Medicinal Chemistry
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Enzymes as Drug Targets

In today's lecture we will examine how enzymes can be target for new drug discovery. As we have seen in the past few lectures enzymes can catalyze the reaction and this can be achieved by the destabilization of the ground state as well as the stabilization of the transition state.

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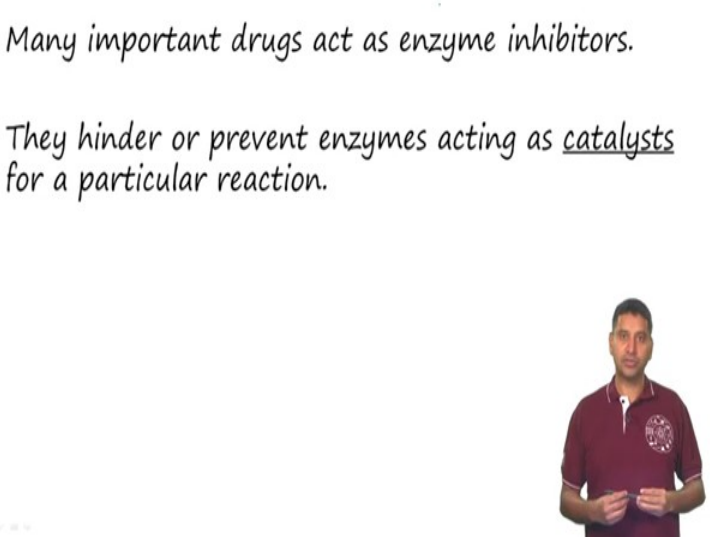

Now many important drugs act as enzyme inhibitors. They hinder or prevent the enzymes acting as catalysts. And what this does is, because enzymes are catalytic in nature a small amount of an inhibitor which can bind or inactivate an enzyme will have a profound effect on the cellular machinery.

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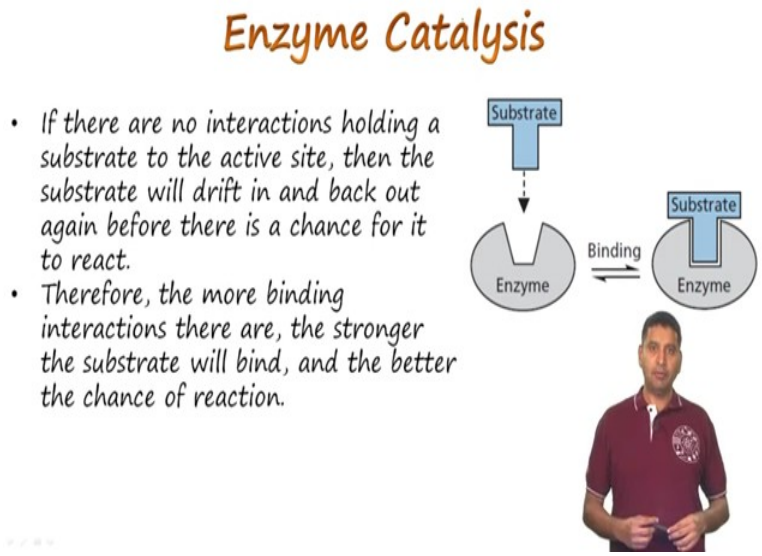

So just to recap, we have looked at enzyme catalysis. So fundamental concept of enzyme catalysis involves the binding of a substrate and we have already looked at the various types of interactions that hold a substrate into the active site. And so in a dynamic situation one can imagine that the substrate binds to the enzyme and then it gets off and then it bind again and gets off and so on and so forth.

And so therefore, the more binding interactions there are stronger the substrate will bind. And of course, that also may mean that there is a better chance for reaction. Because as we looked at earlier, the reactants have to be at the right orientation for the reaction to occur and if it is bound into the enzyme active site in the appropriate manner, the chances of that go up. But this is an equilibrium process.

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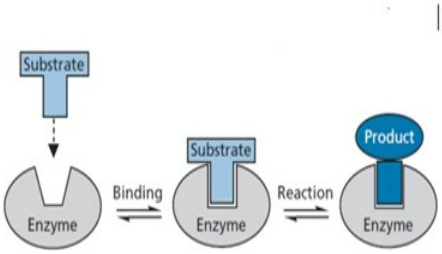

- What happens if a strongly bound substrate gives a product that also binds strongly to the active site?

Now what happens if a strongly bound substrate gives a product that also binds strongly to the active site? As we have looked at earlier, the product must dissociate out of the enzyme. Otherwise, the reaction is not going to go forward.

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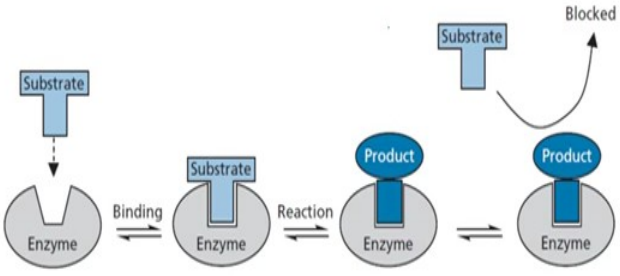

- The enzyme becomes clogged up and is unable to accept new substrates!

So this is one of the concepts that we use in developing inhibitors. That is, we want to understand how the substrate as well as the product bind to the enzyme and how we can design something that will bind tight to the enzyme perhaps and prevent the substrate from being catalyzed further.

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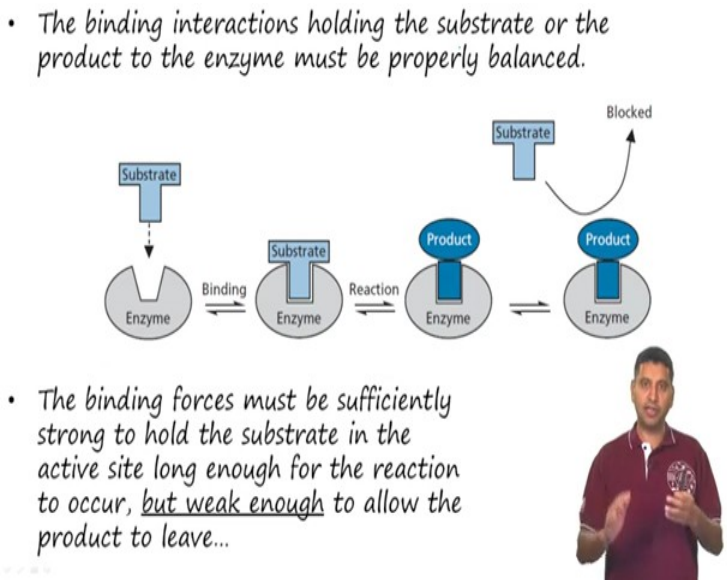

So therefore as we have discussed earlier, the binding interactions that hold the substrate or the product must be properly balanced for an enzyme to function properly. That means that in a normal situation the substrate binds to the enzyme and then there is turnover of the product. And the product must bind very weakly with the enzyme so that it is dissociated. So the substrate must bind strong enough and long enough for the reaction to occur. But if it is too weak, then the substrate will dissociate, there will be substantial amount of dissociation of the substrate and therefore the reaction will not go forward. And the product must dissociate very well from the enzyme, otherwise it would block the further catalysis. So there is a fine balance that has to be achieved in this entire catalysis process.

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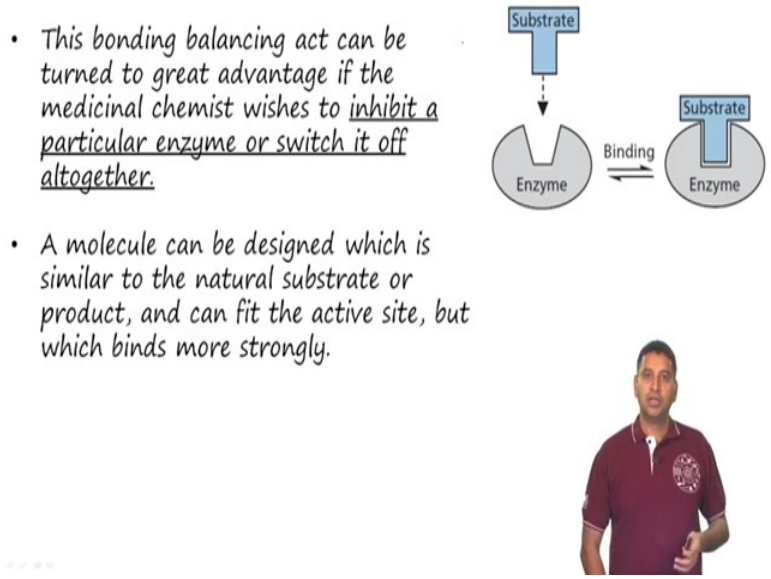

So this bonding balancing act can be turned to great advantage if we are able to design compounds that inhibit an enzyme or switch it off altogether. So what we would like is to design a molecule which is perhaps similar to the natural substrate or the product and which can fit the active site which binds more strongly but does not turn over to give you the product.

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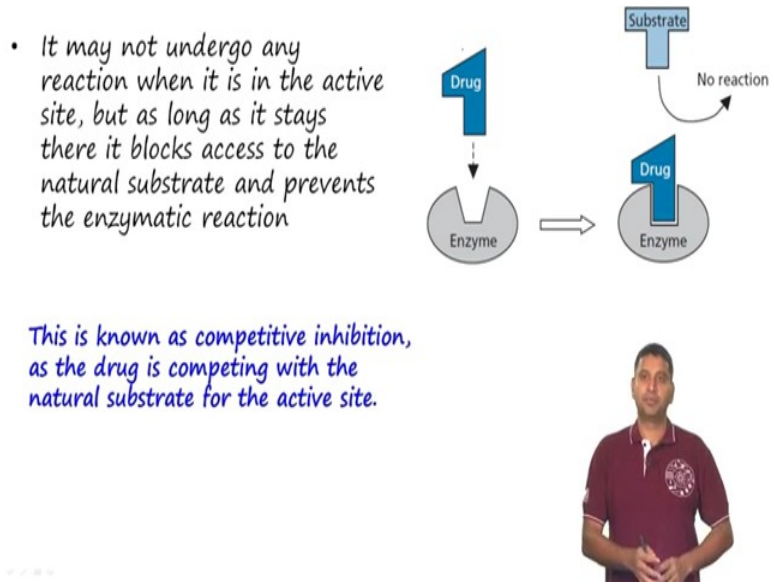

So now if it does not undergo any reaction but it has to stay in the active site long enough so that the natural substrate does not come and bind, this type of compound would then turn out to be an

inhibitor. And if it is able to have an impact on disease, then it is also called a drug. This type of inhibition is known as competitive inhibition because the drug is competing for the same active site with the natural substrate.

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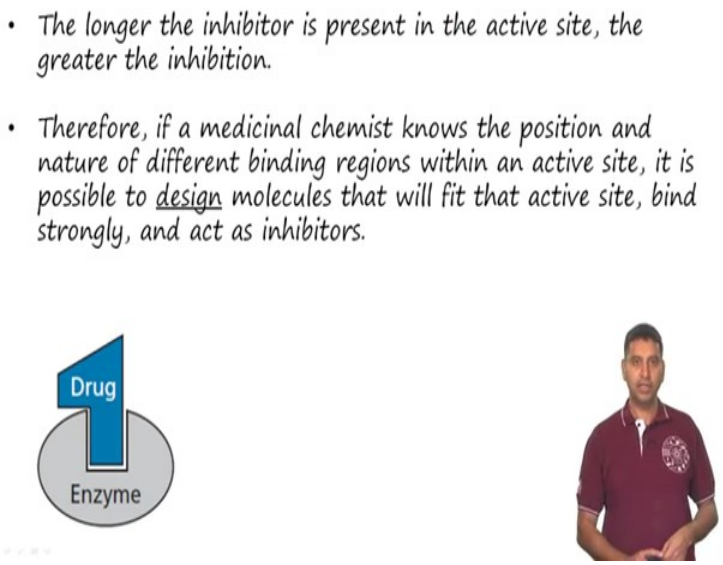

Now if the inhibitor or in this case the drug binds to the active site and it binds for a long time and if the binding is very strong, then the more preferable it would be because the enzyme would not function as well. For this to happen, the medicinal chemist should know the position and the nature of the different binding regions with an active site. And what we need to do is to figure out the nature of interactions in the active site and design molecules which will bind strongly but should not be turned over. And these types of compounds would then act as inhibitors.

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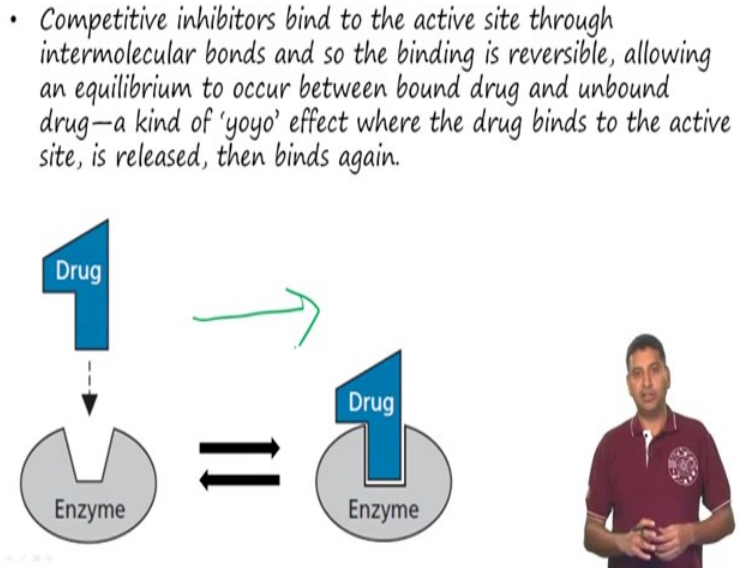

So competitive inhibitors as we have discussed would bind and would also dissociate. So this would be an equilibrium process. So there would be an equilibrium between the bound drug and the unbound drug and this is also sometimes referred to as the 'yoyo' effect where the drug binds to the active site and it is released and then binds again. Much like the substrate there is an equilibrium between the bound and the unbound states. So the stronger the interaction more the equilibrium would be pushed in this direction and perhaps better the enzyme would be inhibited.

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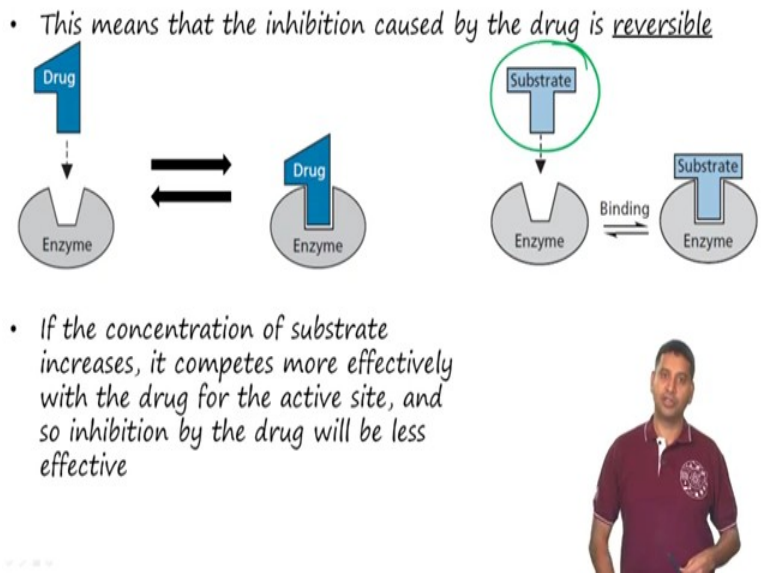

So therefore it is a reversible process and now if you can imagine, if the concentration of the substrate increases, then what happens is that there is more substrate available and the substrate then starts to compete with the drug for the active site. And as we have discussed earlier, the substrate is now going to be turned over to give you the product. And so the efficacy of inhibition of a competitive inhibitor would diminish when the substrate concentration goes up. In other words, inhibition by the drug would be less effective when the substrate concentration is increased.

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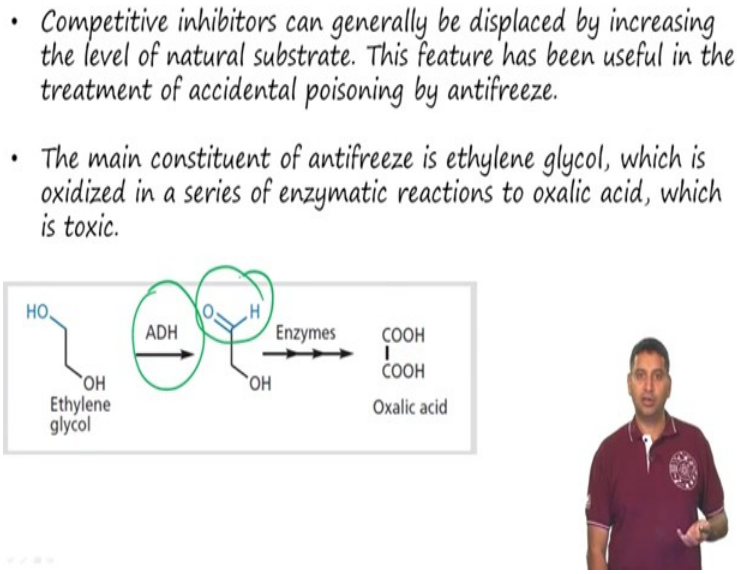

So this feature, the competitive inhibitors can be displaced by increasing the level of natural substrate. But this feature is useful in certain kinds of non-drug discovery kind of situations where for example, one has consumed the poison by mistake. And this kind of poison can be displaced, if the poison is a competitive inhibitor, it can be displaced by the natural substrate. Let us know look at an example of that.

Antifreeze is used in vehicles to make sure that, when the temperature goes very low that the coolant does not freeze. So the typical antifreeze, it is used as ethylene glycol and ethylene glycol is oxidized by alcohol dehydrogenase to produce this aldehyde here which then is further metabolized to give you oxalic acid. Oxalic acid unfortunately, is quite toxic to humans. And so if somebody consumes ethylene glycol by mistake, then what happens is that eventually it is going to produce oxalic acid which is going to be quite bad for the person.

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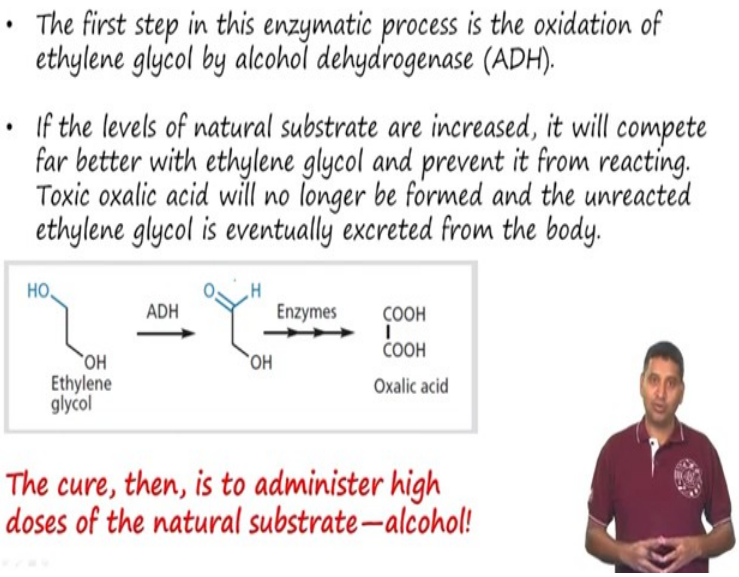

So like I discussed earlier, the first step is the oxidation of ethylene glycol by alcohol dehydrogenase. Now, if ethylene glycol is a competitive inhibitor, then as we discussed earlier the concentration of the natural substrate can be increased so that we would prevent the enzyme from turning over ethylene glycol and ethylene glycol can then be excreted from the body. So the cure therefore is to administer high doses of the natural substrate which is ethanol.

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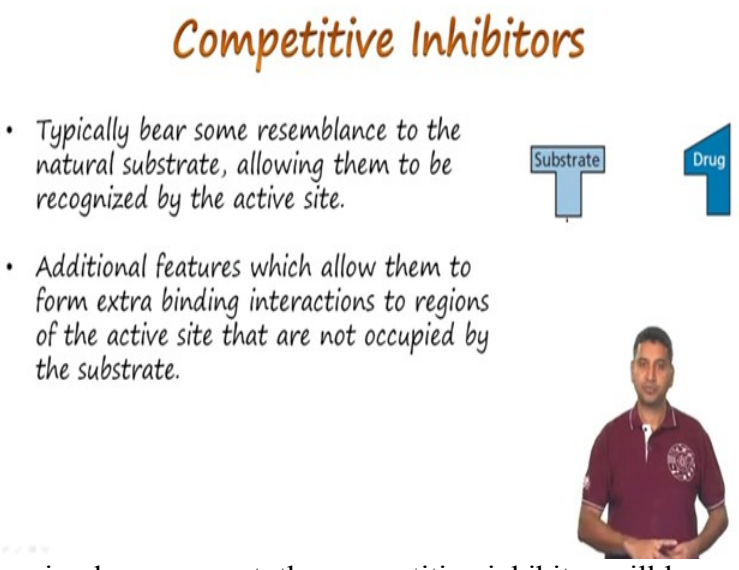

Now as we can very simply reason out, the competitive inhibitor will have some resemblance to the natural substrate. This allows for whatever binding sites or binding interactions that happen

in the active site, those interactions will also happen in the inhibitor. Now we can also increase additional features which allow extra binding interactions in the region so that can bind to the, so that the drug or competitive inhibitor can bind much stronger.

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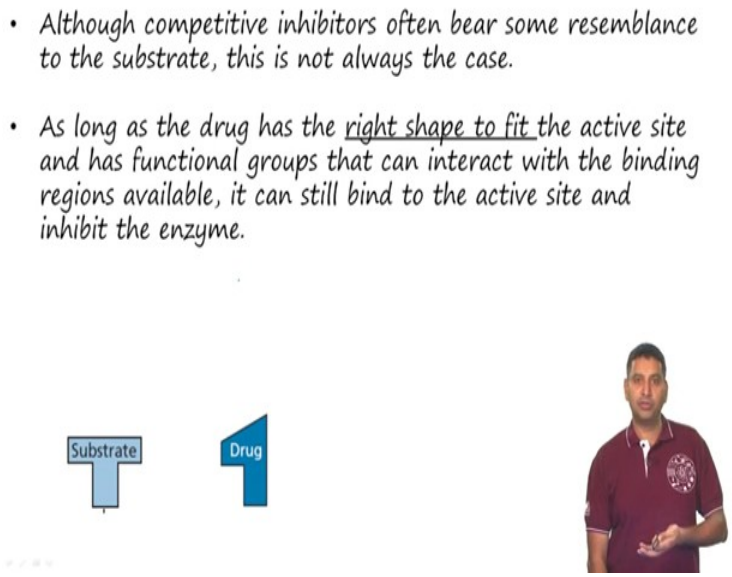

But it is not always necessary that this structure of the inhibitor should resemble the substrate. What we need to understand is that the active site is basically a region where there are number of interactions that are possible. And so if we are able to mimic a compound or if we are able to synthesize a compound which is able to fit in a manner such that these interactions can be maximized, then it is possible for us to develop an inhibitor without any structural resemblance to the natural substrate.

So therefore as long as a drug has the right shape to fit in the active site and has the functional groups that can interact with the binding regions, then it can still bind to the active site and inhibit the enzyme. So there are several situations, where the drug has no resemblance to the natural substrate.

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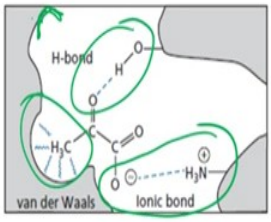
- Therefore, it is possible for drugs with a totally diff erent skeleton to the substrate to act as competitive inhibitors.
- Such drugs may bind to a combination of binding regions within the active site, some of which are used by the substrate and some of which are not.



So therefore a totally different skeleton is entirely possible and for this we need to be able to understand how the natural substrate binds to the enzyme and what are the major binding interactions that occur. So we have looked at previously this example of three major interactions that occur, which is a hydrogen bond, an ionic bond and a vander Waals interaction.

Now, if I can design a compound wherein all these three interactions are actually possible, then in principle this compound must be able to inhibit this enzyme. And not just that, in addition, if there is let us say an extra interaction here that one could exploit, then we would be able to design something that binds even tighter.

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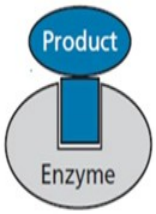
- It should also be remembered that the product of an enzyme-catalysed reaction is bound to the active site before it is finally released, and so it is possible to have enzyme inhibitors which resemble the structure of the product more closely than the substrate.



Of course, it should be remembered that the product of an enzyme-catalyzed reaction is also bound to the active site before it is finally released. So it is also possible to have enzyme inhibitors which resemble the product more closely than the substrate.

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- Some competitive inhibitors bind to the active site, but do not compete with the substrate.
- How can this occur?

Sometimes competitive inhibition occurs but the inhibitor does not compete with the substrate. How can this occur?

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- The answer lies in the fact that the active sites of several enzymes bind a substrate and an enzyme cofactor.
- Therefore, it is possible to have competitive inhibitors that occupy the binding region normally occupied by the cofactor, and so the competition is with the cofactor rather than the substrate.

The answer lies in the fact that the enzyme active site binds a substrate but also binds an enzyme cofactor. We have already looked at previously the roles of cofactors. So therefore, it is possible to have a competitive inhibitor that binds to the active region not by the substrate but that is the region where the cofactor binds. So therefore it can be a competitive inhibitor of the enzyme but not for the natural substrate but for the cofactor.

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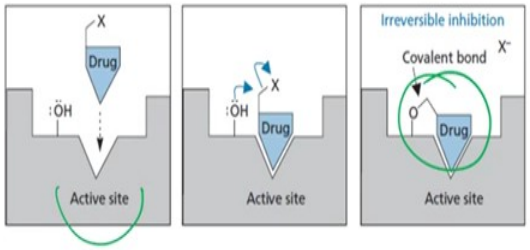
Irreversible Inhibitors

- Irreversible enzyme inhibitors can form a covalent bond to a key amino acid in the active site and permanently block the affected enzyme



Now let us look next at what is known as irreversible inhibitors. As the name suggests there is no equilibrium that happens. So if you are able to push the equilibrium towards the right, then the reaction is irreversible in nature. This is what we have learned in our fundamental chemistry courses. That an equilibrium that is pushed towards one direction is an irreversible process. Now irreversible inhibitors can act by actually making a covalent bond to the, perhaps to the key amino acid that is involved in catalysis.

Now since covalent bonds are extremely strong and they are difficult to break, this can perhaps permanently block the enzyme. So an example here that we can look at is how this active site is modified by a covalent bond by the drug and irreversibly inhibits it.

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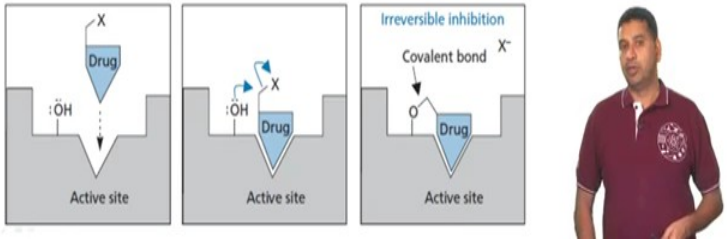
- The most effective irreversible inhibitors are those that contain an electrophilic functional group (X) capable of reacting with a nucleophilic group present on an amino acid side chain.
- Invariably, the amino acid affected is either serine or cysteine , because these amino acids are oft en present in active sites and contain **nucleophilic groups** (OH and SH respectively) that are involved in the mechanism of many enzyme-catalysed reactions



So the most effective irreversible inhibitors are those that contain an electrophilic functional group. So typically nucleophilic amino acids are serine or cysteine. So serine has a hydroxyl group whereas cysteine has a thiol. We have already looked at previously how thiols which are excellent nucleophiles. And many thiol residues can have, can be deprotonated to a significant extent in proteins. These can react with electrophiles.

Similarly serine, despite being an alcohol can actually be a good nucleophile if it is appropriately placed in an active site. And so if one can design electrophilic center such that it can go in and react with either the serine residue or a cysteine residue, then it is possible that a covalent bond can be formed by nucleophilic substitution reaction to give you irreversibly bound drug.

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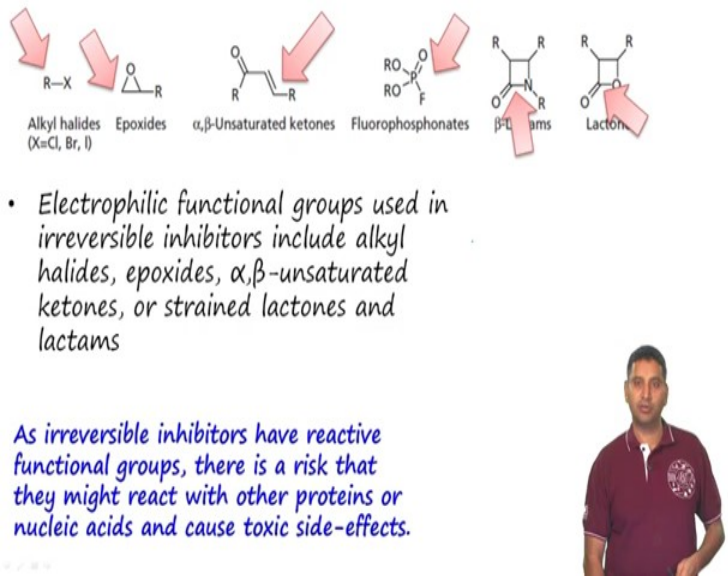

Some of the electrophilic groups that are used in irreversible inhibitors include alkali halides which can undergo S_N2. Epoxides, as we have looked at earlier epoxides are quite strained and therefore releasing them is quite favorable thermodynamically. And so it is possible that an epoxide can react with active nucleophilic residue. We also have alpha, beta-unsaturated ketones or aldehydes which are substrates for attack, for Michael attack. Then another electrophile is a fluorophosphonate. And two other examples are lactams and lactones. We will look at some of these examples later.

So binding site for these residues is shown over here and these are the functional groups that can react irreversibly. Now the problem is that irreversible inhibitors, since they have these reactive functional groups, there is a risk that they may react little bit indiscriminately and they may cause toxic side-effects. So if the biding of the inhibitor to the enzyme is not very selective or specific, then what can happen is that the electrophile can indiscriminately react and it can covalently modify number of active site residues inside the cell which is not going to be useful.

So therefore one has to design these compounds in such a manner that they would be able to go and bind to the correct enzyme in the right amount so that it is able to irreversibly inhibit it, which is a major challenge.

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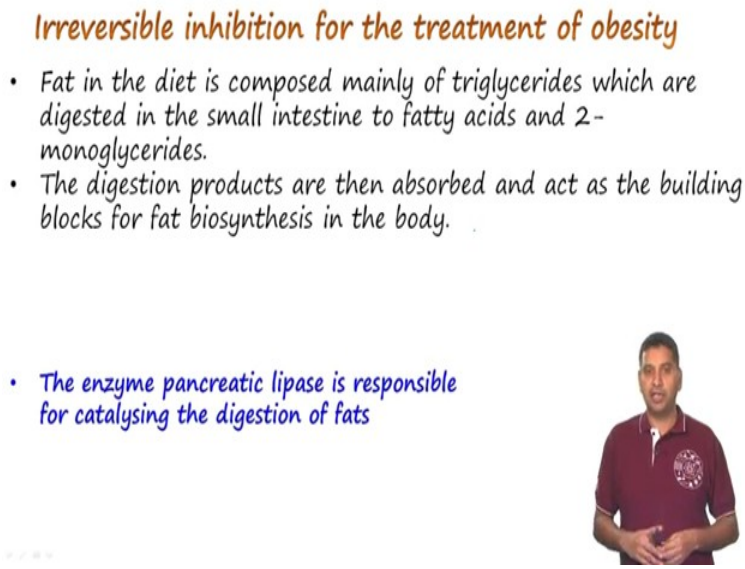

So let us look at an example here that is used is for the treatment of obesity. Obesity is a phenomenon where there is a very high amount of body to mass index which is basically, that the person has very, has a huge body weight for the height that they are. And so one of the features of this, is that there is a very high level of fat content in the person. So now fat in the diet is composed mainly of triglycerides which are digested in the small intestine to fatty acids and to produce two monoglycerides. The digestion products are then absorbed and act as a building block for fat biosynthesis. One of the enzymes that is responsible for catalyzing the digestion of fats is the pancreatic lipase.

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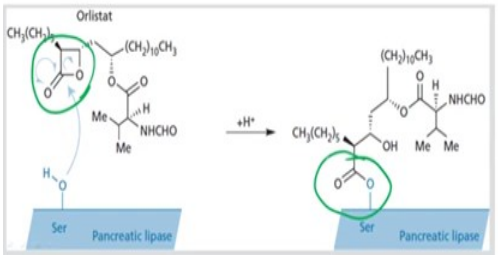
- So, inhibition of this enzyme will result in reduced absorption of glycerides and fatty acids from the gut.
- Consequently, less fat will be synthesized in the body.
- Orlistat is an anti-obesity drug that acts as an irreversible inhibitor of pancreatic lipase as a result of the presence of an electrophilic 4-membered lactone group.



So pancreatic lipase since being an important enzyme, then what one could think of is to inhibit this enzyme so that you can reduce the absorption of glycerides and fatty acids from the gut. And perhaps that would help with reduction of body weight. So if less fat is synthesized in the body, that would help in addressing obesity.

So this drug Orlistat is an anti-obesity drug and it has a four membered lactone ring. And lipase typically have an active serine residue and so therefore this can, the serine residue attacks the lactone, opens it up and forms a covalent bond with the serine residue. So this is an example of how one could use this irreversible inhibition to develop anti-obesity drug.

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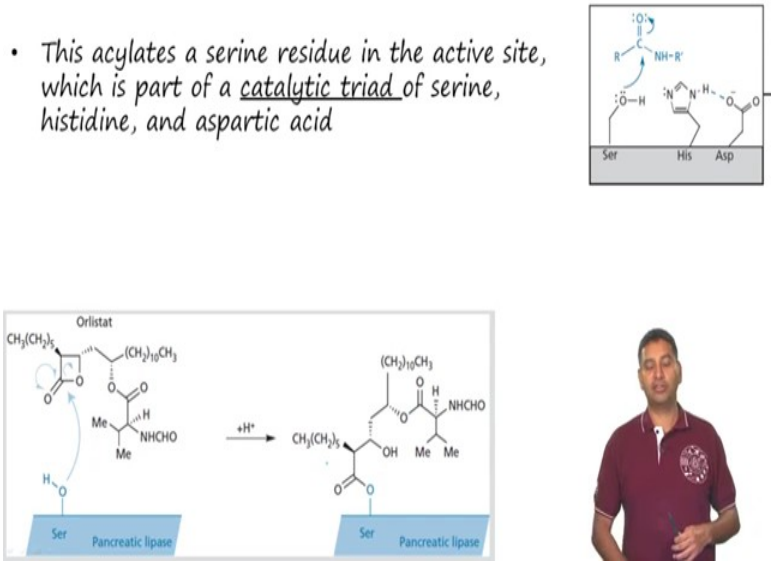

And just to recap, the serine residue has the catalytic triad of serine, histidine and aspartic acid which we have discussed earlier. And so what this triad does is that it provides the right kind of environment wherein a nucleophilic serine can act and subsequently acid based catalysis occurs to actually catalyze a reaction such as hydrolysis. So in this case it is a covalent reaction with a lactone to produce an ester.

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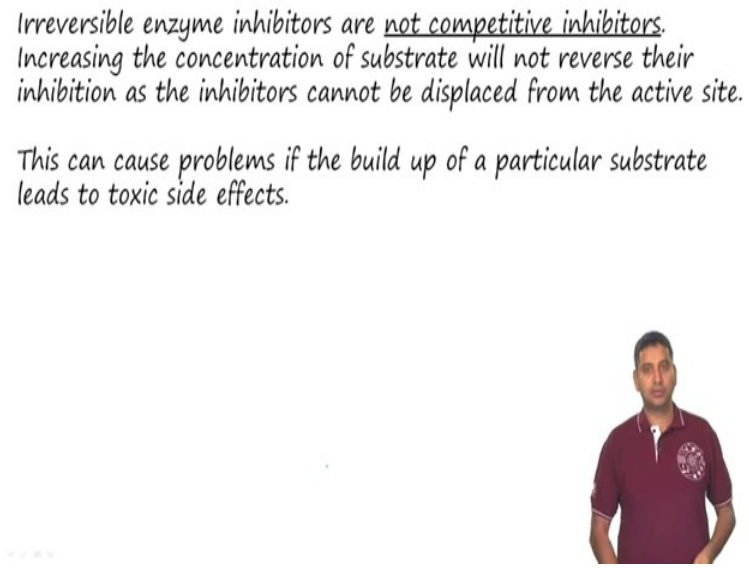

Irreversible enzyme inhibitors are not competitive inhibitors. So you can think about conceptually because there is no equilibrium. Once the covalent bond is formed, it does not go

back. So therefore these inhibitors cannot be displaced from the active site by the natural substrate. Of course, this can also cause problems because if there is a particular substrate that goes up in concentration because it is not turned over, and the substrate is toxic to the cell, it can also lead to side-effects.

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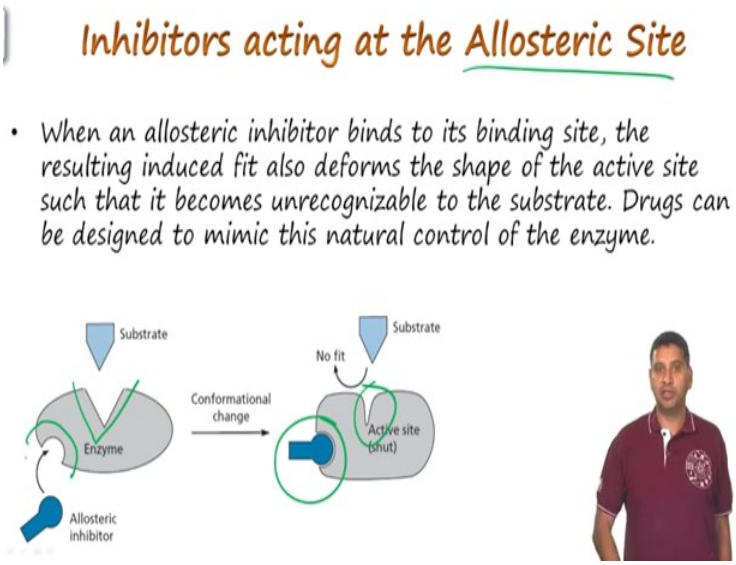

Now there is a third major class of inhibitors which are those that act at the allosteric site. So we have already looked at what allosteric site is. Allosteric site is nothing but it is a site which is far away from the active site. And what happens is that once there is a binding that occurs at the allosteric site which leads to a conformational change that in turn leads to a change in the active site which prevents the substrate from binding to the enzyme.

So here is how it is represented pictorially. So here is the allosteric site. Once the inhibitor binds to it, it leads to a change in the shape which then prevents further substrate from reacting. So therefore drugs can be designed to mimic this allosteric site so that you can inhibit the enzyme activity.

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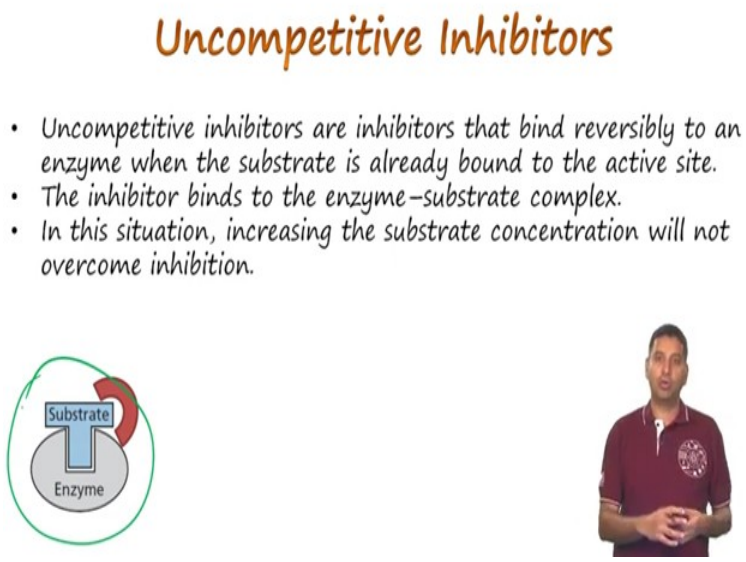

Next class is uncompetitive inhibitors. Uncompetitive inhibitors are nothing but inhibitors that bind reversibly to an enzyme but the only difference is that they bind only when the substrate is already bound to the active site. So here is the pictorial representation of a substrate bound to an enzyme. And so an uncompetitive inhibitor will bind to this complex. So, in this situation increasing the substrate concentration will not be able to overcome inhibition because after the substrate binds to the enzyme only then the uncompetitive inhibitor binds.

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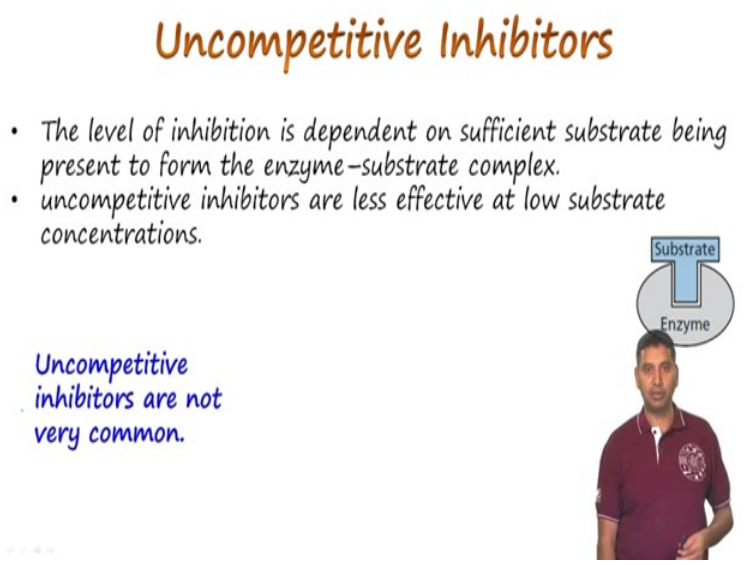

The level of inhibition is dependent on sufficient substrate being present to form the enzyme-substrate complex. So if there is not much of substrate present, then uncompetitive inhibitors are not effective which typically occurs at very low substrate concentrations. And just as a survey uncompetitive inhibitors are not very common in medicinal chemistry.

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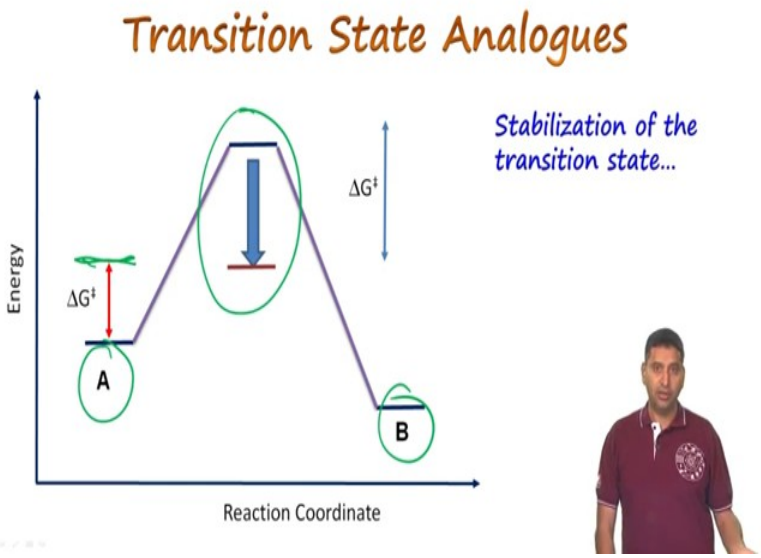

The last concept in terms of developing inhibitors are these concepts of transition state analogues. So we have looked at this energy diagram several times in this course and essentially what it tells you is that when there is a reaction A going to B, let us assume that it is, the delta G is negative here, what happens is that the catalysis can occur if you destabilize the ground state or stabilize the transition state.

And the destabilization of the ground state happens during binding. And the stabilization of the transition state also happens in the active site. And now when both of these occur, the energy profile is such that there is a tremendous acceleration in the rate of the reaction. So fundamentally for the enzyme to be functional, it has to bind strongly to the transition state. So the hypothesis that we can undertake is that the enzyme binds preferentially to the transition state or stronger to the transition state compared to the starting material or for that matter the product.

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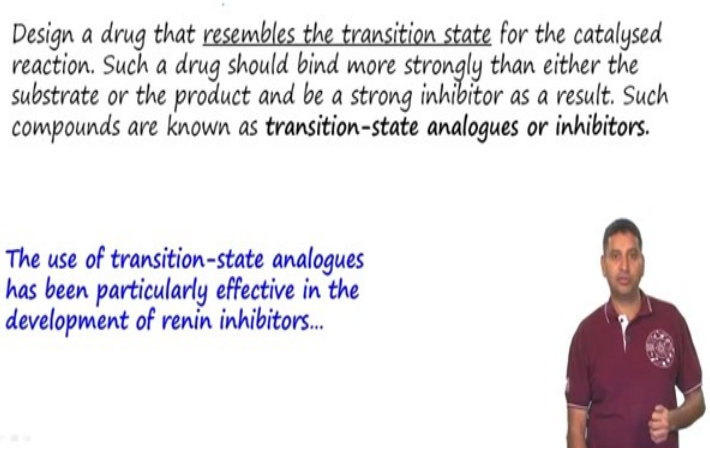

So if we can design a drug that resembles the transition state during the reaction, then such a drug would bind more strongly than either the substrate or the product. Such compounds are known as transition state analogues or inhibitors. So transition state analogues, the example that we can look at is they have been, have worked very well in the development of renin inhibitors.

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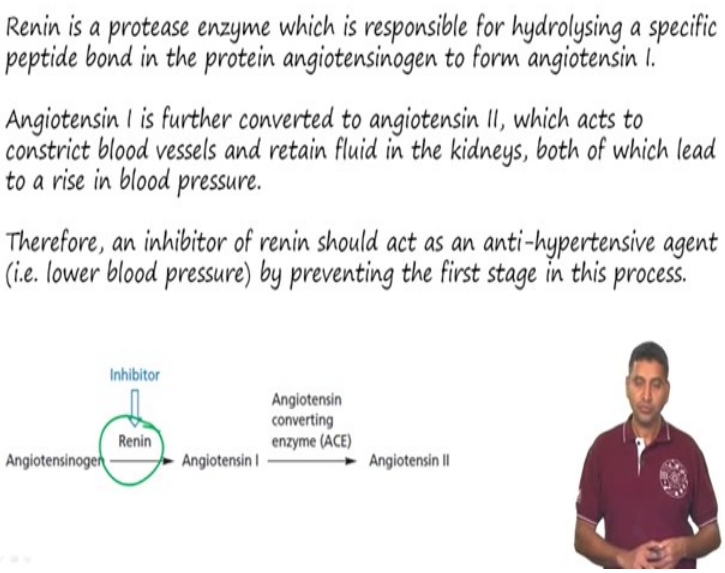

So renin is a protease enzyme which is responsible for hydrolyzing a specific peptide bond in the protein angiotensinogen. And then when this angiotensinogen which forms angiotensin I.

Angiotensin is further converted to angiotensin II and what happens is that this results in the constriction of blood vessels and further fluid is retained in the kidneys. Now both of these effects lead to an increase in the blood pressure. So an inhibitor of renin should act as an anti-hypertensive agent which basically the effect that we would see is lower blood pressure. And that would happen by inhibiting the first step of this process.

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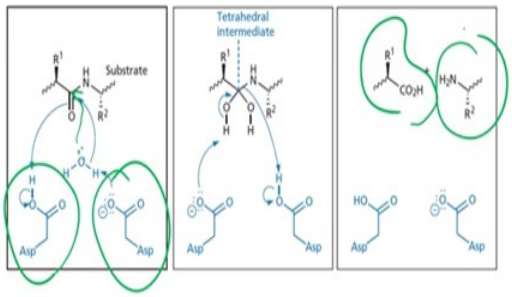

Renin contains two aspartyl residues and a bridging water molecule in the active site which are crucial to the mechanism by which an amide bond in the substrate is hydrolysed

So now renin consists of two aspartyl residues shown here. And one of them is deprotonated and there is also a bridging water molecule which mediates the reaction. So what happens here is this water molecule, activated water molecule attacks on the amide which then opens up and forms a tetrahedral intermediate. So this tetrahedral intermediate subsequently collapses to give you a free carboxylic acid and an amine.

So knowledge of this mechanism is very useful for us to understand what could be the transition state of the reaction. So one can imagine that when water is attacking this amide, there is a situation in the barrier, in the height where there would be a high energy transition state that would be produced. And so that species that is present would resemble the diol or the tetrahedral intermediate more than the starting material.

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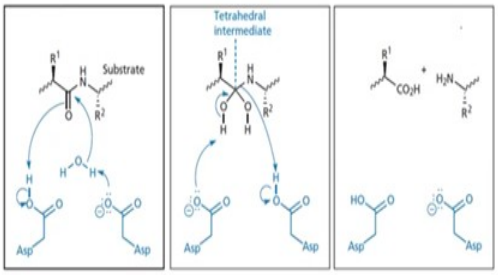

In the first stage of this mechanism, a tetrahedral intermediate is formed. In order to form this intermediate, the reaction mechanism has to proceed through a high-energy transition state, and it is this transition state that we wish to mimic with a transition state analogue.

So therefore one can imagine that the high energy transition state is what we would want to mimic and the high energy transition state would look like the diol or the tetrahedral intermediate. And one can think about this as the starting point for developing a transition state resembling molecule.

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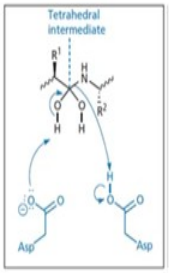
However, it is not possible to isolate such a high-energy species in order to study its structure; so how can one design a drug to mimic it?



Of course these types of tetrahedral intermediates are extremely unstable and we are not able to isolate them. And so we are stuck, how do we design a drug to mimic it?

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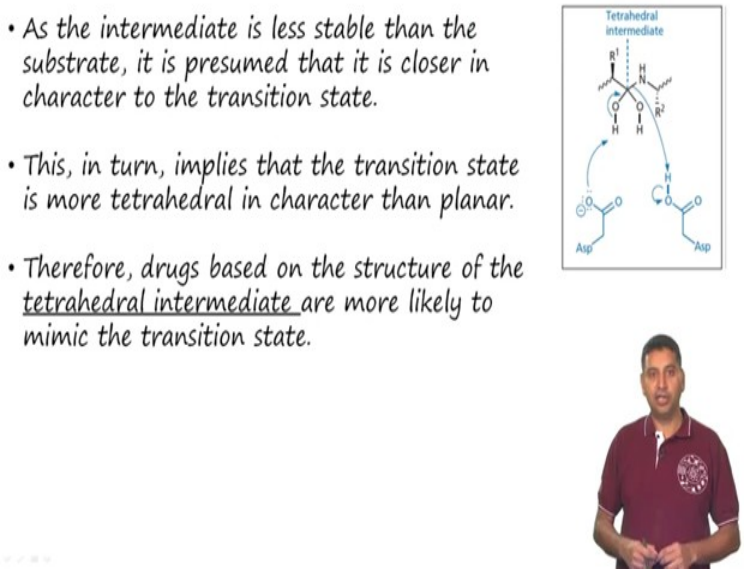

So what we would do is basically, since the intermediate is quite unstable and we have assumed that it is closer in character to the transition state, so what we need to do is to look at structure of compounds which are looking very much like the tetrahedral intermediate.

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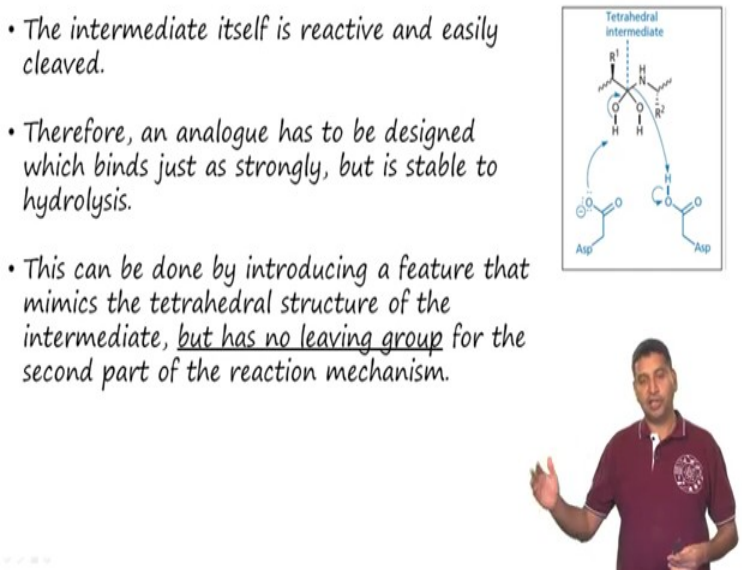

So an example here, of course the intermediate itself is reactive and easily cleaved. And therefore an analogue has to be designed which binds just as strongly but does not undergo hydrolysis to produce any product. So what we need to do is to introduce a feature that mimics the tetrahedral structure but has no leaving group for the second part of the mechanism.

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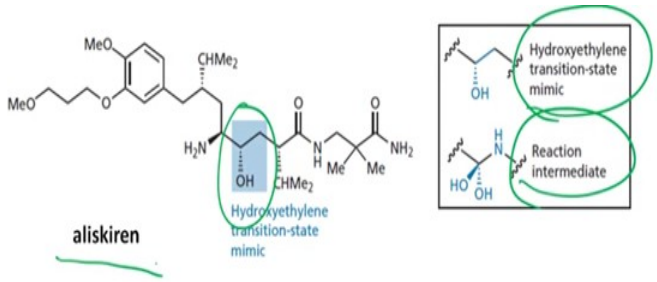

Renin contains two aspartyl residues and a bridging water molecule in the active site which are crucial to the mechanism by which an amide bond in the substrate is hydrolysed

So the way this is achieved is to use a aliphatic alcohol. So as you can see this alcohol is flank by a relatively polar group such as amine and there is also other features of this molecule which can bind to renin fairly well. So the hydroxyethylene group can be used as a transition state mimic for the reactive intermediate which is the tetrahedral intermediate in this case. And so this drug aliskiren works very well to inhibit renin's function.

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Suicide Substrates

- Transition-state analogues can be viewed as bona fide visitors to an enzyme's active site that become stubborn squatters once they have arrived.
- Other, apparently harmless, visitors can turn into lethal assassins once they have bound to their target enzyme.

Such agents are designed to undergo an enzyme-catalysed transformation which converts them into a highly reactive species that forms a covalent bond to the active site.

So transition-state analogues can be viewed as bona fide visitors to the enzyme's active site. And then what happens is that they stay there and they become stubborn squatters. Whereas what we

can also do is to use some harmless, let us say harmless visitors. And we can turn them into perhaps lethal assassins. And how this can be done is by using compounds known as suicide substrates. So such agents are designed to undergo enzyme-catalyzed transformation, not to the product but into a highly reactive species that can then form a covalent bond to the active site and inactivate it.

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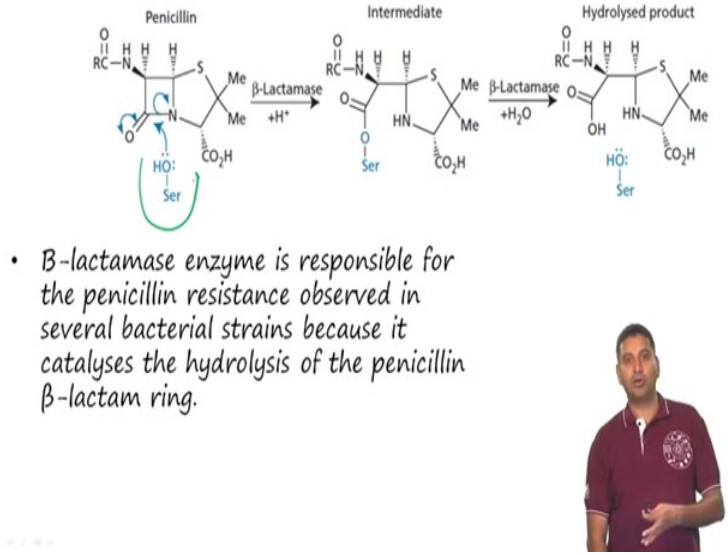

So the example that we look at is this beta-lactam class of drugs. Penicillin which was discovered many years back is inactivated by this enzyme called as beta-lactamase. And therefore penicillin resistance is observed in several bacterial strains because of the presence of beta-lactamase. So beta-lactamase enzyme has an active serine residue which can open up that lactam as shown here. So if we were to design a suicide substrate, what we need to do is to mimic this reaction but form a compound that does not, is not turned over further.

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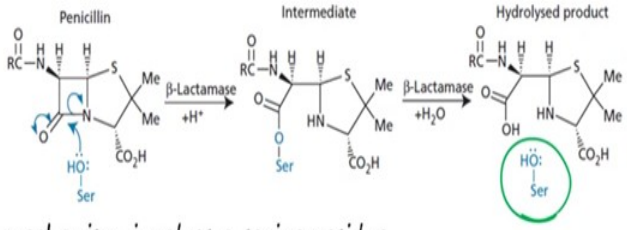

- The mechanism involves a serine residue in the active site acting as a nucleophile to form an intermediate where serine is covalently linked via an ester group to the ring-opened penicillin.
- The ester group is then hydrolysed to release the inactivated penicillin and free up the active site, such that the catalytic process can be repeated

So the mechanism of this process involves a serine residue as I mentioned earlier which acts as a nucleophile to form a covalently linked molecule via an ester group. Then of course the ester is hydrolyzed to release the inactivated penicillin and now the enzyme becomes available for it to go and react with another molecule of penicillin and inactivate it further.

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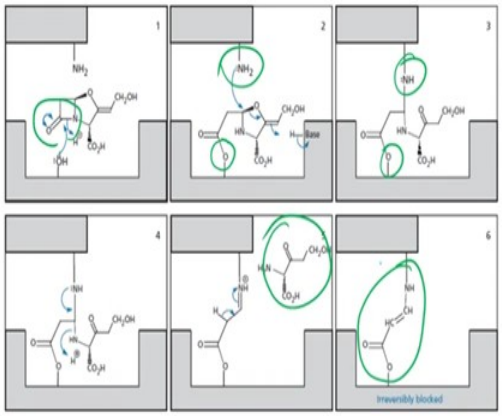

- Clavulanic acid also fits the active site of β-lactamase, and the β-lactam ring is opened by the serine residue in the same manner.

So one can design a compound wherein you can use the information that we have about penicillin antibiotics to actually inactivate the enzyme. So the way this is done is by the example that we are going to look at is clavulanic acid which also fits in the beta-lactamase active site. So

when this molecule reacts, it opens up the lactam and it forms an ester bond over here. But then subsequently another molecule, another polar group that is present is positioned such that or the functional group is positioned such that it can react further and form a sort of a crosslink over here.

And subsequently this molecule can be further cleaved to kick out this product over here. And what happens is that in the process you have generated a crosslink which then inactivates the enzyme because as we know the enzyme active site needs a serine group for it to be effective.

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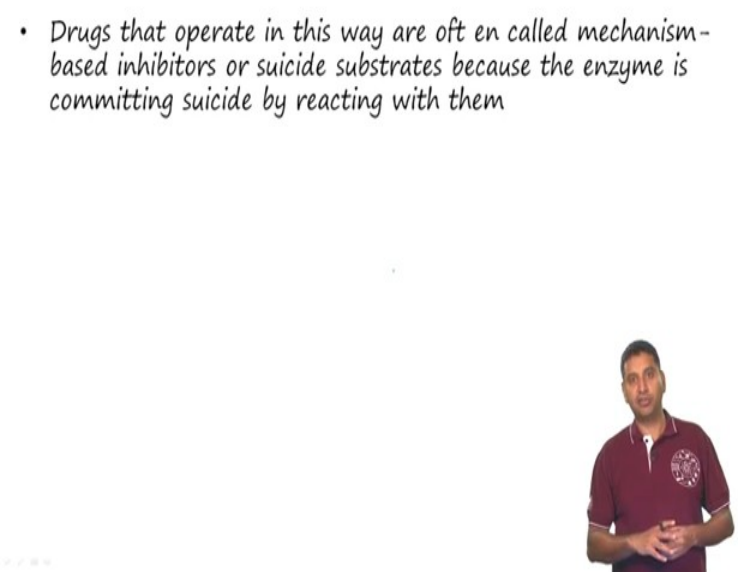

So drugs that operate in this way are often called as mechanism-based inhibitors or suicide substrates. And the reason we have called them as mechanism-based inhibitors is because once we understand the mechanism of the reaction, then one can plug in molecules such that we can inhibit the enzyme or inactivate by using the knowledge of the mechanism of the reaction.

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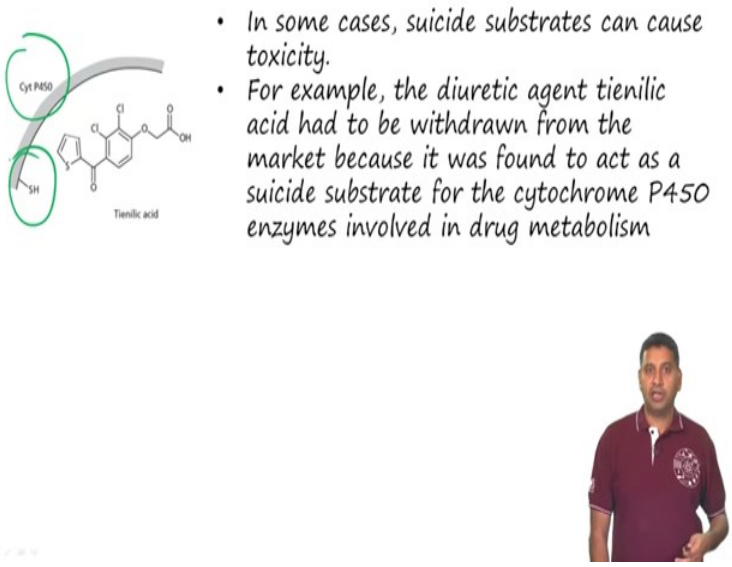

So in some cases suicide substrates can also cause toxicity. So here is an example of tienilic acid which has been withdrawn from the market because it is found to act as a suicide substrate for this class of enzymes known as cytochrome P450. And we will learn later in the class cytochrome P450 enzymes are involved, are very important parts of drug metabolism. And cytochrome P450 have a free cysteine residue among other functional groups and that plays a key role in this toxicity.

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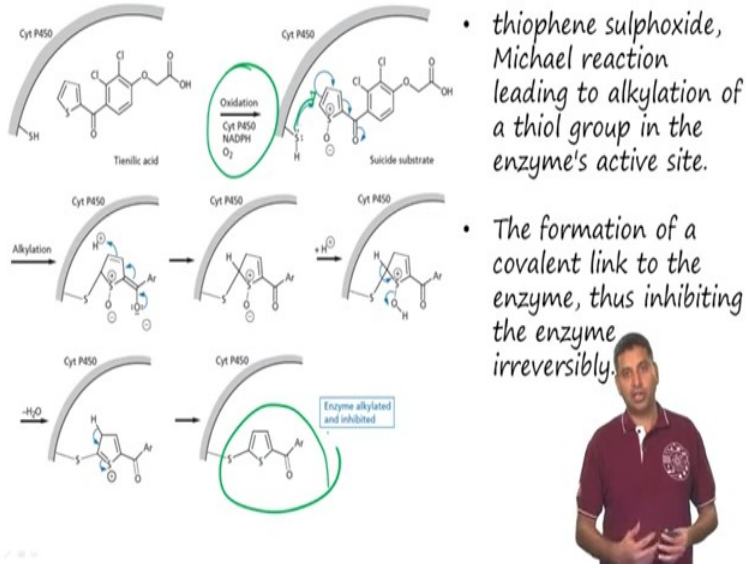

So the mechanism by which this compound acts as a toxin is that first oxidation occurs to generate this sulphoxide which then produces a substrate which is for Michael reaction and subsequently the thiol cysteine residue that is present undergoes Michael reaction and then you

go through a series of intermediates and finally the enzyme is covalently bound to this product. So this covalent adduct does not dissociate any further and so the enzyme is inactivated.

So cytochrome P450 being a very important enzyme for drug metabolism or toxic metabolism, then is inhibited and therefore you will have accumulation of other toxins which can cause harm to the body.

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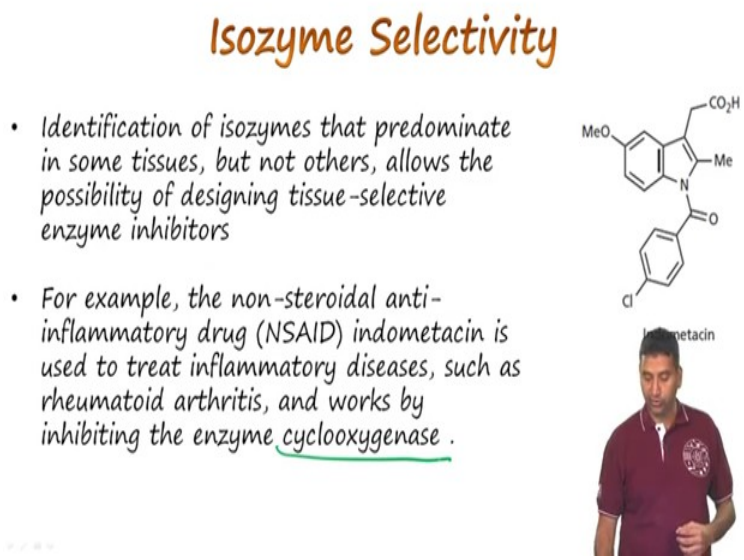

As we know, enzymes that are present in across tissues in a person can vary depending on the tissue. Now what happens is that we can use this information to design tissue selective inhibitors. The example we are going to look at is this very widely used drug non-steroidal anti-inflammatory drug. What this does is that it reduces inflammation and it is used to treat many diseases which are associated with inflammation such as rheumatoid arthritis and they act by inhibiting this enzyme known as cyclooxygenase. Here is the structure of indomethacin.

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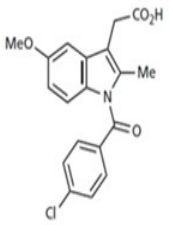
- Cyclooxygenase is involved in the biosynthesis of prostaglandins —agents which are responsible for the pain and inflammation of rheumatoid arthritis.
- Inhibiting the enzyme lowers prostaglandin levels and alleviates the symptoms of the disease.
- However, the drug also inhibits the synthesis of benefi cial prostaglandins in the gastrointestinal tract and the kidney.



Indometacin

Cyclooxygenase is involved in the biosynthesis of prostaglandins. And prostaglandins are responsible for the pain and inflammation associated with rheumatoid arthritis. Inhibiting this enzyme lowers the prostaglandins levels and sort of reduces the symptoms of this disease. However the drug also inhibits the synthesis of beneficial prostaglandins, for example in the gastrointestinal tract or the kidney. So one needs to develop selective compounds that can inhibit only the cyclooxygenase that are present, that are associated with this inflammatory condition and spare the normal functioning of the body.

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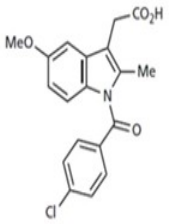
- It has been discovered that cyclooxygenase has two isozymes: COX-1 and COX-2. Both isozymes carry out the same reactions, but COX-1 is the isozyme that is active under normal healthy conditions.
- In rheumatoid arthritis, the normally dormant COX-2 becomes activated and produces excess inflammatory prostaglandins.



Indometacin

It has been discovered that there are two isozymes of cyclooxygenase, one is called COX-1, other one is called COX-2. Both of these carry out the same reaction. But COX-1 is the isozyme

that is active under normal healthy conditions. And so we do not want to inhibit COX-1. Whereas in rheumatoid arthritis which is, COX-2 which is normally dormant, that is it is not active, becomes activated and produces excess inflammatory prostaglandins. So here we need to figure out how to selectively inhibit COX-2 over COX-1.

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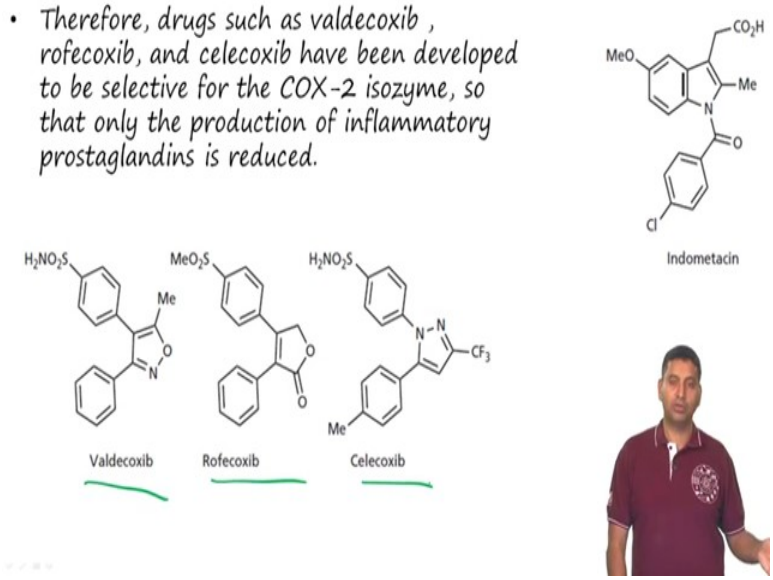

And there are number of drugs which have been developed with this specific purpose of inhibiting COX-2 and the drugs are listed out here. And what these do is that they help in reduction of inflammation only due to COX-2 or preferentially due to COX-2 rather than COX-1.

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- Designing drugs to be isozyme-selective means that they can be designed to act on different diseases, despite acting on the same enzyme.
- This is because isozymes differ in substrate specificity and are distributed differently in the body.

So designing drugs to be isozyme-selective means that they can be designed to act on different diseases despite acting on the same enzyme. This is because isozymes differ in substrate specificity and are also distributed differently in the body.

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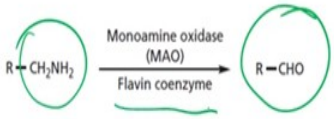
- Monoamine oxidase (MAO) is one of the enzymes responsible for the metabolism of important neurotransmitters such as dopamine, noradrenaline , and serotonin, and exists in two isozymic forms (MAO-A and MAO-B).
- These isozymes differ in substrate specificity, tissue distribution, and primary structure, but carry out the same reaction by the same mechanism



Another example that we will look at is monoamine oxidase. Monoamine oxidase is one of the enzymes responsible for metabolism of important neurotransmitters which we have looked at earlier such as dopamine, noradrenaline, serotonin, etcetera. And there are two isoforms here. One is monoamine oxidase A and the other one is monoamine oxidase B. And what they do is that they convert an amine to an aldehyde, CH_2NH_2 to aldehyde. The isozyme differ in their

substrate specificity, tissue distribution and the primary structure, but carry out the same reaction by a similar mechanism where a flavin coenzyme is actually involved.

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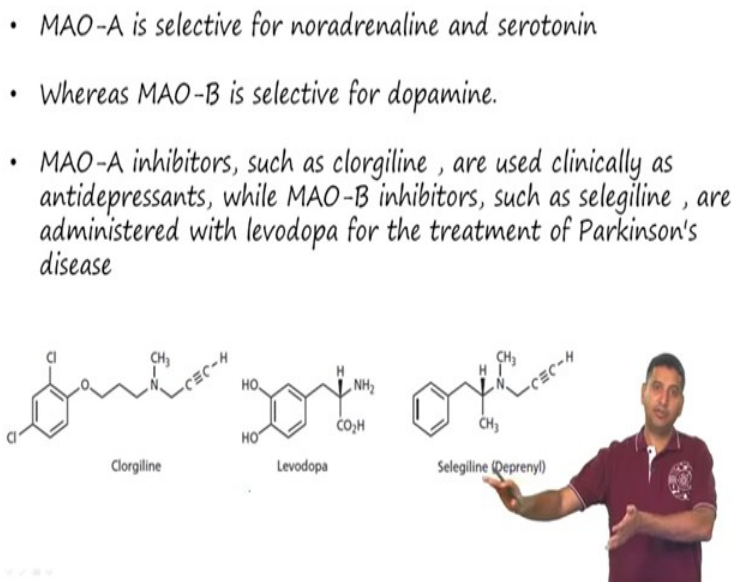

Now monoamine oxidase is selective for noradrenaline and serotonin, whereas monoamine oxidase B is selective for dopamine. So monoamine oxidase A inhibitors such as clorgiline are used clinically as antidepressants whereas monoamine oxidase B inhibitors such as selegiline are administered with levodopa for the treatment of Parkinson's disease. So the knowledge of what the various isoforms or isozymes and how they function is very useful in addressing a particular kind of disease. And one can also develop selective inhibitors for either of these isozymes to have completely different effects inside the body.