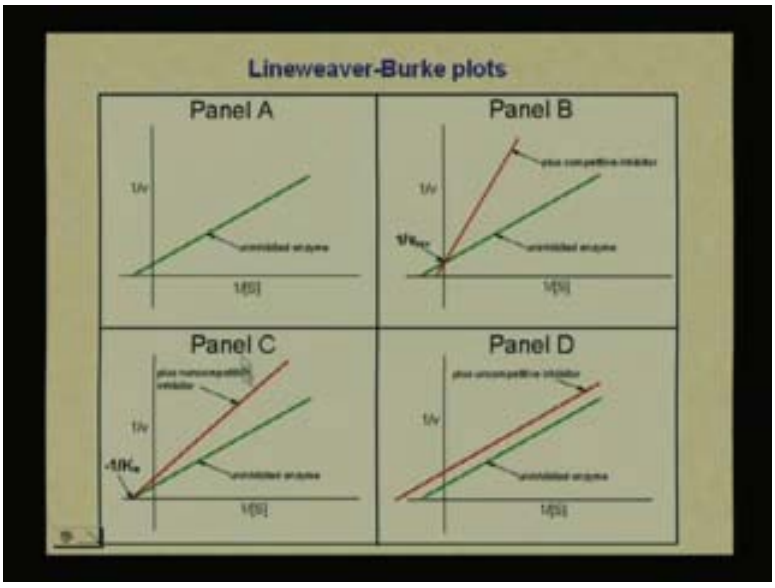

This is the Lineweaver-Burke plot. So straight away from the Lineweaver-Burke plot you can say whether you have an uninhibited enzyme. What is this? A competitive inhibitor, what is this? A noncompetitive inhibitor, what is this? a uncompetitive inhibitor. Here the inhibitor is reacting with the enzyme only. Where is the inhibitor reacting here? Here the inhibitor is reacting on the enzyme and the enzyme substrate. Where is the inhibitor reacting here? Here on the enzyme substrate only. So that is basically the difference.

And one other thing that before we get back to our problem called feedback inhibition. This is something that extremely interesting that happens in our body all the times. Suppose we have a reaction say A→ B→ C. now each of these arrows corresponds to an enzymatic reaction. so there is a particular enzyme acting for the transformation of A to B, another enzyme from B to C and another enzyme from C to D and so on.

So say here we are going from A to Z what happens is this Z usually is an inhibitor for this enzyme that transforms A to B. Here we have particular series of enzymatic steps. the series of enzymatic steps say goes from A to B to C to D and so on and so forth and finally say it has these one two three four five steps, in the fifth step Z is formed. The Z is obviously different from A and it is gone through all these enzymatic steps.

In fact this happens to a particular amino acid from Threonine to Isoleucine. This is the form of amino acid biosynthesis. So actually we have one amino acid form another amino acid. (Refer Slide Time 49:49 min). So this Z is actually inhibits the enzyme that catalyzes the reaction of A to B, this is called feedback inhibition. So essentially if you look at it what is it doing is it is actually blocking its own formation, it is blocking a reaction that is forming itself.

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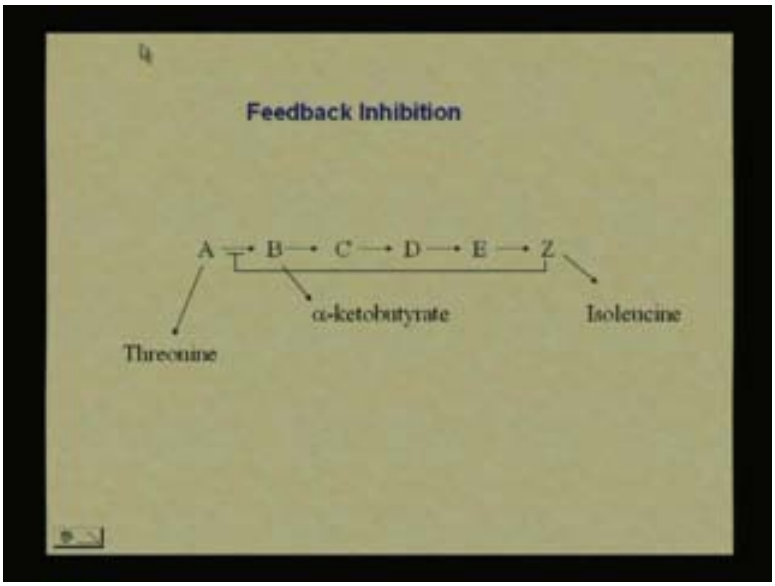

Now this is extremely important in regulation because you do not want a large amount of Z to be formed in the body. Z is being formed by A. so here if we have the A forming B then forming C to D to E and so on then our Z inhibiting this first step means that unnecessarily B C D E are not going to be formed, it does not make sense for them to be formed. So this is exactly what happens. We have Threonine, we have α-ketobutyrate that is formed here and we have Isoleucine. Isoleucine actually blocks this enzyme so that Isoleucine is not formed. But if you think of it is a regulation you do not want too much Isoleucine in the body you do not want even too much Threonine in the body. So there may be some other reaction that inhibits this. So this is exactly what is happening.

We have the Threonine. The Threonine sits in the active site of Threonine deaminease and we have intermediate A B C D finally forming to our Z. Now what does this Z do? In a feedback inhibition process it sits or it inhibits this enzyme in such a manner that it does not allow the Threonine to bind anymore. This is called a halosteric site because it is regulating the reaction. It will not allowing any Threonine to bind, it is inhibiting its own formation. This is called feedback inhibition. (Refer Slide Time 52:08 min)

So if we go back to our slides we will find out that when we plot this data we will get one that looks like this and one that looks like that. So you can work it out and figure out this is minus inhibitor. So what else do you need is actually you need the concentration of A to find out the K_I value. The concentration of A is 5 millimolar, the concentration of B is 0.1 millimolar so you can find the K_I value of A and you can find the K_I value of B because you are going to get modified K_M values or you are going to get a modified V_{max} value. And from that you can determine not only the type of inhibitor you are going to have you have either a competitive inhibitor or a noncompetitive inhibitor.

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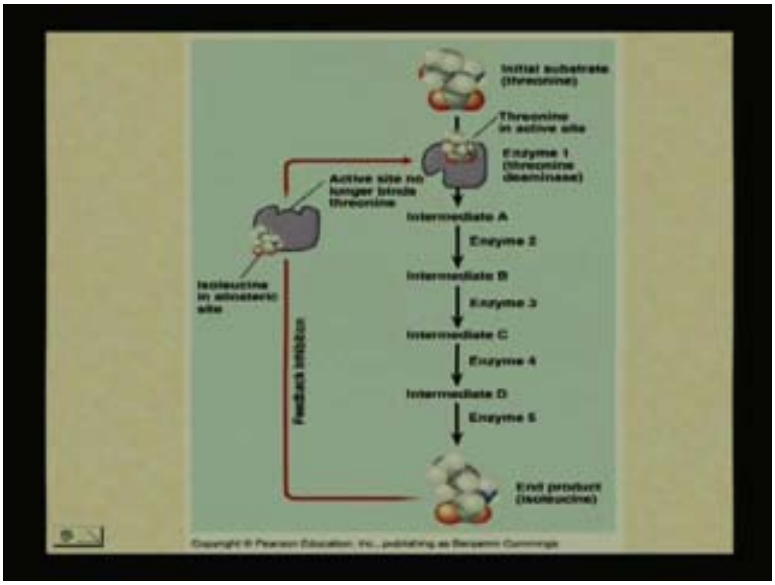

So this is going to be the competitive inhibitor, this is going to be the noncompetitive inhibitor. So we can determine from all these velocity sets what type of inhibition we have and also the inhibition or inhibitor constant of this specific type of inhibitor. So what we learnt in this class is basically enzyme inhibition, how the enzymes are inhibited by the different types of inhibitors. The different types of inhibitors that we can have are reversible or irreversible type. The irreversible means that they have a covalent interaction with the enzyme thus rendering inactive altogether.

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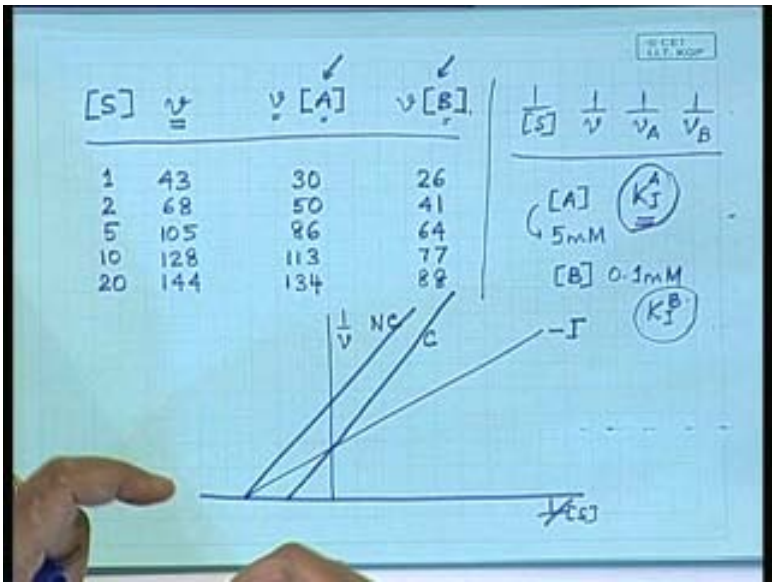

In the reversible type of inhibition we have enzyme interact with the inhibitor in three different ways. Then we have competitive inhibition where the inhibitor acts on the enzyme alone, we have noncompetitive inhibition where the enzyme interacts or the inhibitor interacts with the enzyme and the enzyme substrate complex and we have uncompetitive inhibition where it interacts with the enzyme substrate complex only. (Refer Slide Time 54:13 min)

Then we looked at different types of Lineweaver-Burke plots that we get for each case and how the V_{max}, K_M values change for each set of inhibition. Then we looked at feedback inhibition as to how a product of series of enzymatic reaction can actually inhibit the first step of its own formation and this is very important in regulation. In the next class we will look at specific enzyme mechanisms.
Thank you.