Introduction to Complex Biological Systems
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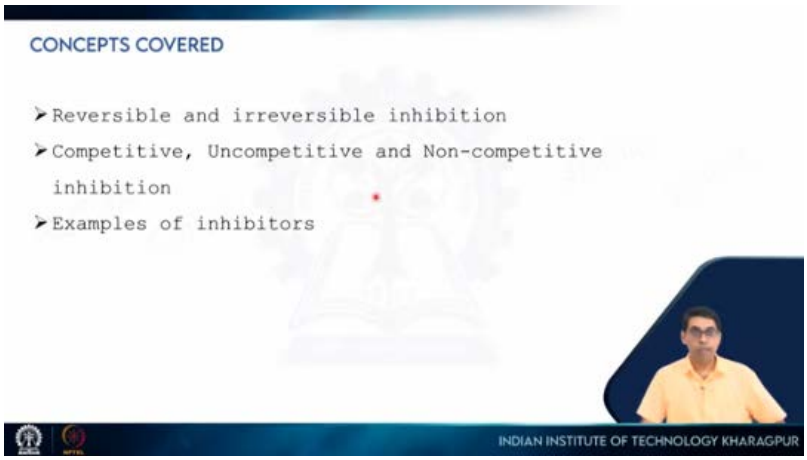
Lecture 19
Enzyme inhibition. Types and mechanism of inhibitors. Design of inhibitors.

Welcome back. So, in this lecture, I am going to talk about enzymes and their inhibitors. So, I am going to talk about several mechanisms of enzyme inhibition, and I will also give you examples of several very important inhibitors. So, I will discuss types of inhibitors.

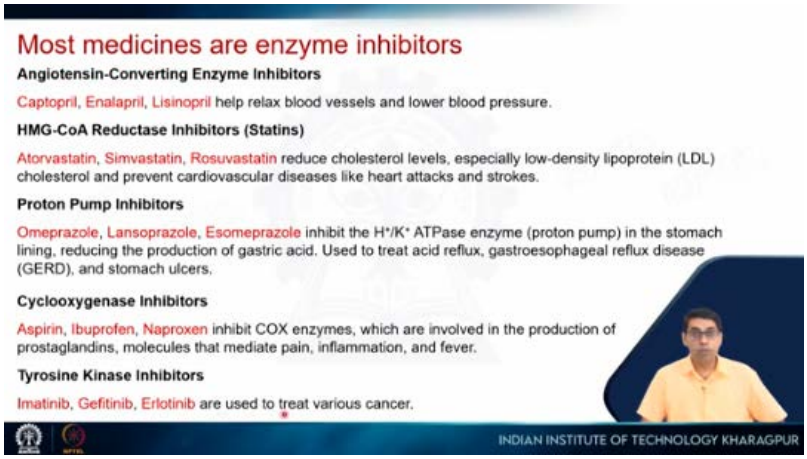
So, there are reversible and irreversible inhibitors. And then, under reversible, there are these major three different types of inhibition mechanisms: competitive, uncompetitive, and non-competitive. And then, I will also give you examples of these different types of inhibitors. So, inhibitors are very important, and it turns out that most medicines that we take on a regular basis are inhibitors of different types of enzymes. So, not all medicines are inhibitors, but most of them are, and I have listed a few examples here.



So, for example, angiotensin-converting enzyme inhibitors are captopril, enalapril, lisinopril. So, these are medicines that help relax the blood vessels and lower blood pressure. So, you will see that many elderly people in your house take some of these medicines for reducing their blood pressure. Then there are statins, which inhibit HMG-CoA reductase. So, these are some of these medicines that reduce cholesterol levels, and again, you will see many senior people taking one of these molecules on a regular basis. When we eat something very oily or something very rich, we might actually get acid reflux or acidity. So, in that case, we take medicines like omeprazole, and its other variants. So,

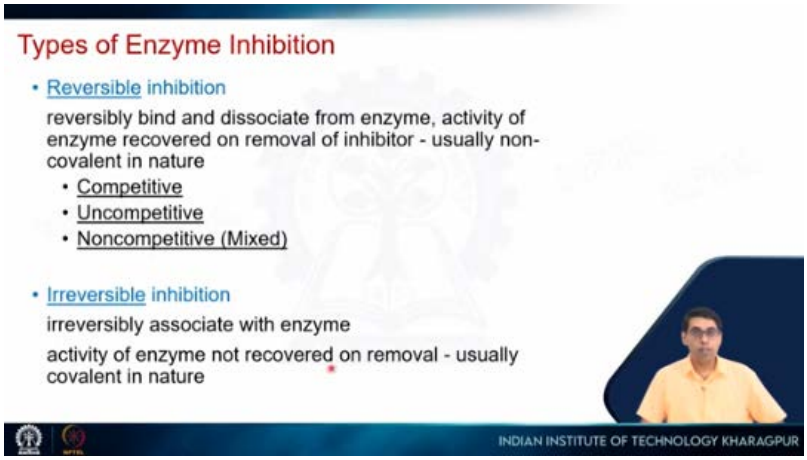
these are proton pump inhibitors, and what they do is reduce acidity. They reduce the proton potassium ATPase enzyme, which is basically a proton pump, and thus they reduce the acidity of your stomach right. Then there are these Cox enzyme inhibitors, Cox's cyclooxygenase inhibitors. So, these inhibitors are something that helps us relieve pain, and these are some names you might have seen. These are very common: aspirin and ibuprofen. And then there are tyrosine kinase inhibitors.

So, we have already seen the example of imatinib and then there are other variants which are used to treat various kinds of cancer. So, I will also talk about some more inhibitors as I go through this lecture. Now, what are the types of inhibition? There are two types of inhibition.

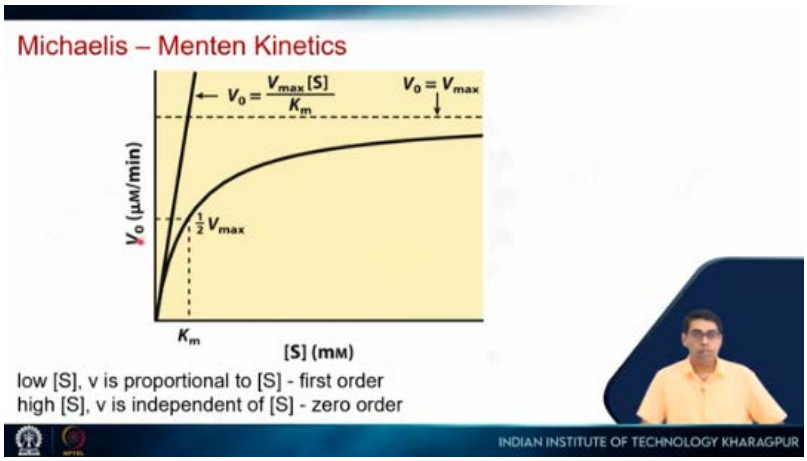


The first one is called reversible inhibition, which means that the inhibitor is not binding the enzyme with a covalent bond. So, the interaction is reversible. So, we can increase the substrate concentration and maybe we can remove the inhibition. But again, this is not always true. So, it turns out that there are three types of inhibitors.

One is competitive, the second one is uncompetitive, and the third one is non-competitive or mixed inhibition. So in all of these cases, the inhibitor can actually diffuse out of the enzyme. Wherever it binds, it can bind, but it can also diffuse out. The second types of inhibitors are called irreversible inhibitors or irreversible inhibition. In this case, the enzyme actually forms a covalent bond and it binds irreversibly.



So the enzyme activity cannot be removed or regained back. So these inhibitors permanently inhibit the enzyme. So let us look back at this. We have already seen this. So this is our Michaelis-Menten curve. What we have also seen is that this Michaelis-Menten equation can be linearized, which was done by Lineweaver and Burk.



$$V_0 = \frac{V_{max}[S]}{K_M + [S]} \rightarrow \frac{1}{V_0} = \frac{K_M + [S]}{V_{max}[S]}$$

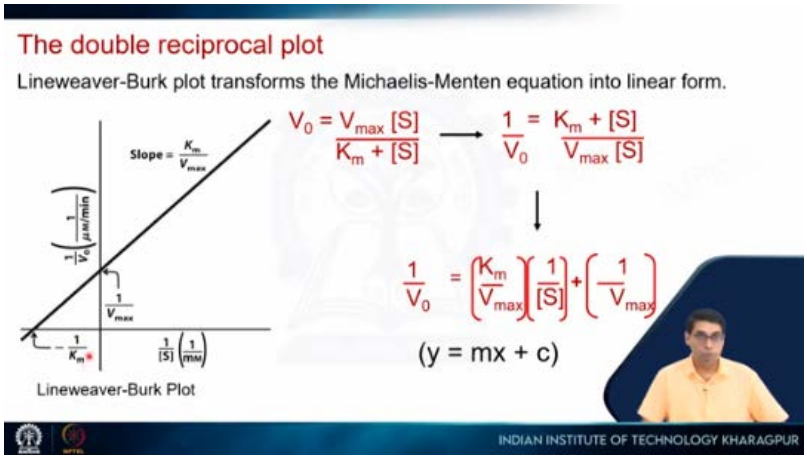
$$\frac{1}{V_0} = \left(\frac{K_M}{V_{max}}\right)\left(\frac{1}{[S]}\right) + \left(\frac{1}{V_{max}}\right)$$

$$(y = mx + c)$$

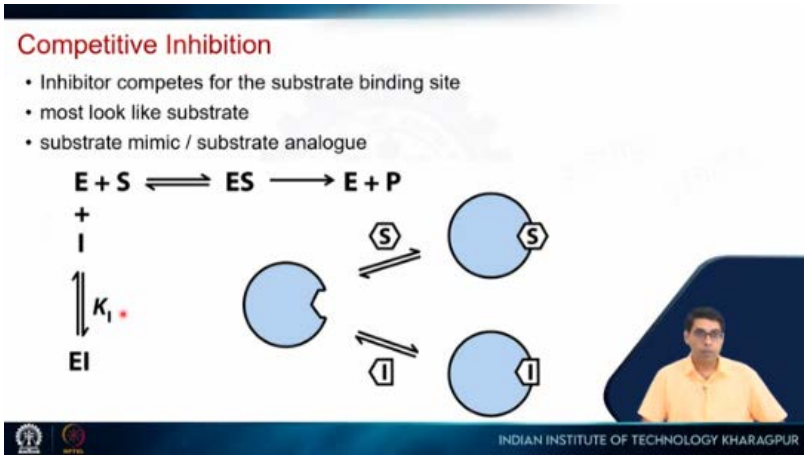
So, what we do is we flip it, we inverse it, and then we divide these two by V_{max} over $[S]$. So, this is what we get. So, 1 by V_0 equals K_m by V_{max} times 1 by $[S]$ plus 1 by V_{max}. So, this is in the form of $y = mx + c$. y is 1 by V_0, x is 1 by $[S]$. It is multiplied by m, which

is the slope of the line, which is K_m by V_{max}, and a constant c is 1 by V_{max}, which gives us the $y - intercept$, and when we plot 1 by V_0 by 1 by $[S]$, it will look something like this.

So, the line of the actual data will be somewhere here, and then we can extrapolate it. To get the $y - intercept$ and the $x - intercept$, and from that, we can determine 1 by V_{max} and 1 by K_m. So, we can get V_{max} and K_m. So, why am I showing you this? Because these plots will be very important or very useful to determine which type of inhibitor we are dealing with. So, what is the first type of inhibition?

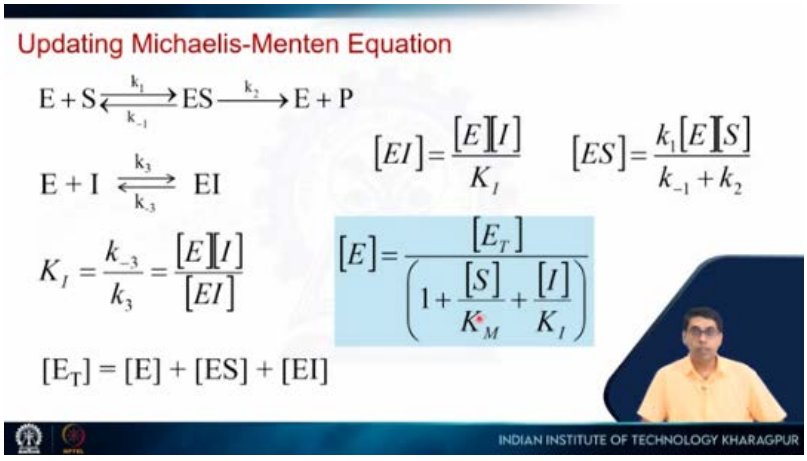


The first type of inhibition that I am going to discuss is called competitive inhibition. So, let us look at this picture. This is our enzyme, and this is the active site. This is where the substrate binds, and then the reaction will happen. The inhibitor looks very similar to the substrate and binds to the same active site.



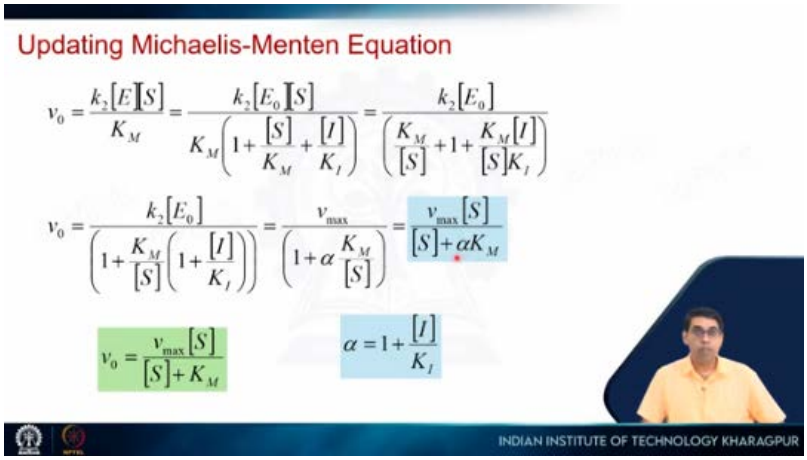
So, if it binds there, no further reaction will happen. So, you can see that the substrate and the inhibitor are competing for the same active site. So, if the substrate is bound, the inhibitor cannot bind, or if the inhibitor is bound, the substrate cannot bind. So, that is why it is called competitive inhibition or a competitive inhibitor, and we can write the equation like this: $[E]$ plus $[S]$ gives you $[ES]$ gives you $[E]$plus P. This is our standard Michaelis-Menten kinetics. But the free enzyme can also bind the inhibitor and form the enzyme-inhibitor complex, and this is given by this K_I or the inhibitor constant. So, this is basically the K_D or the dissociation constant of the enzyme-inhibitor complex.

So now we have to update our Michaelis-Menten equation. So this is the Michaelis-Menten kinetics, and this is the kinetics of the enzyme inhibition. So now we have this additional parameter where K_I equals to K_{-3} over K_3. So again, you can think of this as the dissociation constant of the inhibitor.



So we have this, and we have $[ES]$ like this. So now we have to update all of this because the total enzyme will be equal to free enzyme plus enzyme bound to the substrate plus enzyme bound to the inhibitor. So, if we update it like this, we can actually solve for $[EI]$; $[EI]$ is this, and we can solve for $[ES]$; $[ES]$ is this. So, we can replace $K_{-1} + k_2$ by k_1 as K_M.

So, we can plug in all of this, and we will get this form. So, we are solving. So, we are plugging $[ES]$ by this and $[EI]$ by this. So, we get this form where the free enzyme concentration equals the total enzyme concentration divided by this and we know the substrate and the inhibitor concentrations.



So, again, if we manipulate this V_0 equals to this k_2 by K_m times $[ES]$times $[S]$. We know what $[E]$ is from the previous equation. So, we plug in it here, and then we do some more manipulation. So, what we do is we multiply this K_M here.

So, we multiply it by K_M by $[S]$. So, this $[S]$ we bring it here. So, it becomes K_M by $[S]$. So, this becomes K_M by $[S]$ like this. This cancels out. So, it becomes 1, and this becomes this. So, now if we take K_M by $[S]$ as common, then it becomes 1 plus K_M by $[S]$ times 1 plus I by K_I.

So, this I by K_I is left. This 1 plus I by K_I, this is what we call α. So, this is, you can call it an inhibition constant or some parameter. So, the α value will increase if we increase our inhibitor constant. So, this is α, and that is multiplied by this.

So, our final form of the Michaelis-Menten equation looks like this. So, V_{max} times $[S]$ divided by $[S]$ plus α times K_M so α is this. Now, you can compare it with the original equation, which looks like this. So, all we have done is replace this K_M by α K_M, where α is given by this.

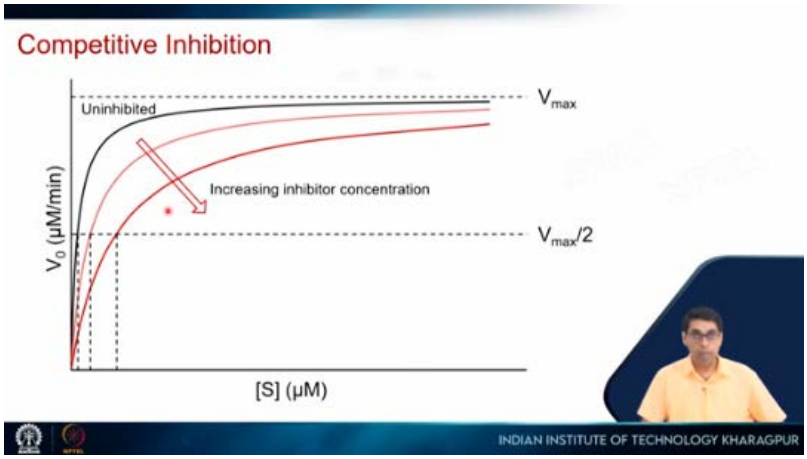
So, if the inhibitor is 0, then α equals 1. So, this equation goes back to this form. But if the inhibitor has some value, let us say inhibitor $[I]$ equals K_I, then this becomes 1 so α will be equal to 2. So, α will have a value of 2 so, the effective Michaelis constant will increase. So, what this equation tells you is that V_{max} will not change, but the K_M will change. And that is expected because if I go back to this, both $[I]$ and $[S]$ are competing for the same active site.

So, if I have some value of $[I]$, if I increase my substrate concentration more and more and more, they will outcompete I. So, I can actually drive the reaction more towards this direction than this direction. So, if I have a large amount of substrate compared to the inhibitor, I can drive this reaction in this direction. So, I can essentially saturate the enzyme by binding all the enzymes by the substrate. So, I can actually reach the V_{max} value.

So, V_{max} is not going to change, but I will need more substrate to reach that V_{max}, because I have to compete out $[I]$. So, my K_M increases, and that we can see if we plot this, the Michaelis-Menten equation into this, where we are plotting V_0 versus $[S]$. So, this black line is the uninhibited Plot for the uninhibited reaction. So, for the same enzyme concentration, if we add a little bit of inhibitor, you can see the line goes like this. So, you see that it will eventually reach the same V_{max}.

If I increase the inhibitor concentration more, it looks like this. So, again, it will eventually reach the same V_{max}, but after a much higher concentration of substrate. What happens toK_M? So, if this is my V_{max}, this is my V_{max} by 2. So, for the uninhibited reaction, my K_M is here.

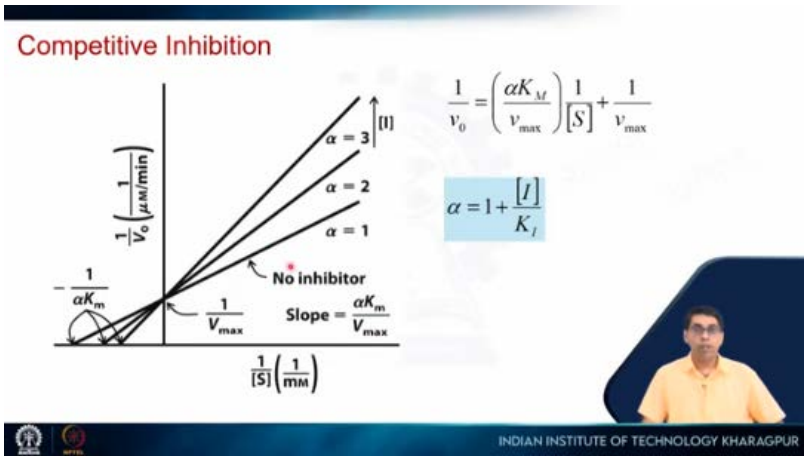
But for a little bit of inhibitor, you can see the $K_M$increases. If I increase the inhibitor, my K_m increases further because my α value is increasing. So, to understand the inhibition, this type of plot is fine. But the Lineweaver-Burk plot is even more convenient. So, instead of plotting it like that, if you plot it in this linearized fashion, then no inhibitor is this.



So, my α equals 1 so, inhibitor $[I]$ equals 0. So, this is 0. So, my α equals 1 so, this α equals 1 line represents no inhibitor, you see.

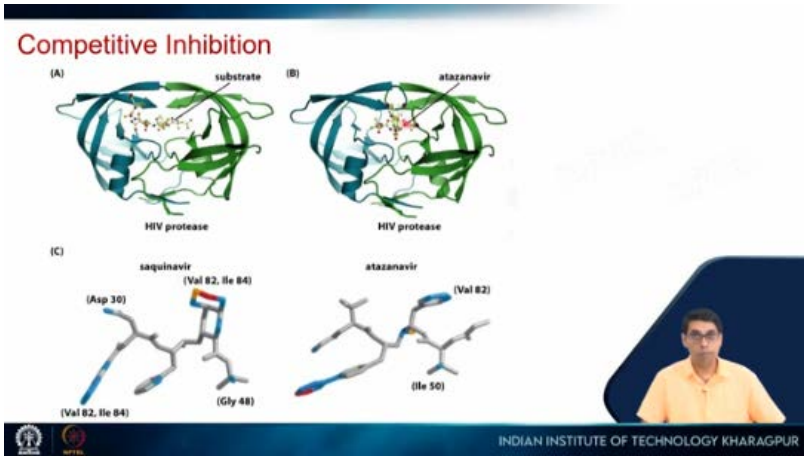
So, this is where I have V_{max}, and this is where I get K_M. So, if I increase my inhibitor, let us say $[I]$ equal K_I, then this becomes 1. So, α becomes 2. See, it goes to the same $y-intercept$, which means my V_{max} is not changing, but now my K_M is increasing. So, if K_M increases, 1 by K_M will decrease.

If $[I]$ equals 2 K_I, then this becomes 2. So, α becomes 3. So, it further increases and V_{max} remains the same, but K_M increases. So, my slope keeps increasing because my α K_M is increasing, while V_{max} remains constant. So, if we have some inhibitor, we do not know where it binds, and we do not know how it binds. But if we do this simple kinetic experiment and we plot the Lineweaver-Burk plot, if we see our V_{max} is not changing, but the slope is increasing, we can tell that this inhibitor is a competitive inhibitor, which means that it is going to bind exactly in the same active site as the substrate.



So, there are many examples. One example is shown here. So, this is an HIV protease. So, in the HIV virus, it produces this protease, and the substrate binds here. So, there are these different types of peptides which have been designed to inhibit this enzyme.

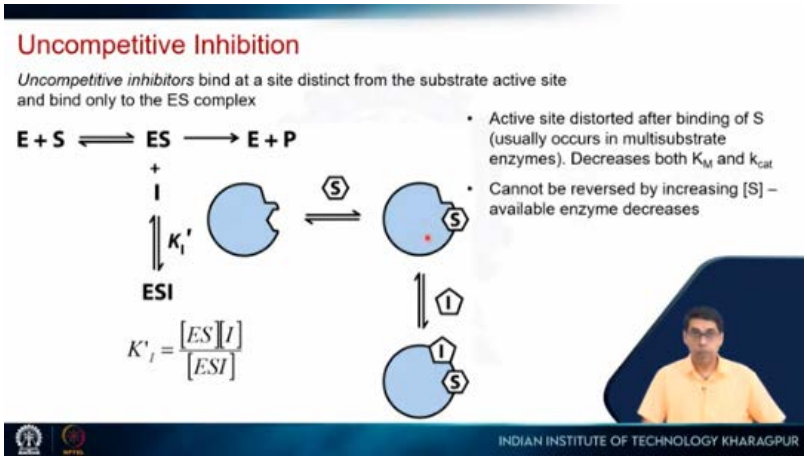
And you can see that Atazanavir, which is this one, binds exactly in the same place as the substrate. So, it is a competitive inhibitor because when it binds, it will not allow the substrate to bind. So, Saquinavir is another peptide, and it is also a competitive inhibitor. So, it will also bind in the same place, in the same active site as the substrate.



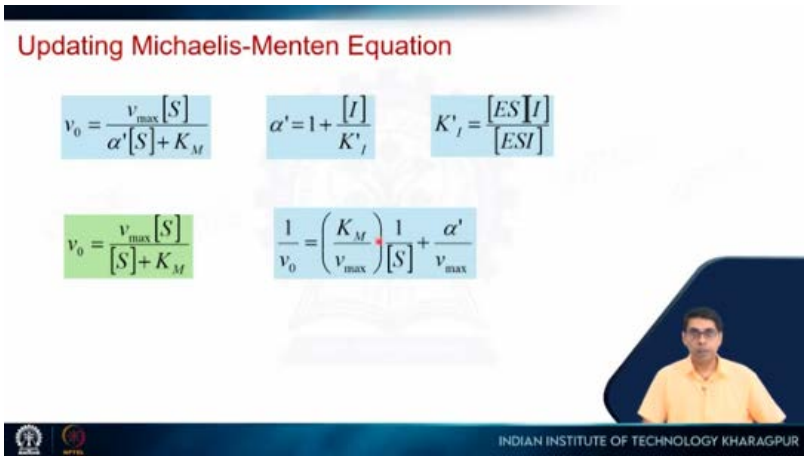
Another type of inhibition is uncompetitive inhibition. So, what happens in this case? This is our standard equation: enzyme plus substrate gives you the complex, which gives you the inhibitor, instead of binding the free enzyme, binds the enzyme-substrate complex and forms this $ESI\ complex$. So, the substrate binds the enzyme, and this can go on to form the product. However, if the inhibitor is present, it will bind this and form this complex, thereby inhibiting the enzyme. So, you can see that somehow the binding of the substrate either induces some change in the enzyme, allowing the inhibitor to bind, or the substrate binding itself creates a pocket where the inhibitor binds.

So, the substrate binding actually facilitates the binding of the inhibitor. Here, you can actually see that by increasing the substrate concentration, I cannot drive this reaction back. So, in this case, we can tell that the enzymes which form like this will not be easily converted to this unless the inhibitor diffuses out by itself. So, I will never gain 100 percent enzyme-substrate complex. So, I will never reach the V_{max} value.

So, here my V_{max} is going to decrease, and that is what we will see. So, if I do the math in the same manner. So, where K_I, so it is K_I' , is defined as this. So, I will define α' as 1 plus $[I]$ by K_I, and I will get this equation.

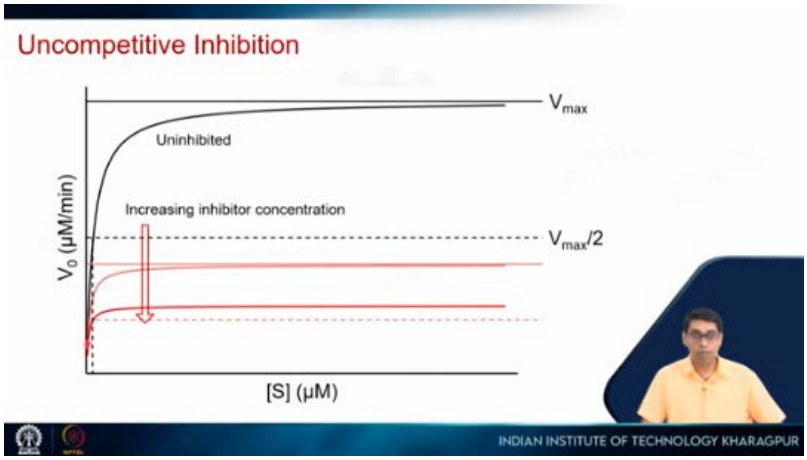


So, in this case instead of α being multiplied to K_M, it gets multiplied to this substrate. So, this is α' and this is your uninhibited equation. So, if I flip it then it will look like this. So, instead of α getting multiplied here, my α' goes here.



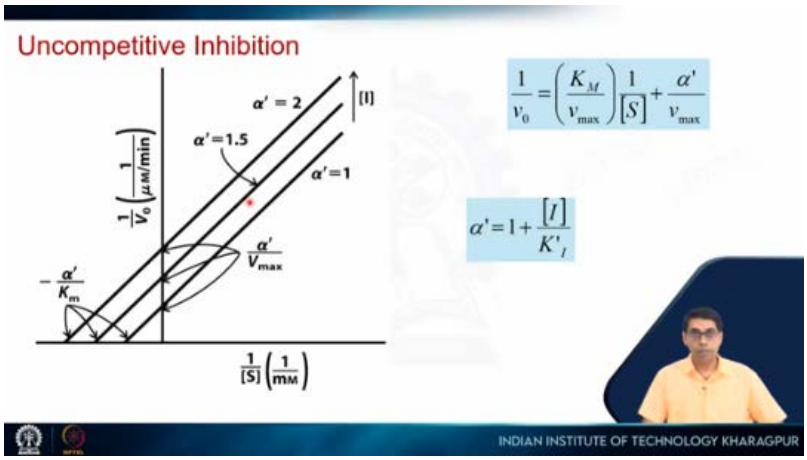
So, if I go to my standard Michaelis-Menten plot, how will it look? This is my V_{max}, which is uninhibited, if I add some inhibitor, my V_{max} actually goes down. So, this red line is the V_{max}.

So, this is my V_{max} by 2. And if I go to the intercept, my K_M will be somewhere here so, actually the K_M decreases. The V_{max} decreases, but the K_M also decreases. But you can see that in this particular plot, it will be very difficult to determine K_M. If I increase my inhibitor constant even further, the V_{max} goes down even further.



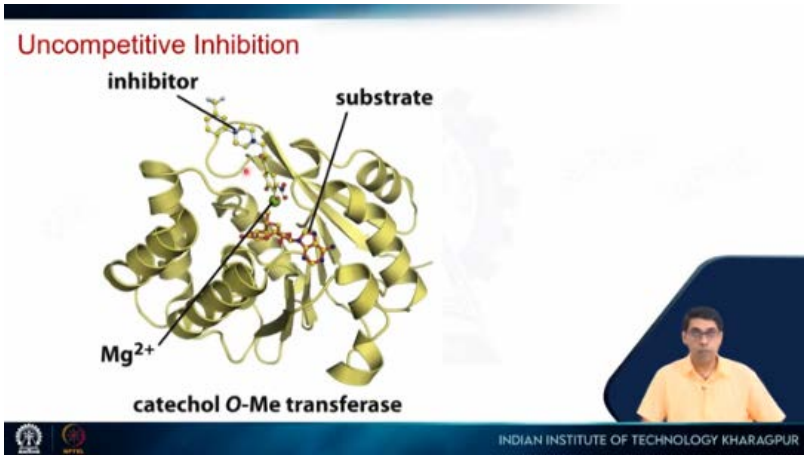
So, my V_{max} by 2 will be somewhere here, and my K_M will be even somewhere here. So, this will become really difficult to estimate what K_M is. But if we go to our linearized plot, the equation is given by this; you can see that $K_M$over V_{max} has not changed. So, even though my effective V_{max} has changed, the y-intercept is changing, the effective V_{max} is changing, but the slope has not changed, which means that for no inhibitor, if it looks like this, as I keep on increasing my inhibitor concentration, the line will remain parallel to this.

So, this is a very good characteristic of uncompetitive inhibition. So, if you see something like this, you can tell that my inhibitor is binding to the enzyme-substrate complex. So, one example of that is this particular inhibitor, which inhibits catechol O-methyltransferase. So again, this is the enzyme; you can see it is all α-helical and there are some beta-strands on the backside and this is the substrate.

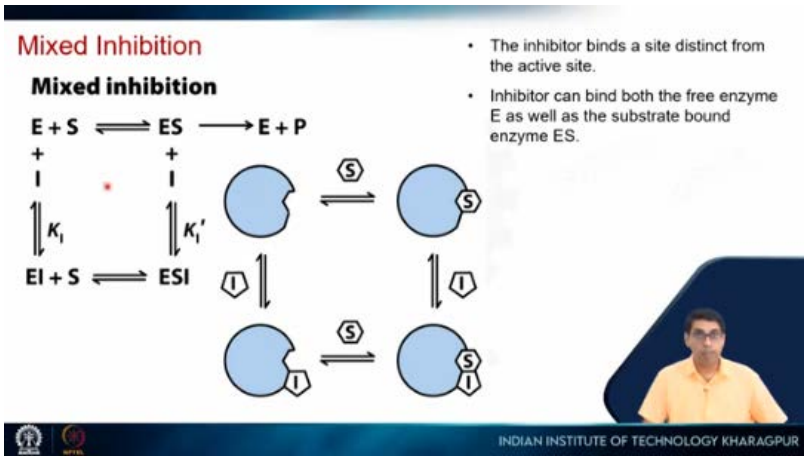


So, once the substrate binds, it creates this pocket where the inhibitor binds. So, the inhibitor actually interacts with this magnesium ion, which is interacting with the substrate,

and then it is also interacting with these residues of the enzyme. So, the binding of the substrate is important because then only this pocket is created where this inhibitor binds. So, it binds to this enzyme-substrate complex.

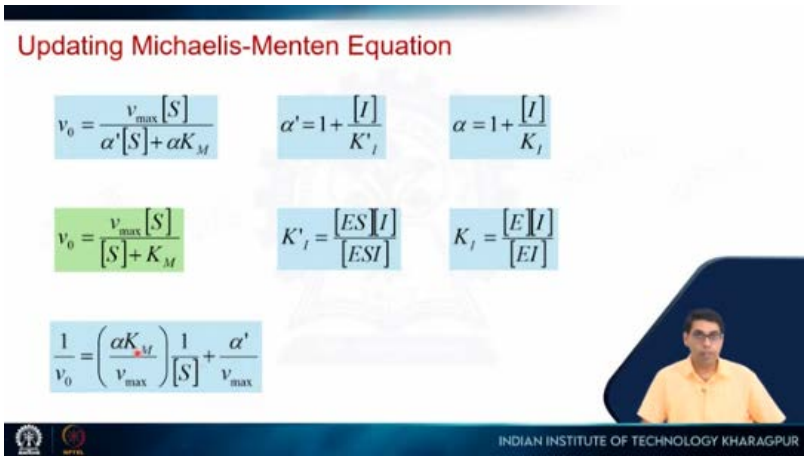


The last one that I am going to discuss is called mixed inhibition. So, in this case, what we get is the inhibitor can bind the free enzyme, it can also bind the enzyme-substrate complex, and they can also interchange. So, in this case, you will see that I will get an α for this, I will get an α' for this, and my Lineweaver-Burk or my Michaelis-Menten equation will be updated, which is basically a combination of the previous two, the competitive and the uncompetitive.

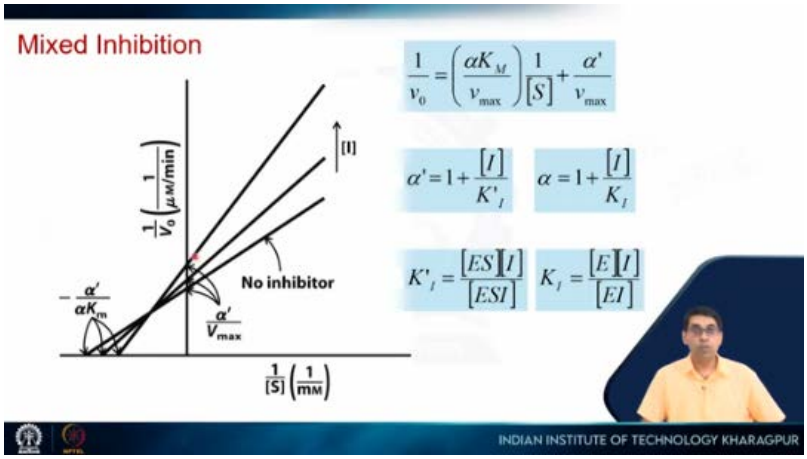


So, α gets multiplied to K_M, and α' gets multiplied to the substrate, and these two are defined here where K and K_I' are defined. So, this is exactly the same as before. So, if we linearize this equation, it will look like this. So, my K_M is getting multiplied by α, and my

1 by V_{max}, gets multiplied by α' . So, here, my slope will increase, and my y-intercept will also change.



So, you see that this is not here; it has shifted, and how it will look will depend on the combination of the relative values of α and α'. So, if you find something like this, then you can predict or estimate that your inhibitor is following a more complex mechanism where it is mixed inhibition. So, these are all reversible inhibitors. We also have inhibitors which are irreversible inhibitors, and here I am giving you some examples of irreversible inhibitors. So, irreversible inhibitors permanently bind the enzyme.



So, they kill the enzyme permanently because they form a covalent bond with the enzyme. Penicillin and all the penicillin class of molecules, so the related beta-lactams, are antibiotics. So, they irreversibly inhibit the bacterial transpeptidase enzyme. So, the transpeptidase enzyme is important for the synthesis of the bacterial cell wall. So, if we can

inhibit this enzyme, we can prevent the formation of the cell wall, and that will lead to cell lysis and the death of bacteria.

So, penicillin is an irreversible inhibitor. Another inhibitor is 5-fluorouracil, and we are going to look at the structure of the enzyme and this inhibitor in the next slide. So, this gets metabolized into a compound. So, this is not the inhibitor. Once a patient takes this molecule, it gets metabolized into a compound which forms a covalent complex with thymidylate synthase.

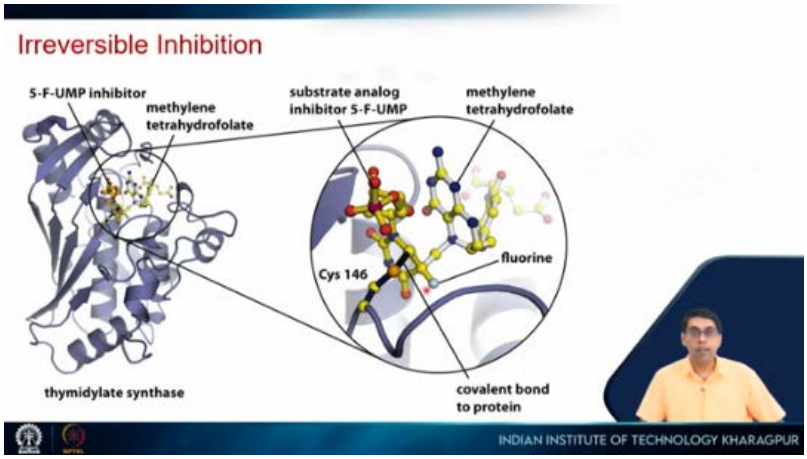
So, this is the molecule which helps in the synthesis of thymine and it binds this irreversibly, which means that it will actually interfere with the formation of DNA and hence cell division. So, 5-fluorouracil is used to treat different forms of solid tumors. Another molecule is allopurinol. So, this is a very common inhibitor.

So, it gets metabolized into oxipurinol, which inhibits xanthine oxidase. So, what is this enzyme? This enzyme is involved in the production of uric acid. Now, there are people, older people, who have certain enzymes that do not work or something goes wrong.

So, uric acid is increased, and then that uric acid gets deposited in different joints, like the toes, and that causes pain in those regions. So, those are called gout attacks. Now, if we can reduce the level of uric acid, we can actually treat patients who have gout attacks, and allopurinol, which is an inhibitor, inhibits xanthine oxidase and prevents the production of uric acid. So, let us look at this 5-fluorouracil. So, this is the enzyme thymidylate synthase, and the inhibitor is bound here, and the enzyme is also bound to methylene tetrahydrofolate.

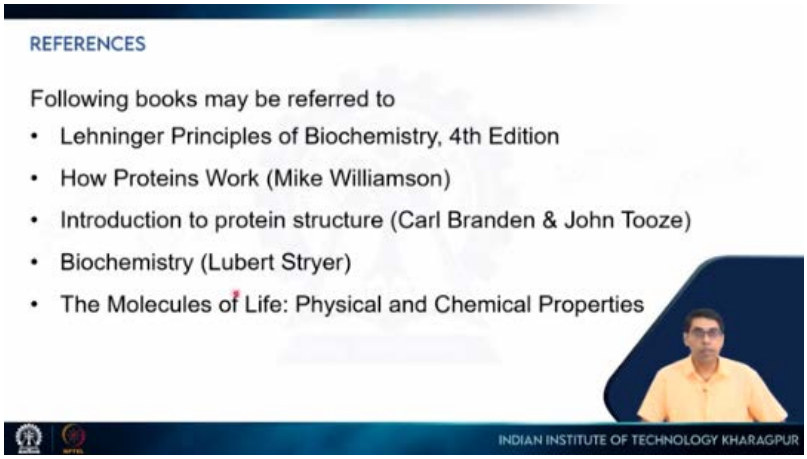
So, tetrahydrofolate is the one which will ultimately donate this methyl group to uracil and result in the formation of thymine. So, one of the substrates is bound here. So, it is actually a cofactor and we have the inhibitor which is bound here. So, if we zoom into this region, it looks like this. You can see that it is forming a covalent bond with this cysteine 146.

So, once this forms a covalent bond. So, it is bound to this, it is also bound to this cofactor, and these two will permanently remain in the active site of the protein. So, this enzyme molecule is for all practical purposes, it is inactivated. So, that is why these are called irreversible inhibitors because these inhibitors will go to the active site, use the same reaction mechanism as the enzyme uses, and form a covalent bond.



So, once this covalent bond is formed, no further reaction happens. And since no further reaction happens, this enzyme is permanently, so this inhibitor is permanently bound to the enzyme active site.

So, again, these are some of the books that you can follow. You can go through Lehninger's Principles of Biochemistry, and you can also go through Molecules of Life. So, these are the two primary books that I have been using so you can, but you can use any of these books.



Thank you for now.