Enzyme Science and Technology
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Module - X
Enzyme Inhibition Kinetics
Lecture - 42
Enzyme Inhibition (Part - I)

Hello, everyone. This is Dr. Vishal Trivedi from Department of Biosciences and Bioengineering IIT, Guwahati and what we were discussing? We were discussing about the different properties of the enzyme, in the course Enzyme Science and Technology. So, so far what we have discussed? We have discussed about how you can be able to produce the enzyme by cloning the enzyme into a suitable vector, followed by we have also discussed about the different types of methods and approaches how you can be able to purify the enzyme.

And, then we have also discussed how you can be able to perform the enzyme assets and subsequent to that in about the previous module we have also discussed about how you can be able to utilize the enzyme assets to design the different types of inhibitors. And, when we were discussing about the Enzyme Inhibitor approaches, we have discussed about the (Refer Time: 01:36) approaches, where you are actually going to screen the compounds and you may not need the structure of the enzyme or the inhibitor.

And, you will be able to get the inhibitor to you know inhibit the enzyme and then we also discussed about the targeted approach where we have discussed about the ligand base approach, receptor base approach or the computer aided inhibitor design. So, in if you have designed an inhibitor you would like to understand the mechanism of his action.

You would like to understand how the enzyme is inhibiting the protein enzyme and how you these inhibitors can because once you know the mechanism of the enzyme inhibition you can be able to work on that mechanism and you can be able to improve the activity of those enzyme those inhibitors.

So, in this today's lecture we are going to discuss about the how you can be able to study the inhibition of the enzyme and how you can be able to do the different methods to

understand whether the enzyme inhibition is reversible or reversible and all the kind of aspects. So, in this lecture we are going to recover that particular aspect.

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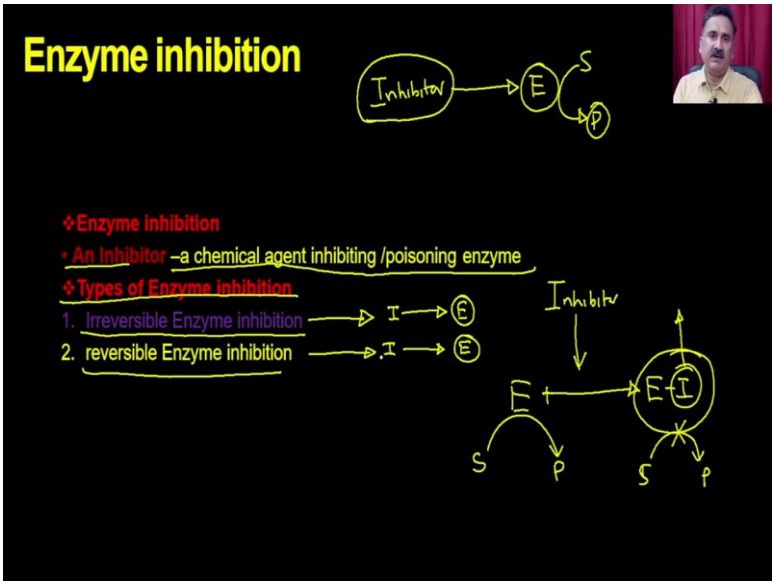

So, as you can say that the enzyme inhibition so, once you have developed the inhibitor, right you have developed a inhibitor it is actually going to block the enzyme and that is how the enzyme will not be able to convert the substrate into the product, right. Now, this inhibitor could be of multiple types. So, what is the inhibitor?

An inhibitor is a chemical agent which is inhibiting or poisoning the enzyme which means it is actually blocking the enzymes in such a way that your the enzyme will not be able to convert the substrate into the product. Depending on the type of inhibitions the enzyme the inhibition could be of irreversible inhibitions or the reversible inhibitions.

Irreversible measure means it is going to be a permanent type of inhibition once you end add the inhibitor it is actually going to block the enzyme and then this it is going to be a irreversible process. Whereas, in the case of reversible inhibition the enzyme inhibitor will actually going to inhibit the enzyme, but if you do some treatment or if you do some other method it is actually going to you know reduce or it is actually going to relieve the inhibitions.

So, the first question comes how you will be able? So, suppose you have designed an inhibitor right and you have tested that onto the enzyme, right. So, suppose you have

tested it on the enzyme and you will find that it is actually forming a complex with the enzyme and because of that the enzyme is unabled. So, in when the enzyme is active it is getting converted the substrate into the product, but when the enzyme is inhibited, it is not being able to convert the substrate into the product, right.

So, it will not be able to convert a substrate into the product, but this information is not complete until you know whether the inhibition or the inhibition is reversible or the irreversible. So, the first thing what you have to understand is whether the inhibition is reversible or the irreversible. So, how we can actually be able to use the different types of methods to understand this?

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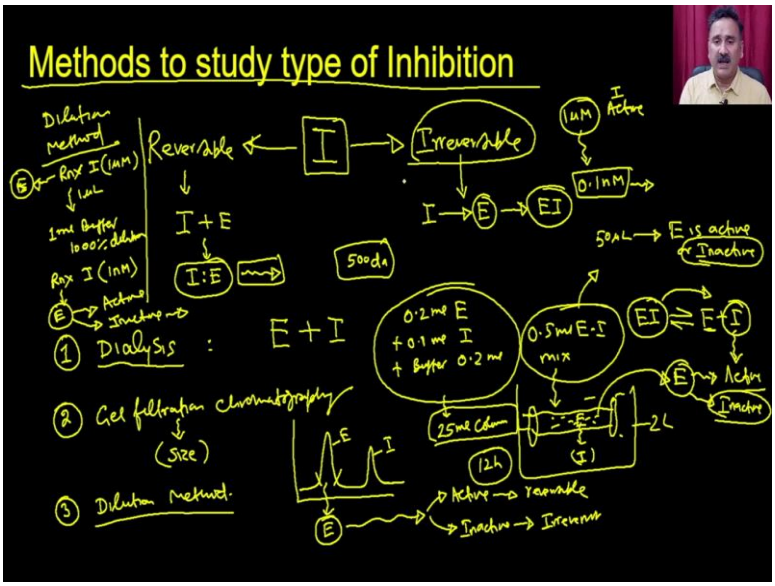

So, the enzyme. So, inhibitor could be reversible or irreversible, right. Now, in an irreversible enzyme inhibitor the enzyme inhibitor is actually going to interact with the enzyme and that is how it is actually going to make a permanent complex, which means it is going to be a permanently lock the enzyme into this particular transformation.

Whereas, in the case of reversible inhibition what will happen is that you have an enzyme and when it is actually going to interact with the inhibitor you have an inhibitor when it is interact with the enzyme it is actually going to form the enzyme inhibition complex, but that inhibit that complex is being mediated mostly by the known covalent interaction such as hydrogen bonding, mineral wall forces and electrostatic interactions.

So, this is reversible which means if you add for example, if this is being mediated by the third-bit interactions you can actually be able to change the pH and if you change the pH this inhibitor is not going to be bind the in enzyme or even the inhibitor is bound to the enzyme, it is actually going to be changed. The other thing what you can also do is you can actually be able to do the other kinds of treatment and that is how the enzyme inhibitor complex can be broken down and that is how the enzyme can be reactivated its activity.

So, how there are many methods what you can actually be able to use to understand whether the inhibition is reversible or the irreversible. First thing what you have to do is first method is you can actually be able to do the dialysis ok. So, what you can do is and I am sure you all know about dialysis, right.

So, what we do is we take the enzyme ok and then you add the inhibitor ok and take this whole thing for example, if I take the 0.2 ml of enzyme and then if I add the 0.1 ml of inhibitor ok and then you add the buffer right for example, you add the buffer of 0.2 ml ok. So, this means this is the total 0.5 ml enzyme inhibitor mixture ok.

Now, what from this 0.5 ml what you can do is you can just take out the 50 microliter and you check whether the enzyme is active right that we confirm that the inhibitor is working and inhibitor has inhibited the enzyme. So, it is not active, right. So, if that is why the enzyme is not active, right. So, in active or inactive, right. So, if the enzyme is inactive you will know that the inhibitor is working.

Now, what you can do is just take this and put it into a dialysis bag ok. So, you put the dialysis clips and put it into a dialysis bag ok. So, you take this 0.5 ml and put it into a dialysis bag right and put it into a beaker right with lot of buffer right. So, you can put like for example, 2 litre buffer.

So, what will happen is that when you put it into the 2 litre buffer, the if it is a if it is a reversible inhibition right because you have reduced because you have increased the volume the enzyme inhibitor complex is actually going to be get because you know that enzyme inhibition complex is having the equilibrium with enzyme and inhibitor, right.

So, if you increase the volume the equilibrium will go into this direction, ok. And because of that the enzyme is actually going to be get dissociated from the inhibitor

because the concentration of this complex is going to be lower down and as a result the it is actually will go into this direction.

And, as soon as it will go into this direction the inhibitor will actually going to be removed from the inhibitor from the enzyme and when the inhibitor is going to be removed and since the inhibitor is of a small molecular weight mostly the inhibitors are in the range of 500 dalton, right they will come out. So, inhibitor will come out and we will present in the outer environment ok. And, because of that the enzyme is you are going to get a free enzyme.

So, enzyme will remain inside and the inhibitor will come outside and that is how the enzyme and now, what you can do is just after for example, if you do this at for 12 hours ok. And after that what you can do is just take out the enzyme and ask the same question whether the enzyme is active or inactive. And, if the enzyme is active ok then the inhibition is reversible, if the enzyme is still inactive ok, if it is enzyme is inactive then you will know that the inhibition is irreversible.

Because the inhibitor enzyme complex is not being mediated by the reversible forces. It is being mediated by the irreversible forces such as covalent bond and all those kind of things. So, in that cases the enzyme is not inhibitor will not get going to be dissociated from the enzyme and that is how you can be able to know this. So, one method is dialysis. You can actually be able to do dialysis.

The 2nd which is still the same simpler method, but it is more refined. So, what you can do is you can do the gel filtration and I am sure still you remember when we were talking about the gel filtration chromatography that gel filtration chromatography is actually going to resolve the molecules based on the size. So, what will happen is that you take the you again make a mixture of 0.5 ml and then you load this mixture onto a gel filtration chromatography.

So, what will happen is the on the gel filtration this 0.5 is actually going to be loaded onto a 25 ml of column ok. So, because you have loaded into a 25 ml column the complex is again going to be get diluted. And as a result, the enzyme inhibition complex is again going to be dissociated into the enzyme and inhibitor. And, as a result what will happen is that inhibitor is going to be separated from the enzyme.

So, what will happen is you will going to see the two peaks. You are going to see a peak which is for the enzyme and you will going to see another peak which is for the inhibitor. So, this is for the peak for the inhibitor, this is the peak for the enzyme when you run it on the gel filtration chromatography.

So, now what you can do is just take this whole peak, you can collect and you are going to get the enzyme. And, that you can actually again ask the same question whether the enzyme is active or whether it is inactive? If it is active, then the inhibition is reversible right because you have removed the inhibitor and you can be able to remove the inhibitor.

If it is inactive, then you can be able to say that the inhibition is irreversible because you still have the any use you have collected the enzyme fraction, but still the inhibitor is present because inhibitor is of a small molecular weight. So, it will be get separated from the enzyme. So, this is the two method what you can actually be able to use very nicely. The 3rd method what you can do also is you can actually do the dilution method.

So, what the dilution method is? The dilution method will say I will try here ok. So, dilution method. Now, what you can do is in the dilution method suppose an inhibitor is active at 1 micromolar concentration ok. So, suppose the inhibitor is active at 1 micromolar, but it is not active it is not active if you convert the enzyme if you change the concentration at 0.1 nanomolar ok. This means if you do a dilutions, it will not going to be active.

So, at this time it is active the inhibitor is active which means it is actually going to inhibit the enzyme, but this concentration it is not active because it is very very low right you will going to have. So, what you can do is you can just make the reaction mixture right, this reaction mixture right and you keep the inhibitor at 1 micromolar ok. Now, you take the aliquot of the enzyme and ask the question whether the enzyme is maybe active or inactive? So, in this particular concentration the enzyme is going to be active.

Now, what you can do is you just take 1 micro litre of this ok and put it into 1 ml of buffer. This means you have actually done the 1000 percent dilution ok. So, when you do the one 1000 times dilution, what will happen is the inhibitor is 1 micromolar here the reaction mixture is actually going to have the inhibitor which is now in the 1 nanomolar, right because you have diluted it by the 1000 times.

Now, if this again you take the sum amount and you take the enzyme from here and you do the activity. So, if it is active, then you will say that the inhibition is reversible. If it is still inactive right, if you got the enzyme which is inactive then you will say that it is a irreversible.

So, dilution method is not confirmatory result ok. Because sometime what happens is when you dilute the concentration the buffers to 1000 volts or something the enzyme it is quantity also is going to be reduced and in that case it possible that the enzyme may not be very active.

So, dilution method is being done once you have the confirmatory test from the dialysis as well as the gel filtration chromatography. So, dilution method is a quick method because it is just takes you know lot of time because dialysis will take 12 hours, gel filtration chromatography also will take some time.

But dilution method is going to just you have to set up the reactions take the 1 micro litre, put it into 1 ml, mix it and then you take the small aliquot and check whether the enzyme is active or inactive. If the enzyme is active the inhibition is reversible, if the enzyme is inactive, then the inverse in the inhibition is irreversible.

So, dilution method is a quick method, but it is having its own drawback that sometime when you do a 1000 times dilution or 100 times dilution, the enzyme concentration also will be very low and in at that particular concentration the you may not get a very good readable count. So, these are the some of the method analytical method, but you can actually be able to use to study whether the type of inhibitions. Once you know the type of inhibitions the type of inhibition could be reversible or the irreversible.

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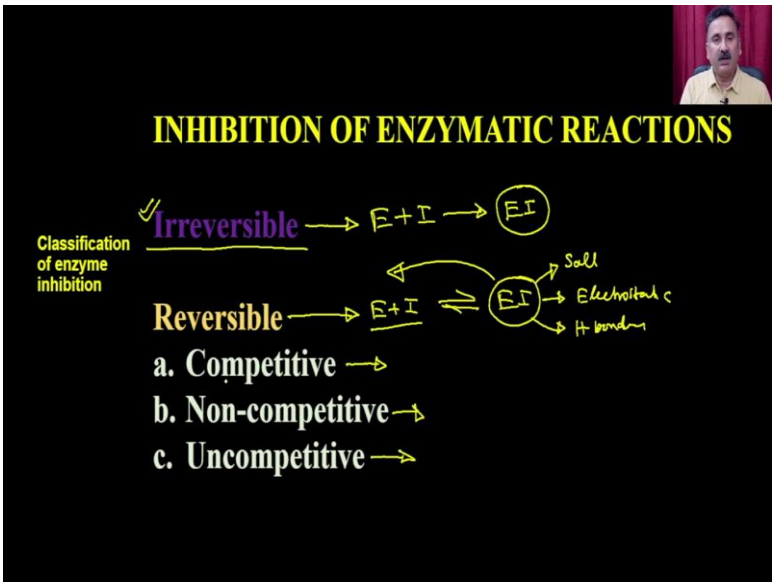

So, we have the irreversible inhibitions where the enzyme is actually going to make a complex with in inhibitor and this complex is the going to be mediated by the irreversible forces such as covalent bond and what other kinds of interactions. So, this is not going to be get dissociated whereas, in the case of reversible inhibitions the enzyme is going to interact with the inhibition and it is actually going to be reversible.

So, in this inhibition this enzyme inhibition complex is going to be mediated by the reversible forces such as salt bridge interactions it is going to be mediated by the electrostatic interactions and it is going to be mediated by the hydrogen bonding and so on. So, because these interactions can be broken down the inhibition can inhibitor can actually get broken down and give you the free enzyme and inhibitor gain.

So, in the reversible category you have three different types of inhibitions – you can have the competitive inhibition, you can have non-competitive inhibition and you can have the uncompetitive inhibition. So, first we will going to discuss about irreversible inhibitions and then we were going to discuss about the reversible inhibitions.

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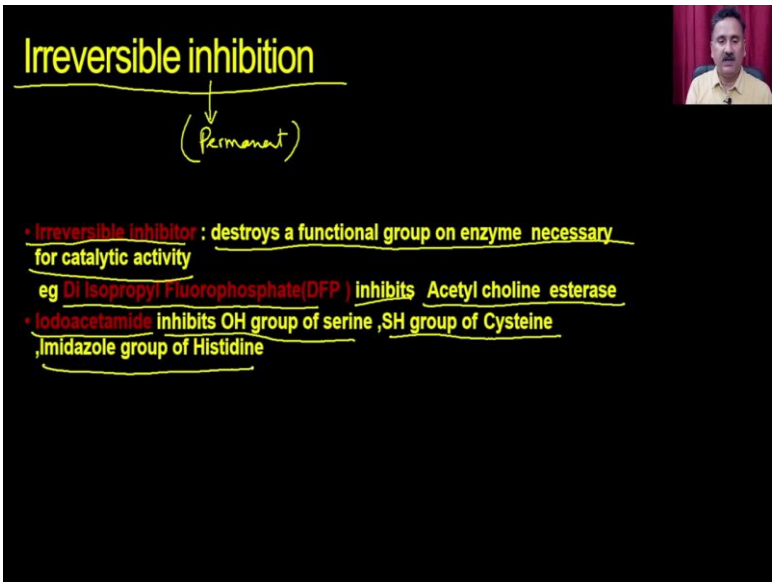

So, irreversible inhibitor or irreversible inhibition as the name suggests it is actually going to be permanent and it is not going to be get affected whether you dilute the enzyme, whether you do gel filtration chromatography whether you dialysis some knitting. Once you add the inhibitor, it is actually going to might make a complex with the enzyme and that is how it is actually going to destroy the activity.

So, irreversible inhibitor destroyed a functional group on the enzyme which is necessary for the catalytic activity. For example, Di Isopropyl Fluorophosphate or DFP which inhibits acetyl choline esterase ok. The another example is iodoacetamide and the iodoacetamide inhibits the OH group of serine and SH group of cysteine and imidazole group of the imidazole group of the histidine.

So, they are mostly the irreversible inhibitor are actually going to go and bind or destroy the functional groups onto the enzyme and that is going to be irreversible damage and that is how the enzyme will not be able to recover it is activity even if the inhibitor is present or not present around.

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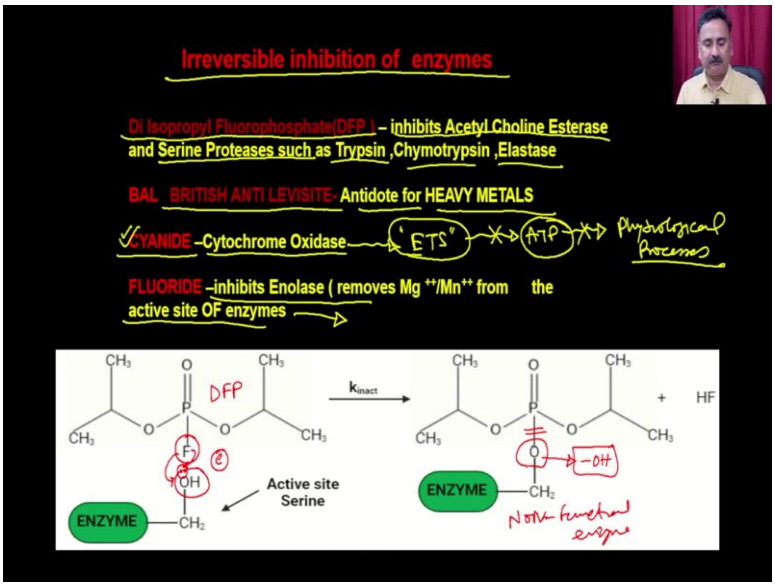

There are some examples of irreversible inhibition of the enzyme. For example, Di Isopropyl Fluorophosphate DFP which inhibits the acetyl-choline esterase and serine proteases such as trypsin, chymotrypsin and elastase. Then we have the British anti-levisite which is a antidote for the heavy-metal toxicity.

Then we have a cyanide, right. You remember that the classical case of cyanide that cyanide nobody know the taste of cyanide because cyanide is very very toxic poison, right and why it is poison because it is actually having an irreversible inhibition of cytochrome oxidases. And, cytochrome oxidase is a very important component of electron transport chain.

So, if the electron transport chain is going to be blocked by a molecule it will actually stop the production of the ATP. So, there will be a no production of ATP and as soon as that there will be a no production of ATP it is actually going to stop the physiological processes, right.

And, that is why the cyanide is going to be very very very potent right inhibitor right because it is going to be irreversible and this because it cannot be you know allow the enzyme to be reactivated or something that is why any kind of poisoning or toxicity by the cyanide cannot be reversed.

Then we have the fluoride. So, fluoride actually inhibits the enolase and it removes the magnesium and manganese from the active site of the enzyme and that is how it is actually going to deprive the enzyme from the crucial co-factors and because of that it is the enzyme will not be able to active.

So, in a typical reaction what will happen is that this is a reaction where I we are actually discussing about how the enzyme is actually how the this DFP is actually inhibiting, right. So, this is actually di isopropyl fluorophosphate. This is a difluoro phosphate and what you see here is this is the fluorine what is present onto this phosphate, right and this is the DFP, right.

And, this is the enzyme where enzyme which actually contains the active site serene ok and it has a serene OH, right. So, you see the lone pair of electron here which is present onto the oxygen of the serene. So, what happen is that when it interacts the fluorine is actually going to be removed, right and this fluorine is going to be removed because it is fluorine is going to be attacked onto the lone pair of the oxygen and as a result the it is OH O is actually going to make a complex with phosphate.

And, now what you see here is that lone pair is also gone and does not have the functionally active OH group, right and this functionally active OH group is very important because once the OH is present it actually can take up the electrons from the substrate it actually can give the electrons to the substrate and that is how the functionality of the enzyme is solely being depend onto this particular functional group.

So, when you added the DFP what the DFP will do is this fluorine is actually going to attack onto this particular oxygen and as a result it is actually going to make a permanent complex, see this is a covalent bond, right. So, if it is making a covalent bond whether you dilute this enzyme, whether you dialyze this enzyme or whether you run it as a in the gel filtration this bond is not going to be broken down and that is why this enzyme will no longer be functional.

So, this is actually going to be a non-functional enzyme because the inhibitor has blocked one of the crucial amino acid and this crucial amino acid could be present in the active site and that is why it is participating into the reaction. So, this enzyme is functional enzyme this function enzyme is a non-functional enzyme. So, this is the more

or less the classical activity or classical way in which the irreversible inhibitors are actually inhibiting the enzyme.

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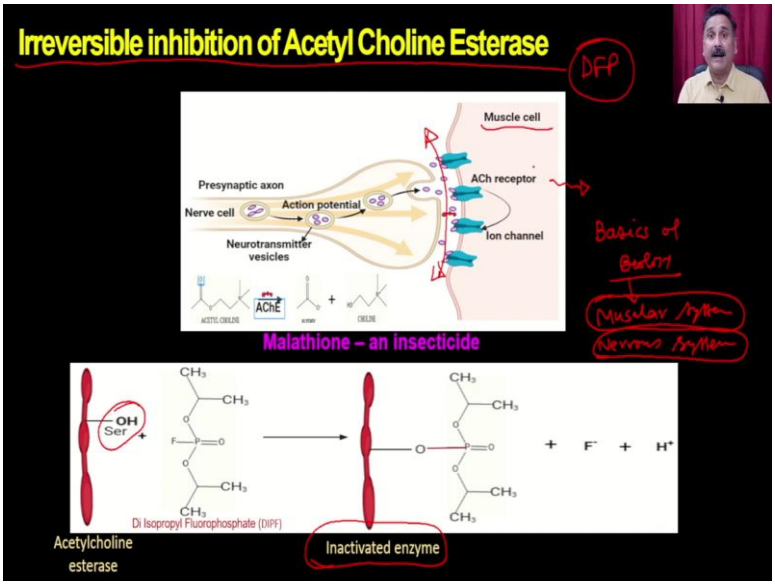

Now, irreversible inhibition of the acetyl choline esterase by DFP, right. So, DFP is a very potent inhibitor. So, what will happen is that when it will actually going to you know inhibit the acetyl choline esterase it is actually going to interfere into the neural transport and because of that it is actually going to stop the communication between the muscle cell and as well as the neurons, ok.

And, as a result it is actually going to stop the activity of the muscle cell because you know that the muscle cells require the neural signal for the and it is their actions, right. If you want to know more about the muscle action what you can do is you can actually be able to go through with some of my course of which is called as the basics of biology where I have discussed about the muscular system and we have I have also discussed about the nervous system ok.

So, how the acetyl choline esterase and the neural transmission works you can actually be able to go through with these lectures and they will actually going to give you the full idea. And, once this is actually going to be blocked, the muscles will not actually going to be contract or relax and all those things and that is how the person is no longer be able to move it is you know body, ok. So, this is what exactly going to happen.

So, acetyl choline esterase will have the functional A active serine and DFP is going to be added it is actually going to make a complex and that is how it is actually going to produce the inactive enzyme. And, this inactive enzyme is actually going to block the you know the transmission of the signal from the nerve cells to the muscle cell and that is how it is actually going to stop the muscle contractions and as well as and so, the person is going to be get paralyzed.

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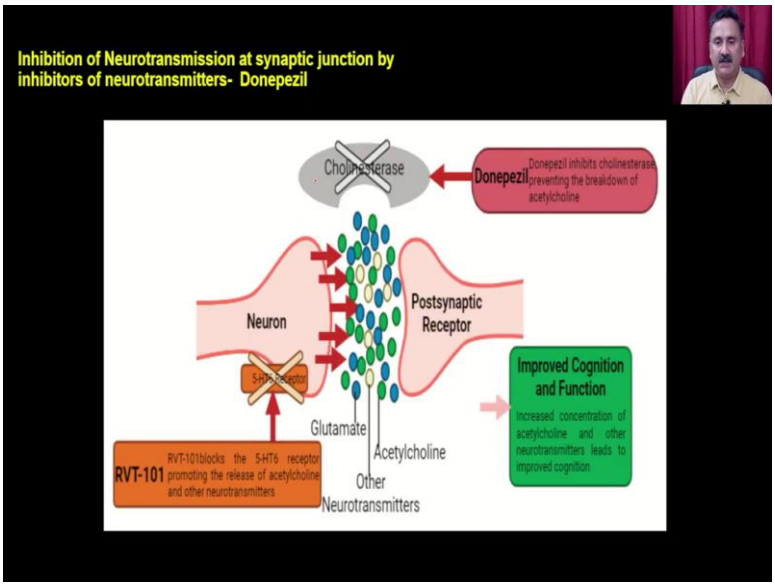

So, cholinesterase this is exactly the same, what we have shown that you are actually going to have the RVT-101 inhibitor. And, that also blocks another receptor which is called as the 5t 5-HT6 receptor which actually promotes the release of acetylcholine and other neurotransmitters. And, once you are actually going to block this particular receptor it is actually going to stop the you know, secretion of the neurotransmitters such as glutamate, acetylcholine and all that.

And, because of that it is actually going to stop the neural transmission between the two particular neurons. And, same the other drug which is called as donepezil inhibits the cholinesterase preventing the breakdown of the acetylcholine. And how it is actually going to be act working because it is because it is going to stop the neural transmissions, it is actually going to increase the concentration of the acetylcholine and other neurotransmitters leading to the improved cognitions.

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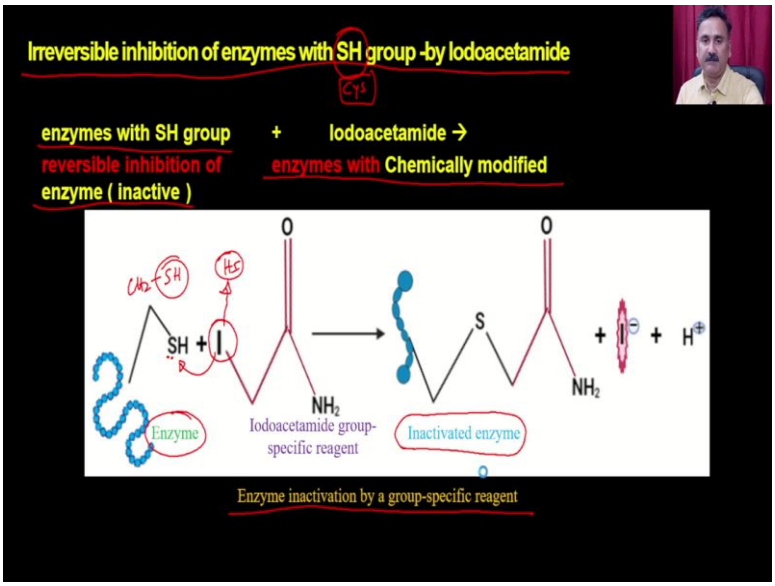

So, irreversible inhibition of the enzyme with the SH group. So, this is another example where the iodoacetamide can be used and iodoacetamide is going to block the enzyme which has the SH group which means it is going to block the enzyme where you have the functionally active cysteine. So, enzyme with SH group such as the cysteine when they are interacting with the iodoacetamide, they are enzymes are getting the chemically modified and that is how the enzyme is getting converted into the inactive enzyme.

So, what happen is that you have the enzyme for example, some enzyme which actually contains the active site cysteine, right. So, it is going to have this right CH 2 SH. So, this is the CH 2, this is SH right and when you add the inhibitor, inhibitor is going you are going to add the iodoacetamide. So, iodoacetamide is going to have the iodine and SH is also again going to have the electrons, right.

So, this iodine is actually going to attack onto these sulfur groups, right and as a result there will be a covalent bond which is going to be formed between the sulfur and the this carbon right and iodine is actually going to be removed, right. So, H I is actually. So, this is going to be removed here right.

Iodine is going to be removed and hydrogen is also going to be removed from here, right and then there will be a covalent bond. Once a covalent bond is formed, this enzyme is going to be get converted into a inactive enzyme. So, these are the some of the examples where you are actually going to you know chemically modify the enzyme.

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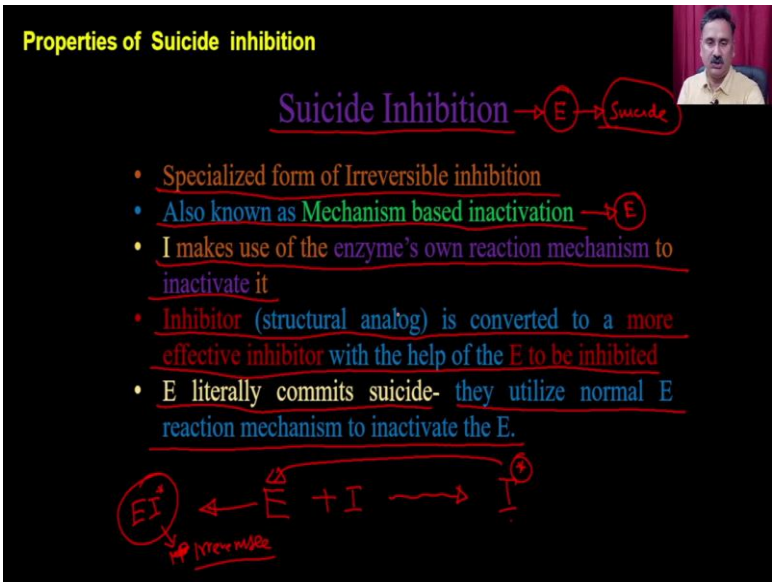

Then we have the another variation of the irreversible inhibition which is called as the suicide inhibitions. So, suicide inhibitions is as a name suggest here when you add the inhibitor, the enzyme will actually going to go for the suicide which means it is actually going to enzyme it is actually going to process the inhibitor enzyme is actually.

So, inhibitor is not irreversible inhibitor. It is actually once it is going to be processed by the enzyme, it is actually going to be get converted into the irreversible inhibition, inhibitor and that is how the enzyme is actually responsible for converting a inhibitor into a suicidal inhibitor.

So, this is a specialized form of irreversible inhibition. It is also known as the mechanism based inactivation's right because here the enzyme is going to be actively participate into the inhibition mechanisms. Enzyme inhibitor makes use of the enzyme's own reaction mechanism to inactivate it. Inhibitor which is a structural analog is converted into a more effective inhibitor with the help of the enzyme to be inhibited.

So, enzyme is literally committing suicide because they utilize the normal enzyme reaction mechanism to inactivate the enzyme. We will take the example then you will understand how what it mean actually. So, what it says is that enzyme is actually going to process the inhibitor and as a result it is actually going to be get converted into it is actually going to process this inhibitor.

So, inhibitor is actually going to be go and bind to the enzyme and then it is going to be processed and that is how the enzyme is inhibitor is going to get converted into a inhibitor star.

And, then as soon as the inhibitor star is going to be formed, it is actually going to attack on the enzyme and that is how it is actually going to form a enzyme inhibitor star complex. This is actually a irreversible ok. So, this is actually going to be irreversible inhibition. This means the enzyme is itself acting on the inhibitor and making on its own depth actually and that is why it is called as a suicide inhibition.

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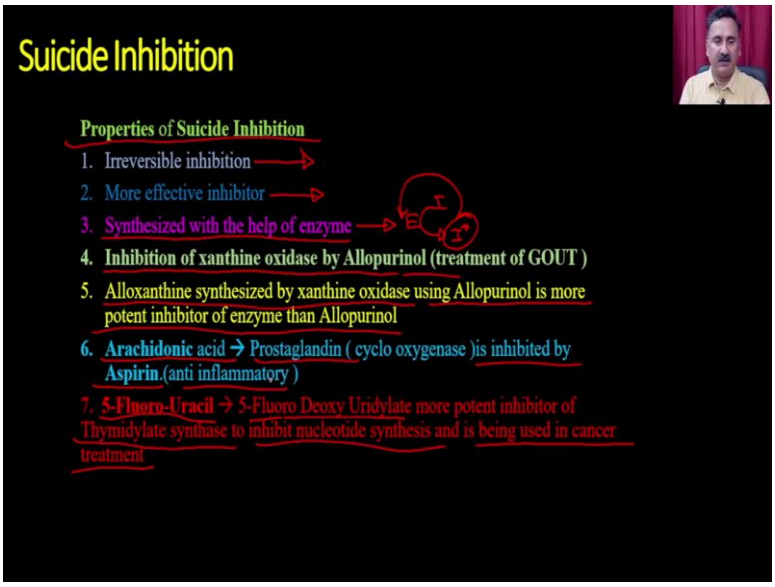

So, what is the properties of the suicide inhibition? It is going to be a irreversible inhibitions. It is a very very effective inhibitor compared to even the irreversible inhibitions where you have the covalent bond. It is synthesized with the help of the enzymes which means the enzyme is actually going to be get convert the inhibitor into a suicidal inhibitor and then this in suicidal inhibitor is actually going to act on to the enzyme.

Examples are inhibition of the xanthine oxidase by the allopurinol which is a treatment of the GOUT. Then we have the alloxanthin synthesized by the xanthine oxidase using the allopurinol is more potent inhibitor of enzyme than the allopurinol. Then we have another example of arachidonic acid getting converted into a prostaglandin which is

going to be catalyzed by an enzyme which is called as cyclooxygenase and it is inhibited by the aspirin.

Aspirin is an anti-inflammatory drug, right and, then we also have an example of 5-fluoro-uracil. So, 5 fluoro-uracil is getting converted into 5-fluoro deoxy-uridine and this 5-fluoro deoxy uridine is a more potent suicidal inhibitor because it is going to inhibit the thymidylate synthesis and it is going to inhibit the nucleotide synthesis and it is being used in the cancer treatment. So, 5-fluorouracil is an anti-cancer drug. So, let us take an example of these some of these mechanisms.

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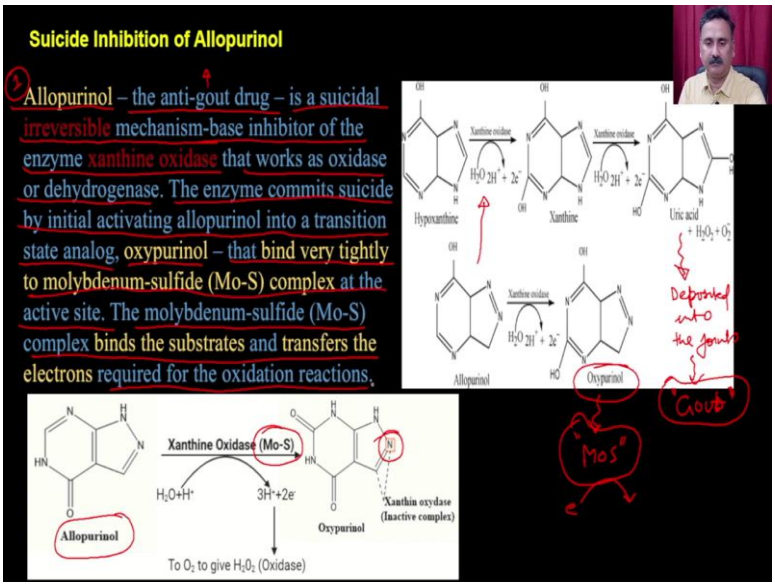

So, first example is allopurinol. So, allopurinol and allopurinol is a drug for the gout, right; gout you know that gout is a disease of the joints, right. So, the anti-gout drug is a suicidal irreversible inhibitor, mechanism based inhibitor of the enzyme xanthine oxidase that works as the oxidase or the dehydrogenase. The enzyme commits suicide by initiating, activating allopurinol into a transition state analog, oxypurinol that binds very tightly to the molybdenum sulfide complex at the active site.

The molybdenum-sulfide complex binds the substrate and transfer the electron required for the oxidation reaction. So, what happen is that in a normal reactions what is the xanthine oxidase is doing is it is processing the hypo xanthine to xanthine and then from xanthine, xanthine oxidase is getting converted into the uric acid and this uric acid is a actually a problem because uric acid is a metabolic by-product.

Remember that when we were talking about the amino acid metabolism, we will say that there are so many you know nitrogen derivatives and other things are going to be produced. So, you will going to produce uric acid, urea and all the other kinds of derivatives. So, uric acid is actually going to be get deposited into the joints, right and that is how it is actually going to cause the disease which is called as gout and mostly it is a sometime it is a genetic disease and other kinds of thing, right.

So, now what happen is that when you have the allopurinol, right. So, allopurinol is a inhibitor of xanthine oxidase. But, when the allopurinol is actually going to be processed by the xanthine oxidase, it is actually going to be get converted into oxypurinol ok. And, oxypurinol is a very, very, very potent inhibitor because it is actually going to bind the molybdenum sulfur center what is present inside the xanthine oxidase.

So, initially the allopurinol does not have any activity or any kind of binding to the molybdenum sulfur center and that is how the allopurinol actually can will not be will not be able to inhibit the xanthine oxidase if the xanthine oxidase will not process the allopurinol to a oxypurinol. So, allopurinol getting converted into oxypurinol and oxypurinol is actually you know attacking onto this molybdenum sulfur center and making a complex.

And, once it is making a complex, the molybdenum sulfur center is actually the active center which is involved into the electron transport or electron movement between from the enzyme to the substrate. And, because of that, it will actually going to stop this in the this particular all these reactions which means it is actually going to stop the synthesis of the uric acid.

And, as a result, it is actually going to give you the treatment for the gout. So, this is what exactly happened. Allopurinol getting converted into the oxypurinol and oxypurinol, it is actually going to have this nitrogen, right and this nitrogen is actually going to interact with the molybdenum and sulfur center, what is present on to the xanthine oxidase. And that is how it is going to make the irreversible complexes.

And, because it is made to make a reversible complex, molybdenum sulfur centers are no longer or molybdenum sulfide center will no longer be able to transfer the electron from the substrate to the other molecules and, as a result, they will not be able to catalyze these reactions.

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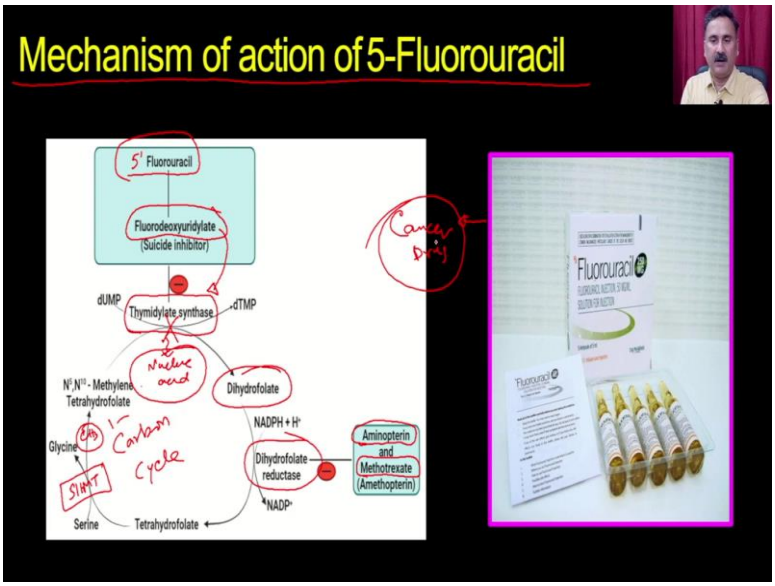

Then we have the mechanism of action of the 5 fluorouracils. So, 5 fluorouracil, so, 5 fluorouracil is getting converted into a fluoro-deoxy uridine, and that is actually a suicide inhibitor. So, this is the carbon cycle, this is the carbon, one carbon cycle, right, through which the nucleotide and as well as the amino acid components are actually exchanging the carbon pool between the. So, you will see that these are the amino protein carbon pool, and this is the amino, the nucleotide carbon pool.

So, when there is a shortage of carbon into the protein pool, the protein can actually take the carbon from the nucleotides. When there will be a shortage of carbon in the nucleotide pool, then they will actually do the reverse. And, these are the some of the enzyme, but actually going to function. So, you have the dihydrofolate reductase, you have the thymidylate synthase and all other kinds of thing, right.

And, at this place, you also have another enzyme which is called as the SHMT, right. So, which actually going to convert the serine to glycine, and that is how the carbon, the CH 3 is actually going to be transferred onto the tetrahydrofolate and form the N 5, N 10 methylene tetrahydrofolate.

And, then N 5, N 10 methylene- tetrahydrofolate, which actually carry the carbon from the serine is actually going to be taken up by the this system and that is how it is actually going to form the dihydrofolate and then dihydrofolate is actually going to be converted into tetrahydrofolate and so on.

So, thymidylate synthase is a very, very active enzyme and it is responsible for the nucleic acid synthesis, right, because it is actually going to be involved into the synthesis of neurotypes and that is how it is actually going to be. So, if you thymidylate synthase, when it is process is the 5 fluorouracil, it get converted into fluorodeoxy-uridine. And, fluorodeoxy-uridine is a suicidal inhibitor for the thymidylate synthase.

And, that is how it is actually going to block the thymidylate synthase and that is how it is actually going to stop the reaction here. This means there will be no exchange of carbon pool between the I mean protein chain and as well as the nucleotide. And, as a result, the nucleotide is synthesis is going to be stopped and that is how it is actually going to be blocked.

The other enzyme which is dihydrofolate is actually going to be inhibited by the aminopterin and the methotrexate. These are the two other inhibitor, but these are not irreversible inhibitor. These are the drugs, right, 5-fluorouracil what people take for the cancer, right,. So, this is a cancer drug.

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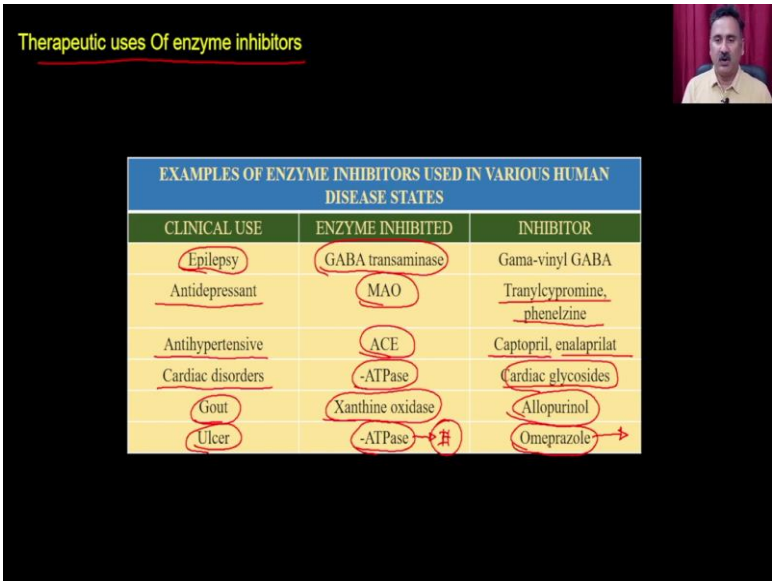

So, apart from the cancer, we have another example, the therapeutic usage of the enzyme inhibitors. You can have the, you know, the clinical use for the epilepsy. So, where you are actually inhibiting the enzyme which is called as GABA transaminase and the inhibitor is called as gama-vinyl GABA, right. Then, we have the drugs for the

antidepressants which is called as MAO and the inhibitor is the tranylcypromine phenelzine.

These are the two different types of inhibitors and then we have the anti-hypersensitive drugs which is acetylcholinesterase. The drug's name is captopril and the enalaprilat drug. Then we have the cardiac disorders. So, there are so many ATPases and then you also have the cardiac glycosides. Then we have a gout. So, gout, you enzyme is xanthine oxidase, the drug is allopurinol.

And, then we have ulcers, right. So, you can have many types of ATPases because these ATPases are involved in the, you know, the proton transport and all that. So, you can actually be able to use the omeprazole, right, and it is very popular, right, omeprazole, pantoprazole's and all the kinds of azole derivatives which you can use and these are the all are irreversible inhibitors for the these class of enzymes.

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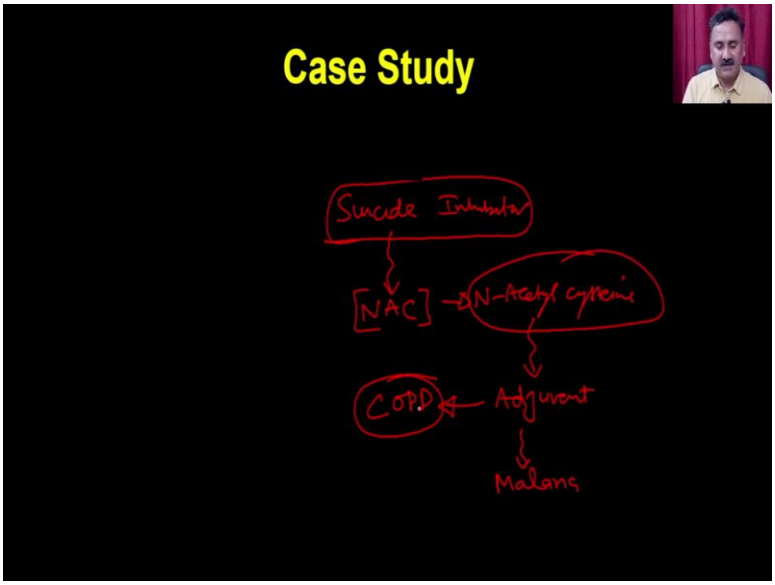

Now, let us see, you know, the case study where we are actually going to study how you can be able to study a suicidal inhibition kinetics and how you can be able to understand the different types of, you know, the parameters and all that. So, the case study is very simple, right, where we are actually going to study about how you can be able to suicide inhibitors, right.

This is a suicidal inhibitor where we are actually trying to inhibit, where we are trying to study inhibitor which is called as NAC, ok. So, NACs full form is N-Acetyl-Cysteine, ok, and it is actually, being we very extensively being used as a adjuvant into the relieving the some of the you know, the damages during the malaria. Or it is also being used in the drug, in another disease which is called as the COPD, ok. So, cognitive pulmonary disease.

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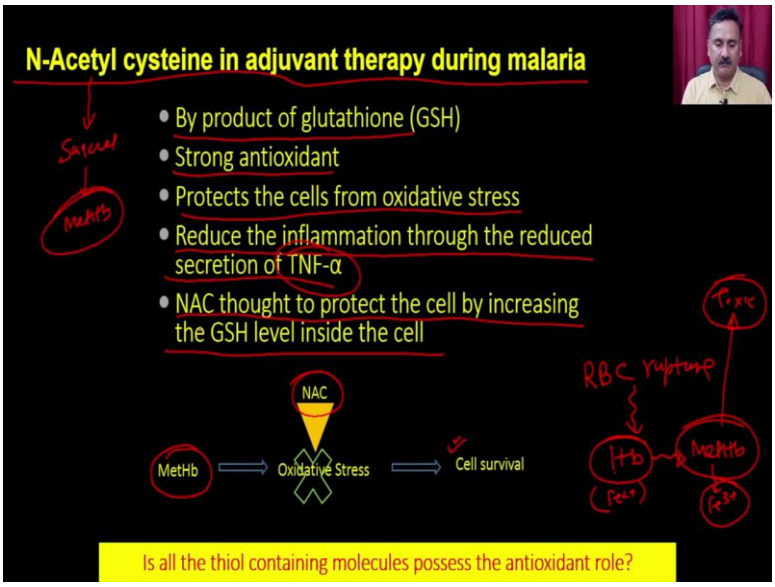

So, N-acetyl cysteine is being used in adjuvant therapy, it is a by-product of glutathione. It is a strong antioxidants. It protects the cells from the oxidative stress, it reduce the inflammation through the reduced secretion of the cytokine, which is called TNF alpha and NAC thought to be protect the cell by increasing the GSH level inside the cell.

So, what happen is that during the malaria, you always have the RBC rupture, right, and when there is a RBC rupture, the RBC is actually going to be released the hemoglobin and that haemoglobin is getting converted into methaemoglobin. So, haemoglobin, you know that haemoglobin is the oxygen carrier, right. And, once that is haemoglobin is getting present into the oxygen environment, it get oxidized.

So, haemoglobin is actually containing the iron as iron 2 plus, right, whereas, when the iron is present as iron 3 plus, it is called as methaemoglobin and, because of this conversion, the methaemoglobin is very toxic, right. So, it actually can cause lot of toxicity issues, right, and that is what, that happens during the malaria. So,

methaemoglobin actually can cause the oxidative stress into the cell and that is how it is actually going to be kill the cells, right.

So, since the NAC was antioxidant molecule, we thought that it may be blocking the methaemoglobin molecule and that is how it may be reducing the oxidative stress and that is how it probably will be promoting the cell survival. So, exploring this particular hypothesis, we discovered that the NAC is a suicidal inhibitor for methaemoglobin.

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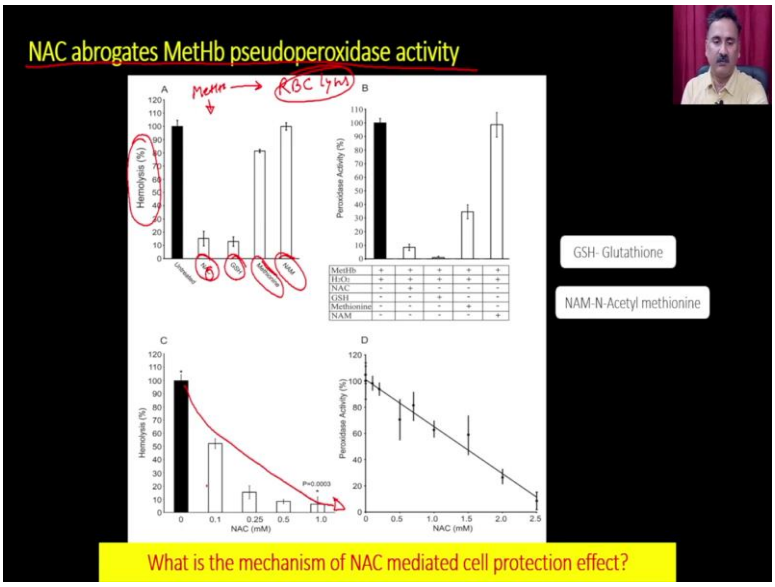

Now, when we want to do this kind of study, we have to actually take lot of control experiments, ok. So, for example, we have taken the, we have studied how the, because you know, methaemoglobin is actually going to cause the RBC lysis, ok, which means it is actually going to. So, we first thing what we have measured is we have measured whether the RBC lysis is getting blocked or not.

So, we did the NAC, GSH, methaemoglobin, methionine and NAM, right. NAM and NAC is having only one difference that instead of cysteine, you have the methionine and you will see that when I remove the cysteine, right, it is actually not protecting the cell from the haemolysis, right. So, there is a haemolysis, even methionine is also not been able to block, but GSH is being able to block because GSH is doing the same function as the NAC, right.

And, then the same thing we have done also that we have found that the, because the methaemoglobin has the peroxidase activity. So, it was actually blocking the peroxidase activity also the same way and then the haemolysis is also been blocked by those dependent T by the NAC.

So, as you increase the NAC concentration, the haemolysis is going to be blocked, same is true for the peroxidase activity. So, this is true that the NAC is actually protecting the cells from the haemolysis and NAC is also protecting the, also inhibiting the peroxidase activity.

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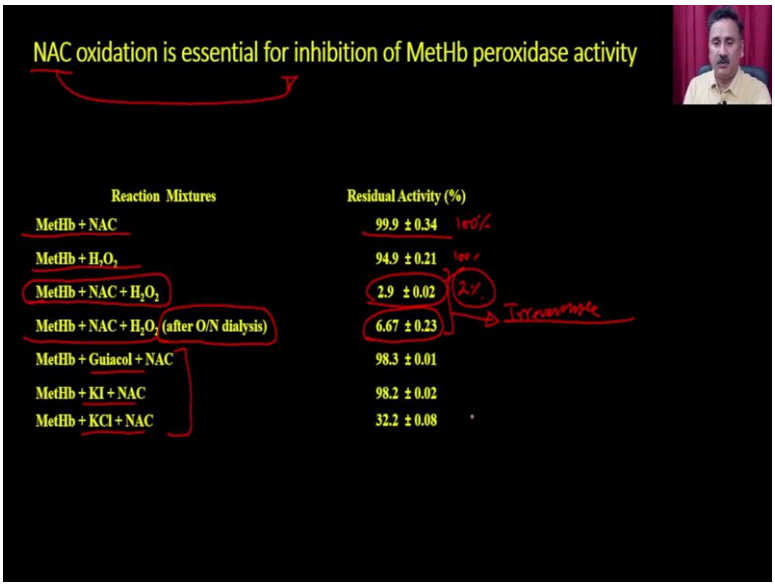

So, then we also ask whether the inhibition is reversible or irreversible. So, this is exactly what we have done and remember, this is the experiment I have just said, right. So, what we did is we took the math, methaemoglobin plus NAC you will see, this is a 100 percent activity what you call, right. Then we did the methaemoglobin plus H 2 O 2, it is 100 percent activity.

Then, we did the methaemoglobin plus NAC plus H 2 O 2, which means this is the complete reaction. Once you did the complete reaction, the activity goes down to 2 percent, right. And, then we took this is 100 percent reaction, we did the overnight dilution dialysis and there is no recovery or enzyme. This means by doing this, we know that the inhibition is irreversible, ok. This means the NAC is irreversible inhibitor of the methaemoglobin peroxidase activity.

And, then we tried the other methods, right. So, we tried like protecting it by the substrates and other kinds of things to understand whether the substrate is actually going to protect. So, it is clear that if you do not allow the methaemoglobin to process the NAC, it is actually going to be get blocked, right. This means it is actually going to be suicidal inhibitor. So, if it is a suicidal inhibitor, we also did the suicidal kinetics.

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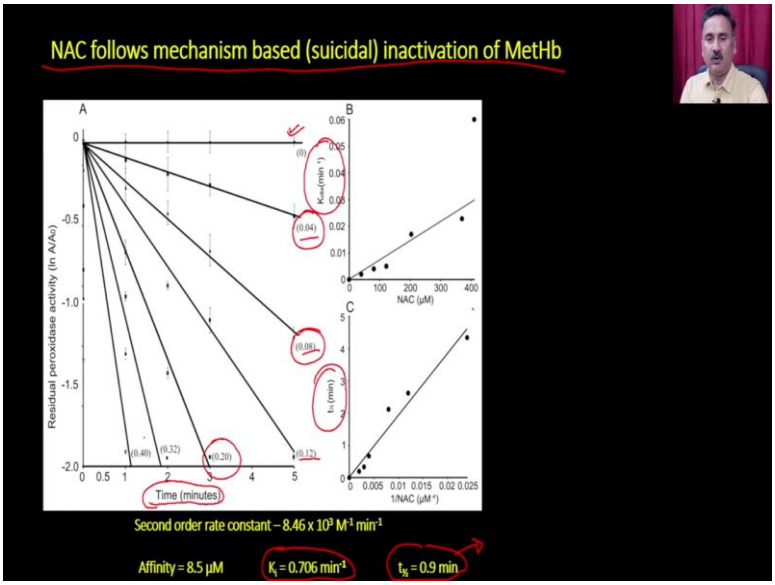

So, this is the suicidal kinetics. So, NAC follows the mechanism based inactivation of methaemoglobin. So, what you do is in the suicidal kinetics, what you do is, you take a reaction mixture and then you are actually going to take out the enzyme at different time intervals, right. Like for example, 0.04, 0.08, 0.12 and all that, you allow the inhibitor to react for these many time points.

And, you will see that the enzyme is fully active. So, 100 percent active, then it is actually going to show you a reduction in change, ok. So, these are the number of times, minutes, what you have actually kept and then you can actually be able to calculate the K ops and you can be able to calculate the T NAC.

So, what is the minimum time required to inactivate the enzyme, ok. And, from this, you will know that the K I for this is 0.76 and the affinity of NAC for the enzyme is 8.5 micromolar and t half in act, which means only 0.9 minute is required for inhibiting the 50 percent of the enzyme.

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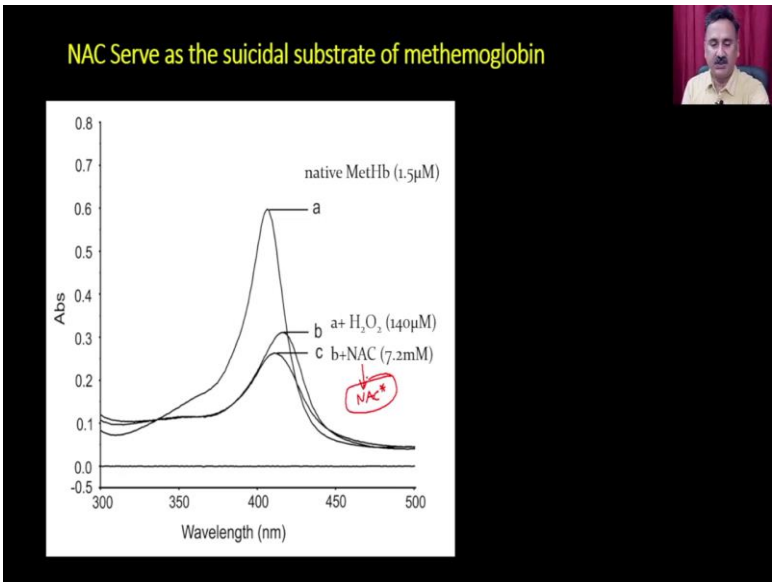

Then we also did the, you know, UV visible spectroscopy to know whether the NAC is been processed by the enzyme. So, it is actually been processed by the enzyme and that is how the NAC is going to be modified by the enzyme.

And, that is how this, in modified NAC is actually going to be the culprit for responsible for the blockage. And that is why this was not happening when we were adding the substrate, because if you add the substrate, then enzyme will process the substrate rather than inhibitor. So, we identified what kind of modification, right.

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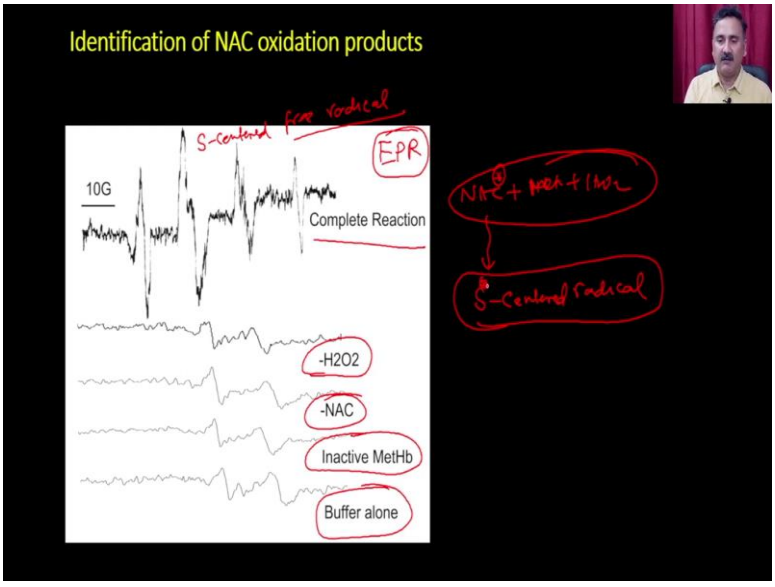

So, what we did is we did an EPR experiment or Electron Paramagnetic Resonance spectroscopy. And, what you found is that there is a sulfur centered free radical, what is going to be produced when you are treating the NAC plus methaemoglobin plus H2O2, ok.

So, that is not present when you are removing the hydrogen peroxide, that is not present when you are removing NAC or when you are adding inactivated methaemoglobin or when you are adding the meth buffer alone. So, this was shown that this complete reaction is producing a free, you know, producing the EPR signal and that EPR signal is, sorry, is actually saying that NAC is getting modified.

And this, what is the modification? Modification is that it is actually forming the sulfur centered radical, ok which means some of the cysteine molecules probably have the sulfur where the you have the single electrons. So, once you have the single electron, it is actually going to go and attack onto the proteins residues and that is how it is actually going to block some of the crucial residues.

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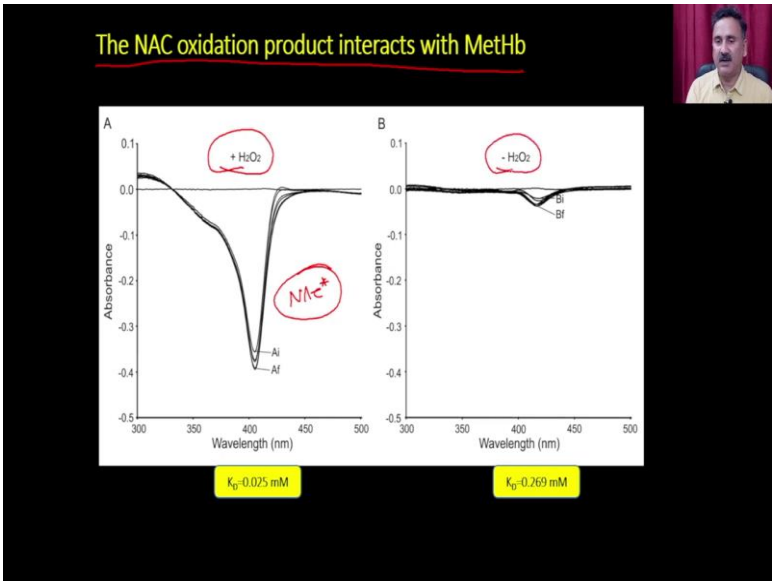

So, NAC oxidation product is actually interacting with the sub enzyme. So, if you add the H 2 O 2, you are actually going to produce the NAC, you know, oxidation product and that is how it is actually going to go and bind. Whereas, in you do not have the hydrogen peroxide, it is actually not going to bind.

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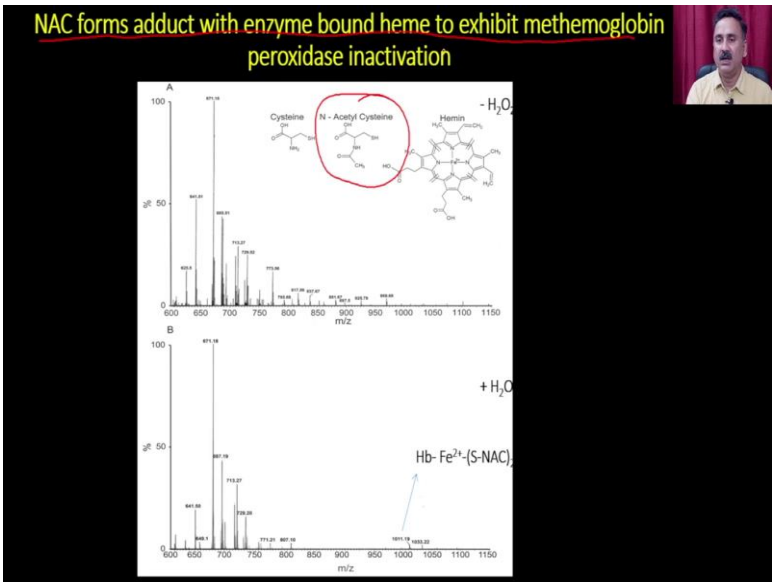

Then what we did is we also identified the what kind of modification. So, we found is that the N-acetyl cysteine is getting modified and this modified product is binding onto the protein and it is actually blocking the hemin. So, methemoglobin has the hemin and it is actually blocking the hemin part. So, hemin is NAC is forming an adduct with the enzyme bound hemin to inhibit the peroxidase activity.

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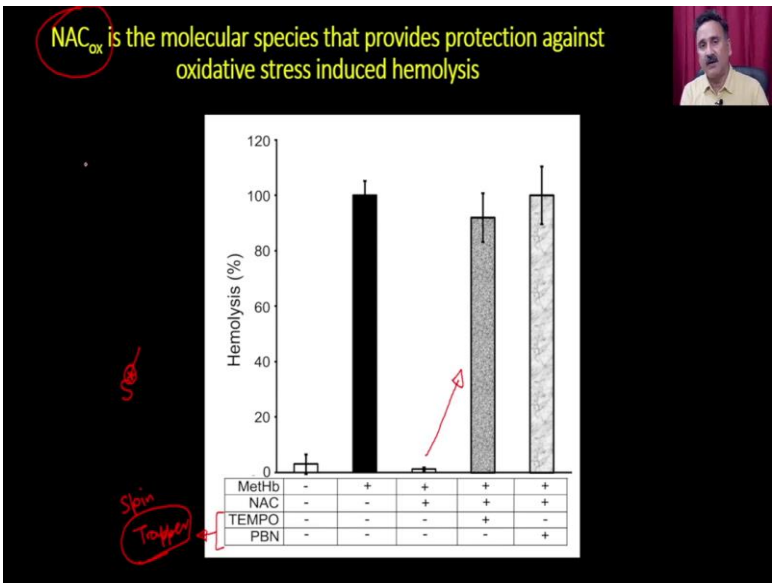

Then we tested this NAC oxidation product and what we found is that when you add actually the spin trappers; so, these are the single electron trappers ok. So, if you add the

NAC along with the tempo or PBN, so, these are the spin trappers actually. So, they will actually go into trap the single electron species ok. So, they are not antioxidant, they are single electron trappers ok. So, for example, if you have a sulfur-centered single electron, they will go and buy it ok. Instead of this, allowing this inhibitor to go and bind to the enzyme.

So, if you have the spin trappers, you will see the haemolysis. If the methaemoglobin NAC is present, but if the methaemoglobin is NAC is present, you will see that if you add these two, there will be inhibit. So, if you have the methaemoglobin, you are going to see a 100 percent haemolysis.

When you have the NAC, you are not going to see the haemolysis, but when you have the activity in the presence of tempo or the PBN, you will going to see that the effect of NAC is actually getting reversed. So, that actually proves that there is a NAC oxidation product which is responsible for the haemolysis.

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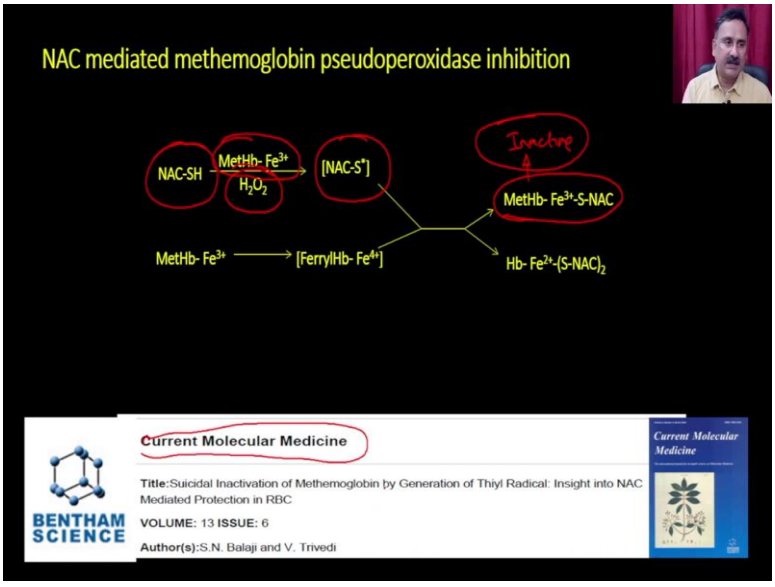

And, ultimately, we have come up with this particular model that NAC is actually having a SH group. So, you will know that the NAC has GSH, right. And, when it is getting processed by the methaemoglobin peroxidase activity with the help of the hydrogen peroxide, NAC is getting converted into the NACs, sulfur-centered radicals.

And, these sulfur-centered radicals are in attacking onto the methaemoglobin and that is how it is actually forming the methaemoglobin iron-sulfur clusters, right and this is inactive. So, this is actually inactive enzyme and that is how it is actually not going to process. If you want to read more about this, you can actually be able to download this particular paper from this particular journal. And, it will actually going to tell you the more details about this.

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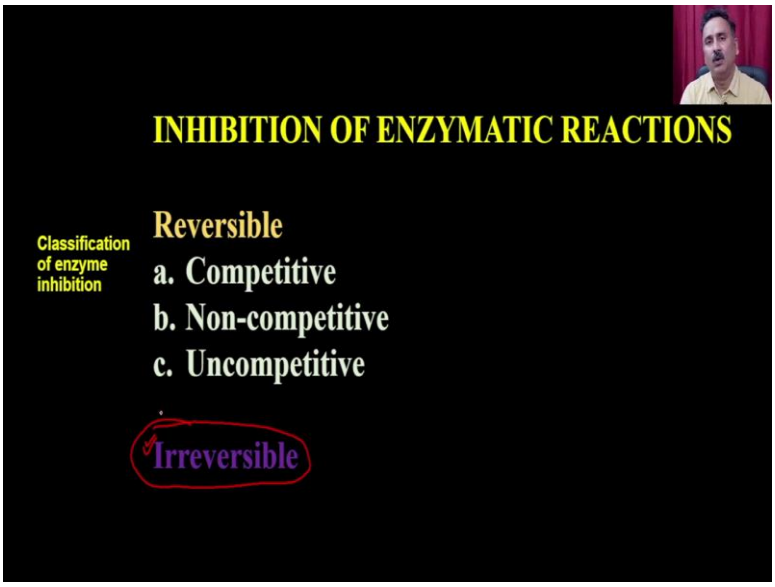

So, so far what we have discussed? We have discussed about the irreversible inhibitions. And, we have discussed also about the suicidal inhibitions. So, inhibitor, when you develop the inhibitor, inhibitor could be of reversible in nature or the irreversible in nature.

And, within the irreversible nature, you can actually have the suicidal in enzymes or suicidal inhibitors or the mechanism-based inhibitors or the non-mechanism-based inhibitor, which means in a non-mechanism-based inhibitors, the irreversible inhibitors, the inhibitor will go and modify the active groups onto the active site.

Whereas, in the case of suicidal inhibitors, the enzyme is actually going to process these inhibitors as such like for substrate and then when it is actually converting the inhibitor into a modified form, that modified form is actually going to have the irreversible binding to the enzyme active site.

And, that is how it is actually going to inhibit the enzyme. So, with this, we are going to conclude our lecture here in our subsequent lecture. We are going to discuss about the reversible inhibitors and we are also going to talk about the enzyme-inhibition kinetics. So, with this, I would like to conclude my lecture.

Thank you.