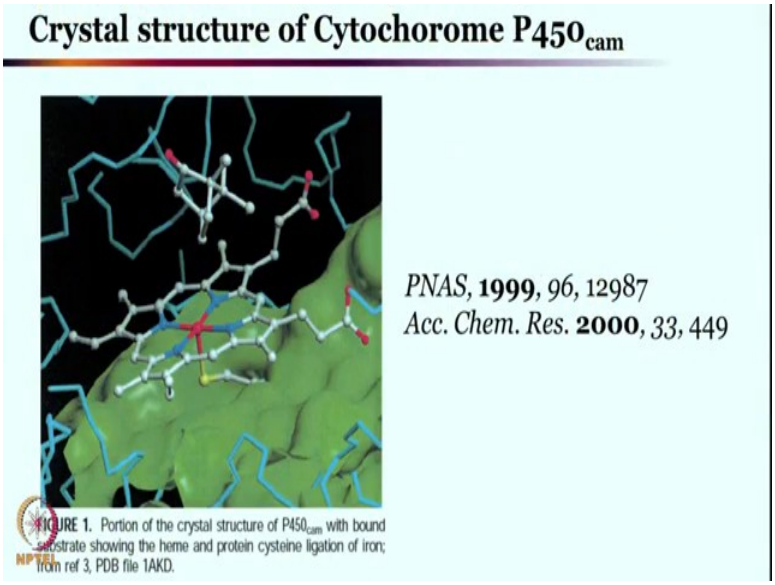
Metals in Biology
Prof. Debabrata Maiti
Department of Chemistry
Indian Institute of Technology, Bombay

Lecture – 26
Cytochrome P450 Part II – Reactions

Hello, welcome back to today's discussion on Cytochrome P450. In the last class, we were discussing this very important metallo enzyme known as cytochrome P450. We have seen a nice and crystal clear structure of cytochrome P450 with the camphor the substrate.

(Refer Slide Time: 00:46)



As you have seen here is the porphyrin ring, iron center is at the middle and there is a proximal site; at the proximal site this cysteine is attached. As you have seen in case of hemoglobin and myoglobin exactly similar porphirine ring is there, but over here you will have histidine as a side chain.

In this case, the cysteine side chain makes it really interesting for its chemistry. As you have seen when histidine is there in case of hemoglobin and myoglobin, this iron center with histidine is capable of reversibly binding oxygen and deliver that oxygen towards the center where it is necessary. In this case histidine is replaced by this cysteine and we will see that upon oxygen binding at this distal site. So, again this is the proximal site, this is the distal site. So, at this distal site upon oxygen binding this oxygen will get

reduced by this iron center and subsequently it will try to react with the organic substrate.

Now, the orientation of this organic substrate in front of the active site will dictate which C-H bond to be hydroxylated or to get functionalized. So, in that particular case, in these cases or any enzyme these side pocket which are not really directly part of the main active site plays crucial role in dictating the reactivity pattern on this substrate. As you will see for a radical type of mechanism, a tertiary C-H is more preferred over secondary and secondary is more preferred over primary.

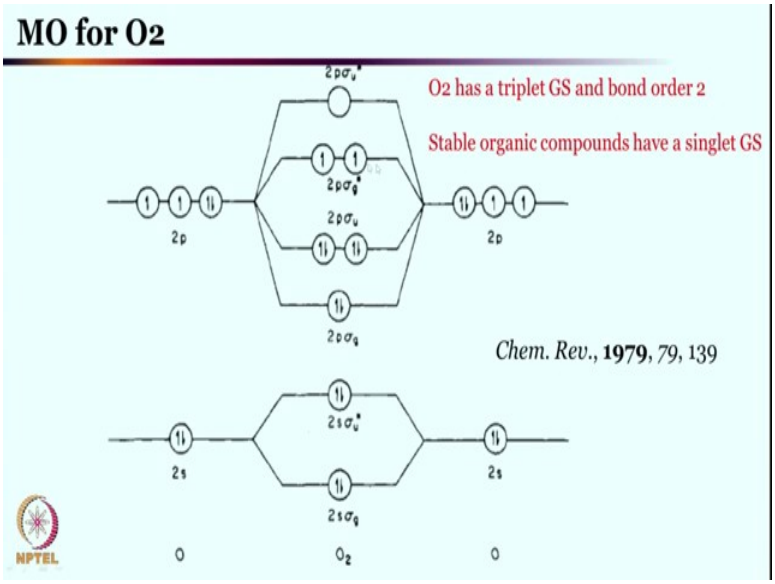
But, all of these cases in case of the substrate binding pocket substrate orientation with respect to the enzyme active site and perfect positioning of these two component that is means the reactive component as well as the reactant that is substrate is quite important. As you have seen cytochrome P450 type of enzyme not only capable of hydroxylating a particular organic substrate, it can also promote a series of related events. Number of reaction or type of reaction that cytochrome P450 can carry out is almost countless. We will see a list of reaction today where cytochrome P450 can play a role and a crucial role.

I hope you got that this is a porphyrin center and iron in the middle. So, heme iron centre with the cysteine binding thiolate binding this is the site which is responsible for the chemistry. We did not show here any oxygen species yet because this is the crystal structure that obtained. Obtaining crystal structure with an oxygen binding is always very difficult because those are very reactive intermediate and the binding may not be too strong and therefore, getting a crystal structure is next to impossible although many attempts has been made to get this crystal structure.

Usually to get this unstable crystal structure one needs to cool down the reaction temperature reaction solution so that the desired other reaction can be slowed down significantly. In addition since these are reactive intermediate that is going to be generated from reaction of iron and oxygen. Eliminating the substrate or replacing the substrate with some another organic substrate which can inhibit the desired reaction; desired let us say in this case hydroxylation reaction that would be quite useful because in presence of the inhibitor may be those desired reaction can be prevented partially or completely.

In particular for cytochrome P450 cases, these are very reactive intermediate that forms at the iron centre. Therefore, preventing a desired reaction is always extremely challenging.

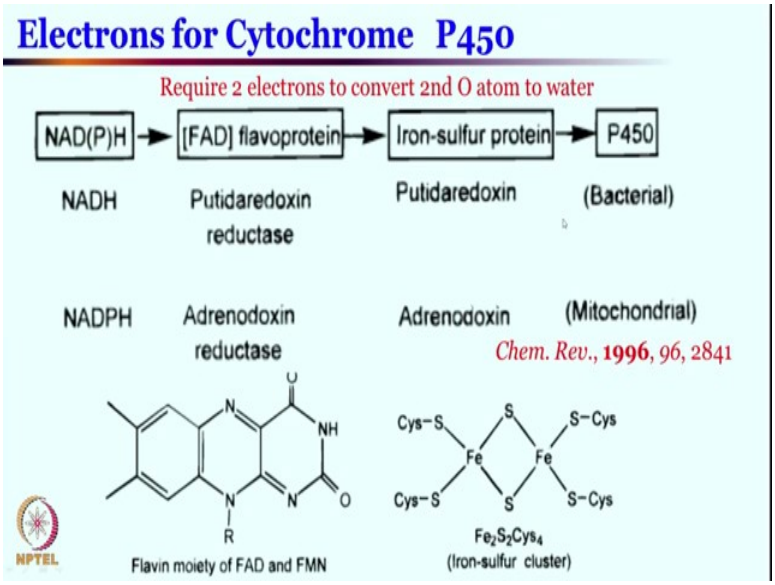
(Refer Slide Time: 05:58)



Let us look at what we have discussed in the last class. Of course, we have discussed about the oxygen molecular orbital and these lone pair or unpaired electron are responsible for the triplet ground state of oxygen and once these oxygen's are getting reduced by one electron that electron will be coming in one of these 2 orbitals right. These are degenerate orbitals. If it is doubly reduced; that means, a peroxo species is from. So, the mono electron or one electron reduced species is called superoxo, 2 electron reduced species is called peroxo. Once it is reduced by 2 electrons, then one electron will come over there another electron will come over here.

So, overall that would be the a molecular orbital diagram of oxygen 2- that is the peroxo. If it is one electron reduced that would be the superoxo or super oxide species.

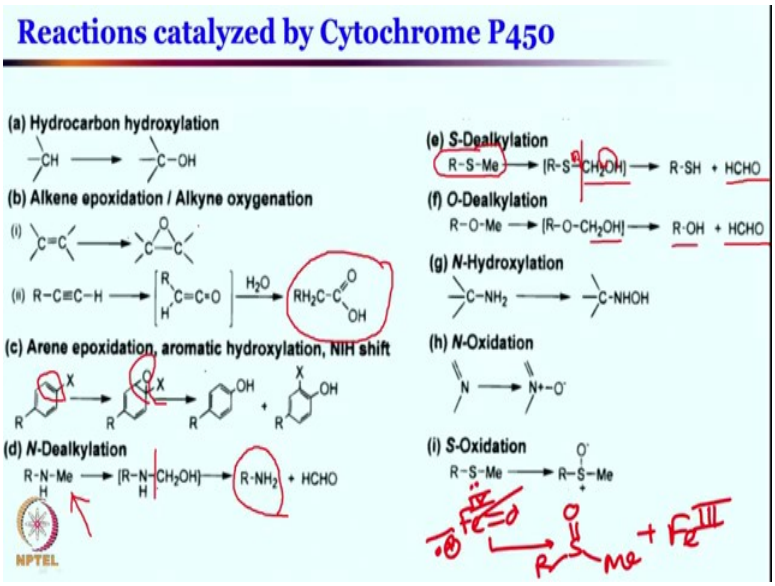
(Refer Slide Time: 06:54)



In the whole process in converting oxygen into some useful compound such as aliphatic substrate can be converted into hydroxylated product, you still need to have a second oxygen atom reduced subsequently to form the quarter. So, these 2 electron that is required to reduce to these terminal or the second oxygen atom to water molecule that is taken care by this relay of electron that happens towards the active site as we have discussed in the last class.

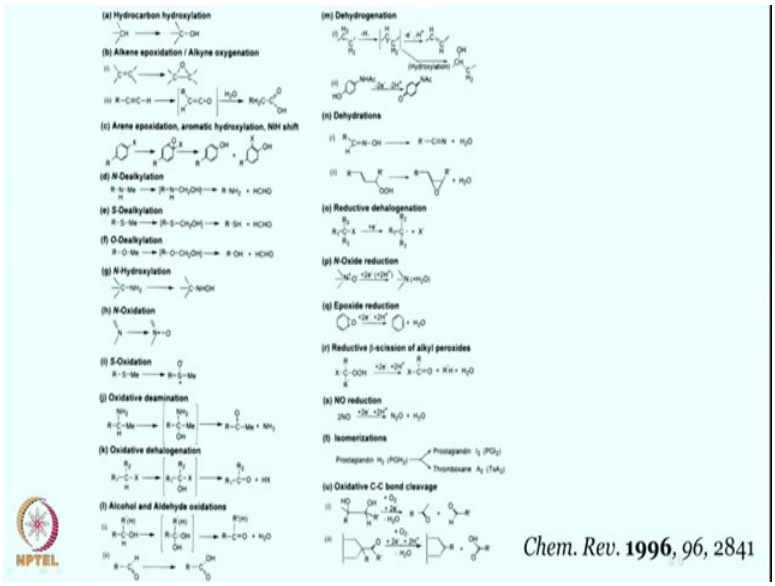
So, the electron hopping or transferred throughout the chain and up to the iron sulfur protein happens and from there on, it provides the electron whenever required for the oxygen reduction process at the heme iron center with cysteine bound center in the cytochrome P 450.

(Refer Slide Time: 07:55)



Let us look at some different type of reaction that we have discussed in the last class. So, if you are taking an organic substrate such as aliphatic substrate, it can be reduced. So, this is this is from a review article.

(Refer Slide Time: 08:15)



We have the review article, yeah, I think one of these review article yeah that is mentioned over there Chem review, 1996, that is the article. Let us go back ok. So, Chem review, this is from Chem review, 1996 where hydrocarbon hydroxylation can be occurred. So, this is a summarize summary of the cytochrome P450 reaction. Here you

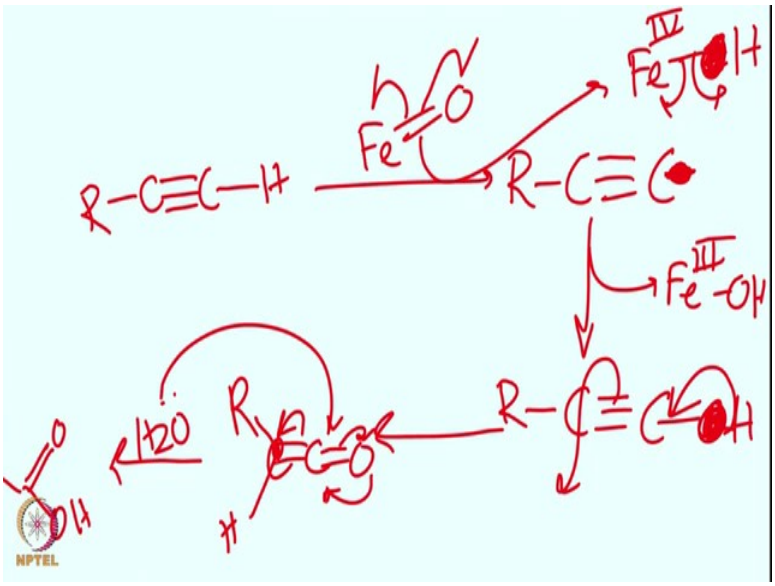
see that a C-H bond can be reacted to give the C-OH aliphatic substrate hydroxylation chemistry.

In this is one of the easy to understand reaction. So, where a hydrogen atom abstraction at this center will occur and the OH iron OH iron IV hydroxo that is gets generated over there undergo rebound to give the C hydroxylation reaction. One can think of taking an olefin as a organic substrate as opposed to this aliphatic sp3 C-H bond.

These iron-oxo species that you have seen getting generated in the last class that iron-oxo species these are high valent iron-oxo species. Iron V oxo, so, you cannot talk it as an iron V oxo, it should be iron IV oxo with porphyrin radical cation being there can transfer the oxygen atom to give you the epoxide species. If you are taking a terminal alkyne let us say phenol acetylene or alkyl acetylene moiety, it can be subsequently transformed to the corresponding terminal carboxylic acid that is quite phenomenal reaction I will say and you see this is and quite interesting ethyl intermediate that is proceeding for the reaction.

So, abstraction of the C-H bond from this and the rebound of the hydroxo and then subsequent rearrangement will give rise to the C=C=O intermediate. So, let me draw that is reaction just quickly.

(Refer Slide Time: 10:27)



So, if we are trying to draw this reaction it will be. So, C triple bond CH we have and then R we do have and let us say these iron-oxo intermediate is there. This should be iron IV oxo with a radical cation which is essentially iron V oxo. So, this will gives rise to the abstraction of the species and we will have R-C radical plus these species will be giving rise to iron hydroxo. This is going to be an iron IV hydroxo. This radical and this hydroxo then combined. So, iron III hydroxo getting generated iron; III hydroxo is getting generated and this C radical and this hydroxy radical will form a bond together. So, overall it will be iron C-OH. So, this is the oxygen that is coming from there.

So, this is the oxygen coming from there and then the protonation would lead to the intermediate where you will see R-H double bond C=O is forming right. So, this is a quite simple and effective reaction by and then upon hydrolysis at this center one can think of reacting it with of course, this will go and that will come back. Overall this is going to give you the terminal acid moiety along with this CH2-OR moiety right. So, what we have seen right now over here is the formation of such terminal oxide species and that gets generated from this from the species right. So, what you have seen this intermediate is reacting to give you R-CH2-CO2H terminal a carboxylic acid is getting generated from this reaction.

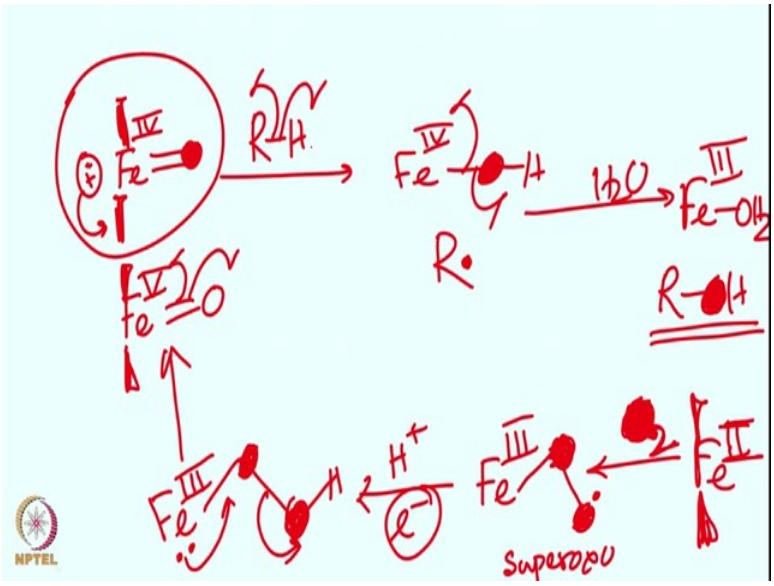
If there is an arene ring; so, arene epoxidation or aromatic ring hydroxylation and along with NIH shift it will happen. So, what happens over here is the oxo species that is over there so, it can undergo electrophilic aromatic substitution reaction at this olefin center to give the epoxide moiety which can then undergo either NIH shift or the overall phenol formation upon subsequent rearrangement and removal of HX right.

If you are taking N-methyl or N-alkyle amine, it could be a secondary amine as is here or it could be a tertiary amine. This amine can have reaction with the iron V oxo or iron IV oxo radical cation intermediate where this is the aliphatic sp3 center this can react and the hydroxy moiety can rebound with it or bind with it. This hydroxo then can undergo further reaction to give you the formaldehyde this CH2=O is unit gets to the formaldehyde and the N-dealkylation occurs in the whole process to give you the aliphatic amine or primary amine, it could be aliphatic and aromatic depending on the R. So, N-dealkylation chemistry can also happen.

Another very interesting reaction which we may have seen in different context is that the S-dealkylation chemistry can also happen as you can see over here. These this thioether R-S-Me can be a can be also reactive towards these high valent iron-oxo species to give you R-S-CH 2 OH moiety. Now, this R-S-CH 2 OH moiety can then further undergo a cleavage of this C-alkyl bond upon further rearrangement found from this unit and so, these N S-dealkylation can also be possible right. So, overall R-SH is getting generated and formaldehyde is coming out from the reaction.

Similar to S-dealkylation and N-dealkylation reaction, one can think of doing O-dealkylation reaction. For example, if you have anisole you can get phenol if you have thioanisole you can get thiophenol and formaldehyde. If you have thio; if you have thioanisole, then this methyl unit once again similar to the S-dealkylation chemistry. It can undergo the sp3 C-H hydroxylation chemistry upon C-H activation or C-H abstraction. In this case, it is a radical mechanism which can give rise to the COH2 OH and R-OH overall HCHO is generated.

(Refer Slide Time: 16:00)



So, we have discussed briefly in the last class. Let me discuss one more time here in brief that is, so, if you have this high valent iron-oxo intermediate, it could be iron IV oxo and then radical cation whatever way you are ready to ready to write radical cation at this center and in the porphirine unit right in the porphyrin unit and you can take an R-H ok. Now, this RH can undergo the reaction with it.

So, overall let us say just for clarity, I would write it down as iron V oxo and the porphyrin moiety over here. So, it can give rise to the iron IV hydroxo right. So, if you are breaking it down, it is going to be iron IV O dot; O dot and this H dot over here picks up to give you that along with R radical formation.

Now these R radical formation then can undergo. So, this is the species not that one you have to draw like that and then further this hemolytic cleavage if you want to draw it. So, this is a iron III of course, it will not stay as it is. Now, over there it will pick up a water molecule from the media and then RH R radical and this radical half a bond will give you the R-OH formation. If you are doing O^{18} labeling in these cases, you will end up getting O^{18} labeling from the oxygen moiety.

So, as you have seen this superoxo this iron V oxo has come in turn form from the iron III hydro peroxo species right where this was also let us say, if it is labeled oxygen or O^{16} or O^{18} depending on where it is coming. In turn that has taken a proton and an electron to and from iron III superoxo, it is getting generated right. In the last class, we have seen and that is in turn getting reacted with iron II and O2. O2 if it is O^{18} labeling; if it is O^{18} labeled O2, then all the way up to the product this O^{18} leveling will be getting reflected.

So, these iron II species, iron II porphyrin complex once it is formed of course, there is always a porphyrin ring. Once this iron II porphyrin ring is reacting with the oxygen, then this oxygen will be getting reduced by one electron to form iron III and the superoxo intermediate. So, this is the superoxo intermediate. This superoxo abstract a hydrogen atom or picks up a proton and electron from the system. Electron is coming from let us say these iron sulpher cluster that we were discussing a moment ago and this electron and proton. So, it forms a first iron III peroxo species, then gets protonated to give the iron III hydroperoxo intermediate.

Now, this iron III hydroperoxo intermediate can then further cleaves to give the hydroxide and the iron V oxo which is necessarily iron IV oxo with a radical cation on the porphyrin moiety. So, porphyrin ring has a electron cloud and conjugated electron cloud that pi electron can be oxidized pretty easily to give the radical cation, as well as the iron V gets a let us say reduced by that electron to give the iron IV radical cation oxo intermediate which is essentially iron V oxo and, for the clarity purpose for our discussion purpose it can be concluded that and then the RH which can be over there can

react through which is sitting right over there can react with iron V oxo to give you iron IV hydroxo and R dot.

Over here a water molecule will react with this R-OH overall to give you overall to give you R dot OH or R-OH molecule. So, this is what the mechanism is for the substrate RH to R-OH formation. So, as you can see RH is getting reacted with this high valent iron-oxo intermediate to give you the R-OH species ok. We have seen this similar mechanism previously just to say that these O18 leveling is also quite interesting.

So, similarly this hydroxylation chemistry; these hydroxylation chemistry as we were seeing over here it can be followed quite easily. It can be followed quite easily and one can follow up this chemistry either on the alkyl, S-alkyl and O-alkyl species or even the N-alkyl species. In case of the N-hydroxylation chemistry similar chemistry can happen. Once again, the one of the H over here will be replaced by OH-H over here one of the H is replaced by OH that is sp3 CH. This is sp3 CH replaced by OH, once again this is sp3 CH is replaced by OH.

This is more of a epoxidation type of chemistry like olefin epoxidation, electrophilic aromatic substitution type of reaction that is happening. If oxide formation and subsequent rearrangement gives that and in case of the alkyne once again the CH bond is getting cleaved and getting hydroxylated as we have just discussed and giving rise to the overall terminal carboxylic acid formation which are I believe quite successful and quite efficient transformation of synthetic arena ok.

So, we have the N-hydroxylation chemistry and the N-oxidation chemistry as you see if you have an amine or equivalent species, it can form the N oxide. If you have sulpher oxidation; of course, sulpher N-dealkylation or S-dealkylation is possible, dealkylation type of chemistry is possible. In addition sulphur can be oxidized further to give you the sulpho oxidation species. So, all these reactivity essentially trying to tell us is that cytochrome P450 is a very reactive intermediate.

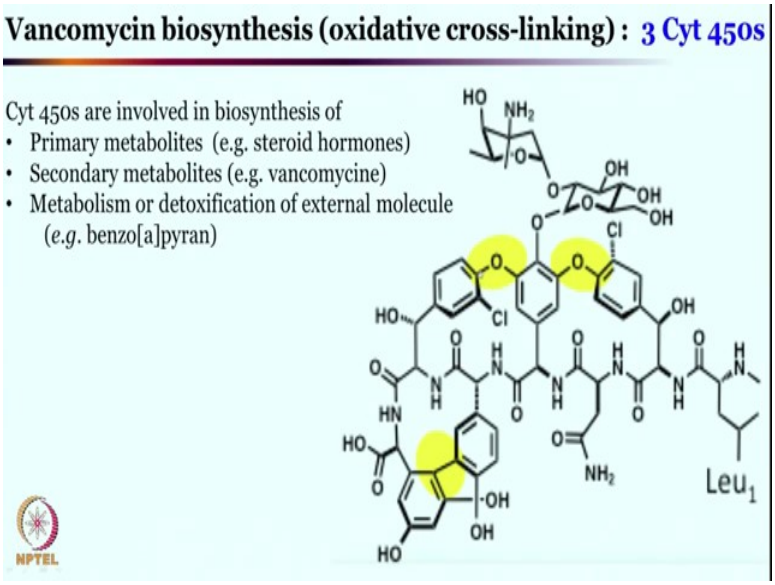
For example, this R-S-Me if it is reacting with l let us say, if it is reacting with S-Me with iron-oxo high valent, so, what can happen that these sulphur this lone pair electron can just oxidize to give you the sulpho oxide sulpho oxide R Me and iron III species right. So, if this was iron IV radical cation radical cation where so, iron V oxo then this upon transferring the oxygen it becomes iron III.

So, these are again very fascinating reactions and it is quite exciting to see that a versatile reaction or a series of great reaction can be performed by cytochrome P450. But, remember the major problem of the cytochrome P450 is really we are having quite a lot of reactivity. As you can see over here this reactivity is not limited to a particular organic substrate. It almost reacts with every organic substrate. So, that is going to be a quite a challenge and minimizing such reactivity a reactivity can be extremely difficult.

So, if you have aliphatic substrate if the it reacts, if you have olefin it reacts, if you have alkyne it reacts, if you have aromatic ring it reacts, if you have N-alkyl it reacts, S-alkyl, O-alkyl, primary amine secondary amine. So, this is primary amine this is secondary amine if you have tertiary amine of course, it will react to give the N-dealkylation product, if you have an even amine it can form S-N-oxidation, it can form the S-oxidation also to give you the sulfur oxidation product not only S-dealkylation product.

So, as you see that even with one substrate different types of reaction it can do. So, it is a very very reactive intermediate and thanks to that iron high valent oxo species. In these cases it is equivalent to iron V oxo species right. So, we will we will get back to that once again little later.

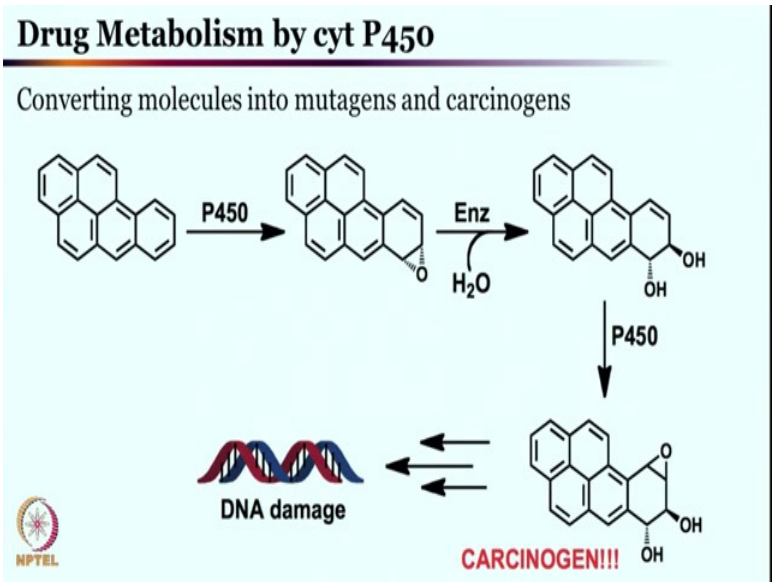
(Refer Slide Time: 24:55)



But, what is important to understand that this cytochrome P450 in turn has helped us in biosynthesis of many different natural product. For example, if one is looking for biosynthesis of the CO bond just like this, one can perhaps think of having an epoxide formation over there and then this phenol which was over here then can attack. So, an epoxide over here and the attack can form the CO bond.

Similarly, one can think of doing the CO bond formation starting from the from the phenol either from this side or from this side; let us say if it is a diphenol then the epoxide formation over there and subsequent attack by the phenol can form this bond. Similarly, a carbon bond can also be formed in this vancomycine by the cytochrome P450. In the 3 units of cytochrome P450 can be active on these vancomycine to gives rise to the reaction product or the final vancomycine synthesis right.

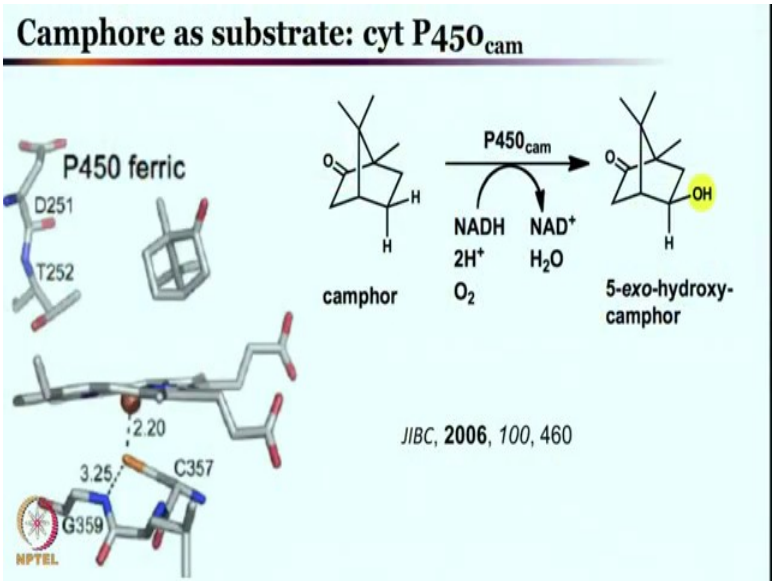
(Refer Slide Time: 26:05)



So, in the last class also we have seen how different metabolite metabol how different organic substrate that is get getting incorporated into our body and then further can be reacted with cytochrome P450. For example, by mistake or by a by a by accident if these benzopyrene benzopyrene molecule is getting incorporated in our body then this highly conjugated and highly the of course, delocalized pi electron system which are relatively easy to react with a with high valent iron-oxo intermediate, it will end up reacting as if like this is an olefin isolated olefin in a way.

So, this olefin can be can be reacted with the with the iron IV oxo or in this case iron V oxo equivalent to give rise to this to this epoxide ring which can then a ring open to give the cis-hydroxylation intermediate which again can give rise to this epoxide formation at this side which is truly isolated olefin double bond and this epoxide formation and further damage further ring opening can ensure that such molecule benzopyrene molecule can interact with the DNA and, during these formation and or via this formation, it can react with DNA it itself it may not be reacting with the DNA, but this species particularly is going to be very reactive or the species derived from this is going to be very reactive and is going to damage or intercalate interact bind with DNA and is going to going to destroy the DNA significantly alright.

(Refer Slide Time: 28:04)



So, so far we have seen that these reactions are quite powerful. Just like last reaction we have seen that in even the camphor type of substrate which can be crystallographically characterized and can be hydroxylated quite easily by utilizing this enzyme alright. We will come back and discuss this reaction beautiful reaction slowly with respect to the cytochrome P450 activity. I hope you will keep studying this and we will start here on the camphor substrate in the next class.

Thank you very much.