Medicinal Chemistry
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Lecture 36
Drug Metabolism
Part III

Welcome back, so in today's lecture we are going to continue to look at drug metabolism. So we discussed previously that you know once a drug enters the body it is going to be exposed to a number of enzymes and these enzymes are going to act on it and they can do a variety of reactions. We looked at a list of reactions last time and how these reactions are going to mainly convert the drug into something that is more water-soluble so that it could be excreted through the kidneys or it can you know be excreted through the bile. So now let us continue to discuss these topics so now let's recap some of the main things that we have discussed in the past couple of lectures.

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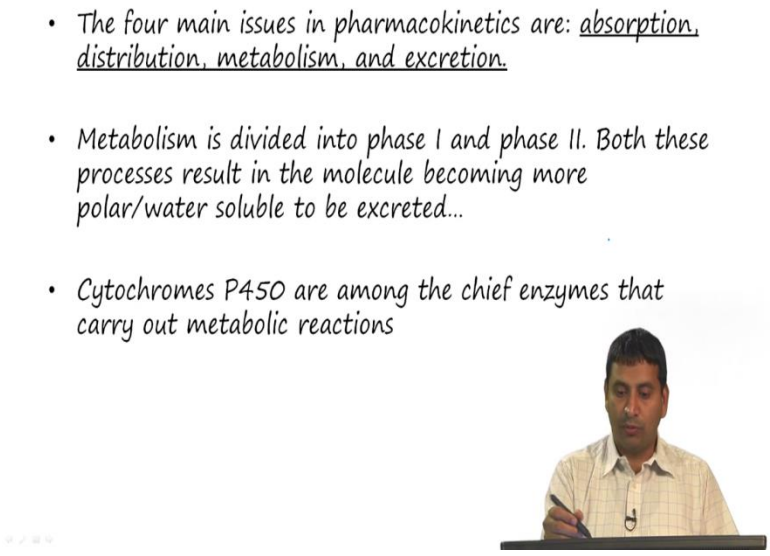

So basically we looked at the concept of ADME which is basically absorption, distribution, metabolism and excretion. And we also saw that there are two phases of metabolism and both these processes typically end up making the molecule more water-soluble so that they can be excreted. And we introduced this enzyme known as super family of enzymes known as

cytochromes P450 which are pretty much non-specific enzymes which can carry out these metabolic reactions.

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So many of the phase 1 transformations are catalyzed by flavin containing monooxygenases, so this is another important group of metabolic enzymes present in the endoplasmic reticulum of the liver cells okay. And this monooxygenases as the name suggest is going to end up taking one oxygen and putting it in a molecule and these are chiefly responsible for many of the oxidation reactions okay, and the targets of these oxidations are nucleophilic nitrogen, sulphur and phosphorus okay and they they prefer these atoms over carbon atoms.

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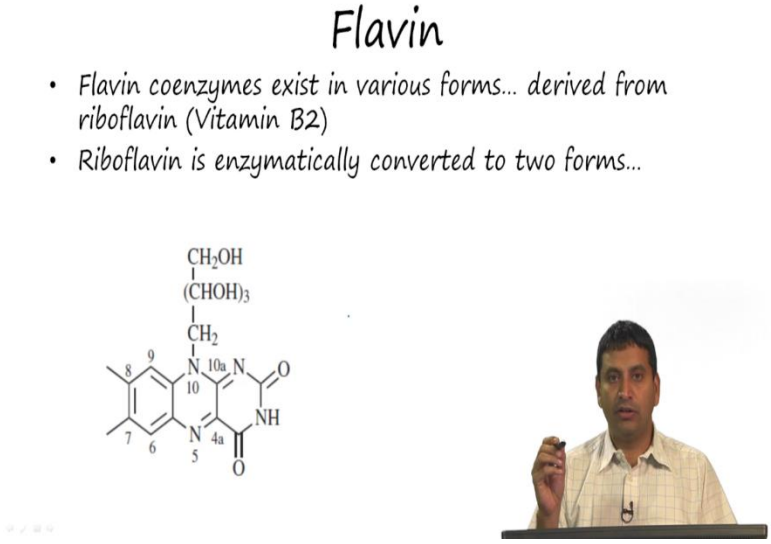

So here is the structure of flavin, so flavin is basically a highly delocalised structure and it is commonly known as vitamin B2 which is riboflavin and riboflavin is enzymatically converted into two forms, so let us look at both these forms now.

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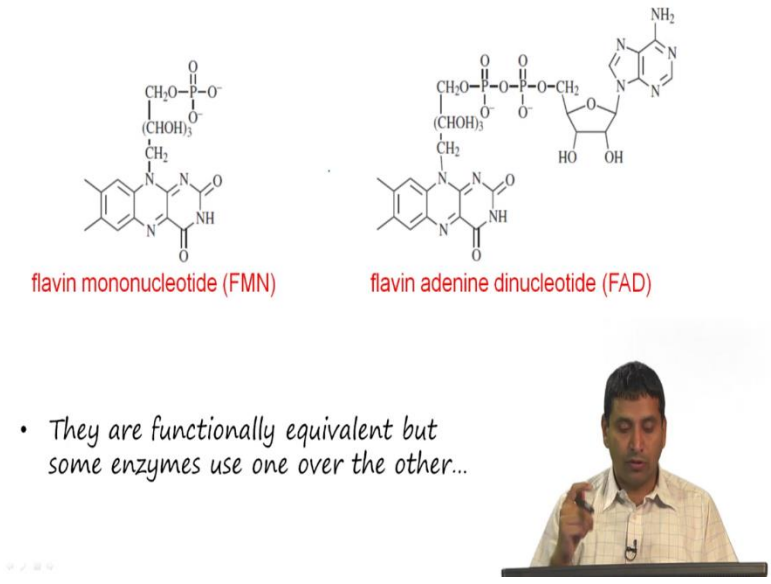

So one is called the flavin mononucleotide who structure is shown here, and the other one is flavin adenine dinucleotide which is FAD. Both these are functional equivalent but some enzymes use FMN and some enzymes prefer to use FAD okay.

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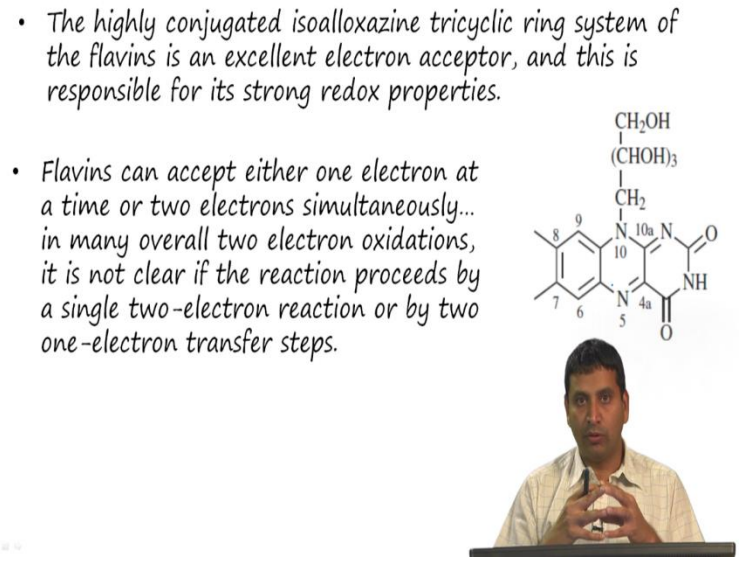

So it is, this molecule contains a highly conjugated isoalloxazine tricyclic system. So here is the tricyclic system right. And it has very strong redox properties, we will look at these very shortly and they can accept either one electron at a time or they can accept two electrons simultaneously okay and overall two electron oxidations, it is sometimes not clear if the reaction proceeds by a two one-electron processes or by one two-electron process okay.

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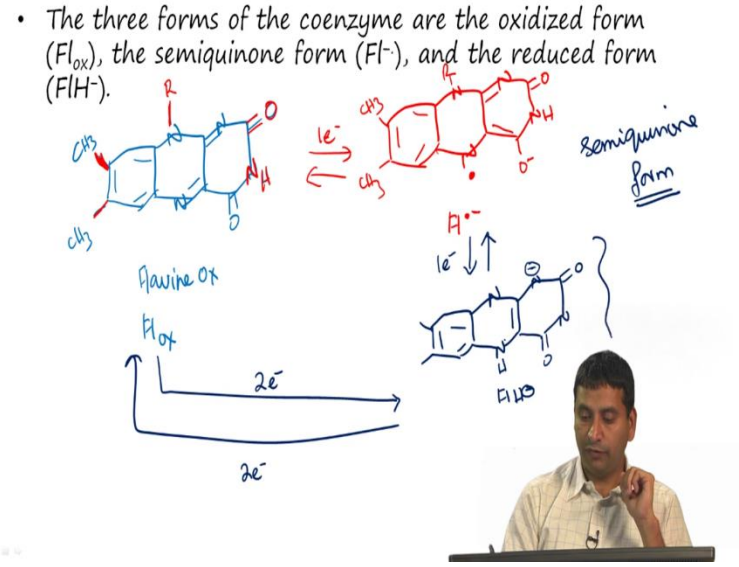

So now let us look at the of the aspects of how this flavin works, so here I am going to draw the structure of flavin on the left. So basically the tricyclic system is as follows, it has the benzene ring and has a nitrogen-nitrogen, right and the 3rd cyclic system is here which has a C double

bond O nitrogen nitrogen C double bond O and this structure has two nitrogens in the centre and this is the methyl group CH_3CH_3, and since it is a highly conjugated system you can imagine there is a double bond here, a double bond here and NH over here okay, this is called flavin oxidised form or Fl_{ox}.

So this group here is R, so this is the main portion of the flavin molecule which contains three rings and it has a number of functional groups, it has a conjugated system right. And once it picks up one electron right, it can assume a similar structure which I am going to draw here. So the benzene ring here remains the same and the nitrogen over here picks up one electron, this nitrogen remains the same and we have O minus here, NH here, C double bond O, this nitrogen remains the same, this nitrogen remains the same. So this intermediate has one electron here as you can see and this is called as Fl dot minus ($Fl^{\cdot -}$) okay. So what happens in this process is that the flavin ring picks up one electron and it just results in rearrangement of electrons to produce a nitrogen-based radical and a enolate.

Now this molecule can further pick up one more electron which we will look at now, so this will pick up on more electron and which I am going to draw over here and these are reversible processes, so you have a benzene ring which remains the same, the 2^{nd} nitrogen is the one where the electron is going to be transferred and you have a C double bond O - N - C double O - N, this becomes a negative charge and the double bond is moved over here and this becomes NH okay. So what ends up happening is that this electron actually communicates here with this ring and picks up electron and produces a full negative charge on this nitrogen on top okay, so this species is called FlH minus right. So these are the three means species that are involved in the flavin redox process.

Now, it is also possible that you can convert this to this by adding two electrons, and you can do the reverse process by subtracting or removing two electrons, so this is how the entire flavin redox process occurs and this is called the semiquinone form and this is the fully reduced form which is FlH minus.

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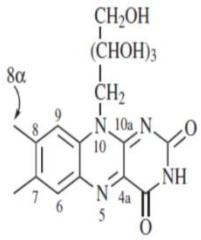
- Although most flavin-dependent enzymes (also called flavoenzymes) bind the flavin with noncovalent interactions, some enzymes have covalently bound flavins, in which the flavin is attached at its 8α-position or 6-position to a histidine or cysteine



Now although most flavin dependent enzymes bind the flavin in a non-covalent manner, some of the enzymes actually bind to it in a covalent way, right. So this covalent bond happens at this 8th alpha opposition or at the 6th position okay, so we will not go into too many details but we will look at one example later where this kind of a bond occurs, and the residue that binds to this is either a histidine or a cysteine.

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- Once the flavin has been reduced, the enzyme requires a second substrate to return the flavin to the oxidized form so that it can accept electrons from another substrate molecule.
- Some flavoenzymes are called *oxidases* and others *dehydrogenases*.
- The distinction between these names refers to the way in which the reduced form of the coenzyme is reoxidized, so the catalytic cycle can continue...

So once the flavin has been reduced, now the enzyme requires a second substrate to return the flavin to the oxidized form so that it can accept electrons from another substrate molecule. So some flavoenzymes are called as oxidases and others are called as dehydrogenases okay. The

distinction between these names refers to the way in which the reduced form of coenzyme is reoxidized okay, so the catalytic cycle can continue. And keep in mind that we looked at the entire the three redox forms of flavin and this is a reversible process and so flavin can get oxidized as well as reduced. And so the mechanism by which this occurs is what we are going to look at in very briefly in next few minutes, but depending on how it goes back to the oxidized form it is called an oxidases or dehydrogenases.

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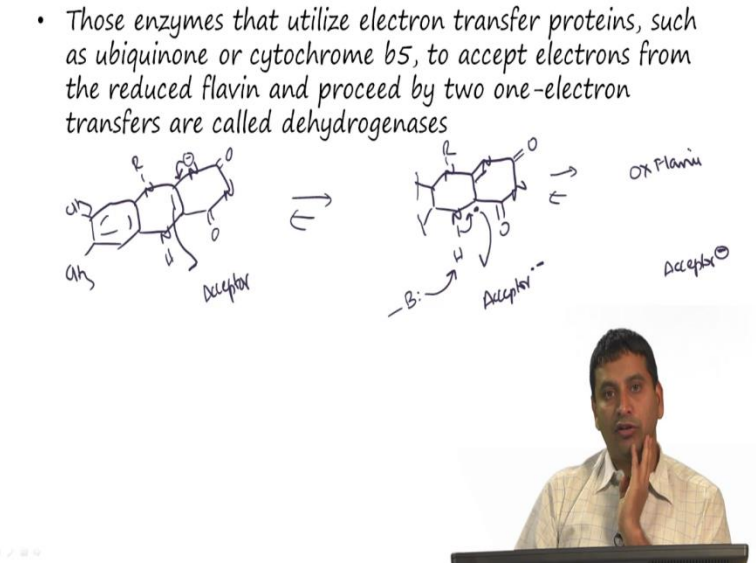

So the enzymes that utilise electron transfer proteins, such as first class of enzymes that we are going to look at are dehydrogenases and these enzymes utilise electron transfer proteins such as ubiquinone or cytochrome B5 to accept electrons from the reduced flavin. So let us now draw the structure of reduced flavin very quickly and so here are the two methyl groups and here is the central ring and here is the 3rd ring of flavin. So there are two nitrogens here, nitrogen is here, nitrogen here, C double bond O and you have N minus NH and we have R okay. So what happens is that you can push 2 electrons in here and this is actually going to donate a single electron okay, so when we are looking at a single electron we always draw a fish hook arrow.

And this is going to be donated to some acceptor molecule and the second species that is going to be produced is N - N, so if you draw the arrows you have a double bond over here with N and then there is C double bond O, N - C double bond O and his carbon actually ends up with a

radical, rest of the molecule is the same and there is an NH popping out here and now you can imagine that there could be a base from a protein which then picks up this hydrogen and it transfers two electrons over here and this in turn given back one electron process to the acceptor dot minus okay. So now the acceptor which is in the form of radical anion becomes acceptor negative charge and you get back the oxidized flavin whose structure I am not going to draw okay. So this is the process by which two one-electron transfer can occur and this is the mechanism by which dehydrogenases acts.

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Oxidases on the other hand use molecular oxygen to oxidize the co-enzyme with the concomitant formation of hydrogen peroxide, now let us look at the ways in which oxidases are going to function.

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- Reaction with triplet oxygen, leading first to the caged radical pair of and superoxide by electron transfer...
- Superoxide, after spin inversion, can undergo either radical combination or second electron transfer from the flavin semiquinone to go directly to the oxidized flavin...

And one of the ways in which this can happen is by reaction with triplet oxygen which leads to the formation of caged diradical pair of superoxide by electron transfer and then superoxide after spin inversion can undergo either radical combination or second electron transfer from flavin semiquinone to go directly to the oxidized flavin okay. So again we will not look at too many details of this mechanism, but at the end of it you will end up producing hydrogen peroxide.

So if we were to look only at the central flavin ring, you have NRNH, this is the same species which is N minus over here C double bond O – NH, then there is double bond O, rest of the molecule remains the same and you have a reaction with O dot dot and this produces, arrow pushing is pretty much the same, we have a fish hook arrow, this O attacks here as again a fish hook arrow and you end up producing a radical intermediate such as this O - O - dot C double bond O - NH double bond O - N and now this forms a double bond, rest of the molecule remains the same right and then this can subsequently get oxidized and produce H_2O_2 and regenerate the flavin okay.

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- The analogous reaction with singlet oxygen to give the flavin hydroperoxide directly, which will only occur if there is a mechanism for spin inversion from the normal triplet oxygen, such as with a metal ion. Loss of hydrogen peroxide gives oxidized flavin.

The analogous reaction with singlet oxygen to give the flavin hydroperoxide directly is also possible and it will occur only if there is a mechanism of spin inversion from the normal triplet oxygen, because normally triplet oxygen is what exists and so this singlet oxygen is produced in situations where there is a metal ion, and again there is loss of hydrogen peroxide to give oxidized flavin. So we will not look into details of this mechanism but this is another way in which you can regenerate flavin after the redox reaction okay.

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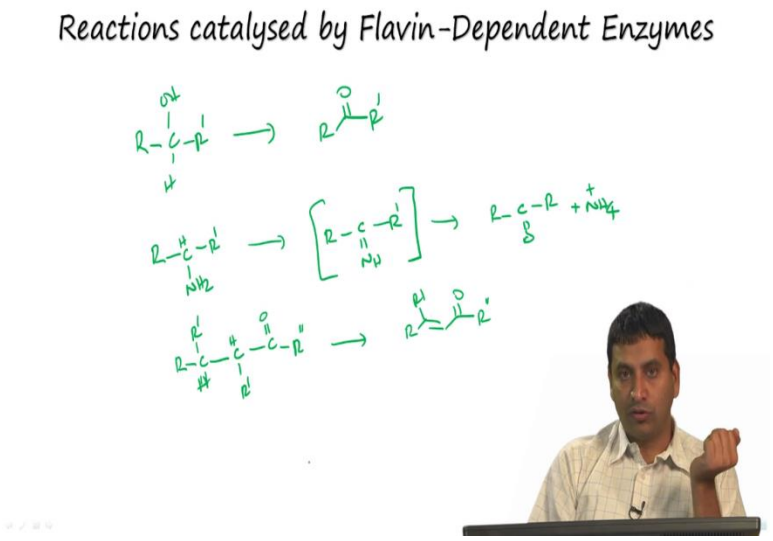

Now there are number of reactions that are catalyzed by flavin dependent enzymes, let us look at some of these. So the first example that we are going to look at is RCOH which is an alcohol going to a ketone okay. You can also have a amines getting oxidized such as this NH_2R prime giving you R - C double bond NH which is an amine which is going to be an intermediate which will then subsequently form R - C double bond O - R which is a ketone + NH_4^+ okay. The third reaction that can happen is an oxidation reaction to produce an olefin. So here you have the example that we are going to look at is CRR prime - CHHR double prime to give you and this carbon-carbon bond is going to form double bond and you end up with this ketone okay, so this is again an oxidation reaction that is catalyzed by flavin dependent enzymes.

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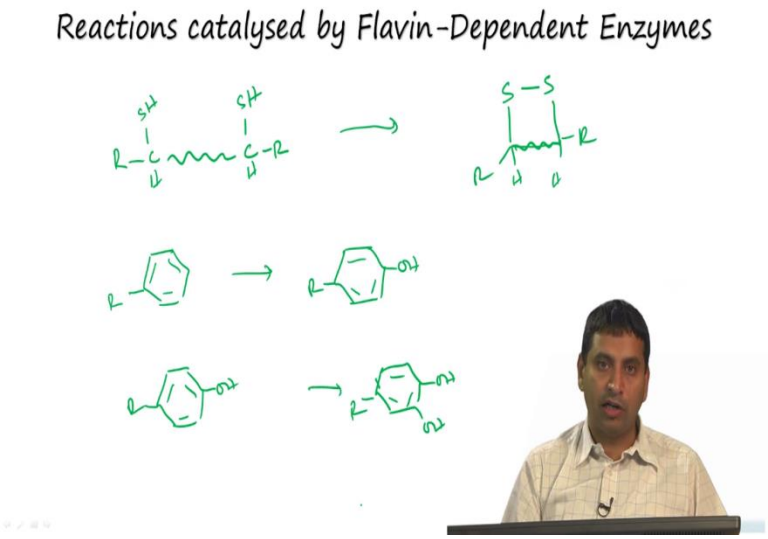

The next class of reaction is the formation of disulphide bond, so you can have RCSH - a long chain maybe with another SH here okay, and this can undergo oxidation to produce a disulphide linkage such as this okay, so this is the next one possible. Flavin dependent enzymes also can oxidize benzene rings to give you the corresponding phenols. We have already looked in the previous lecture and also the oxidation of phenols to catechols is also possible okay, so these are the major reactions that flavin dependent enzymes can catalyze. And as you can see all of these are metabolically very important and therefore flavin dependent enzymes become a very important class of enzymes that are involved in drug metabolism.

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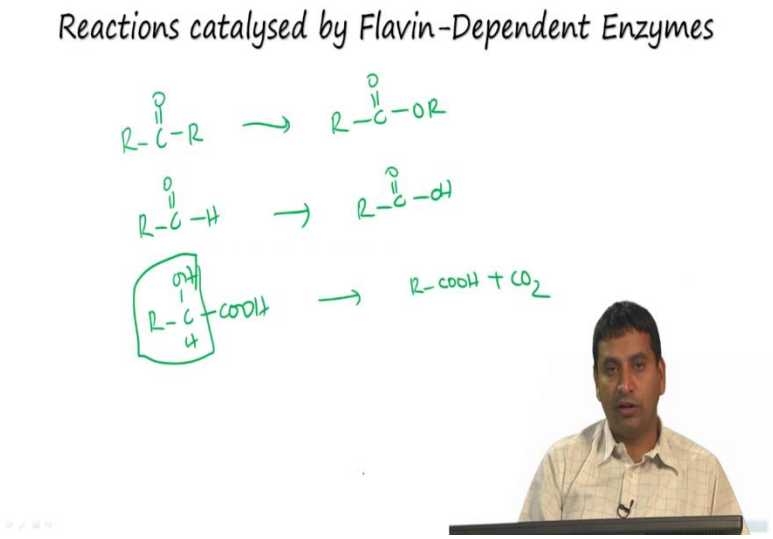

Lastly they also can oxidize carbonyl compounds such as R - C double bond O – R, this is a ketone to form an ester okay, so this ester formation is also possible. You can also form an ester from an aldehyde by flavin dependent monooxygenases, so you form a carboxylic acid from an aldehyde. And lastly you can also have the oxidation of hydroxy acids to produce COOH which is basically this part of the molecule is going to get oxidized and other part of the molecule is going to form carbon dioxide, so all of these are catalyzed by flavin dependent enzymes.

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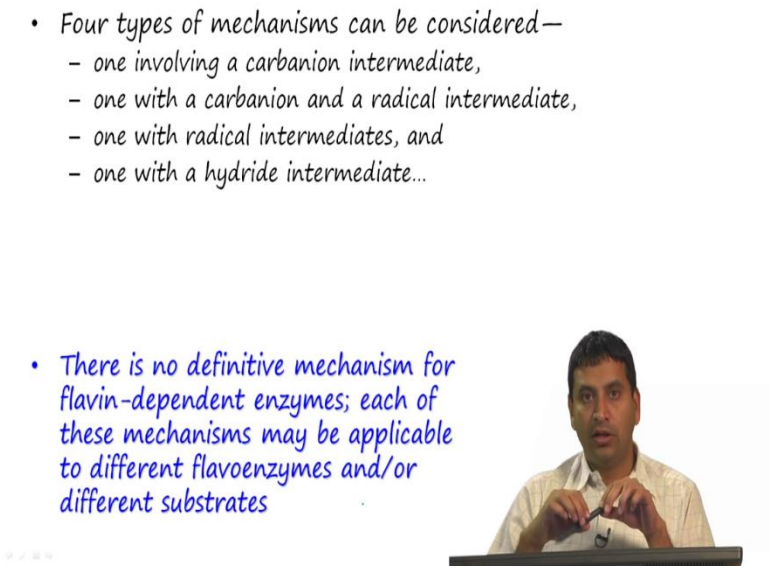

There are four types of mechanisms that can be considered one which involves carbanionintermediate and then one with a carbanion and a radical intermediate and then one with radical intermediate alone and the last one is only with a hydride intermediate. So these are being studied extensively but no definitive mechanism is possible is known, some of these may be applicable to different flavoenzymes and also it also depends upon the substrate. So the principles of this mechanism remains pretty much along these lines but the exact definitive mechanism is difficult to ascertain.

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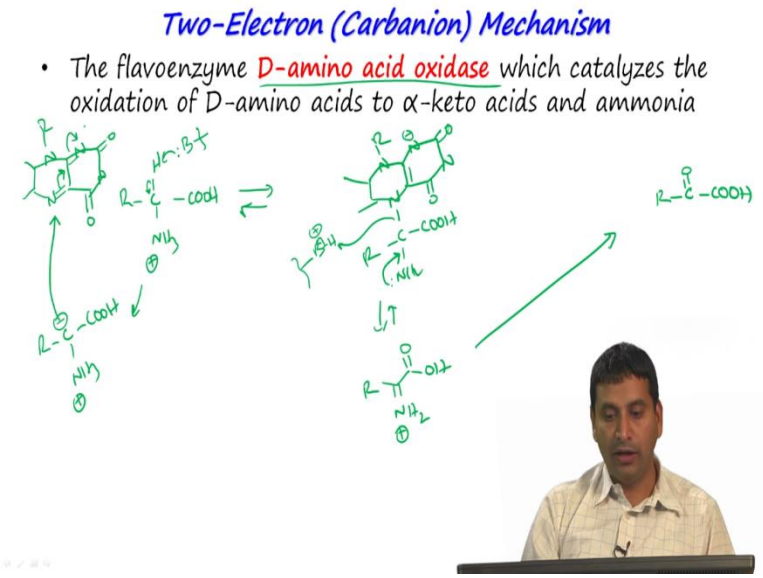

Now let us look very quickly at the two electron carbanion mechanism, so here the example that we are going to look at is D-amino acid oxidase, so let us draw an amino basically it is acid, so basically it is NH_3^+, COOH and this is the hydrogen, and the final product that we are going to get is a keto which is basically acid R - C double bond O - COOH. So the 1st step in this mechanism is a flavin dependent reaction so let us look at that reaction, so if you draw out the nitrogen ring of flavin as shown here and you have a molecule that looks like this, here is the flavin ring so you have N double bond N and another N double bond N, so you can imagine that there would be a base that would come, abstract this hydrogen and this can then be transferred to C minus and that will give you R - C minus NH_3^+, COOH.

And since this carbanion is mixed to a electron withdrawing ammonium ion as well as carboxylic acid, it is possible that it can be stabilised to some extent and then this carbanion can

then react over here and give you this table N minus and so intermediary species that you would be producing would be NCCOOHNH$_2$R and rest of the flavin molecule as we would have drawn it out previously, right okay. So now for this species you would need the base which is here, BH$^+$ which has picked up a proton from her and this nitrogen will probably push in the electrons over her and this goes and picks up a proton and regenerates the base, so you end up with a product that is C double bond NCOOHR right, which then undergoes hydrolysis to give you the ketone okay. So this is the two electron mechanism in a nutshell there are many standards books wherein you can obtain this mechanism okay.

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- Evidence for a one-electron (radical) flavin mechanism comes from a variety of experiments with monoamine oxidase (MAO), a flavoenzyme important in medicinal chemistry
- This is one of the enzymes responsible for the catabolism of various biogenic amine neurotransmitters, such as norepinephrine and dopamine.

So the another one electron mediated oxidation reaction is an enzyme called as monoamine oxidase, this enzyme is really important flavin enzyme in medicinal chemistry. It is one of the enzymes responsible for catabolism of very important neurotransmitters such as norepinephrine and dopamine.

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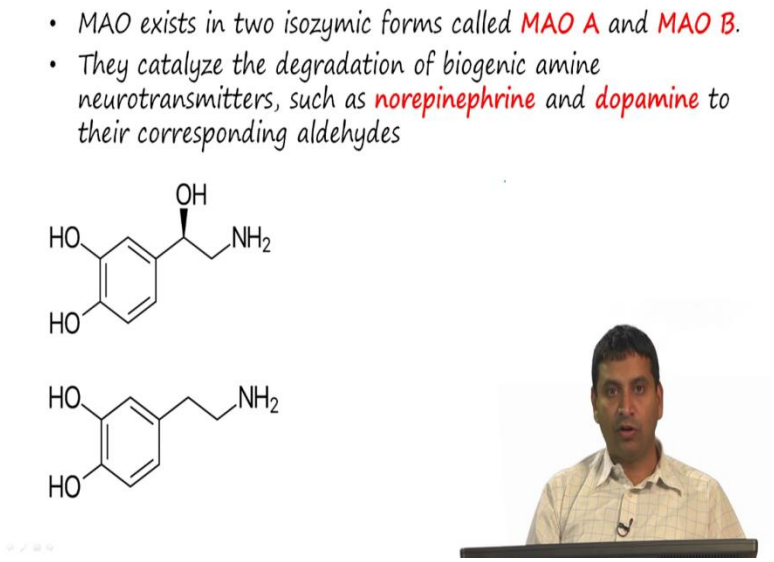

So these are the structures of norepinephrine and dopamine and they are oxidised to the corresponding aldehydes okay. And monoamine oxidise exists in two isomeric forms; MAO A and MAO B, and they catalyse this reaction of which wherin the norepinephrine and dopamine is converted to the corresponding aldehydes.

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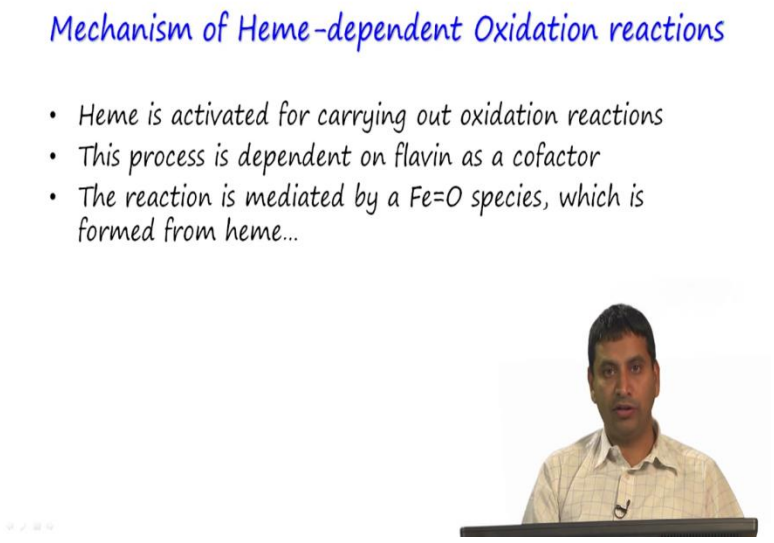

Now we will look at mechanism of Heme-dependent oxidation reactions. So heme is a very important part of we looked at previously cytochrome P450 they are heme containing proteins

and it is useful to carry out many oxidation reactions and of course this process is dependent on flavin as a cofactor okay. So the reaction of heme dependent oxidation involves a Fe double bond O species okay, and this species is formed from heme and that process is mediated by flavin.

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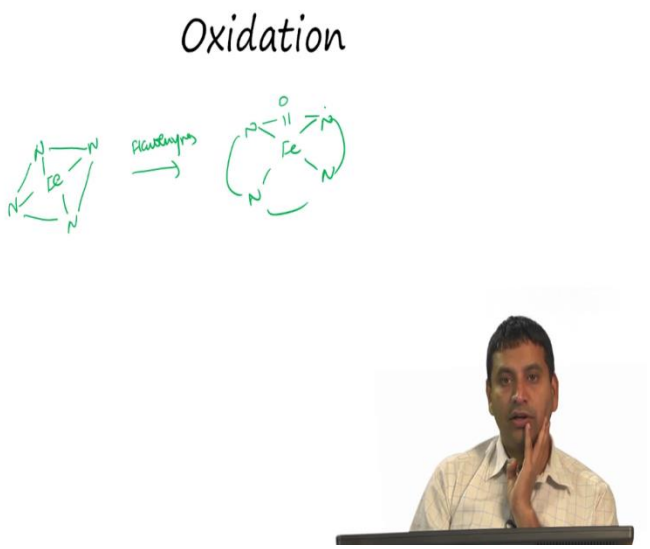

So if we were to look at the structure of heme in a very simplistic manner, basically it is a porphyrin ring with iron in the centre and you have many ligands on the top, so this oxidation reaction is characterised by flavoenzymes to give you Fe double bond O nitrogen nitrogen nitrogen nitrogen right and this is now involved in the major species that is involved in oxidation.

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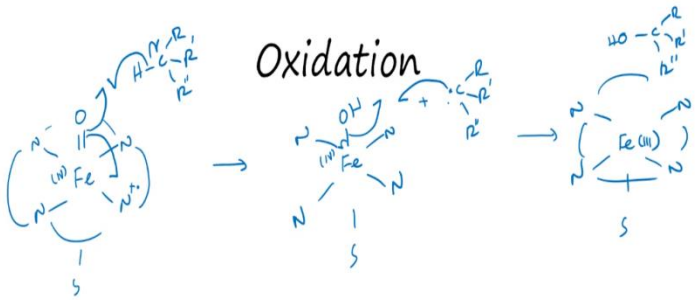

So let us look at the species again, so we have Fe double bond O with the porphyrin ring over here. Again as I mentioned this species is obtained from heme by flavin dependent enzymes. So now if you have alkane for example, R prime - R double prime, this reacts with the heme oxidised Fe double bond O with a radical mechanism so you have a radical that is produced over here and this first is picked up one of the electrons goes here and a second electron goes here and this carbon hydrogen bond undergoes reaction again to produce a carbon centered radical okay.

So we will produce Fe - OH right and now the oxidation state of iron here is +4, nitrogen nitrogen nitrogen nitrogen and the product that is formed is CRRR, which is basically a radical. And now there is an oxygen that is going to be transferred over here, the second bond breaks this is the bond that is formed and then this is going to go back into the iron system and you get Fe(III) right. And of course in this all this there is a cysteine residue that is bond to the iron, right and you get OHCRRR okay.

So this is a general principle by which heme dependent enzymes work so the first step you have the formation of a iron oxo species which has cation radical on it, which then is able to involve itself in radical reactions with an alkane for example, we end up with a carbon-based radical which then reacts further with OH dot that is transiently produced to give you the alcohol and it gives you an Fe (III) complex okay.

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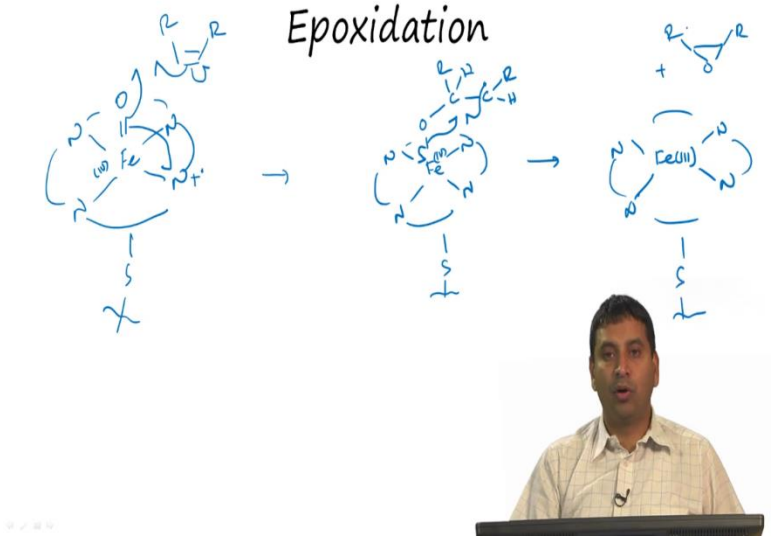

Using the same principle the epoxidation reaction also occurs, so again we should look at just the key steps of this mechanism. You have Fe double bond O, you have the nitrogen nitrogen nitrogen nitrogen okay and as I mentioned earlier there is a cysteine residue over here which is going to anchor itself to the iron and you have an olefin R - R and as I mentioned there is a radical cation over here so you have the formation of radical species, you have formation of another radical species and this is now donated over here

The second electron is given to the radical cation or the nitrogen and end up with Fe - O carbon R - H carbon - R radical – H, rest of the molecule is the same, iron is still in oxidation state four continues to remain in oxidation state four and you have a thiol residue that is attached here. And once this is formed, now there can be reaction of this radical with oxygen in the following manner, this donates one electron, this donates the other electron and then this goes back here to give you Fe (III) porphyrin with the sulphur remaining here and the product is the epoxide okay, so this is broadly speaking the mechanism by which epoxidation occurs.

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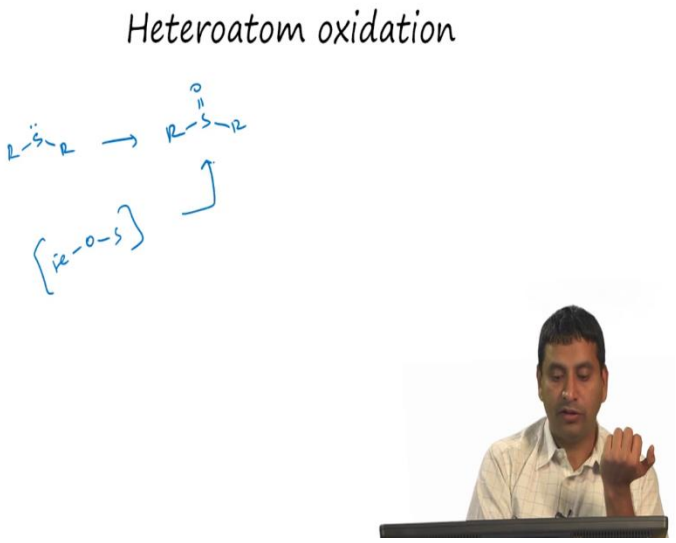

In a very similar manner you can also have hetero atom oxidation such as R - S - R going to R - S double bond O - R okay. So I am not again going to go into the details of the mechanism but you can imagine that there is going to be a radical that is formed on the sulfur because you have a lone pair on sulphur, which can donate one electron and once that radical is produced, it again forms bond with oxygen to produce a Fe - O - S type of intermediate which then rearranges and gives you the product okay.

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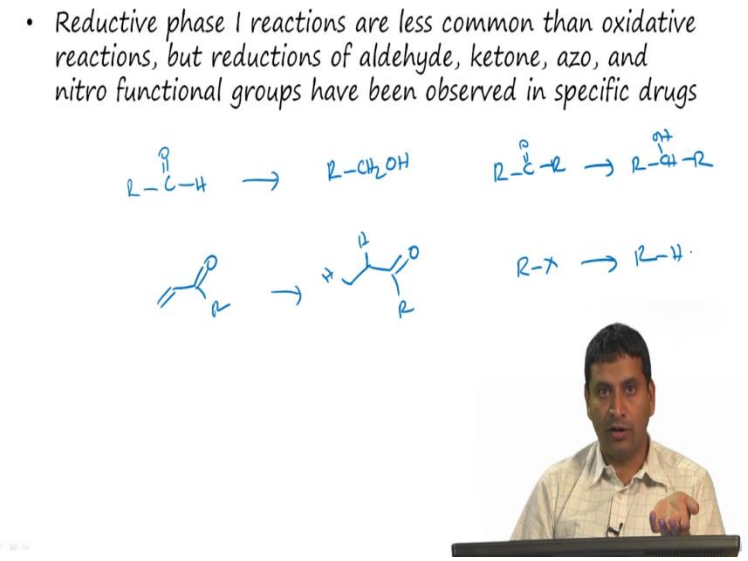

So these are all examples of oxidative phase 1 reactions, but there are also several reductive phase 1 reactions but they are less common and the main reductions that occur are of aldehyde

ketone, azo and nitro functional groups, so let us look at some of these examples very briefly. So you have an aldehyde which is going to give you R - CH_2OH similarly you have a ketone which will give you a secondary alcohol okay. And you can also have alpha beta unsaturated compounds such as Michael acceptor which can and give you R over here so that is hydrogen has been added over here, you can also have alkyl halides giving you R - H. So all these are formally reduction reactions and they can all occur in reductive phase 1 reactions but they are less common.

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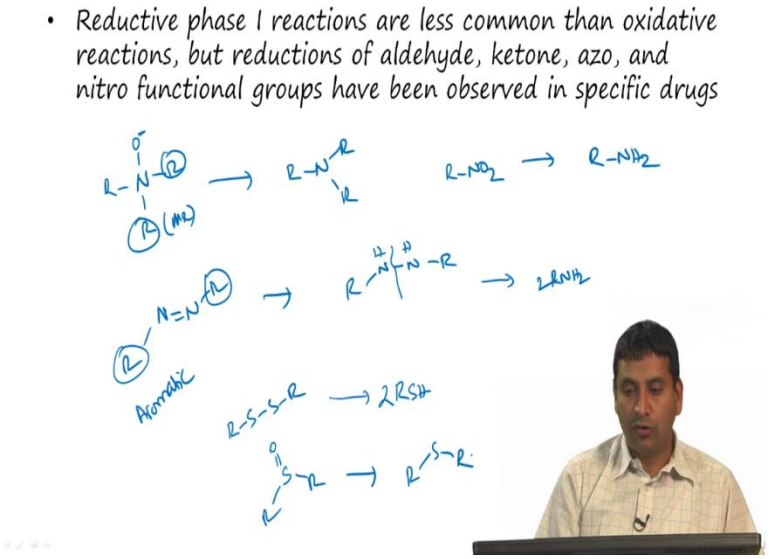

As I mentioned earlier you can also have nitrogen base compounds undergoing reduction so you can have *N*-oxides which we looked at previously going to R - N - R - R and these are typically methyl groups that are quite common. You also have nitro groups which can go all the way to RNH_2 okay, you can also have azo compounds such as this giving you RNNHR so all these are reduction reactions and this can further undergo reduction to give you two moles of RNH_2 okay. And these R groups can actually be aromatic rings as well and those are going to give you the corresponding aromatic amines.

Of course we know that disulphides are very common. Disulphides can also be reduced to form two moles of thiols and sulphoxide which are S double bond O can also reduce to form R. So again these are all possible but they are less common compared to oxidative reaction.