Organic Chemistry In Biology And Drug Development
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Lecture - 17
Concept of Enzyme Inhibition
(Contd.)

After the completion of the reversible inhibition cases, now we will go to the irreversible inhibition

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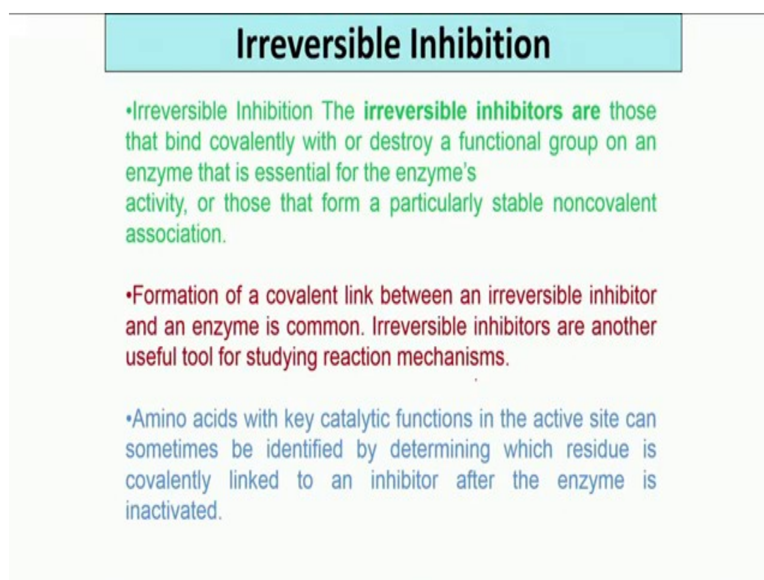

Remember the basic difference between reversible and irreversible inhibition is the formation of a covalent bond in case of irreversible inhibition whereas there is no covalent bond formation in case of reversible inhibition.

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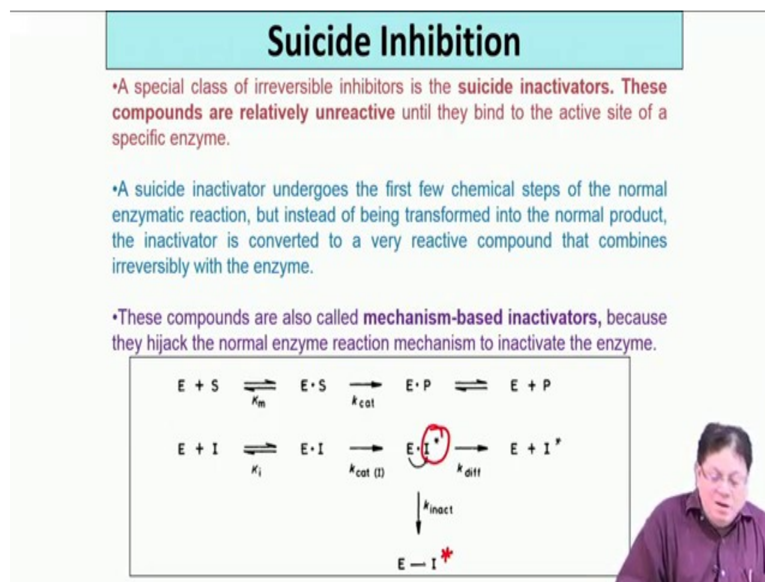

Now, I have already told you that there are two types of irreversible inhibition. One is called Suicide Inhibition.

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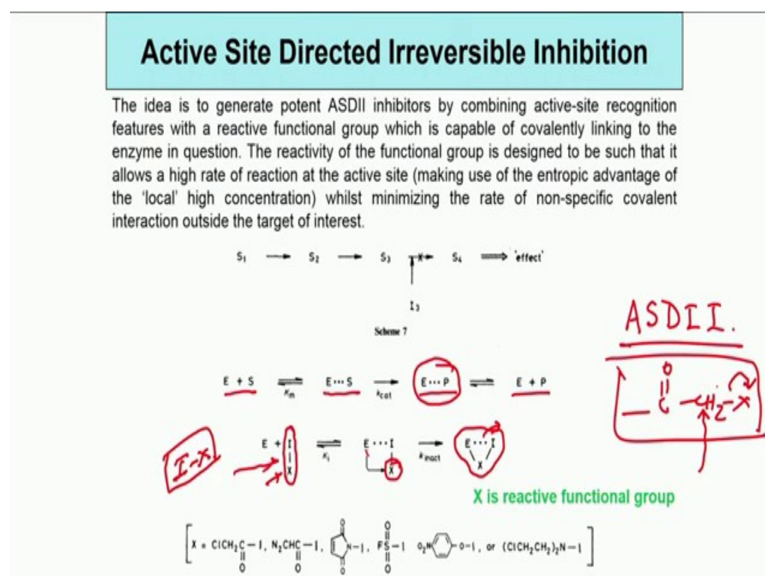

And the other is called active site directed inhibition. Before we go on to the suicide inhibition, we will talk about the Active Site Directed Irreversible Inhibition; it is abbreviated as ASDII.

Now, what happens here? The name suggests that something is active site directed; that means, you have a molecule which is having a directional property, and this property is directly utilized to inhibit the enzyme. Basically I can give an analogy; it is like somebody wants to kill another person. So, he has a gun and he shoots the person straight away at the victim's active site. What is the active site? The active site in a person is mostly the heart; if the heart stops then everything stops.

So, heart is the active site. If you consider this, then active site directed irreversible inhibition means you should aim straight at the active site. Schematically how it is shown? It is shown that E plus S normally goes to the ES complex. Then it actual actually goes to enzyme product complex, and then that goes to E plus P. Usually for Michaelis Menten equation, we generally do not consider this fact since we assume that is a very fast dissociation.

In active site directed inhibition, you have a molecule which is represented as I-X; this X is the one which is attached to the inhibitor that is extremely reactive. The whole assemble is complementary to the active site of the enzyme. The complete inhibitor is I-X not only I. This is the inhibitor, but the inhibitor already has a very reactive functionality, but it has got again electronic and geometric complementarity with the active site of the enzyme.

So, now this I-X goes and binds to the enzyme at the active site because it has got this complementarity. But at that point, after binding, the enzyme realizes the mistake. It has got a lot of reactive functionalities, like lysine NH_2, thiol from cysteine, OH which comes from threonine or serine, or acid functionality as carboxylate.

So, this X functional group is a very reactive group. So, that reacts with any one of the amino acid residues that are present in the enzyme active site. Then there is a covalent bond formation between E and X because you are doing a reaction now. So, even if I tries to go out, it won't be able to do so as it is now bound to the enzyme via this X-E bond.

This is known as the active site directed inhibition; that means, here the molecule goes binds and it already has a very reactive functionality (X) and then while it binds, the enzyme amino acid residues which are extremely reactive functionalities, immediately reacts with X and in the process E gets tied up covalently with this IX molecule.

So, even if this portion is broken now, but your enzyme is now covalently attached to this IX. What are some of the examples? See some of the examples are like this: Suppose it is

containing a carbon with a leaving group attached to another carbonyl like $COCH_2X$. We know that any leaving group attached to a carbon which is adjacent to a carbonyl is highly succeptible to S_N2 displacement; rate of S_N2 displacement is very high for such substrates. So, based on this part $COCH_2X$, you can have different types of X, these are all different examples of Xthat are given here. So, you can have different reactive functionalities attached to this inhibitor and the inhibition can take place. But remember the enzyme has not done any normal reaction that it was doing to the substrate, that reaction has not taken place; it is just the like a magic bullet; it went in and then it forms a cross link with any of these amino acid residues.

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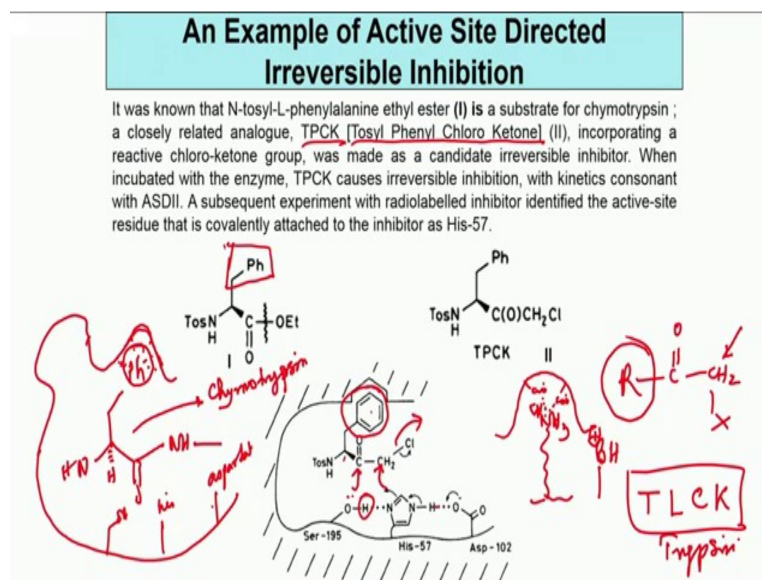

I told you that most of these inhibitors are based on systems like this. And this is R they will be all inhibitors because this is very reactive. Now, if you want to design a particular inhibitor for a particular enzyme then you have to think of what could be my R.

Now, suppose if we have chymotrypsin in mind. We know chymotrypsin hydrolyses amide bond from the carboxy site, where the carboxyl belongs to an aromatic amino acid like phenylalanine, tyrosine or tryptophan. It was also found by people that chymotrypsin can also hydrolyze esters. It not only hydrolyses amides the peptides, but it can hydrolyze esters like the ethyl esters of this, but you have to maintain the factor that it only recognizes aromatic amino acids.

So, in general what happens? You have a system that is required for hydrolysis by chymotrypsin. You have a system like this and this is also important that that the amino acid should be in the L configuration. Chymotrypsin is a serine based enzyme and it has serine, histidine and aspartate in the catalytic triad.

But, apart from that, it must have some hydrophobic pockets where phenyl or aromatic groups can be stabilized by π-stacking interactions or hydrophobic interactions. And because of the presence of this pocket which only recognizes aromatic groups, chymotrypsin is specific for aromatic amino acids. Trypsin hydrolyses only the basic amino acids like lysine or arginine. Why it is so specific? This is because the pocket in trypsin is no longer hydrophobic. Trypsin recognizes groups like say NH_3 plus (lysine or an arginine) here so, I can just represent by BH plus because they will be all protonated.

So, they go and first fit to the enzyme. So, a trypsin enzyme must be having a site where lot of carboxylates are there so, that you can make salt bridges between NH_3 plus and CO_2 minus. So, there will be lot of salt bridges. So, that is why trypsin recognizes basic amino acids because of the presence of a pocket which ensures salt bridge formation or electrostatic interactions; chymotrypsin has a hydrophobic pocket, which recognizes only aromatic amino acids.

Now, I want to design an active site directed inhibitor of chymotrypsin. How do I do it? This is the mechanism which involved the charge relay process. Carboxylate group of that aspartate takes up the hydrogen from the imidazole and that activates this nitrogen as a base that takes up this hydrogen and this is supposed to go and attack the carbonyl. But if you do not take the amide here, if you take a CH_2Cl instead of taking an ester or an amide, then two things have to be considered which are important. If this serine attacks the carbonyl, it cannot break a C-C bond as it is difficult to break; earlier there was C-N bond in the same position. Secondly the more important point is that now this imidazole is very close to this CH_2Cl; this methylene carbon which is extremely susceptible to nucleophilic attack, it is very close to that.

So, now the charge relay sequence will be like this: O minus takes the hydrogen, this goes here and the nitrogen is activated. So, instead of taking the proton from serine, because that is now useless, even if it takes the proton there is no productivity, since the serine cannot attack

this carbonyl; so the nitrogen instead of taking the hydrogen, it goes and attacks this carbon and breaks the carbon chlorine bond.

So, what is the result of this process? The result of this is that you are forming a carbon nitrogen covalent bond with this molecule. So, this is a very good example of active site directed irreversible inhibition. Why active site directed? Because your molecule has a system which is recognized by the enzyme, so the inhibitor goes and goes binds to the active site.

Then the normal cycle wants to take place, but that cannot happen here. Since it has got a reactive functionality $COCH_2Cl$, now the imidazole goes directly acts as a nucleophile and attacks this carbon and forming a carbon nitrogen bond. So, this is the first example. The compound is called Tosyl phenyl chloro ketone, abbreviated as TPCK. This is text book chemistry; this chemistry now has appeared in many textbooks. Since you now have this background knowledge, if I ask that how to make an active site directed irreversible inhibitor of trypsin?

You may now replace this phenyl alanine group and instead of taking this TPCK (tosyl phenyl chloro ketone), you take tosyl lysyl chloro ketone which is abbreviated as TLCK. This is an inhibitor for trypsin and TPCK is an inhibitor of chymotrypsin. Both are active site directed, because firstly both the molecules have reactive functionality; secondly they also have the structural feature that is necessary to be recognized by the pocket which is present in the active site.

The next one is Suicide Inhibition; this is another ivery interesting topic. Here what happens is that the inhibitor looks very similar to the substrate. And what the enzyme can do? Since the inhibitor is very similar to the substrate. Now it is no longer I and S; it is now designated as the product. So, here the inhibitor is converted into another molecule. What this enzyme is doing? It is doing the same reaction that it does on the substrate, but in the process, it makes a molecule which is highly reactive. Before the reaction, the inhibitor was not that reactive, after reaction it became very reactive. And then as it is so reactive, it immediately forms EI^*. So, a super reactive, covalently bonded species is formed.

So, basically it, I can make an analogy. If you have read the story of Frankenstein, you would know that a scientist wanted to bring life to a dead person. So, he instilled the life into that dead person and the dead person ultimately killed the scientist. So, it is a very similar

process; the enzyme initially thinks that I is my friend like the substrate. So, the inhibitor goes and enzyme embraces it with both arms; for friends we generally exchange words, similarly here the enzyme does the normal reactions, thinking that it is a very friendly molecule. But, unfortunately after doing the reaction, this molecule becomes an enemy and finally, kills the enzyme by forming a covalent bond.

So, that is why it says, that as if the enzyme is committing suicide and that is why this is called suicide inhibition. It is also called mechanism based enzyme inhibition which is another name given to this process, because the whole thing is depending on a particular mechanism that how I is converted to I^*. So, this is also called mechanism based irreversible inhibition, but the more common name is suicide inhibition.

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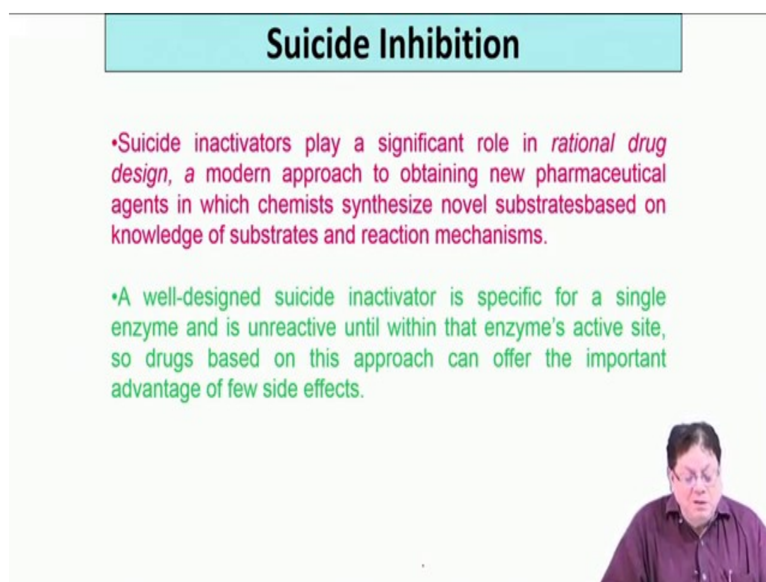

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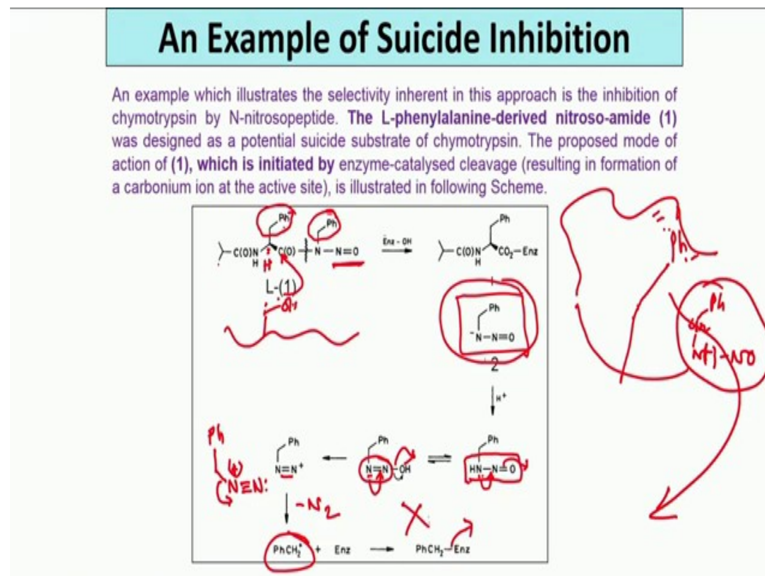

I told you that enzyme inhibitors are very important from drug design point of view. Many of our drugs (nearly 60% of the drugs) are actually inhibitors of some kind of enzyme or receptor. Now in case of cholesterol, we may have the disease of having high cholesterol. I want to modulate and lower the activity of the enzymes which are involved in the biosynthesis of cholesterol. But I do not want to shut off the biosynthesis of cholesterol totally; complete shut off means that cholesterol is not produced at all in my body. Cholestrol in needed for our body as cholesterol has some vital roles to play; from cholesterol we get the steroid hormones in our body cholesterol also constitutes the membrane that surrounds our cell that keeps the cell contents intact.

So, you cannot completely shut off cholesterol biosynthesis. Like if somebody is suffering from higher diabetes, you have to maintain the level of the glucose at a certain value. If it goes down from the normal value, that may also lead to problemsin some cases. So, in those cases, a reversible inhibition is better; because you take the drug, it modulates the activity of the enzymes. So, that your cholesterol level or sugar level is maintained at the optimum (what is expected) level; and then when the drug is metabolized, next day you take another pill to maintain the concentration of the drug in your body.

But, the important thing is that you need reversible inhibition. You do not want to completely shut off the cholesterol biosynthetic pathway or your glucose metabolic pathway.. But in some cases you need this irreversible inhibition. Remember reversible inhibition can be

reversed; the inhibition can be overcome by adding excess of the substrate. But for irreversible inhibition, you are destroying the enzyme, whatever molecule is attached covalently to the that enzyme, you cannot recover; its activity is already gone. It has formed a covalent bond, so the active site residue is gone.

But where is it required? It is required when we want to really destroy the enzyme totally. For example, when we have a bacterial infection. Suppose there is a particular enzyme in the bacteria which is very essential for the bacteria to grow. So, then we target that enzyme in the bacteria and it will be much better if you have this type of irreversible inhibition, because you want to kill the bacteria; that means, you want to kill the enzyme. You want to completely shut off the pathway that the enzyme follows.

So, I will quickly go to the example again, a text book example. This is not a drug molecule, this is a text book example about how to design a suicide inhibitor. You know again chymotrypsin was the model because the chymotrypsin structure and its mechanism are well known; it is available in plenty, at a cheap rate, so, all these studies initially were done with chymotrypsin.

We know chymotrypsin recognizes aromatic amino acids. So, all these examples are based on phenylalanine; that is the easier one to work with. This is nitrogen, that is at the carboxyl side of the phenylalanine. You have the NH carbonyl bonded to isopropyl and here instead of the amine CONH, this nitrogen is also having another benzyl group instead of H. This is actually benzyl. We call it phenylalanine because we think it is phenyl derivative of alanine, but you can also think of this as a benzyl derivative of glycine. So you can think that this may be called benzyl glycine, but unfortunately from the very beginning it was called phenylalanine.

So, you have now two CH_2Ph groups each having L-configuration, and this nitrogen is attached to a nitrosyl group (NO). So, what you have is *N*-nitroso *N*-benzyl phenylalanine derivative and show it to the enzyme. What was the idea of the scientist who was doing this? The idea was that since the enzyme was phenylalanine having the same catalytic triad,

The serine OH was supposed to do an attack on the carbonyl carbon and break the carbon nitrogen bond (peptide bond) . Here it is N-nitroso N–benzyl anion as the by-product. Because you have this benzyl group in the correct configuration; so there was no problem. So, it goes and it binds to the active site and then via this catalytic triad mechanism, hydrolysis of this carbon nitrogen bond occurs.

Now, what happens to this species? The species will be initially nitroso benzyl amine anion. So, that will now take the proton so, to make it NH. So, what you get now N-nitroso benzyl amine. Now this type of functionality (N-nitroso amine) is very unstable as per the concepts of organic chemistry. So, you see now the reactivity of molecules come in to play. As a result, it tautomerizes to the diazo oximino form.This diazo compound is also not very stable.

Now, this lone pair of electron delocalizes which releases the OH that comes out as water and this forms this diazonium cation. $PhCH_2N$ triple bond N and these nitrogen is plus. In this structure, all the atoms are fulfilling the octet, but this is also not stable.

Now, these N triple bond N can come out as nitrogen, which is a very stable molecule. So, in the process, you are generating $PhCH_2$ plus which is a very reactive electrophile. Any alkyl group with plus will be a very reactive electrophile. So, whatever nucleophilic amino acids are there in the active site; that can react with this enzyme active site and form covalent bond with the benzyl group.

Firstly, it is an irreversible inhibition because you are forming a covalent bond with the inhibitor molecule. Secondly, this is not active site directed, because here the normal reaction has been done by the enzyme.

The enzyme hydrolyzes this carbonyl carbon nitrogen bond. So, that normal reaction has happened and ultimately what is created is a monster (in terms of reactivity). This is a monster that ultimately creates a very electrophilic benzylic cation. Whatever nucleophilic amino acid is there in the active site of the enzyme, it will now capture this electrophile and in the process the enzyme will be inhibited.

Now, the scientist who made this molecule had this idea in his mind. In research we have many things in mind, but that does not work. Here apparently it was very well thought out proposal. So, he made this molecule, showed it to the enzyme (chymotrypsin); unfortunately he did not observe any inhibition. Why did that happen? That was the question that why there was no inhibition. Whether this reaction failed? No he found that, this reaction was going on; that means, this part was released and this part was forming the acyl enzyme complex and finally, this was converted to the CO_2H.

But it failed to inhibit the enzyme. What happened here? Since he was a brilliant scientist, he realized what is happening here. Here the part which is attached to the enzyme is the benzyl

part of the L-phenylalanine; that Ph group is bound to the enzyme. And the reactive species that was generated was $PhCH_2$ and then you have NH and NO; that had no connection with the enzyme.

So, by the time, all these reactions take place, it comes out of the enzyme pocket. So, it has gone out of the enzyme because there was no binding attachment of this species with the enzyme. The part which was attached to the enzyme hydrophobic pocket was this phenyl group. So, the other part was free to be released and by the time all these rearrangement reactions took place, that reactive part was outside the enzyme. Since it was outside the enzyme, so, it could not destroy the enzyme. So, this did not work.

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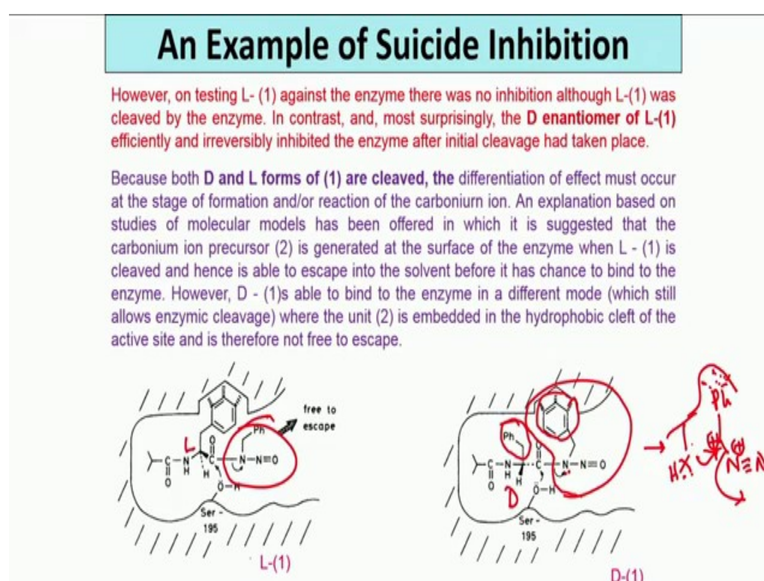

This is the one I was telling. This phenyl was being attached to the enzyme hydrophobic pocket; the other part is free to escape from the enzyme active site. But as I said, he was a brilliant scientist. So, what he did? He did a slight change.

He changed the configuration of this carbon. He made it a D-phenylalanine derivative instead of L-configuration. Remember that proteins are all made up of L amino acids. So, it is natural that the enzyme will also recognize only L amino acid containing molecules. It will not recognize the D amino acids; that means this phenyl now cannot bind to this hydrophobic pocket since the configuration is D. Earlier this hydrogen was α (below the plane) and you can think of this phenyl as above the plane. The active site is somewhere here.

So, now when the phenyl is above the plane, hydrogen is below it, the phenyl can go and fit in to the active site. But when you take the other configuration (the hydrogen is up and the phenyl is down), it cannot go and bind to this active site.. In the earlier case (when it was L), this benzyl was sitting by the side of this α carbon, there was perfect match in stereo chemistry, so this benzyl group got bound to the hydrophobic pocket easily keeping the other part (N-nitroso linked benzyl moiety) free to escape.

When you made the configuration D, then this molecule binds to this enzyme, but via the phenyl group of the N-nitroso part now. We know that nitrogen can actually flip back and forth. Nitrogen configuration is not a fixed one; whatever is required, it will do that. So, if it requires an up phenyl, then the nitrogen lone pair will be down and the phenyl will be up. So, this phenyl now replaces the other phenyl of phenylalanine in terms of binding to the hydrophobic poccket. So, now that aromatic ring of the N-nitroso linked benzyl group is interacting.

Now what is the effect of that? This aromatic ring of the N-nitroso linked benzyl moeity is no longer free to escape from the active site because the phenyl is bound to this point. So, whatever time it takes it will take, but this is already attached by weak interactions. So, it cannot escape from the enzyme active site. So, this goes out, you make the electrophilic carbon here. And now if there is a nucleophile in surrounding amino acid, then that is going to react with the this benzylic carbon. So, this is the mechanism of this reaction.

Now, this is an example of suicide inhibition. Because as I said in suicide inhibition, the substrate and the inhibitor go and bind, then the enzyme does the normal reaction. Normal reaction means chymotrypsin is supposed to hydrolyze an amide bond and that has happened. But after the hydrolysis, the species that is generated is extremely reactive. And because of this switch over from L to D, this benzyl group is now bound to the hydrophobic pocket; that means, this reactive species is now bound.

So, whatever chemistry that takes place after this, even if it takes some time, that does not matter, because the reactive species is still bound to the enzyme. And then the enzyme nucleophile will attack this benzylic carbon and forms the C-X bond. So, this is the story of reversible and irreversible inhibition. I think now, we have completed this inhibition part.

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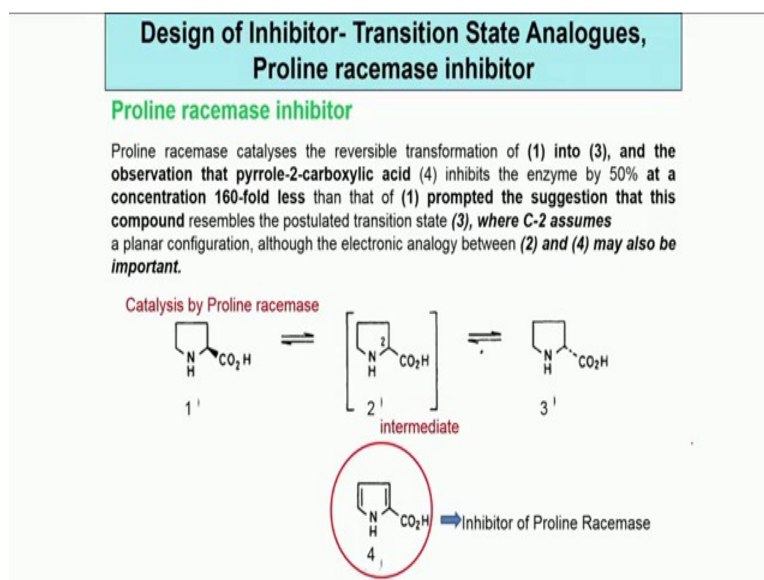

So, next day we will see more designing part because this forms the basis of our drug design, which is our next module that is related to whatever we are talking now. So, we have to clarify all doubts about enzyme inhibitions and then we will go to the nucleic acids, once this is over.

Thank you very much.