Biochemistry
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Lecture - 09
Enzymes III

In the last class, we spoke about enzyme kinetics and how we have specific active site of the enzyme and how we form the enzyme substrate complex.

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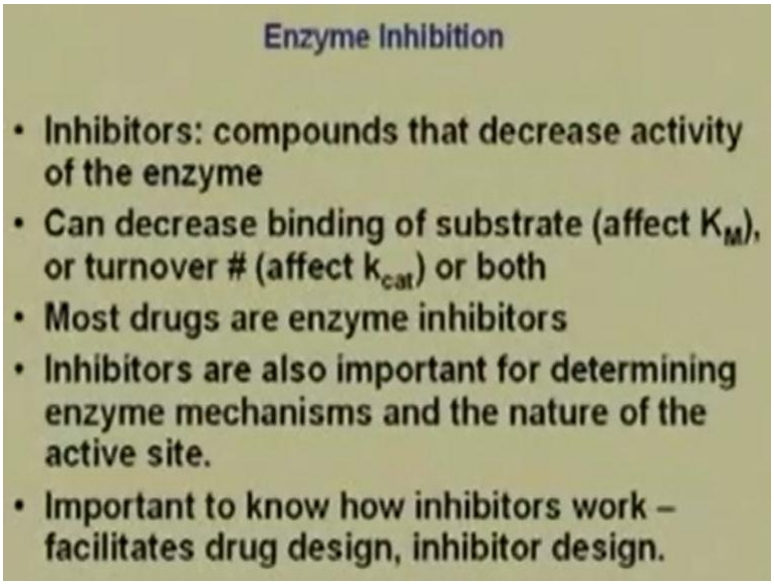

What we are going to speak about in this class is enzyme inhibition. Enzyme inhibition is a very important factor in determining drugs or specific compounds that are going to inhibit the action of definite or specific enzymes. So, apart from knowing the structure and once we know the function there are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

Now what we want to do is it means that we want to stop the particular reaction that the enzyme is catalyzing. So, these inhibitors are compounds that decrease the activity of the enzyme. Now 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 doing what? They can affect the Km value or the Kcat value or both of the values.

So, we have to decrease the binding of the substrate, if an inhibitor of an enzyme has to act So, we want to stop the enzyme from forming the enzyme substrate complex as simple as that. 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 enzyme So,mehow. Now most drugs are enzyme inhibitors. Because there is certain bodily function that goes wrong which is why you have a specific activity of an enzyme in the wrong fashion So, what would you want?

You would want So,me drug that is going to inhibit the enzymes So, it does not act in a fashion that it is acting right now. For example, if you have antibiotics what does it do? It inhibits enzymatic reactions of the bacteria that have form the infection. So,, inhibitors are also, use to determine enzyme mechanism and also, for the nature of the active site. Of course, it is extremely important to know how these inhibitors work because this can facilitate drug design and inhibitor design.

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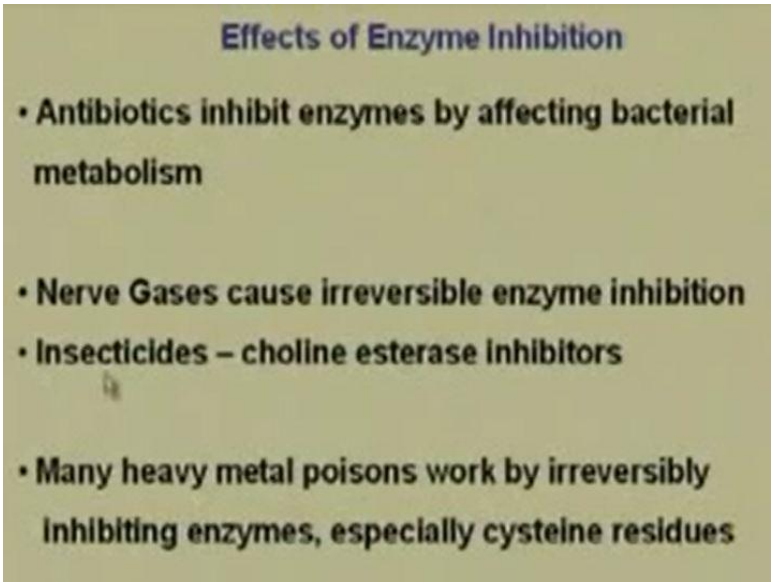

For example, what are the effects of enzyme inhibition? The antibiotics what do they? They inhibit the enzymes by affecting bacterial metabolism. Okay, So, they act on bacterial enzymes and So, what you need to know? You need to have inhibition of those enzymes So, they do not form infection for you for example if you look at nerve gases. Nerve gases actually cause irreversible enzyme inhibition. So, that obviously you know they are extremely dangerous.

They stop a particular in fact choline esterase only that transports your nerve signals okay, So, that is lost. The activity of the enzyme is completely lost. For insecticides, they are choline esterase inhibitors again from the name you can see that it acts on an ester. Then again many heavy metal poiSo,ns they work irreversibly inhibiting enzymes especially cysteine residues. So,, how do we study enzyme inhibition? Now what are the different types of enzyme inhibition?

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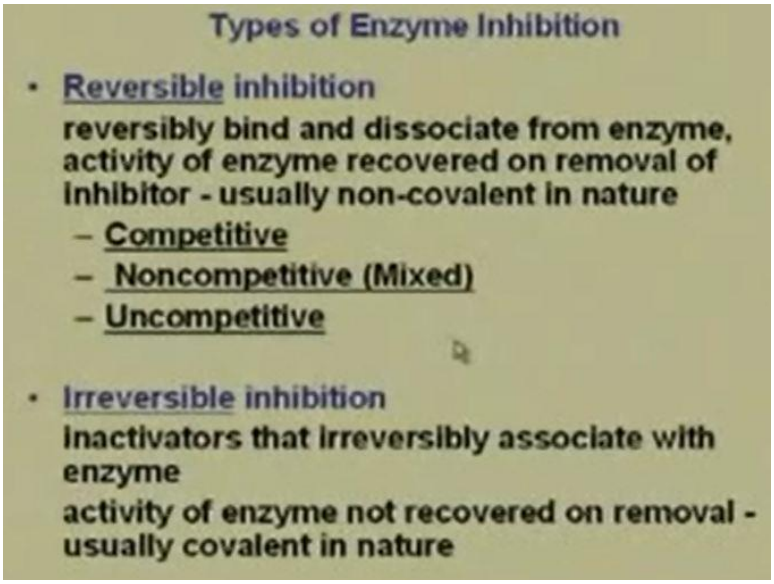

Actually, there are two different types. We have a reversible inhibition. Now reversible inhibition can be of three types. What are these? In reversible inhibition as the name implies the inhibitor can bind and disSo,ciate from the enzyme and usually in most cases, the activity of the enzyme is recovered on removal of the inhibitor and the asSo,ciation as we studied in the previous class is usually non-covalent in nature. You just have a lose asSo,ciation of the enzyme and the inhibitor just like you have the enzyme and the substrate.

The reversible inhibition can be three types. You can have competitive inhibition, noncompetitive inhibition and uncompetitive inhibition. We will see how these three different types are going to change the overall activity of the enzyme and how actually they occur? So, these are the three different types of reversible inhibition. The features of reversible inhibition again are there is a reversible binding and disSo,ciation from the enzyme and on removal of the inhibitor the activity of enzyme is recovered and the asSo,ciation is non-covalent in nature.

We can have irreversible inhibition in this case you understand that what you are going to have is the inactivators or the inhibitors in this case irreversibly asSo,ciate with the enzyme usually forming covalent bonds. So, once the covalent bond is formed you know it is extremely difficult to break a covalent bond, So, there will be irreversible inhibition of the enzyme and the enzyme will be rendered inactive that is not the case with reversible inhibition.

Once the inhibitor is removed, because you have a loose asSo,ciation once the inhibitor is removed the activity of the enzyme is recovered. But in the case of irreversible inhibition, you have inactivators that irreversibly asSo,ciate with the enzyme and the activity of the enzyme is not recovered on removal it is usually covalent in nature. The main two different types are reversible and irreversible and under-reversible inhibition we have competitive, noncompetitive and uncompetitive.

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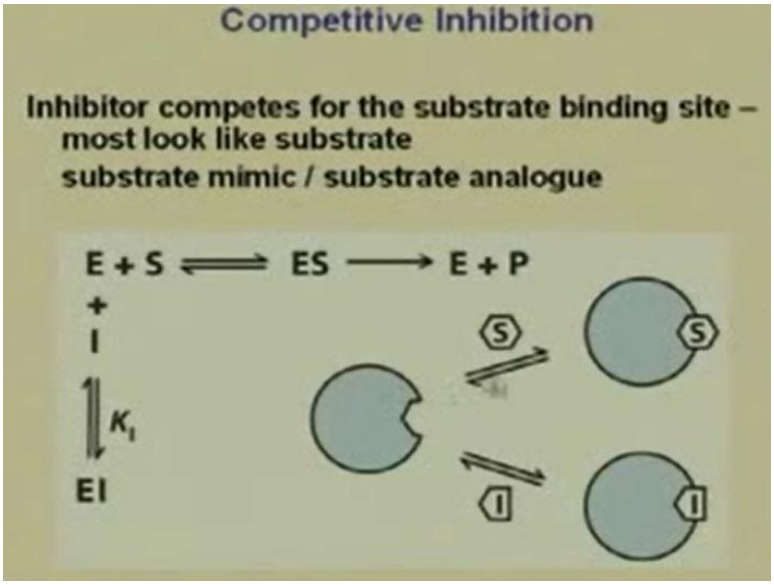

What is competitive inhibition? Competitive inhibition is competition basically. We have a competition where the substrate or rather the inhibitor looks like the substrate. So,, it is called a substrate mimic or a substrate analogue. So,, the inhibitor competes for the substrate binding site. Why does 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 such as particular residues in there. These specific residues are there for a specific reaSo,n, So, it is going to bind to a particular region of a substrate. 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.

The inhibitor competes for this site. It competes for the substrate binding site and obviously therefore it must look like substrate also, if it has to compete for the same site since the active site is So, specific, it has to look like the substrate which is why it is also, called as a substrate mimic or a substrate analogue. So, if you want to design say an inhibitor that is going to act on this particular active site of this enzyme, you have to design So,mething that looks like a substrate. So, that what do you do? You have to fool the enzyme basically in to believing that it is binding the substrate but it is binding the inhibitor.

What are you doing? You are decreasing the activity in a sense of the enzyme. So,, what do we have in our enzyme kinetic set. We have an E + S go to the ES which goes to the E + P So, this is a normal reaction. What we have here is a normal reaction but now I have an inhibitor what So,rt of an inhibitor a competitive inhibitor. 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. Now, since the enzyme inhibitor complex is in equilibrium with the enzyme and the inhibitor and I know it is a loose asSo,ciation.

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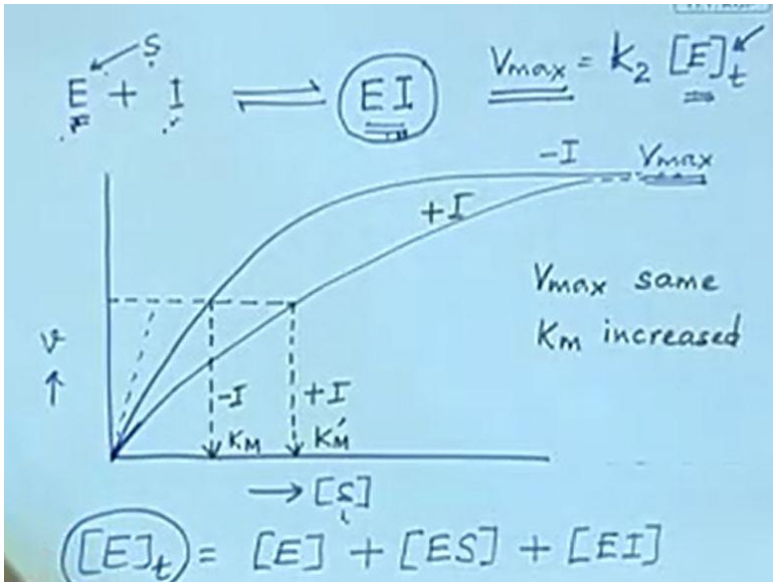

So,, I have an enzyme + the inhibitor form an enzyme inhibitor complex. Now what is going to happen in my normal kinetic set this is what I get right. If I have the inhibitor how is this going to change? It is going to change in a fashion that since I have an equilibrium here the enzyme inhibitor complex also, disSo,ciates to the enzyme and the inhibitor So, ones the enzyme is available again the substrate can form the complex with the enzyme substrate.

So,, this is my Vmax. If I increase the substrate concentration to a large enough value my enzyme substrate complex can then form. Why? Because my enzyme inhibitor actually has an equilibrium this is a loose asSo,ciation So, I can have a free enzyme disSo,ciated from the inhibitor So, if I add a large amount of substrate I can have the enzyme actually reach the same value of Vmax. What do I mean by that? What is my Vmax?

It is my k2 total enzyme concentration. My total enzyme concentration is fixed. Now 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, disSo,ciate to form back E. So, the total enzyme actually now is the free enzyme + enzyme substrate complex + enzyme inhibitor complex right.

So, that is my total enzyme but since we have an equilibrium here what can happen is I will not start off with the same velocity as I had when I had no inhibitor So, this is minus inhibitor. If I

had inhibitor what is going to 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 my velocity is going to be less it is decreased. So, once I have a decrease it is going to be this but it is eventually going to reach Vmax why because the total enzyme is the same.

If the total enzyme is the same this Vmax at So,me point in time all the enzyme will actually be able to of course the Vmax is the same because the total enzyme available is the same right. That is extremely important So,mething that you have to understand to know what is happening to Vmax. 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 Vmax is going to be exactly the same because the total enzyme available is the same. What is going to happen to my Km? What is my Km? I go to half of Vmax now which is approximately this point here. So,, this is my Km value without the inhibitor. This is my Km value this is minus I, this is + I. So,, what has happened to my Km it has increased So, I put a Km say prime for this So, my Vmax same. Km increased for what for competitive inhibition of the enzyme.

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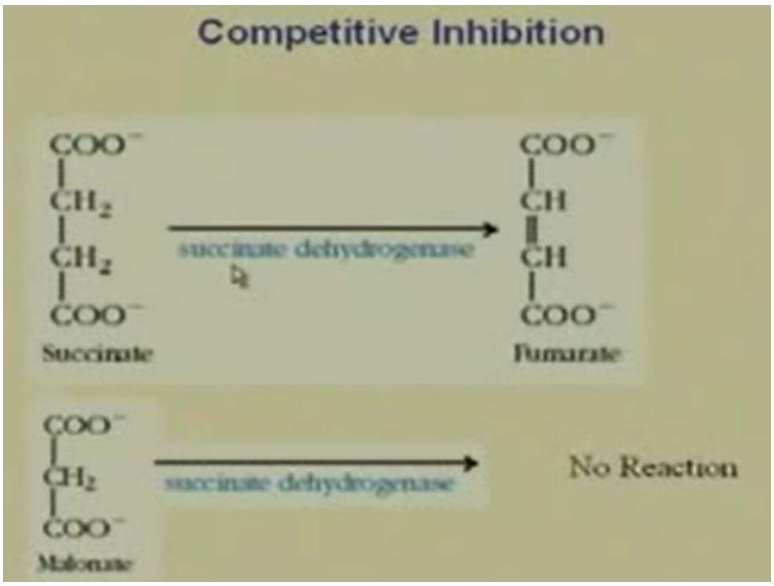

We go back to the slides now, I have this on the slide also, for example this is a very specific example that I have here. This is an example of succinate dehydrogenase. What does dehydrogenase do? The name implies that it is going to dehydrogenate succinate So, here is succinate here are the hydrogens is going to form the double bond. So,, if I tell you that there is a specific enzyme call succinate dehydrogenase and what reaction is it going to work on. Obviously, it works on only succinate and it removes the hydrogen to form a double bond here.

It does not work for Malonate. What is the difference? Just the CH2 So, the succinate dehydrogenase active site is just enough to fit this it does not fit this. It is that specific. Each enzyme is this specific. In competitive inhibition when we speak about it So, this could act as an inhibitor for succinate dehydrogenase is not going to be any reaction it is just going to sit there probably.

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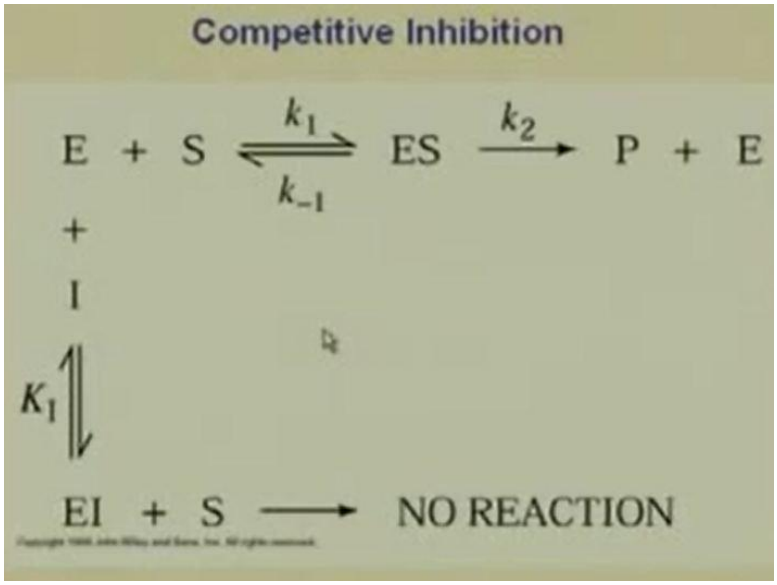

Getting back to our kinetics, what do we have? This is what I was talking about. There is no reaction with the enzyme inhibitor complex. Why is that in competitive inhibition? Because it is already occupied the site there it is sitting in the same site. So,, for competitive inhibition if the enzyme or the inhibitor is sitting in the same site that the substrate is sitting for example you are sitting in a chair So,mebody else cannot occupy the same chair as simple as that.

So,, the inhibitor is sitting in the enzyme pocket where the substrate is supposed to be So, that is exactly why it is acting as an inhibitor. So,, we have our overall kinetics with the k1 and the k-1 and k2 values that actually looks like this. You can also, work out kinetics or Michaelis-Menten kinetics for this expression okay. Because now we have an additional KI that we have to consider here anyway that is going to change or the whole expression for the kinetics.

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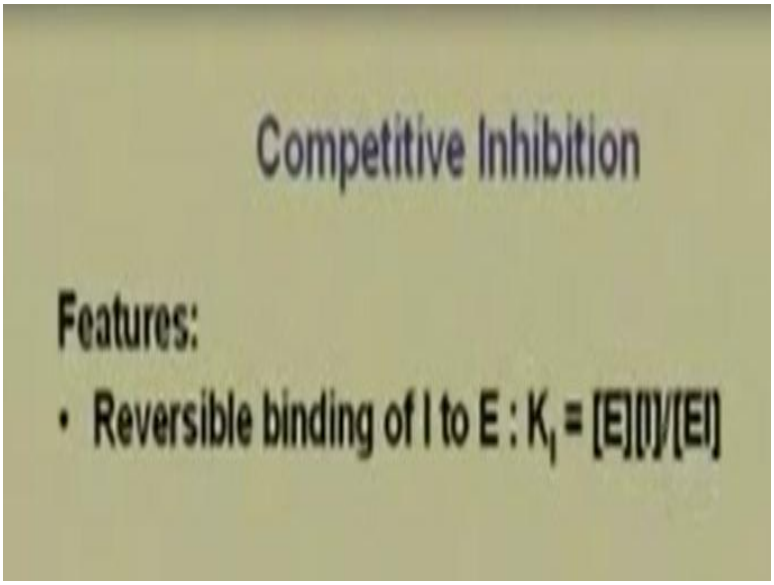

What are the features of competitive inhibition there is reversible binding of I to E what is the KI it is the EI and EI complex? So,, what complex am I talking about? Is this asSo,ciation or disSo,ciation?

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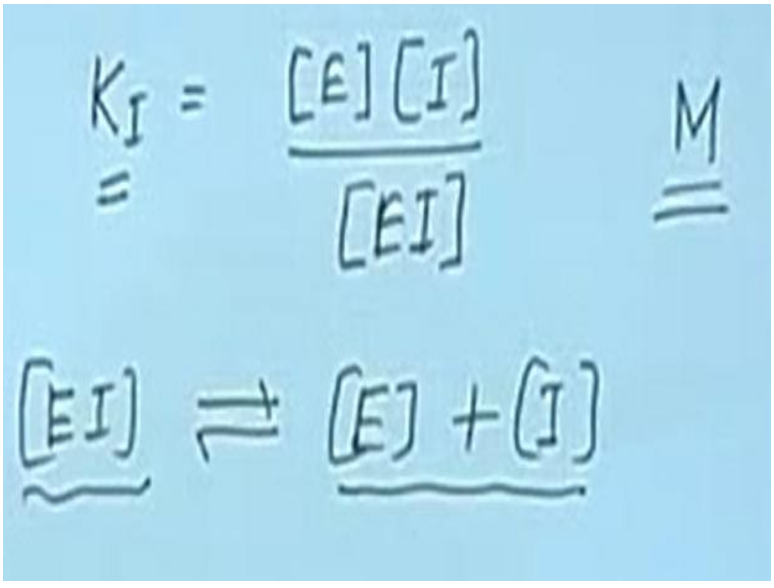

I am saying KI is equal to [E][I] by [EI] disSo,ciation. So,, my [EI] actually I do not have to put the concentration in the brackets in there. What is my KI? It is the product of this and this. What is the unity for KI a mole unit M right?

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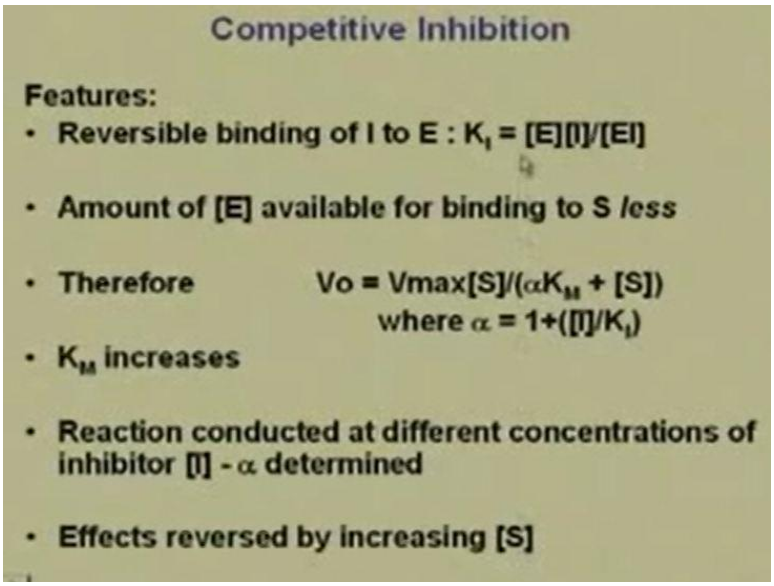

What are the features do we have? The amount of enzyme available for binding to substrate is less why because the inhibitor has taken So,me of the enzyme but effectively the total enzyme is the same. What we have to now look at is the expression for V. How does that change? The Km value has an alpha asSo,ciated with that now okay. The Km value is not as Km anymore it is alpha Km because the Km is being modified and we saw how it is being modified.

The Vmax is the same. The Vmax does not change why? Because the total enzyme is the same this alpha is given by this expression. It is one + the inhibitor concentration divided by KI the alpha is always greater than one. What does this mean? It means that the modified Km in the presence of the inhibitor is always greater than the value Km value for the uninhibited enzyme. So,, we have an enzyme reaction that has no inhibitor and an enzyme reaction with an inhibitor we have a modified Km value that is larger for competitive inhibition.

So,, we have an increase in the Km. The reaction conducted at different concentrations of inhibitor and I can find out alpha. We will see how we do that? So,, these are the specific features. I have the amount available of enzyme less because So,me of the inhibitor is bound to

the enzyme. I have a modified Michaelis constant because of the presence of the inhibitor which is going to keep the Vmax the same but my Km is going to change. I can determine the value of alpha which you we will be doing later on.

How do I have to determine alpha? All I have to do is do the reaction how did we do original Michaelis-Menten? We just conducted our reaction at different substrate concentrations. Now I have to conduct my experiment at different inhibitor concentrations and the effects reversed how by increasing the substrate amount. So, as So,on as this equilibrium is going to release So,me of the enzyme So, if I increase the substrate concentration to a large of value then the free enzyme can react with the substrate to form the normal products in the normal way it would.

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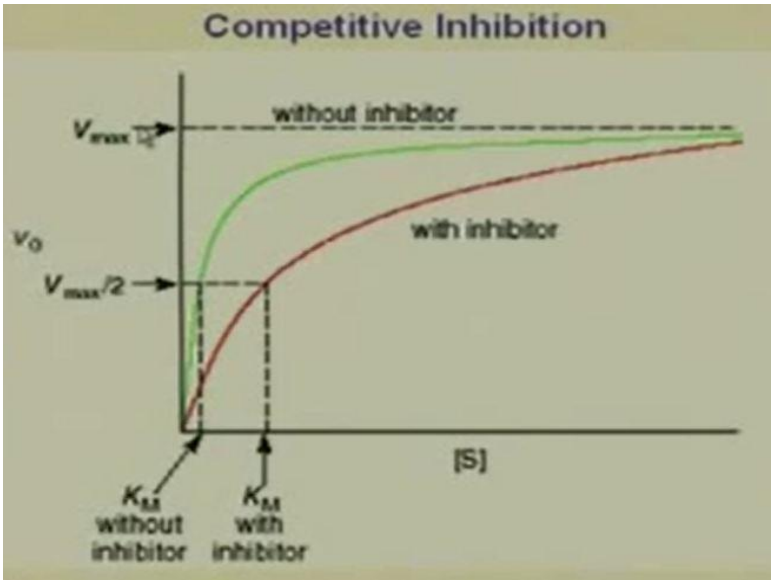

This is what we have. This is the case the green line is what? It is without the inhibitor the normal Michaelis-Menten constant that you would see or the normalize Michaelis-Menten kinetics that you would see which eventually gets to Vmax. With the inhibitor, we have a slow reaction because So,me of the enzyme has been taken up by the inhibitor and we know that if we want to calculate Km I have to consider half of Vmax So, this Vmax corresponds or this Km corresponds to what without the inhibitor and this Km corresponds to with the inhibitor. Now how does this chain change our Lineweaver-Burke plot? What is the Lineweaver-burke plot?

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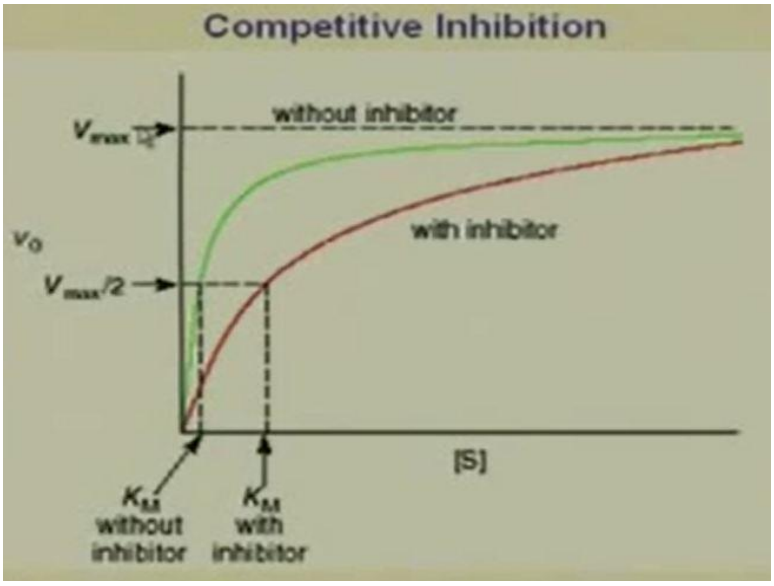

We have a one by V0 versus a one by [S]. Let us work on that now.

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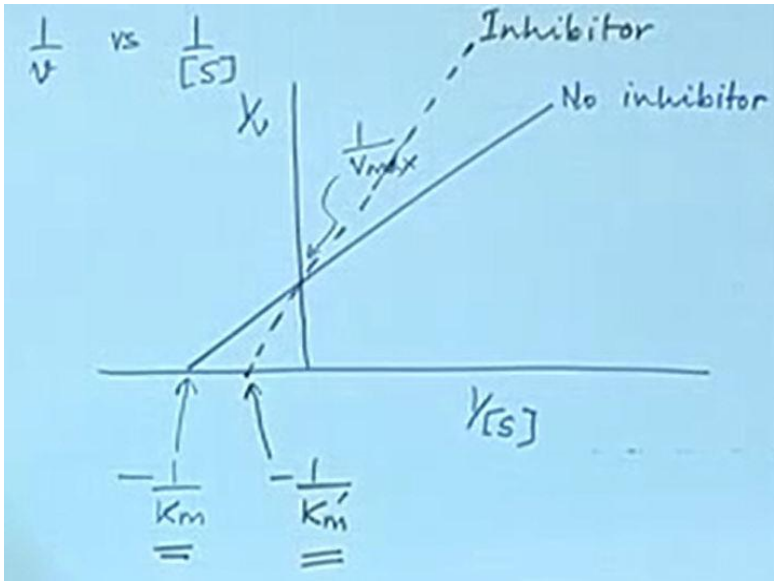

So, I have a one by V versus one by [S]. One by V, one by [S] what is this? No inhibitor. What is my Y intercept? One by Vmax. What is this? Minus one by Km. Now tell me, slope will but the Vmax is the same the slope increases but the Vmax is the same. So,, I have inhibitor now I have the same Vmax one minus Km prime. So,, I have no inhibitor in one case. In the next case So, what can I now determine? I can determine my Km values for the uninhibited enzyme and the Km prime value for the presence of the inhibitor and I can also, find out what Vmax is.

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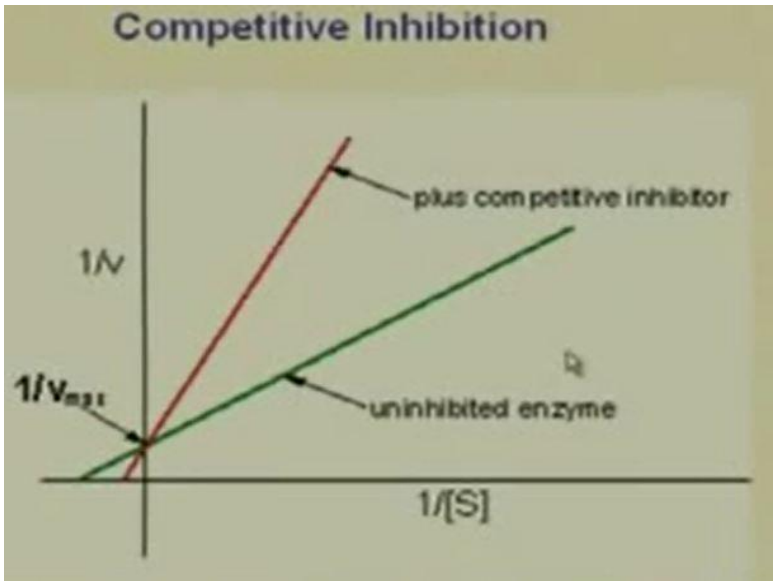

So,, let us get back here that is exactly what we have. So,, we have the uninhibited enzyme + the competitive inhibitor. This is one by Vmax and we know what these two values are now what are they? They are the Michaelis constant for the different sets of reactions that we have So, that is competitive inhibition.

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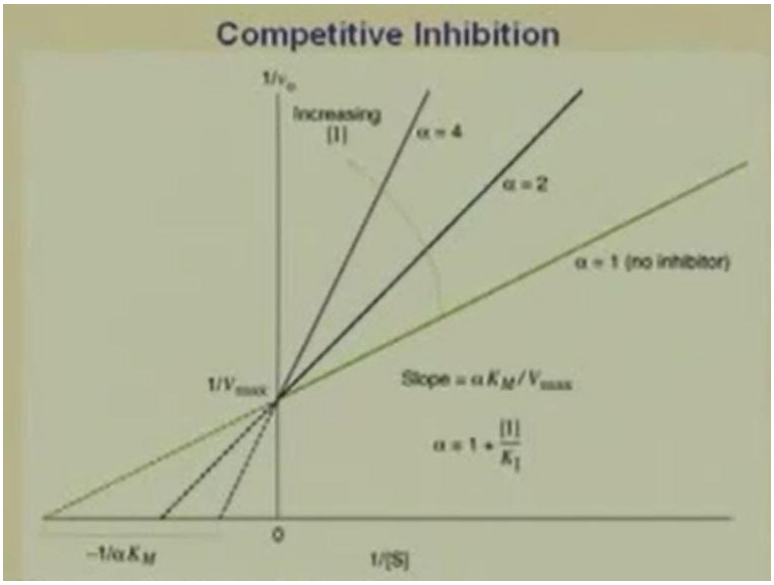

Now remember that this alpha that we spoke about was what? One + I by KI So, when alpha is one it means the concentration of I is zero. If the concentration of I is zero it means that I have an uninhibited reaction So, my alpha is one which essentially means that my Km is what I would have in a normal Lineweaver-burke plot. So,, I have no inhibitor here. This is my original minus one by Km. We increase the slope with inhibitor right.

Now If I add more of the inhibitor the slope is going to increase even further because my Km is going to increase even more. We did this just for one set but if we do this for a series of sets you have to remember that your Vmax remains the same So, the intersection or the intercept on the Y-axis is going to be the same but for increasing inhibitor concentrations you are going to get increasing slopes and if you know the inhibitor concentrations you can actually work out what alpha is because you know the modified alpha Km.

Then you can find out from the inhibitor concentration you can find out the value of KI. If know what the inhibitor concentration is in each case, I can find out what alpha is. Then from the alpha, what can I find out I can find out what KI is. So, usually this experiment is done for increasing a different concentration of inhibitor and you get steeper steeper curves for competitive inhibition.

So,, as So,on as you look at a graph like this which is the Lineweaver-Burke plot for competitive inhibition. Now we are going to look at a different type of inhibition So, what are the features of competitive inhibition? The features of competitive inhibition are that the enzyme are the substrate and enzyme complex is formed the inhibitor is competing for the same site right. In the event of this what is happening? The Vmax is the same.

The maximal velocity attained can be the same. Why? The total enzyme concentration is the same. What about the inhibitor? What about the Lineweaver-Burke for the inhibitor? It is going to have a steeper slope. Why because we are going to have increase Km So, that completes competitive inhibition. This is a type of reversible inhibition.

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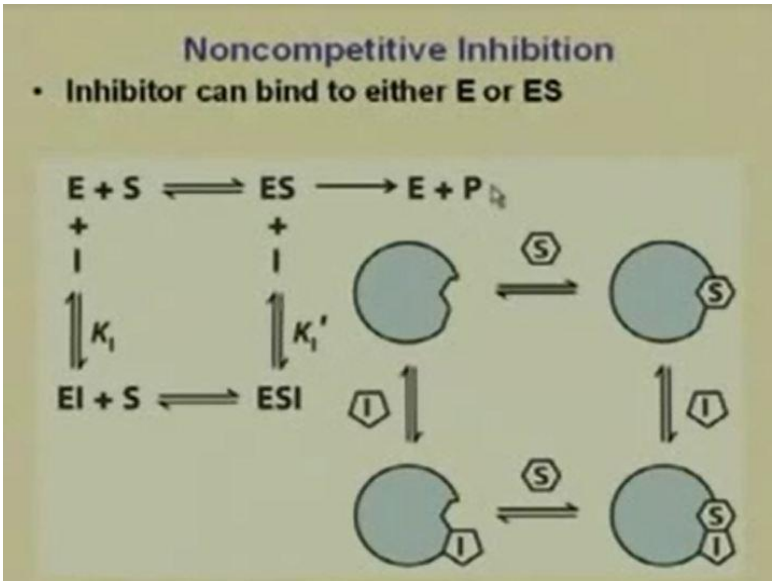

Now we do noncompetitive inhibition. What is the difference here? The inhibitor not only binds to the enzyme but also, binds to the enzyme substrate complex. So,, it is not competing with the same site okay. It is competing or it is rather binding to a different site So, let us look at the picture here. We have the enzyme and we have the substrate then we form the enzyme substrate complex this enzyme substrate complex can bind the inhibitor and So, can 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 now what does this do? It makes basically what it is doing is it affects the formation of the product. The inhibition of a reaction is going to affect the formation of the product So, it is going to affect how the enzyme or how substrate is eventually going to the product. You have to have the enzymes substrate complex that is going to disSo,ciate to form the product.

So, neither will the EI complex give you the product neither will the ESI complex give you the product. It is only the ES that is going to give you the product. So,, we only have to have this complex that is going to give us the product this is not going to give the product this is definitely not going to give the product okay because the inhibitor sits at such a way or it acts at the active site in such a manner that the catalytic reaction cannot take place.

The catalytic reaction takes place only if the active site and enzyme is bound to the substrate in this manner only. The inhibitor sits here So, it prevents the substrate or it prevents product formation basically. The feature of noncompetitive inhibition is that the inhibitor can bind to either the enzyme or the enzyme substrate and it is only the enzymes substrate complex that eventually will form the product So, this is what we have.

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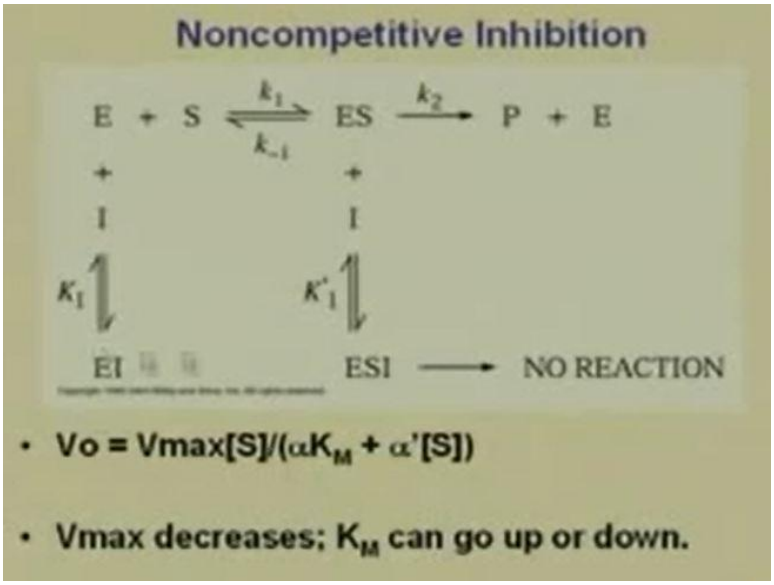

So, we have EI So, we have this again and this ESI is also, not going give any reaction. It is only the ES that is capable as it is giving us the reaction now what we have is we have a decrease in the Vmax. Why? Why do we have a decrease in the Vmax? Because now what is happening is all of the enzyme is not available for product formation initially for the competitive reaction what happen was the total enzyme was available.

If the total enzyme is available at So,me point or the other, then I can form the product but if I have inactivated the enzyme in a fashion it is not available for formation then I will have a Vmax decrease what is that look like.

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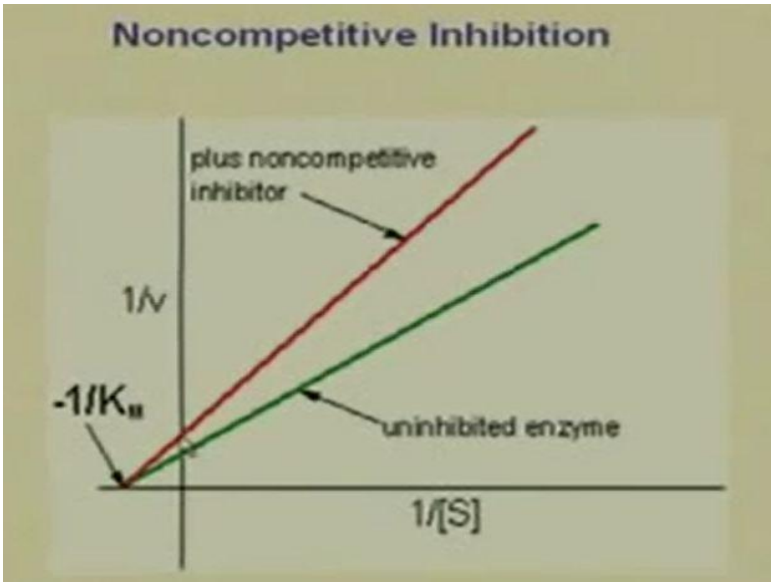

This is what it is going to look like. The Km usually is the same. What we have is? We have an uninhibited enzyme and we have a noncompetitive inhibitor. Let us go back to our drawing sheet here

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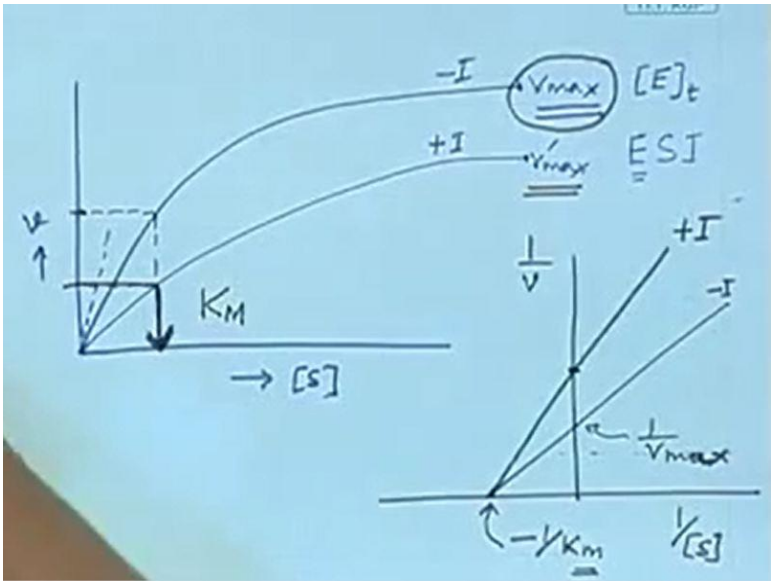

What we are talking about in terms of our substrate concentration and our velocity in our normal reaction we have this. So,, this is our Vmax right and we can find our initial velocity. When I have an inhibitor the start is always going to be slower always obviously you have an inhibition for the reaction So, the start of the reaction is going to be slower. In the competitive case what happened? It reaches the Vmax. Why because the total enzyme was available.

In this case what actually happens is if this ternary complex is formed So,mehow all the enzyme is never available for the total amount of reaction to go. So,, you never reach this Vmax. So,, you reach a lower value here So, this is the Vmax reached with the inhibitor. Now what happens this Vmax and this Vmax are half of this Vmax is going to be where So,mewhere here half of this Vmax is going to be So,mewhere here right.

So, what happens in this case is that the total of the Vmax that could be attained for an uninhibited enzyme is not reached but the Km value which is what? Half of the Vmax and the half of this Vmax So, this is where we have the inhibitor this is where we have no inhibitor So, this is usually the same. Now if I look at the Lineweaver-Burke plot what am I going to have? I have an uninhibited enzyme minus I.

I have now an inhibitor added with the same Km what is again my intercept here? One by Vmax. What is my intercept here? Minus one by Km. My initial velocity is going to increase always why because I have an inhibitor now So, my line is always going to be above the uninhibited enzyme right. My Vmax now has decreased So, what happens to my one by Vmax. It increases sits So,mewhere here. What is my Km? Same + noncompetitive inhibitor is that clear.

So, for the competitive inhibition, I have a case where the Vmax is the same the Km is different. For noncompetitive inhibition, I have the Km same the Vmax different okay So, I have here my noncompetitive inhibition where and why are the lines of the inhibitor always above the uninhibited enzyme because the velocity is or the reaction velocity is always slower in the presence of an inhibitor okay So, when plotting the one by V it has to be higher.

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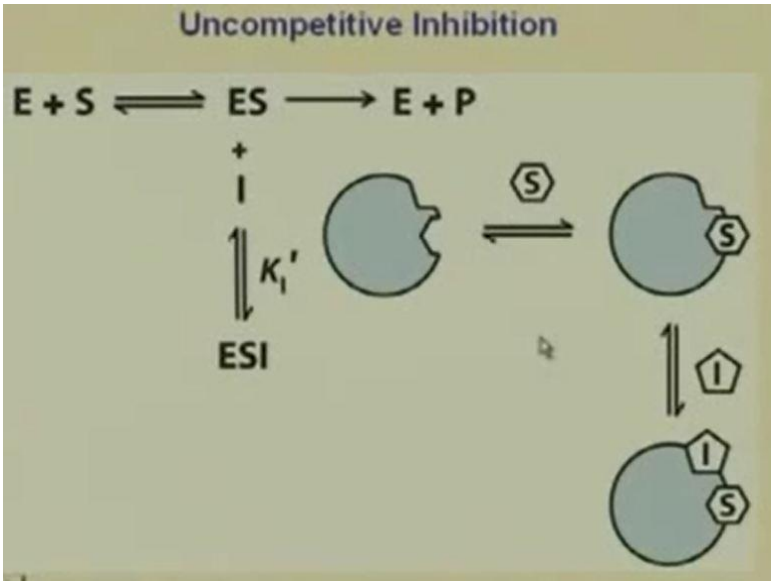

Now uncompetitive inhibition, what is uncompetitive inhibition? Uncompetitive inhibition does not react with enzyme at all. There is no complex between the enzyme and this inhibitor. This inhibitor only reacts with the enzyme substrate complex. So, after the enzyme substrate complex is formed will this inhibitor act So, you understand the distinction between the each of the reversible inhibitors in competitive inhibition the reaction was only with E.

In noncompetitive inhibition, the reaction is with E or ES. In uncompetitive inhibition it is only with ES that is the difference between these. 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 can disSo,ciate into the E + the P. So, this is our enzyme, this is our substrate that sits here and we have the enzyme substrate inhibitor ternary complex look like this. Again, it is the enzyme substrate that is going to form the product So, this is basic difference between these.

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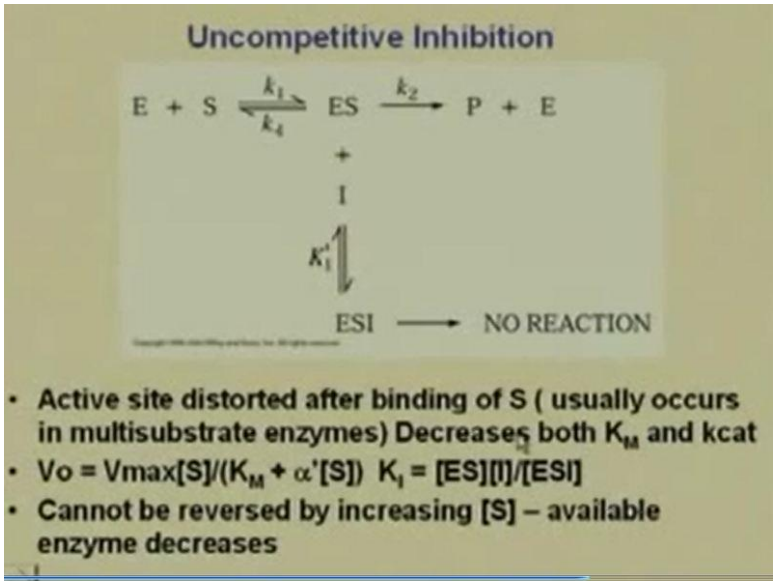

So, what happens is again this ternary complex of this ESI is not going to give us any reaction. The active site is distorted after binding of the substrate this usually occurs in multisubstrate enzymes. Where you have more than one substrate actually formed or like the bisubstrate reaction that I showed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

So, if the Kcat is decreased what is going to happen to the Vmax? It is going to decrease and the Km also, is affected. 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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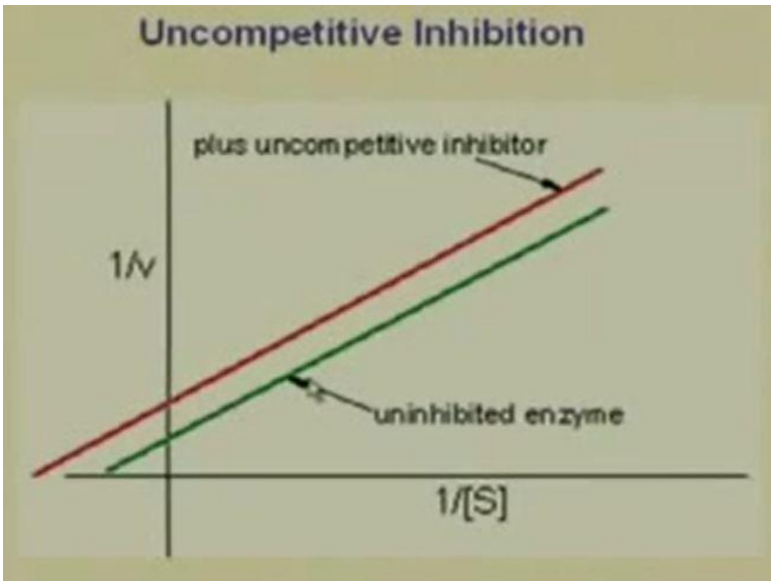

It is going to be a parallel line. Why? First of all, you know that this red line that is the line with the inhibitor is always above the uninhibited enzyme because the reaction is now slower but what happens to our Km? What has happened to our Km?

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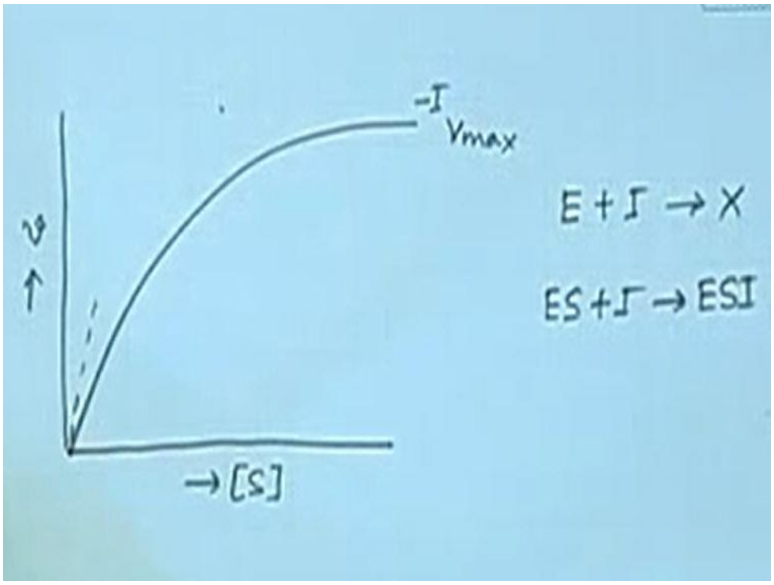

We have let us go back to our Michaelis-Menten. This is minus inhibitor, this is my initial velocity this is my velocity, this is my substrate concentration. What am I saying? This Vmax will not be attained. Why? Because the enzyme is not available the total amount of enzyme is not available for and the Kcat is also, decreases the Km also, is affected but the difference here is that your E + I this does not take place there is no E + I here. There is only ES + I to form ESI right.

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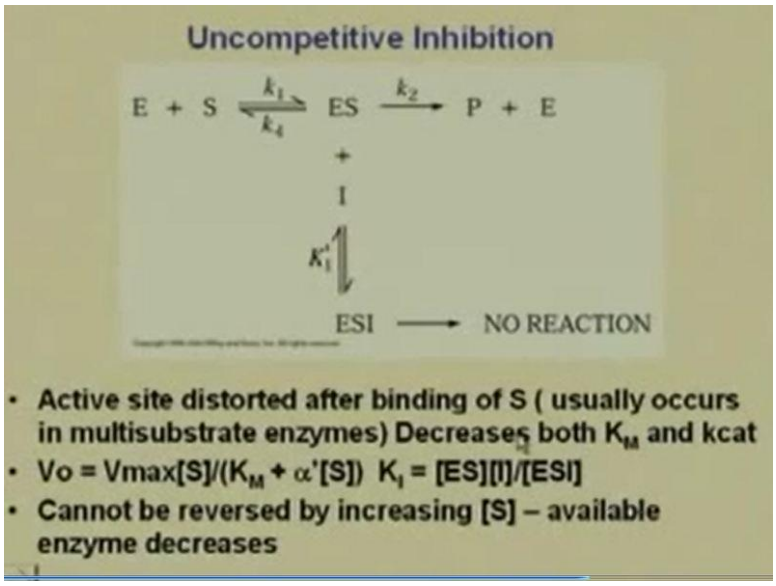

Let us go back here, E + I do not happen here, that happens for noncompetitive inhibition. When we have ES here, ES + I will give you the ternary complex ESI and there is no reaction. It usually has an effect on the Kcat value where your Vmax is decreased. Why does this Vmax decrease? Because the available enzyme actually is decreased.

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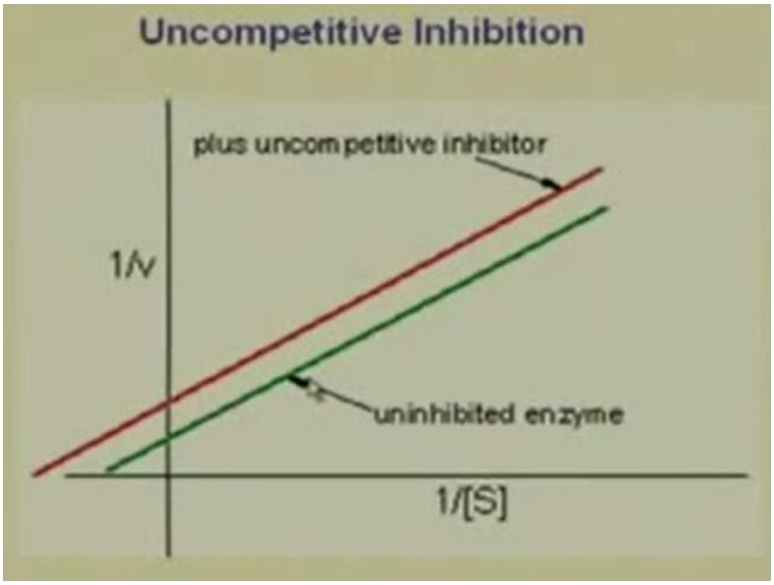

What you have is? You have an increase in the initial velocity due to the presence of the inhibitor and a parallel line for the uncompetitive inhibitor what happened for the competitive inhibitor you have the same Vmax So, you had an intersection on the Y-axis for the noncompetitive set you had an intersection on the X-axis because the Km was the same. In this case for the

uncompetitive inhibition there is no such intersection at all. You have a parallel line with uninhibited enzyme.

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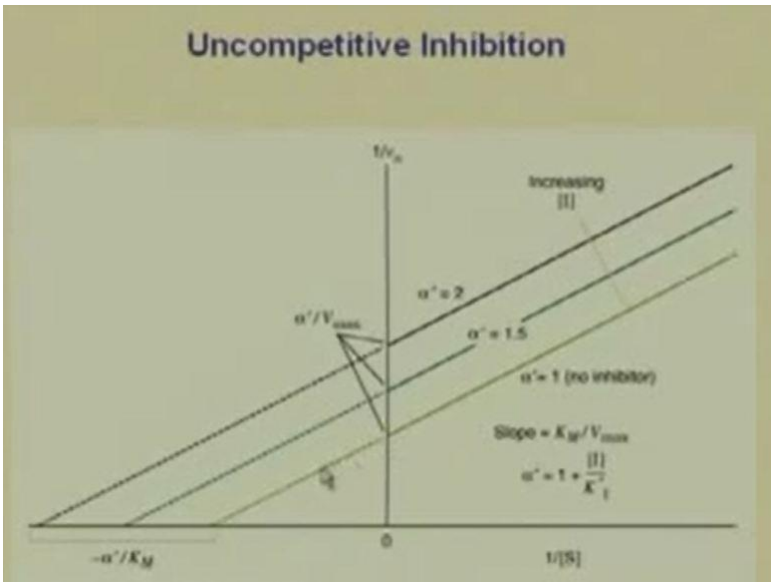

So, when you increase the inhibitor concentration the first line corresponds to no inhibitor and each of the additional line that you have here correspond to increasing concentration of I. Why do we have increasing concentrations of I here? Because this V0 is going to be more for an inhibitor compare to one without inhibition. If you add more of the inhibitor that is going to decrease the initial velocity even more So, you will have an even larger value for the V0 right.

You if you get parallel lines for your inhibition 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 what is it? It is competitive inhibitor. If you have an intersection on the X-axis, it is a noncompetitive inhibitor. Now if we go back to the problem that we were doing in the last class then I gave a set of V0 and V values and S values.

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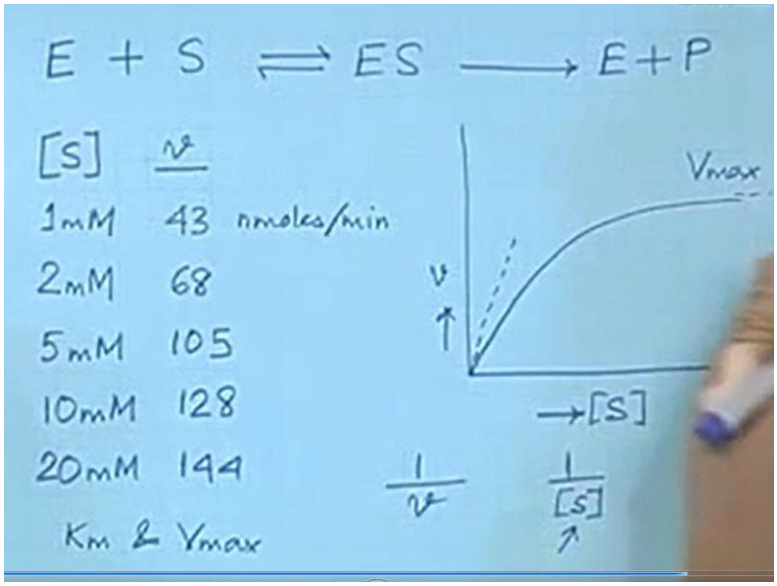

Okay So, we have got a series of S values and a series of V values.

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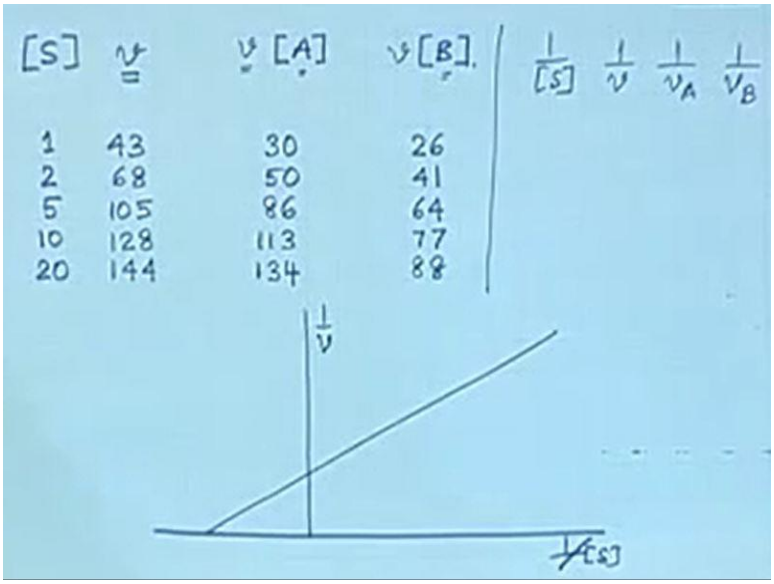

Now we introduce an inhibitor here So, we have [S] values and we have V values for an uninhibited enzyme. If I put in an inhibitor [A] and an inhibitor [B] okay, what do I need? I need the velocities for the presences of [A] and the velocities for the presence of [B] for the same substrate concentrations. So, say we had a value one, two, five, ten, twenty the velocity for no inhibitor 43, 68, 105, 128, 144. With the inhibitor [A] we have 30, 50, 86, 113, 134 for the inhibitor [B] we have 26, 41, 64, 77 and 88.

You see how the initial velocities have decreased than the uninhibited enzyme. Okay now what we are going to do is we are going plot the Lineweaver–Burke plot for this case. So, now if I have these [S] concentrations what information do I required to plot my Lineweaver-Burke plot I need one by [S], one by V, one by VA, one by VB. So, from this plot then we can determine what type of inhibition we have So, I am going to get a plot like this for one by [S] okay now before we plot this let us just complete what we had here.

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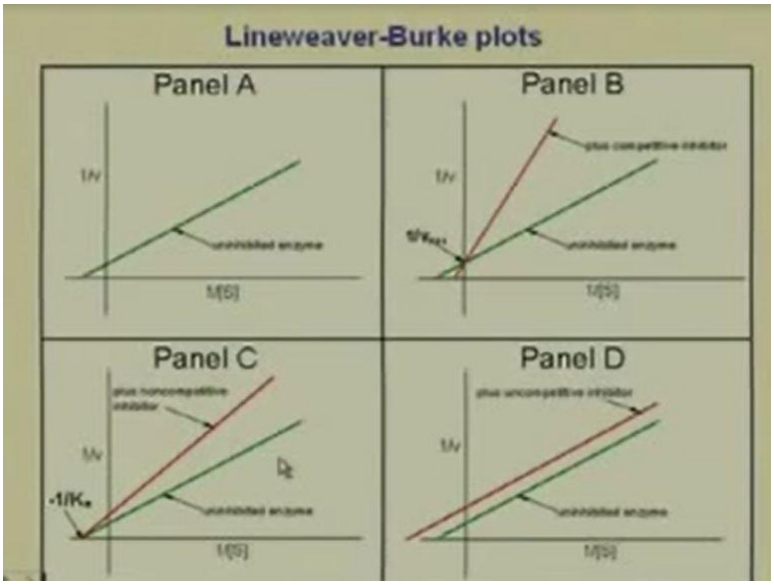

So, this is the Lineweaver-Burke plots. So, straight away from the Lineweaver-Burke plot what can you say? You can say whether you have an uninhibited enzyme what is this? A competitive inhibitor. What is this? A noncompetitive inhibitor. What is this? An uncompetitive inhibitor. Panel B-where is the inhibitor reacting here with the enzyme only, Panel C- where is the enzyme acting here on the enzyme and the enzyme substrate. Panel D- where is the inhibitor acting here on the enzyme substrate only okay So, that is basically the difference.

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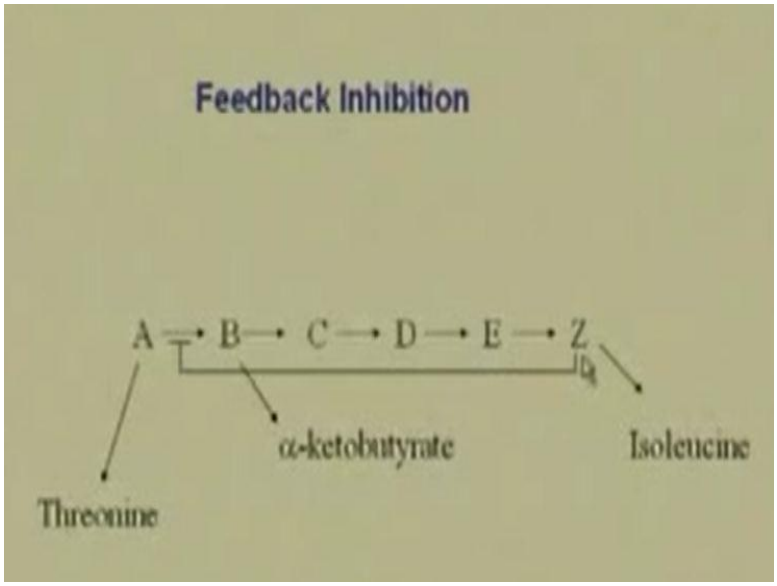

One other thing before we get back to our problem called Feedback inhibition. This is something that is extremely interesting that happens in our body all the times. Suppose we have a reaction say A going to B going to C now each of these arrows corresponds to an enzymatic reaction. So, if there is a particular enzyme acting for the transformation of A to B another enzyme from B to C another enzyme from C to D and So, on. So, say we are going from A to Z here what happens is this Z usually is an inhibitor for this enzyme that transforms A to B.

Let me repeat that, we have a particular series of enzymatic steps here. The series of enzymatic steps say goes from A to B to C to D and So, on So, forth and finally say it has these 1, 2, 3, 4, 5 steps. In the fifth step Z is formed. The Z is obviously different from A it has gone through all these enzymatic steps. In fact, this happens for a particular amino acid threonine to isoleucine okay this is amino acid biosynthesis.

So, we have one amino acid actually form another amino acid. So, this Z actually inhibits the enzyme that catalysis the reaction of A to B this is what is called feedback inhibition So, essentially if you look at it what is it doing it is actually blocking it is own formation. It is blocking a reaction that is forming itself. Now this is extremely important in regulation. Why? You do not want a large amount of Z to be formed in the body.

Z is being formed by A So, if we have the A here forming B then forming C, D and So, on then our Z inhibiting this first step means that unnecessarily B, C, D, E are not going to be formed it is does not make sense for them to be formed So, this exactly what happens. We have threonine we have alpha 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.

It is a regulation you do not want too much isoleucine in the body you do not want even too much threonine. So, there must be some other reaction that inhibits this okay So, this is exactly what has happened.

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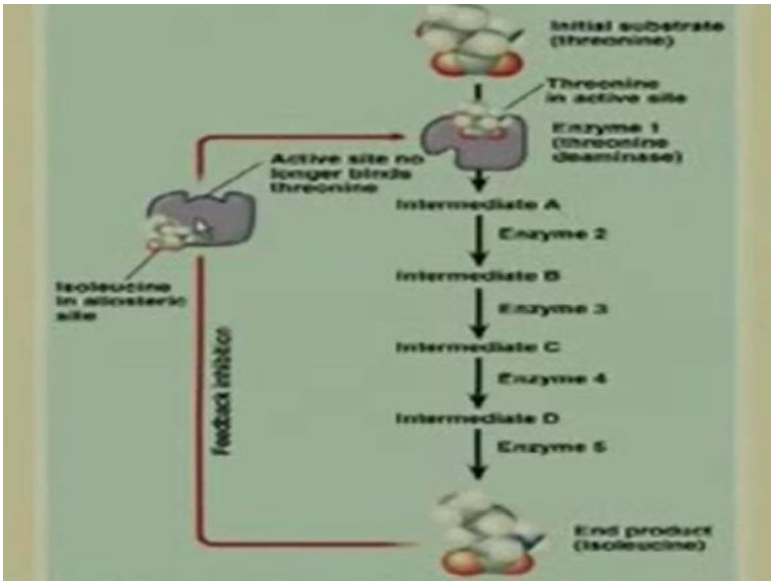

We have the threonine. The threonine sits in the active site of threonine deaminase and we have intermediate A, B, C, D finally forming our Z okay. Now what does this Z do in a feedback inhibition process it sits or it inhibits this enzyme in such a manner this is called an allosteric site because it is regulating the reaction in a manner that it does not allow the threonine to bind anymore. In not allowing threonine to bind it is doing what? Inhibiting it is own formation. This is what is called feedback inhibition.

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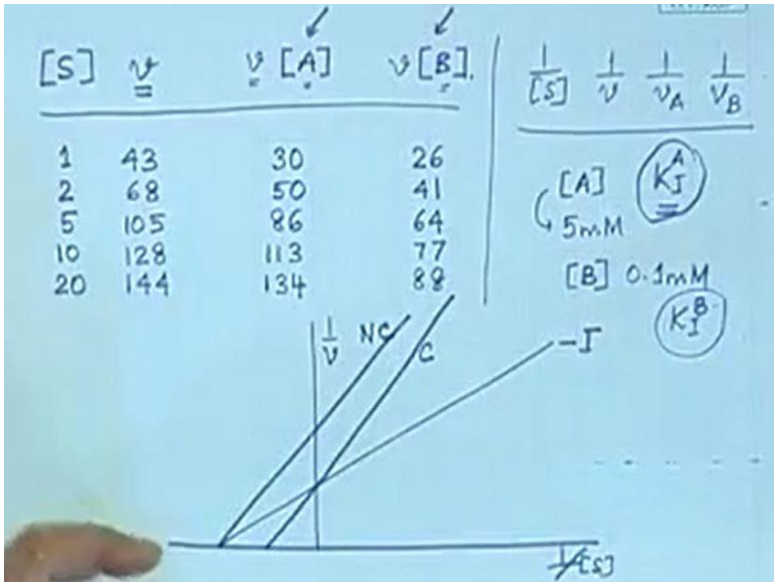

So, if we go back to our slides that when we plot this data we will get one that looks like this and one that looks like that. So, you can work it out and figure out this is minus inhibitor So, one of these what else do you need actually. You need the concentration of [A] to find out the KI value the concentration [A] is 5 millimole the concentration of [B] is 0.1 millimole.

So, you can find the KI value of [A] you can find the KI value of [B]. How? Because you are going to get modified Km values or you are going to get a modified Vmax value and from that you can determine what not only the type of inhibitor you are going to have either a competitive So, this is going to be the competitive inhibitor this is going to be the noncompetitive inhibitor.

We can determine from all these velocity sets what type of inhibition we have and also, the inhibition or inhibitor constant of the 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 that we can have are reversible or irreversible type. Irreversible means that they have a covalent interaction with the enzyme does rendering it inactive altogether.

In the reversible type of inhibition, we have the enzyme interact with the inhibitor in three different ways. We have competitive inhibition where the inhibitor acts on the enzyme alone. We have noncompetitive inhibition where the enzyme interacts with or the inhibitor interacts with

the enzyme and the enzyme substrate and we have uncompetitive where it interacts with the enzyme substrate complex only.

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