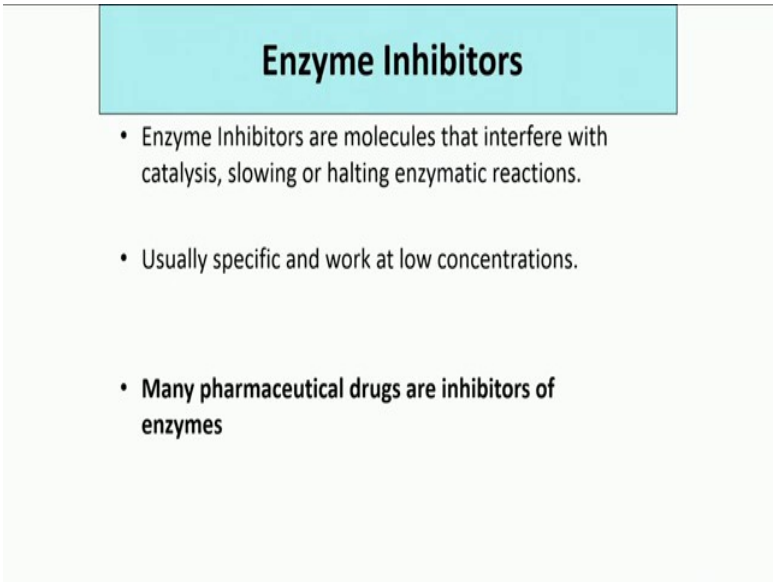
Organic Chemistry In Biology And Drug Development
Prof. Amit Basak
Department of Chemistry
Indian Institute of Technology, Kharagpur

Lecture - 16
Concept of Enzyme Inhibition

Now in this session, we will talk about the Enzyme Inhibitors.

(Refer Slide Time: 00:31)



Enzyme inhibitors are molecules (usually small molecules) that interfere with the catalysis by slowing down or sometimes halting the entire enzymatic reactions. So, inhibitors of an enzyme are basically molecules which modulate the activity in a negative sense; that means, the activity of the enzyme decreases and sometimes the enzyme is totally inhibited, it cannot show any of its catalytic activity.

So, these are called inhibitors. Usually they are very specific because every enzyme has its own typical active site. The active site has a geometric aspect as well as an electronic aspect. To understand this, we have to recapitulate what an active site is. Earlier the lock and key model which was proposed appeared to be a mechanical concept which suggested that the shape of the active site will decide which molecule comes and fits there. But I told you about the induced fit that a substrate may not perfectly fit in; what happens here that it adjusts a

little and there are other interactions; not only there is geometry complementarity, there are other weak interactions that stabilizes this enzyme-substrate complex.

These weak interactions are hydrogen bonds, hydrophobic interactions, π-stacking and the saltbridge or what are called ionic interactions. Thus these inhibitors will also be very specific, you cannot have a very diverse array of molecules that will inhibit a particular enzyme. Only a few classes of molecules can inhibit a certain enzyme.

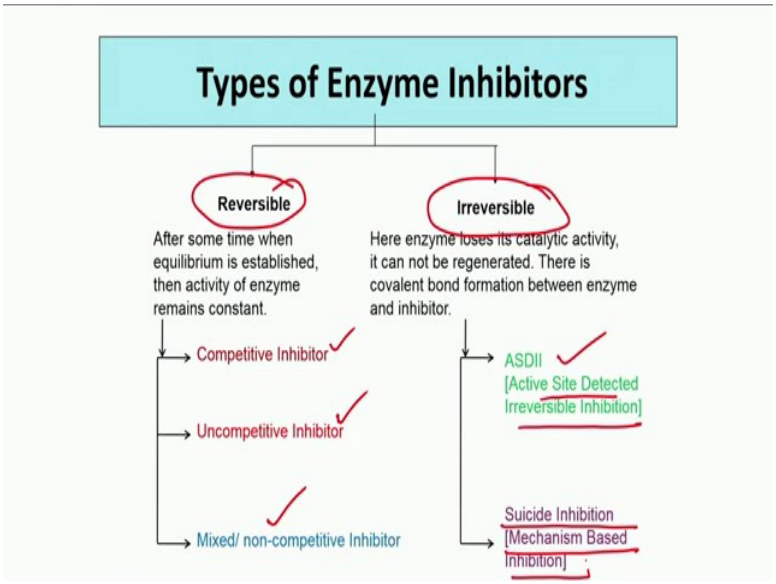
Now, why should we be bothered about these enzyme inhibitors? That is a question. Why do you want to lower the activity of an enzyme? When I introduced this course, I told that in our body there is a perfect harmony between the receptors and the enzymes which are working together hand in hand. And this harmony sometimes breaks down.

What does it mean? That sometimes some enzymes are over-reactive means; it is over reactive, if it's activity is very high; that means, if an enzyme is produced in large amount. Then what will happen? Since that enzyme catalyzes a particular reaction, so upon greater production of the enzyme, the product will be produced in large amount. A classic example is that many people suffer from high cholesterol, what does it mean? Cholesterol is being synthesized in the body by a series of enzymatic reactions.

But somebody is having a high cholesterol means his cholesterol level has to be lowered down. So, in that case, what do you have to do? You have to now modulate the activity of the enzymes which are involved in cholesterol biosynthesis. This is just a single example, but there are multitude of examples where enzyme inhibitors are actually pharmaceuticaldrugs and they inhibit a particular type of enzyme. About 60 percent of the drugs are all inhibitors of enzymes or receptors.

Remember there is a difference between receptor and enzyme; both receptors and enzymes have active sites, but in receptor there is no reaction that takes place where as in case of a enzymes, after the binding, there is a reaction. In receptor after the binding; there is a signal transduction, signal passes from one place to the other.

(Refer Slide Time: 04:43)



Now, let us see how many types of inhibitions are possible. There are, basically two types of inhibitions. One is reversible another is irreversible. The name suggests that in one case, if you add this enzyme inhibitor along with the substrate; you will observe that whatever the rate was obtained without the inhibitor, now it will be slowed down in presence of the inhibitor. Reversible means if you can remove the inhibitor again the enzyme will regain its activity.

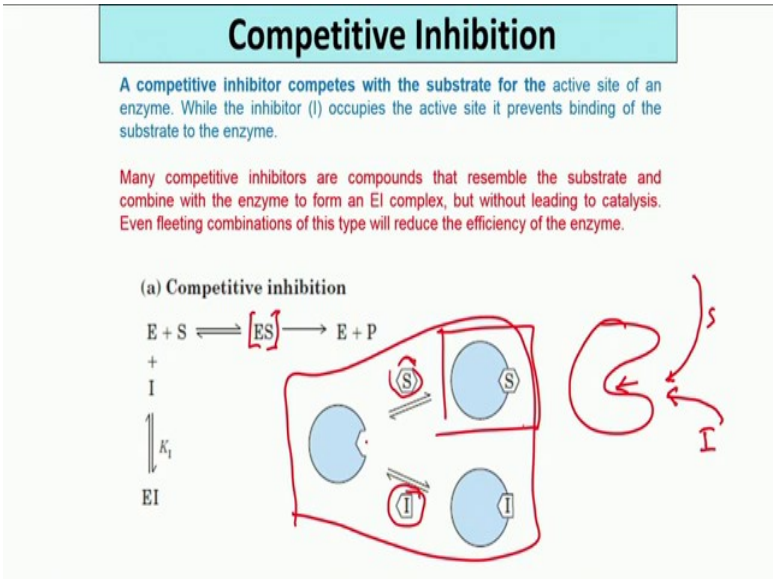
That means, the reactivity of the enzyme which is being perturbed by the molecule can be again restored if you remove the inhibitor. How can you remove the inhibitor? If the inhibitor does not form any covalent bond with active site residues of the enzyme. If it does not form any covalent bond, then it will be easy to remove the inhibitor because the only interactions between the enzyme and the inhibitor are the weak interactions.. Thus it is possible to remove the inhibitor in a reversible inhibition because the inhibitor is not bound to the enzyme by covalent bond(s).

In case of irreversible binding, these molecules that we are talking about as inhibitor, they go and bind and then form covalent bond(s) with any amino acid residue in the active site. In that case, it is very difficult to get back the enzyme in the native form because the inhibitor is already tied up with the enzyme by a covalent bond. So, covalent bond formation or non-formation decides whether it is reversible or irreversible.

Now let us consider the reversible one again; you have usually three classes of reversible inhibitions. One is competitive inhibitor; next is uncompetitive inhibitor; and third one is called either mixed or non-competitive inhibitor.

And in case of irreversible inhibition, you have two types here, one is called active site directed irreversible inhibition and the other is called suicide or mechanism based inhibition. So, let us see what these things are.

(Refer Slide Time: 07:19)



In case of competitive inhibition which is actually a class of reversible inhibition, there is no formation of covalent bond with the enzyme. So, here the steps are like this, that if there is no inhibitor, it will be enzyme plus substrate (earlier we have seen this kind of reaction scheme) to give you ES and then ES breaks down into E plus P.

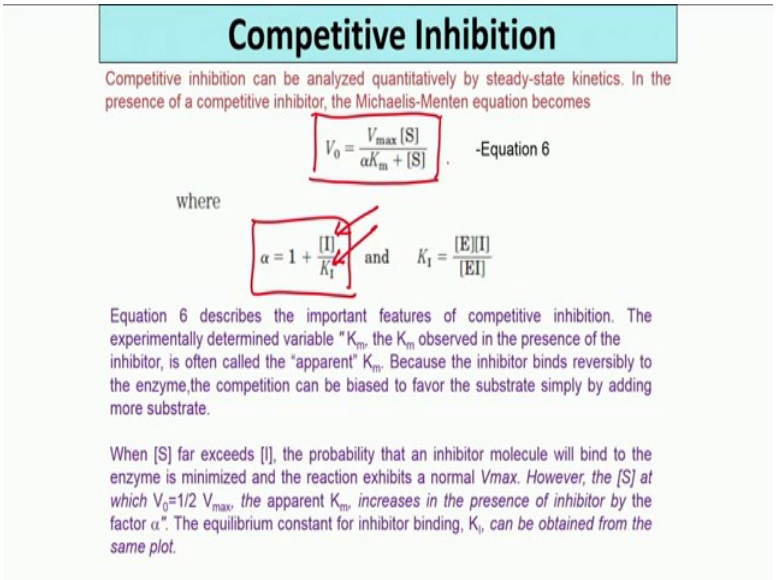
The inhibitor here is a competitive inhibitor. So, in terms of geometry and electronic complementarity, that must be matching to some extent to the substrate.as it also goes and binds to the enzyme. However, in this case they are competing for the same active site of the enzyme. Suppose this is the enzyme and this is the active site. So, what happens? The substrate wants to bind here and then get converted to the product and at the same time, the inhibitor also competes to bind at the same active site. So, basically both these molecules are trying to occupy the same active site possessed by the enzyme. It is like having two persons trying to occupy the same seat in one chair.

So, there will be competition between the two persons to sit on the chair. So, this is known as competitive inhibition; a competition between the two molecules (natural substrate and the inhibitor). And schematically we can that you have this enzyme, this is the active site and this is your substrate on which reaction takes place. So, substrate goes and binds like this, but at the same time there is another molecule which tries to go into the active site. This is because this molecule has also got the shape somewhat similar to the inhibitor and it also may be stereoelectronically complementary to the active site; thus it could have some fruitful interactions with the active site.

So, this can, also at the same time, bind to the active site of the enzyme. How the enzyme loses is efficiency? Some of the active sites now will be occupied by the inhibitor. At a particular time point, since both can bind, so both are competing with each other, but there is only one available site to bind. So, that actually lowers the concentration of the E-S. If you have two enzyme molecules, then both will offer their active sites to the substrate. In presence of the inhibitor, what will happen if we have two molecules of enzyme? Suppose one site is now occupied by the inhibitor, another site is occupied by S.

So, effectively, you are lowering down the concentration of ES complex; that means, you are effectively lowering down the rate of the enzyme reaction; that means, the catalysis will be slowed down.

(Refer Slide Time: 10:23)



This is called competitive inhibition and accordingly, the Michaels Menten equation takes a different form. We are not going into the mathematics, but earlier you remember, in the absence of enzyme inhibitor, it was V_0 equal to V_{max} multiplied with S, divided by K_m plus S. We have to put a constant that is α and this α depends on the inhibitor concentration and also it depends on the K_I that is now called the binding constant of the inhibitor.

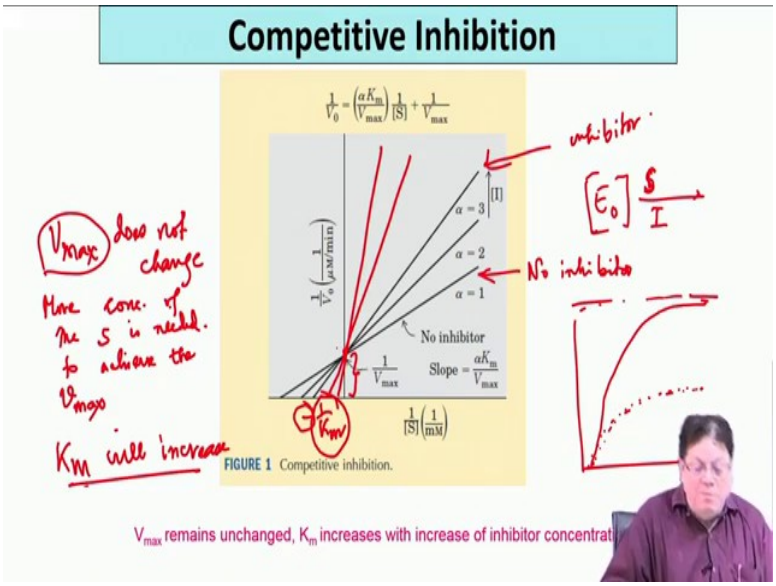
Like earlier you had K_m which gives the dissociation of the enzyme-substrate complex, K_I gives the dissociation constant for the enzyme-inhibitor complex. So, that is the equation that it follows. Now because it is a competitive inhibition, supposing inhibitor concentration and the substrate concentration are same to start with, but then you start putting lot of substrates into it.

So, at some point of time suppose you have thousand times excess of the substrate and so, the inhibitor concentration will be very low. That means, most of the active sites at that time will be occupied only by the substrate molecules and not the inhibitor. Because the inhibitor will not get any chance to bind to that. Suppose there are chairs, and these chairs can be occupied by students belonging to two age groups; there are 100 students who are aged 10, who are and there are only two students who are aged 15.

So, atanyparticular point, most of the chairs would be occupied by the students of the age group of 10 because they are larger in number. So, a similar case happens here that if you have large amount of substrate, then your active sites now will be more accessible to the substrate only.

The inhibitor does not get chance to sit in the active site or to put it in another fashion, the probability of having an inhibitor binding to the active site will be much less if you have large excess of substrate concentration.

(Refer Slide Time: 13:12)



What is the consequence of that? That means, virtually you can remove the inhibition potential of the inhibitor by allowing large amount of the substrate. Now what that happens? Remember if you have started with a particular enzyme concentration and initially you are adding substrate and also inhibitor.

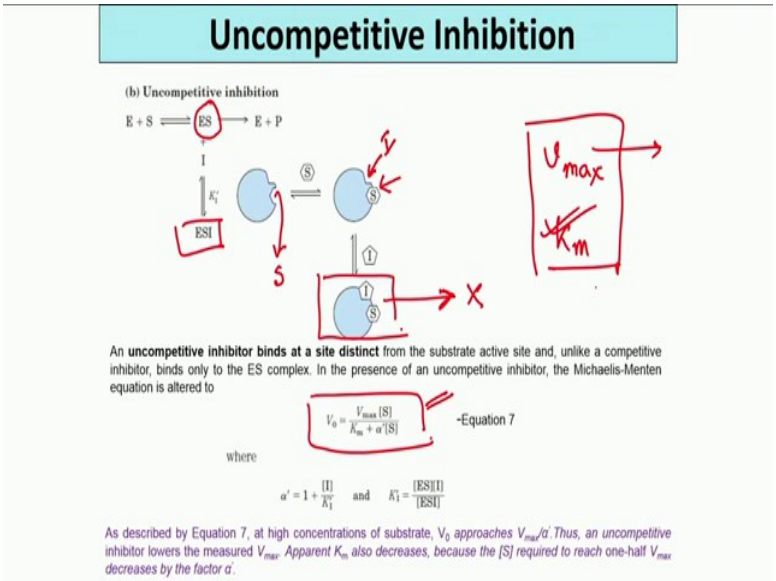
Now what happens if you want to now have a double reciprocal plot (which is also called the Lineweaver Burk plot) like what I said last time?. If there is no inhibitor; that means, that α is 1 then it takes the form of the normal equation. I can show you the equation again; if α is 1; that means, it is the original equation, V_0 equal to $V_{max}[S]$ divided by K_m plus S. This next line is in absence of any inhibitor.Now, what happens? As you increase the substrate concentration, initially your maximum velocity ($V_{max)}$ was much less because your inhibitor was present. But when you have large amount of substrate, ultimately, you will get a line which will be very similar to the line which was obtained when there was no inhibitor. When there is an inhibitor in competing amounts like the substrate, then it will be lower down the V_{max}, but when you have large amount of substrate compared to the inhibitor, you will ultimately reach the V_{max} that was obtained in absence of the inhibitor; that means, the value of V_{max} remains the same in a competitive inhibition. What changes now in presence of inhibitor, is that you need greater concentration of the substrate, more number of substrate molecules, to reach the V_{max} value. So, the substrate concentration should be more in order to overcome the inhibition, but the value of V max remains the same. Only thing that you need

is more concentration of the substrate to achieve the V_{max}. Since you need more substrate concentration to achieve the V_{max}; that means, you need more concentration of substrate to achieve half of V_{max}. What is the significance of the substrate concentration needed to reach half of V_{max}? It is called the Michaeli's Menten constant (K_m).

So, what will happen to K_m? K_m will increase because you need more substrate concentration to reach half of V_{max}. But V_{max} value does not change. You remember the double reciprocal plot without inhibitor is this line and now in a competitive inhibition what happens? As you draw the graph $1/V_0$ versus 1/S, you will see that the lines will intersect the Y-axis at the same point and the intercept is $1/V_{max}$. The V_{max} remain same so; that means, they will all cross this Y-axis at the same point, but what happened to the K_m? The K_m will increase; remember this is -1/ K_m. So, if K_m increases; that means 1/ K_m decreases. So, what happens? In presence of inhibitor, now we have some concentration of inhibitor,, your line will slowly go to this side because this is reciprocal of K_m. So, as K_m increases, the value of value of 1/ K_m decreases; this minus sign may be little bit problematic, but remember because this is in the minus side of the X-axis.

So, we are just talking about the value of K_m. So, value of K_m will increase; that means, $1/K_m$ will decrease. So, the lines will be like this. So, as you increase the concentration of the inhibitor, you will get further lines like this. So, by doing this Lineweaver Burk plot you can actually determine whether this is a case of competitive inhibition or not. So, that is all about competitive inhibition.

(Refer Slide Time: 19:06)



Now, let us come to this uncompetitive inhibition. This also belongs to reversible inhibition category. Now what happens here? The enzyme always has one active site for the substrate. But here the enzyme has got another active site, which was not fitting to the inhibitor to start with. What happens here? When the substrate binds to this enzyme, then this other active site undergoes a conformational change to accommodate the inhibitor; or I can say that the second active site is now more complementary to the structure of the inhibitor.

So, now once a substrate is bound to the active site, it creates a perfect fit for the inhibitor at a particular region and then that binds there. So, unlike the competitive inhibition, here both inhibitor and the substrate are bound to the enzyme. I repeat to make it little bit more clear. So, what happens here? There are two binding sites within the enzyme; one is for the substrate, but for the inhibitor binding site, it is still not really complimentary to the inhibitor. So, inhibitor has to wait, it cannot bind straight away to the enzyme. So, first the substrate goes and binds and as substrate goes and binds there, then the other pocket becomes perfectly fitting to the inhibitor. So, inhibitor cannot bind until the substrate binds to the enzyme.

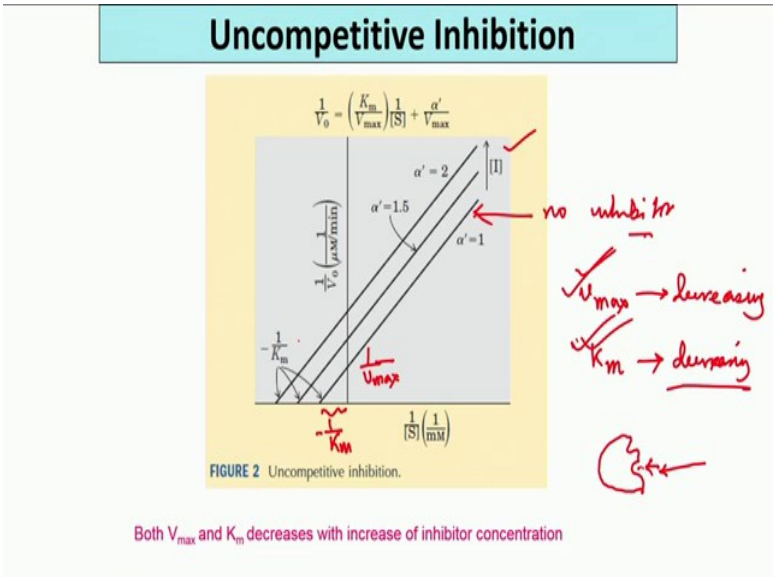
So, this is formed and that is this ESI. So, E plus S goees to ES; some of the ES will be decomposed to the enzyme and the product, but at the same time, some of the inhibitor also can bind to this ES and form this ESI complex. The problem with this binding is that now there is virtually no reaction from this species where both the inhibitor and the substrate are bound. Now can you say why there is no reaction? Because after the substrate is bound, the

inhibitor comes and binds which induces some conformational change, I said this is induced fit and nobody gives you the perfect space; you have to earn your space in this world.

Although it is true that from beginning there is a space for the inhibitor, but when inhibitor binds there is some conformational change that happens to the enzyme and that actually perturbs or prevents the enzyme from doing any reaction on the substrate. So, the bottom line is that ESI does not react. So, your effective concentration of ES is decreased because some of the ES binds to I which go to ESI and that does not react. So, there is reversibility here. So, it goes back to ES again and then E plus P. So, the important thing is that I (inhibitor) binds only after the binding of the substrate and the second important point is that this enzyme-substrate-inhibitor (ESI) ternary complex is catalytically inactive.

Here the equation takes this type of form, V_{max} [S]is the numerator (which remains the same), but earlier, in the denominator, it was the K^m which was multiplied by α, in this case, K_m plus α' multiplied to S. Do not be too worried about how this equation has been derived; if you can go to the book by Voet and Voet, you will see the derivation of this equation. Now the bigger question is that what will happen to the V_{max} and your K_m? In case of competitive inhibition, V_{max} does not change, it remains the same; but K_m increases; that means, there is less binding of the enzyme substrate.

(Refer Slide Time: 23:51)



So, in this case what happens? The graph is here. So, in this case, you will get graphs like this, this is the line where there is no inhibitor.

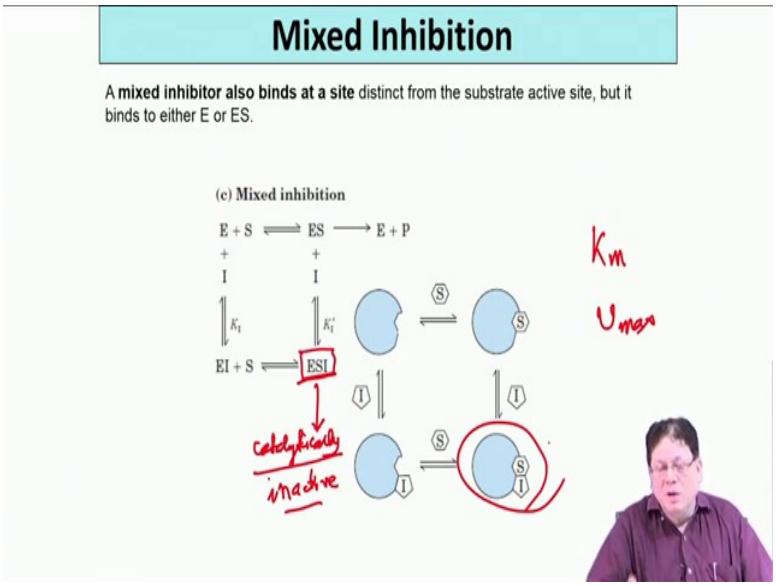
And now as you add the inhibitor which is working in an uncompetitive fashion, then what you are seeing that the V_{max} is changing because the intercept is changing; Since V_{max} remains as $1/V_{max}$. So; that means, since this $1/V_{max}$ is increasing as you add inhibitor, V_{max} is decreasing. Why is V_{max} decreasing? Because the less effective concentration of ES is there; because some of the ES will be bound to that I; now what happens to the K_m? As you increase the inhibitor concentration, the lines are going towards the left.

So, this is $1/K_m$ initially where there is no inhibitor present, but as you add the inhibitor, the $1/K_m$ value increases; that means, K_m decreases. So, here V_{max} decreases and K_m also decreases. Why K_m decreases? I said that there are basically two sites, but one restriction is there that unless the substrate is bound here the enzyme cannot get itself tied with the other active side. So, here basically what happens?.

The induced fit of both the two things together make the complex very stable. So, if the complex is very stable, then dissociation of S is becoming less effective. So, chances of dissociation are reduced. This means your K_m is decreasing; this implies that the enzyme is having the substrate sitting in the active site for a higher amount of time because the binding association is now higher. K_m decreases means your association of the enzyme substrate complex is higher.

So, that is why you get these types of lines. If you have an inhibitor in hand, firstly you may check whether there is any inhibition and then we try to see whether the inhibition is competitive or it is an uncompetitive inhibition? And there is one more kind of inhibition which is called the mixed inhibition or that is also known as non-competitive. Actually non-competitive is a particular type of mixed inhibition. What happens in mixed inhibition? Here there are two active sites again to start with, but unlike the uncompetitive one, here both the active sites are already present. So now, the inhibitor binding is not restricted, that first the substrate will bind and then the inhibitor has to bind; there is no such situation here. The steps are like this: E plus S can go to ES; I can bind to the ES complex or I can bind with E; it is not dependent on the substrate binding.

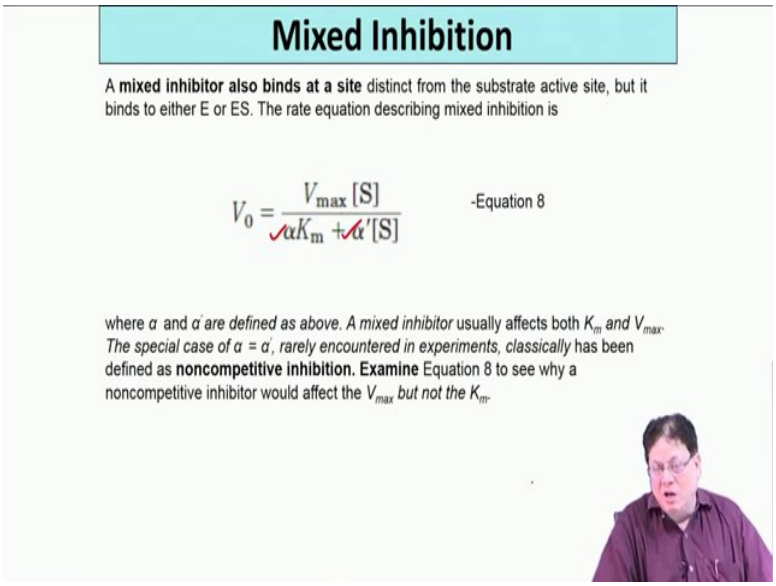
(Refer Slide Time: 27:49)



So, that can go to EI; and the substrate can bind to EI to make ESI. You notice the difference. In an uncompetitive binding, substrate binds followed by the inhibitor, in case of the mixed inhibition, the substrate and the inhibitor binding are independent of each other. But this factor remains that this ESI is catalytically inactive. Now what will happen to that K_m and V_{max}?. So, you will see that basically there is no change in concentration because both the active sites are already filled and the binding is independent of each other. So, K_m should remain the same because both the bindings are independent of each other.

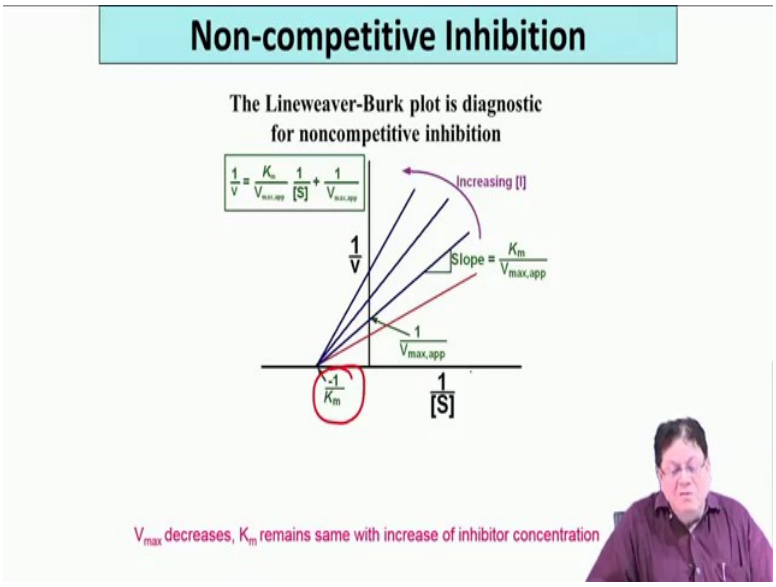
But your V_{max} will decrease because ESI is catalytically inactive form. So, some of the ES complex is converted to the ESI and that is why the V_{max} will decrease.

(Refer Slide Time: 29:01)



And you will see the curve; in this case both K_m and S having this α and α'. If α is equal to α', then that is a case of non-competitive inhibition. So, non-competitive inhibition is a particular case of mixed inhibition where these two constants α and α' are same.

(Refer Slide Time: 29:30)



And in that case, you will now see another type of graph. Here K_m remains the same as I told you; why K_m remains the same? Because the binding of inhibitor and substrate are independent of each other; but V_{max} will decrease.

So, these lines represent the uninhibited one, and slowly as you increase the concentration of the inhibitor, these go upward and that means, your $1/V_{max}$ is increasing; that means, V_{max} is decreasing. So, these are the three types of inhibitions for reversible cases. We will go to the irreversible cases in the next session.

Thank you.