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

Lecture – 25
Cytochrome P450 Part I – Introduction

Hi. How are you doing? Today we will discuss Cytochrome P450. This is a really good enzyme, this is a very very important enzyme, right. It can do many reactions, many different type of reaction, almost any oxidation reaction that you can think of, I think it can be done by this enzyme.

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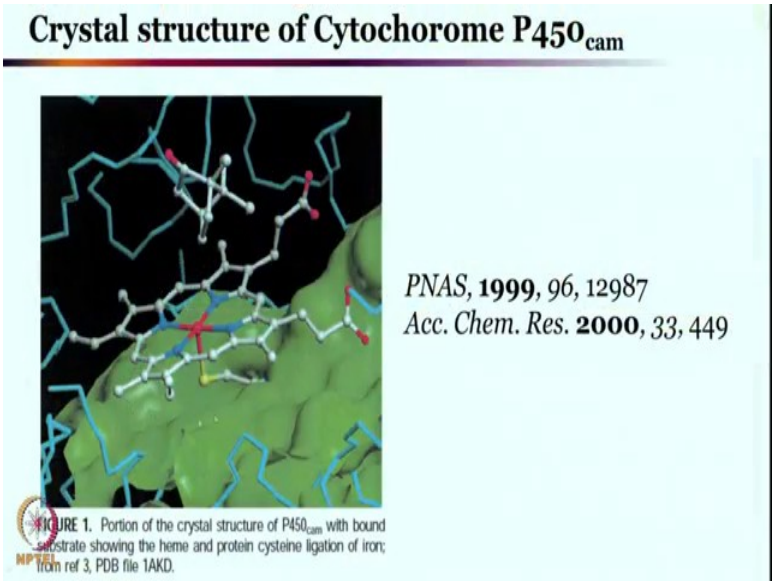

It is having a porphyrin centre at the heart of it. You have seen the similar type of structure in let us say hemoglobin, myoglobin and say protoporphyrin 9 type of species, ok. This is a porphyrin centre. But one of the difference now you are seeing is there is a methionine unit, right, cysteine sorry not methionine there is a cysteine, right. And in front of it the active site structure shows that there is this organic substrate which is going to get hydroxylated. So, this is the camphor ok.

All there are many different version of this enzyme. Essentially, the one great thing that it does is very simple hydroxylation chemistry it can do effectively at the aliphatic center. Obviously, there are many other reaction that it can do very effectively, but other than hydroxylation reaction those reaction we will discuss in a moment. So, this is the

organic substrate that is sitting, right in front of the iron site there is the cysteine, binding and as you can imagine oxygen molecule will come and bind over here and then it will react because oxygen will get reduced by these iron species, those species are very reactive oxygen intermediate, those reactive oxygen intermediate will end up reacting with this organic substrate.

So, what we are trying to tell you here is this metallo enzyme in presence of an organic substrate is capable of doing oxidation reaction, right. So, if you have an aliphatic substrate in front of this metalloenzymes cytochrome P450, it will be oxidized. And this is precisely the reason why lot of let us say drug, medicine that we take prescribed medicine that we take these enzymes are so good that they can react with those medicine and medicines normal function can be complicated or it does not allow the medicine to react at a desired location, before medicine does its job, it is starts working on the medicine itself. So, this is a big problem for the pharma industry that presence of cytochrome P450 enzyme.

It is such a universal reactor that almost every organic substrate perhaps can be reacted with it ok. It has many implications this enzyme has many implications in biosynthesis of many different molecule including those we will see biosynthesis of vancomycine which is a secondary metabolite, also primary metabolite and different, also different other materials if we accidentally inhale or accidentally consume in our body they can start reacting with them.

And if some time what happens if those material itself may not be that very problematic, let us say not carcinogenic, not having any bad effect, but upon reacting with cytochrome P450 it create some intermediate which is becoming now carcinogenic in nature. So, this enzyme is really important to understand how it is so reactive that it reacts virtually with let us say everything, right that we take. And therefore, we need to understand the chemistry of the cytochrome P450 in greater detail. It is simple chemistry, but very powerful and effective chemistry, right.

Just to tell you again that this is a porphyrin center, there is a proximal site where the sulfur binding is there, there is the distal site where oxygen binding will take place and along with the different protein residue there is the organic substrate that is hanging in

front of the iron oxygen species as if like it is perfected to react with the iron oxygen species that will be getting generated at this site.

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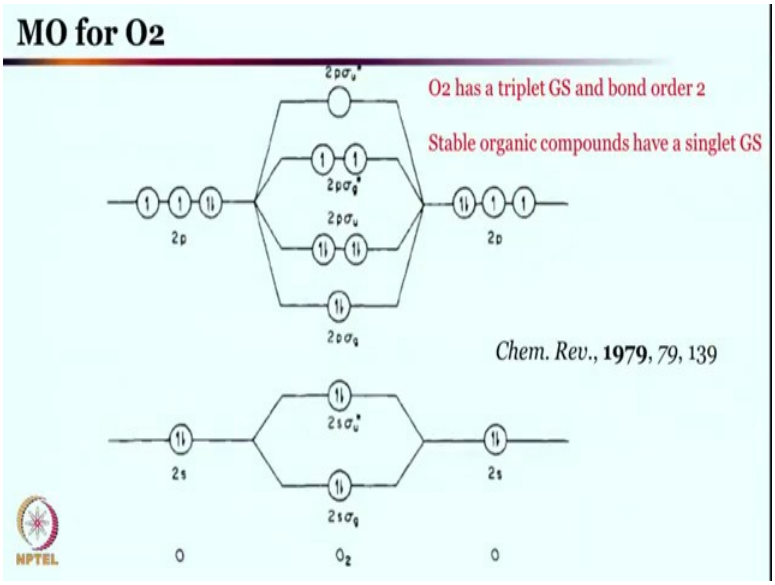

We will see that in a moment. Before that I think we did not discuss this a MO for oxygen molecular, orbital diagram of oxygen as you have noticed previously that it is having these two unpaired electron at the antibonding orbital and this is the triplet ground state and with a bond order 2. So, as you can see over here there is 6 electron minus this 2 electron that is 4 electron divided by 2, so that would be bound order 2. This is a triplet ground state for oxygen, but most organic compounds have singlet ground state that is quite phenomenal that that oxygen can have a triplet ground state and still it is so popular and so important and because of its triplet ground state I think a lot of reaction that can be operated while reacting with the metal center, right.

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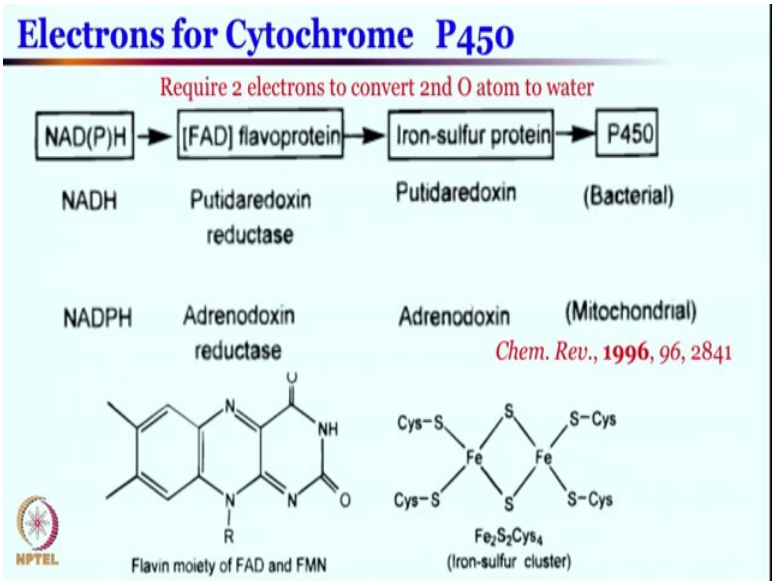

Let us look back at the processes. Of course, cytochrome P450 requires 2 electron to convert the second oxygen atom of the oxygen molecule into water. Where are those 2-electron coming from? As we have discussed in the electron transfer cases these are coming; these are coming through the chains of events that is happening over there. So, essentially iron sulfur cluster that we discussed in the electron transfer protein is the one which is delivering the electron to cytochrome P450, but before that these electrons are getting hopped in from NADPH from this flavoprotein and then to iron sulfur protein and this is what the flavoprotein looks like all of them are acting as electron transfer site ok.

So, electron is hopping from this center to that one to that one and to that one, right, finally, to the cytochrome P450. So, it is a chains of events that are happening to provide the desired electron to the cytochrome P450 sites ok.

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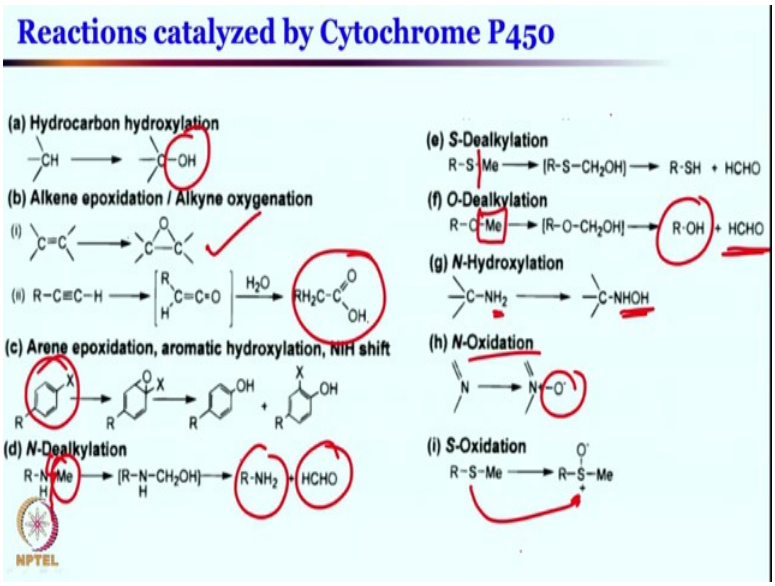

Let us look at some of the chemistry that can be done by utilizing the cytochrome P450. As we mentioned there are plenty of reaction that can be done by utilizing this chemistry. So, it is capable of doing substrate hydroxylation chemistry. For instant, if you are having a substrate like organic substrate, it can go and give you a desired product that you would need.

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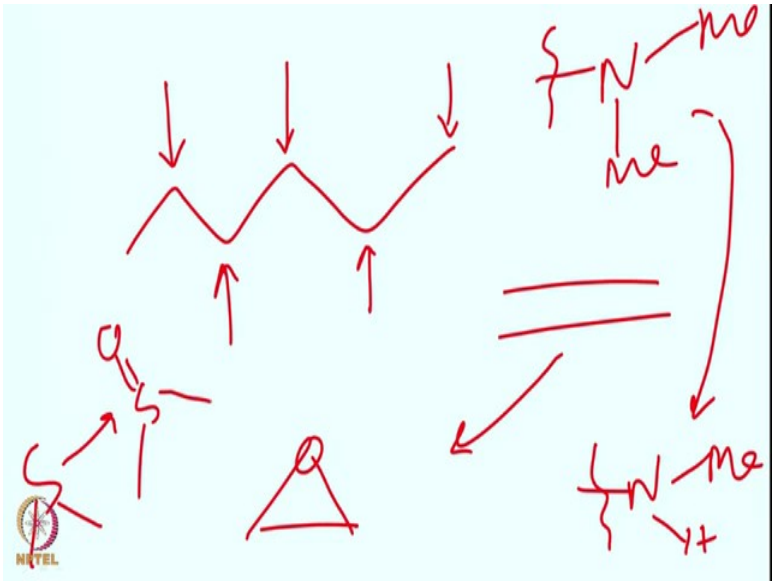

If you have an aliphatic substrate for example, there are different sites of which can be reacted with these iron oxygen species. So, substrate aliphatic substrate hydroxylation is possible.

If you have the olefin, olefin can give you the olefin epoxidation product. If you have N alkylated product substituent such as let us say N N dimethyl substituent, it can react form the informal species and then undergo a further cleavage to let us say give you N H and methyl. From, if you have the sulfur containing compound you can get the sulfoxide species. Many different reaction that can be happening over here, so most importantly you can also get this reaction quite going with these places for example, S dealkylation chemistry is also possible where this dealkylation of this moiety will be possible O dealkylation is possible this whole unit can be dealkylated to give you first of all hydroxylation and then this alcohol formation.

So, the alkyl part is forming the formaldehyde in these cases. You can have the N-hydroxylation chemistry, as you can see here N-hydroxylation chemistry can happen N-oxidation can happen, as you can see over here sulfur oxidation can gives you the sulfoxidation product and overall your aliphatic substrates C-H bond be primary secondary or tertiary all can be hydroxylated to give the COH product. Olefin as we mentioned can give you the olefin epoxidation product.

The alkyne can form into the carboxylic acid, terminal carboxylic acid. Even a benzene ring can form an epoxide to give you finally, phenol when with the transfer or the rearrangement of the phenol. N-dealkylation reaction can be also possible where this methyl group is turning into the formaldehyde. And R NH part is becoming R NH_2. So, it is a flavor of reaction, it is a series of reaction that it can be done, that cytochrome P450 can do.

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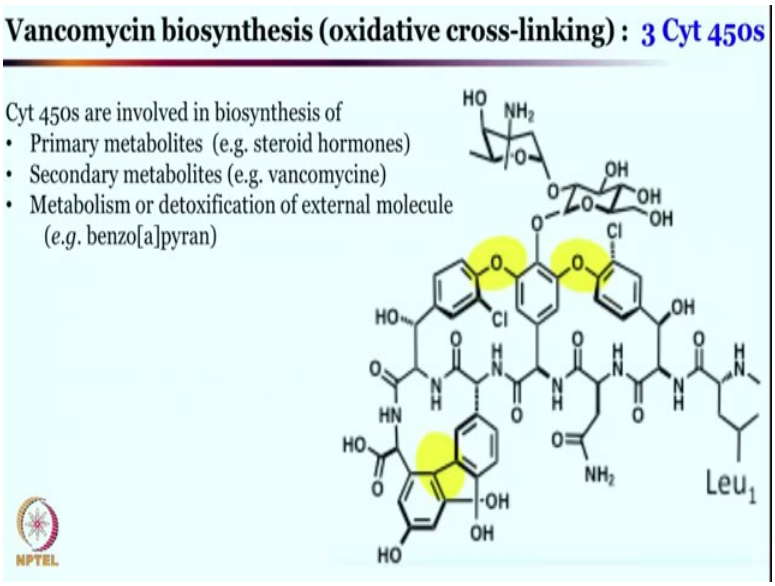

As you can imagine all these reaction put together, if all these things are happening, let me go back if all these things are happening these reactions are going to complicate the whole spectrum of the reaction that can happen in our body for example, right. So, cytochrome P450 is an enzyme not to be messed with too much, so any drug molecule that any pharmaceutical industry has to design they have to take care that these many different varieties of reactions are not easily happening or even if they are happening the molecule still remain active even after this reaction.

So, that is a big challenge because cytochrome P450 can do many reaction that can be unheard of that can be unthinkable by the chemist perhaps they are involved in designing the drug molecules, right. So, this is a very very important enzyme once again and can give rise to the many many important factors that can be happening in the enzyme, right.

So, we will keep this in mind many different reaction system, many different possibilities that which coming into the picture. Well, this is the vancomycine the you know antibiotic of the last resort. Even in antibiotic in these cases we will see that these the important carbon oxygen bond formation and carbon bond formations are taking place by the cytochrome P450. So, the biosynthesis of vancomycine is controlled by the cytochrome P450, in facts 3 units of 3 cytochrome P450 comes into the picture one is for there, another is for there and another is for here.

So, cytochrome P450 are involved in biosynthesis of different primary metabolites, bolites. For example, steroid hormone secondary metabolites such as vancomycine and of course, it is also involved in metabolism as you were mentioning of different drugs of different medicine and it can involve in toxification or detoxification of external molecule, right. So, if any external molecule is coming into our body that can be detrimental for our body because or sometime it can convert into something good even if bad things are coming in.

So, of course, it can do a lot of bad things because unknowingly since it is cannot discriminate too much, it is so reactive, but of course, it is involved in many other important enzyme synthesis or you know important compound synthesis such as even this vancomycine synthesis biosynthesis. So, we will see in a moment also.

So, these sort of CO bond formation and CO bond, CC bond formation, CO and CC bond formation are taking place with the help of cytochrome P450. So, you can imagine this porphyrin oxygen chemistry is extremely effective and that is why we need to understand this chemistry a little bit in molecular level so that we know what exactly is going on in these cases.

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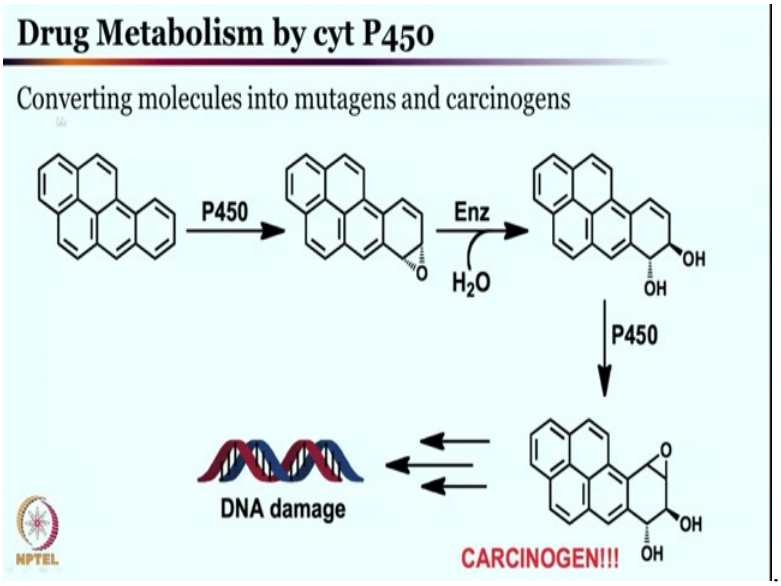

For instant, if by accident this by benzopyran is getting incorporated in our body this benzopy bipyron can be you know can be involved in oxidation reaction by utilizing cytochrome P450. So, therefore, molecules can be converted some time into mutagens

and carcinogens. This is not by design of cytochrome P450, but cytochrome P450 once again is so reactive if any foreign molecules are getting in, it can start acting on those molecule. For example, it can epox from the epoxide as it is shown over there, cis hydroxylation species it can form, subsequently further epoxidation chemistry and overall once these species are forming these are not really great species, right.

So, these are the molecule which are carcinogen and it can intercalate it can interact with DNA, it can end up damaging the DNA and can give extremely dangerous intermediate, it can lead to different diseases and deadly diseases, right. So, this compound itself is not carcinogenic or not harm too much harmful for our body, but in presence of cytochrome P450 which is there anyway and therefore, this will start reacting with those cytochrome P450 and then will deliver a material which in turn can damage our DNA which is not that great news for our body.

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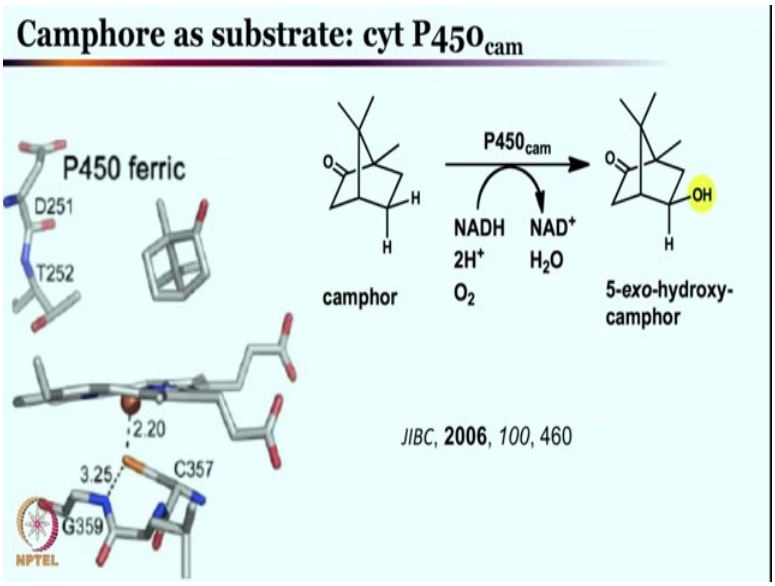

So, cytochrome 4 P450 is a great enzyme once again, but it does perhaps more than what we would imagine and that is where it is so dangerous and we need to understand and design things according to the cytochrome P450's mean.

Well, this is that camphor structure we were talking earlier. So, this is the camphor which is bound, right in front of the porphyrin iron center. As you can see the iron center is little bit outside oxygen is not bound with it yet, as you have seen in case of hemoglobin and myoglobin upon oxygen binding this iron 2 plus center will move inside

the cavity of this porphyrin and that species which is generated upon oxygen binding and the electron transfer, superoxo and the subsequent reduction we will see that it can affect on even on camphor to give the camphor hydroxylated product, right. So, these are quite important reactions and we will be seeing that these reactions will have a overall say in how these processes are going on.

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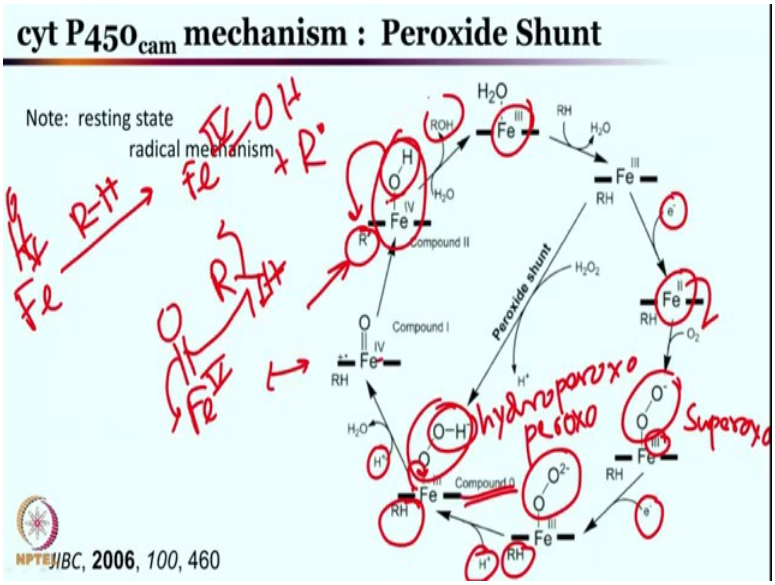

So, today one more slide we will discuss here which will be discussing how these reaction mechanisms are happening ok. So, to summarize over here that we have seen many different reactions are happening. Why so many different reactions are happening? What is the mechanism of these reactions? That is what we are now trying to see. Well, the resting state of the enzyme is this iron III aqua species, the organic substrate that we were discussing in the last slide let us say this is the organic substrate it can then come in and resides in the substrate binding pocket right in front of the distal site or right at the distal site where iron oxygen binding will take place. So, this is what is happening iron aqua molecule.

Let us say just for drawing it is RH over here which is sitting at the distal site and you still have a iron III. Center iron III center will not be reacting with the oxygen, iron III water molecule has to go out once this organic substrate is coming into the picture ok. This organic substrate comes into the picture and resides in front of the distal site of the iron site. So, RH comes in water goes out one electron as we were discussing there are

overall 2 electrons involved into this process, now one electron in this whole process comes in and this iron III as you have seen, this electron is coming through hopping from different centers this electron is now reducing this iron III center to make it iron II plus. This reduced structure is now ready to react with oxygen ok.

The substrate is sitting right in front of this iron center oxygen it reacts as you have seen in case of hemoglobin and myoglobin also. This iron II plus reacts with oxygen to give you iron III plus and superoxide intermediate. So, one of the electron from the iron II + is now getting transferred on the oxygen molecule to give you iron III + superoxide species.

Well, in the case of hemoglobin and myoglobin this was a completely reversible process and that is how it transport and delivers oxygen in different part of the body in the in case of let us say hemoglobin, but in this case we do see that there is a organic substrate sitting very close to this ok. It is quite interesting and this is the step why then subsequent reaction goes on, because these are reversible step, this is a reversible step, but from there on an another electron transfer occurs.

So, this is once again it is from one iron center gives only one electron the second iron center as you have seen from the iron sulfur cluster this electron is coming through a hopping of electron from different sites including those other oxygen's, other organic molecule containing site which are good for the electron transfer. The second electron; first electron reduce it make it active, second electron reduce the superoxo species to make it iron III peroxo species. This is iron III +, this is peroxo species, it got reduced to peroxo oxygen if you look at from the beginning oxygen itself is one electron reduced at this space and at this pace second electron reduced, but more importantly iron in this step remain iron III +.

Now, a proton can transfer or proton can add with this iron III superoxo to form this iron III hydroperoxo intermediate. This is also known as compound 0 in the literature compound 0. These iron III hydro peroxo species is quite interesting because this is the one which will be responsible for chemistry that will be happening next. So, RH it is still there, substrate is still sitting right over there, another proton that comes in that converts this OH into water. So, what you have seen this oxygen atom, two oxygen atoms are there and from this oxygen molecule two oxygen atom, out of the; out of the two oxygen

atom one oxygen atom is converted into water. So, now, this is the water molecule this part goes out and this iron III now is forming iron V, essentially this is iron V. It is a porphyrin radical cation with iron IV plus that goes on in there, I will come back to that in a moment and the water molecules forms over there, right.

So, from there on its the substrate that reacts RH, reacts with iron IV oxo to give iron IV hydroxo and ROH, RO water replaces ROH to gives back the general species. Let me discuss how this compound 0 to compound I is forming, this is compound I and then this is known as the iron IV hydroxo porphyrin species is known as compound II, right. So, we will see that in a moment, what we are trying to see how these reactions are happening.

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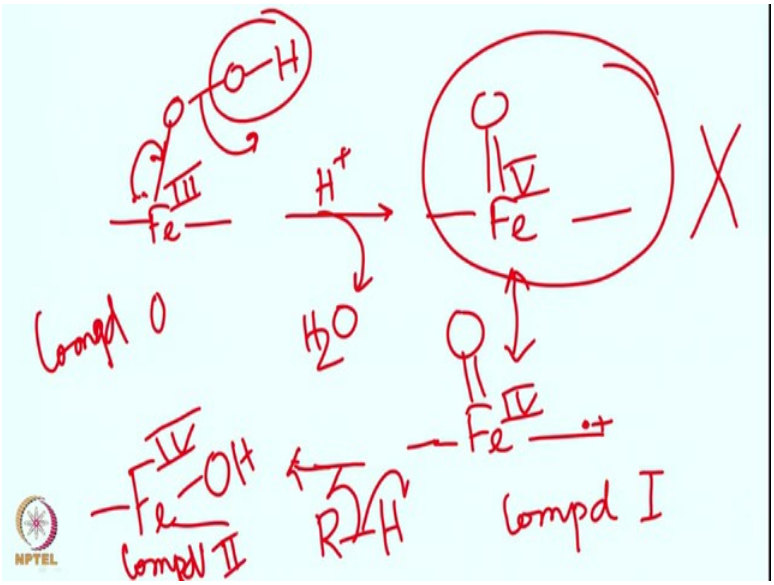

So, how do you have seen you are there that you have a iron III hydro peroxo intermediate. Now, this hydro peroxide intermediate with a porphyrin center this is compound 0, right. So, from there what will happen that it can take one proton to give you water molecule, right. So, this goes on as water molecule. So, this part gives you the water molecule. So, essentially 2 electron from iron is going and kicking out the water molecule, the hydroxide comes out, hydroxide binds with proton to give you the water molecule.

So, this should be then iron V double bond O, right. In these cases what happened that reduction potential is such that it is not really iron V oxo, it is actually iron IV and the

porphyrin unit is giving out one electron iron IV radical catalyze forming and then oxo species is forming ok. This is why this is called, this species not this is why this species is called compound I. So, this is compound 0 and this is compound I.

As you have seen that 2 electrons are coming overall for this process, one electron from the porphyrin ring another electron from the iron center that is why these species is not the right presentation, this is the right representation where you see this is a iron IV oxo species, but it is a radical cation on the porphyrin moiety, right. So, this is compound I and from this compound I as you can see well for chemistry purpose it is perhaps easier if you think that this is iron V oxo, although it is not iron V oxo.

Subsequently what will happen the organic substrate RH will come out and then let us say this oxo will attack on the radical mechanism and this iron these 4 oxo species will give then iron IV hydroxyl with a radical cation is there this would be called compound II ok.

So, once again let me clarify it. It is easier for chemists to think that this is the species, although this is exactly the species if you just for a moment think that this is the species it gets little easier, this is little easier to understand for us. Let me get back once again, ok.

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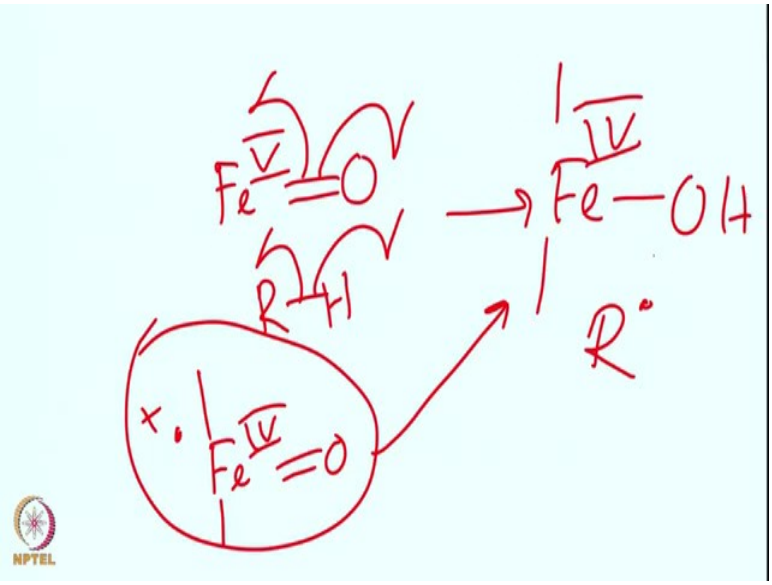

So, what we are saying that if you have iron V oxo and then you have let us say RH, it is easier to think that this is going to iron IV and this is how it is that iron IV hydroxo is form along with RH. Of course, you in this case that is what is happening, but during these cases you still have this porphyrin unit.

Now, in these cases in this compound II, this is not the radical cation, this is actually the iron IV hydroxo that is over there. Originally this was iron IV oxo with radical cation this species this is not the species, but that is what we are drawing from this species that is the species that is forming. The porphyrin is no longer actually radical cation. So, this is the species known as that compound II, compound II, right.

So, let us go back once again. What we have seen then? We have seen that this iron III over here it is getting reduced by one electron to give the iron II species, these iron II species reacts with oxygen to and so one of the electron comes in into the oxygen it reduced to the superoxo. So, this is the superoxo species. Superoxo intermediate, and iron becomes iron 3 because it gives up one electron. Another electron comes in this is the peroxo intermediate that is getting generated over there, this is now remained iron III, so this electron is coming from outside.

So, gives the iron electron. RH in sitting over there nothing has happened like a good guy it is sitting over there, nothing happened. The protonation on this peroxo gives you the hydro peroxo species, so this is the hydro peroxo species, this hydro peroxo peroxo species this hydro peroxo iron III hydroperoxo known as compound 0.

Once again RH nothing happening here, one proton comes in these goes out at OH; in this case as I was saying 2 electron is pumping in one from iron III +, one from the porphyrin ring, so it is iron IV oxo iron IV oxo with a radical cation on the porphyrin, right. So, this intermediate which is nothing but iron V oxo species this intermediate is actually responsible for reacting with RH. So, it will take off one H dot. So, to make it iron OH and then it quenches one of the electron this quenching happens on the porphyrin. So, the R dot sits over there and porphyrin, now radical cation gets quenched and it is just porphyrin, so, it is essentially iron IV oxo.

In other word, you can think of that that iron V oxo upon reacting with RH giving you iron IV hydroxy and R dot, that is what its easy to think, but anyway you should not draw this as the iron V oxo because this is the iron IV plus with a radical cation on the

porphyrin. So, overall this is how the radical mechanism is getting initiated. This is where you see that a radical is being formed and that is quite powerful radical. This is the radical which will then can let us say for hydroxylation reaction a rebound of hydroxo will happen on the R dot to give you the ROH intermediate and will get the product formation.

Well, will come back to that in the next class. What we have seen in briefly then, in cytochrome P450, we have beautiful chemistry, beautiful hydroxylation chemistry, N-dealkylation chemistry, selfoxidation chemistry, olefin epoxide chemistry, cyclisation chemistry if required, N-hydroxylation chemistry, what not. I mean we have seen quite a lot of chemistry that can be done by this enzyme and this is not only a great enzyme sometime it is too great and can create problem for us, even for the medicine that we take can become or can be very problematic in presence of this enzyme. So, we need to learn the better designing of the drugs because cytochrome P450 is always there, it is going to be always reactive, we need to learn how to prevent our medicine to get affected by this enzyme.

We will come back to cytochrome P450 once again in the next class. Keep studying, the book to follow as I said Lippards and Berg Bioinorganic Chemistry, Principles of Bioinorganic Chemistry, keep studying.

Thank you very much.