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

Lecture - 28
Cytochrome P450 Part IV – Role of Cystine ligand and Distal charge relay

Hello welcome back to the discussion of Cytochrome P450, in the last class we have seen the reaction mechanism and this is really an exciting mechanism.

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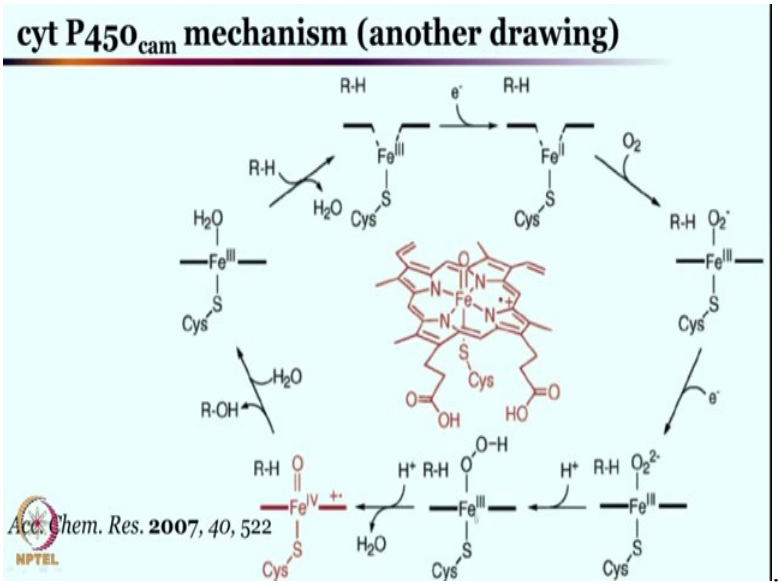

Where you have seen that iron hydroperoxo is being formed and then oxygen, oxygen bond is cleaved in the process to give the high valent iron oxo species. Now you must be wondering what is the difference between the let us say haemoglobin, myoglobin and cytochrome P450. Of course, in case of hemoglobin, myoglobin, you have up to this iron superoxo species, but none of these steps are happening in hemoglobin and myoglobin.

Now, what really differentiate hemoglobin, myoglobin with and that of the cytochrome P 450, yes you have got it right. The cystine this axial ligand that proximal ligand compared to the histidine that we have seen in case of hemoglobin let us say right. Another thing is of course, there are electron transfer processes and proton transfer processes which can also be there, but in mainly in cytochrome P450 I think this axial

ligand is varying and that makes a lot of difference. If this axial ligand was histidine this oxygen oxygen bond cleavage may not have been that easy.

Let us look at therefore, then what is the role of this cysteine ligand for this oxygen-oxygen bond cleavage.

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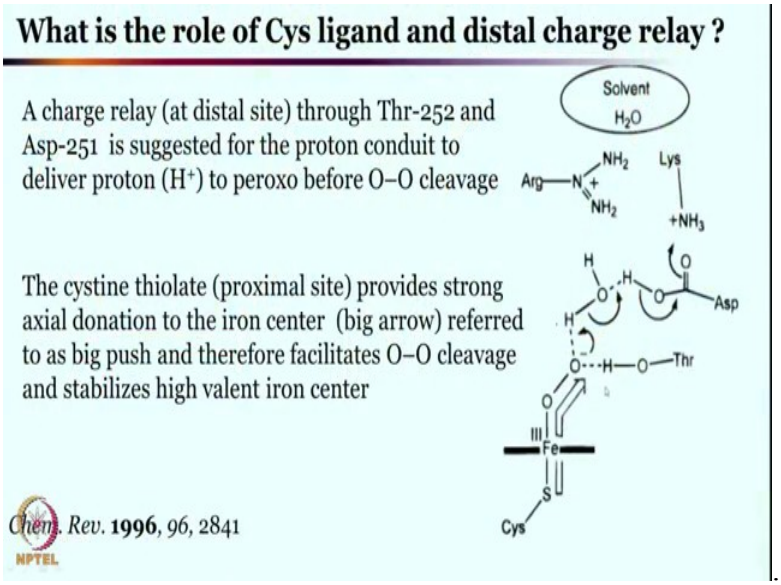

So, overall as you can see that this cysteine ligation to the iron 3 center actually helps in a multiple way, one of the thing that it definitely helps that for cytochrome C there are lot of side chain and this proton conduit that helps overall in protonating such a iron 3 peroxo intermediate right. So, this iron 3 peroxide intermediate is getting protonated with the help of this proton conduit.

So, this charge relay through this threonine 252 and aspartate 251 it is suggested for the proton conduit to deliver proton to peroxo before oxygen oxygen cleavage ok. So, this peroxo unit itself without this protonation that be the peroxo unit itself; that means, without this protonation it cannot undergo the oxygen oxygen bond. So, this peroxide unit itself without the protonation cannot undergo the oxygen oxygen bond cleavage. So, the peroxo unit has to be protonated in the process and that protonation is helped by this side chains different different side chain that is present over there. You see there is a big relay process that is happening by which this protonation is taking place right. So, the side chain definitely helps in the overall process of the cytochrome P450 mechanism.

And more importantly, this cysteine can push electron through this S sulphur iron and through the iron oxygen bond and therefore, the cleavage of the oxygen-oxygen bond becomes much more facet has it been the histidine then this push would have been missing and therefore, this oxygen-oxygen cleavage would have been much difficult. So, the histidine is not a negatively charged ligand, the negatively charged ligand cysteine thiolate helps overall in breaking the oxygen oxygen bond. So, the cysteine thiolate provides strong axial donation to the iron center referred to as big push and therefore, facilitates the oxygen-oxygen cleavage and stabilizes high valent iron center.

Overall not only this cysteine big push cleaves the oxygen-oxygen bond it also helps in stabilize the high valent iron oxo intermediate that is going to be formed at this iron center.

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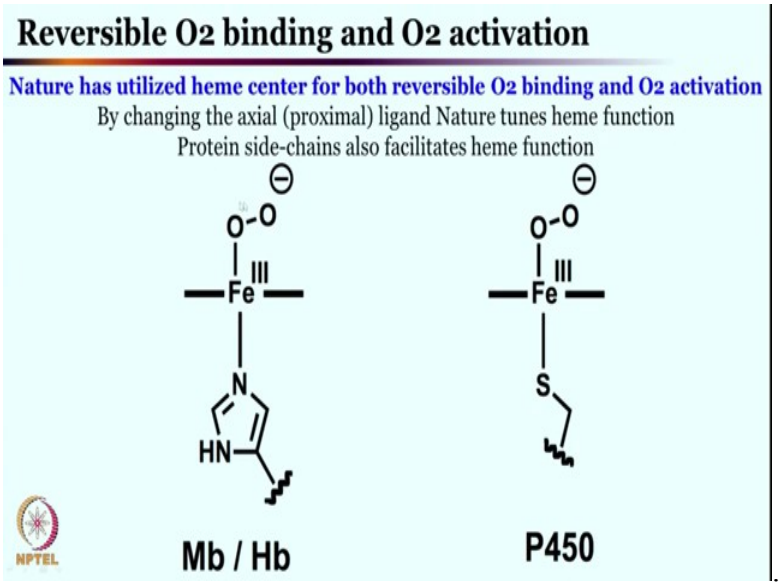

So, just to summarize this part therefore, the role of this cysteine thiolate versus hemoglobin myoglobin this histidine is quite crucial. Nature has utilized heme center in both the cases as you can see both in hemoglobin and myoglobin and cytochrome P450 there is heme center for both reversible oxygen binding and oxygen activation. If you are looking carefully for the hemoglobin myoglobin this is a reversible oxygen binding there is no substrate sitting close to this hemoglobin and myoglobin therefore, just very efficiently it can transport oxygen without doing any oxygenation chemistry with the any organic substrate.

So, this is the same species the common intermediate for both hemoglobin, myoglobin and cytochrome P450, despite having the common intermediate this case it is just transport oxygen or reversibly binds oxygen it does not do any oxygenation chemistry.

On the other hand cytochrome P450; obviously, forms the same intermediate, but it engages the substrate as you have seen many different substrate in taking the oxygenation reaction forward right. So, this is quite amazing by utilizing the same cofactor iron porphyrin nature has decided to do completely different function in one case it is transporting oxygen in another case it is making the oxygen reactive to do the oxygenation chemistry.

In other word by having or by changing the axial ligand from the histidine to cysteine it is possible to tune the heme function and therefore, both the oxygen-oxygen cleavage and the stabilization of iron oxo species which are high valent species can be possible in cytochrome P 450. As you have seen protein side chains also helps overall in the process to provide the required environment for the protonation and overall process of the oxygen oxygen cleavage as well as the substrate binding, substrate orientation, everything helps to make cytochrome P450 and very effective and effective a very effective enzyme.

So, I hope it is now clear that why nature has chosen the different axial ligand for different enzyme. In case of cytochrome P450 it is one axial ligand everything else remain same, in case of hemoglobin myoglobin it is another axial ligand and you have seen the reason behind it ok. Now we are going to see the you know reactive intermediate in case of cytochrome P450 well as you have noticed that we have proposed a number of intermediate.

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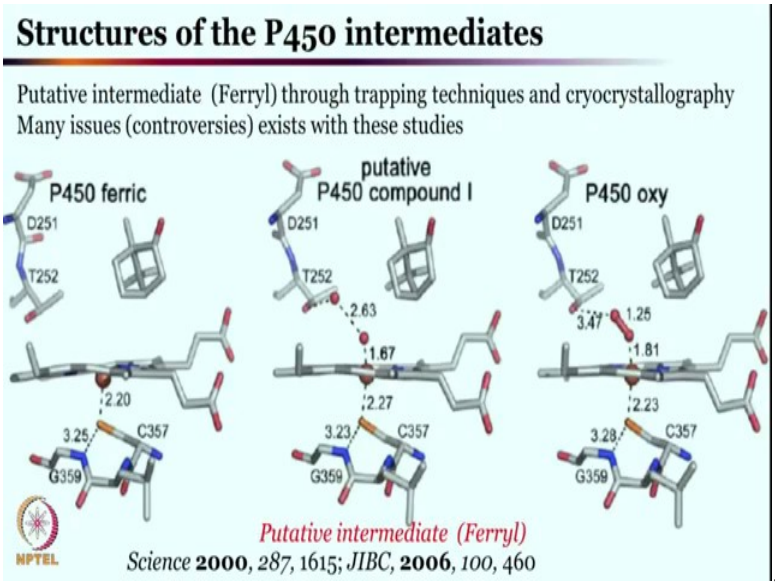

Now, is there any support in favor of those proposed intermediate, all these intermediates are going to be extremely difficult to crystallize only I think reliable data you can get perhaps through the spectroscopic technique for instance resonance Raman (Refer Time: 08:43) EXAFS or UV visible low temperature definitely is one of the most characteristics characteristic feature or characteristic thing you can get, but many attempt has been made to make these material crystalline and use different trapping techniques by which the intermediate perhaps can be trapped.

Now there is cryo crystallography lowering the temperature and doing the crystallography under super cool condition, but then these techniques has it is own limitation and more importantly if the crystal structure or crystal quality is not great enough the conclusion that can be drawn from those study can be problematic ok. There are many issues, controversies, caveats of these cryo crystallographic studies and the trapping technique that has been used for the cytochrome P450 intermediate studies, but nonetheless still it can say light about the putative intermediates. Once again these intermediates are crystallographically although some of them are characterized, but still they are not free of debate that is particularly because the resolution the thus the resolution that one can get.

And therefore, the conclusion that can be drawn at a particular iron oxygen center or iron centers regarding it is regarding it is existence I think can be questionable. Well, despite

having that let us see this is a porphyrin iron center as you have seen this crystal structure before iron is sitting outside, because this is not in the plane yet it gets inside the plane only upon oxygen binding and then reduction to the superoxo ok, it is only gets into the plane only upon oxidation to iron III + and binding with oxygen. So, this is the crystal structure we have seen before and here is the organic substrate camphor that is place to right in front of the active site.

Now it has been proposed that there is iron IV oxo intermediate and it has been crystallographically characterized. Now once again there is a debate, but that debate is whether this is really iron IV oxo or an aqua molecule. Although the crystal structures can be informative in this case it cannot resolve beyond down that this is a high valent iron oxo intermediate, but it looks quite reasonable though as you can see the bond length and bond length is shorter, but perhaps not short enough and overall it seems like it is the intermediate what has been suggested for the cytochrome P450 compounds ok.

There is yet another intermediate which shows that iron center is bound with oxygen. So, these are all remain quite interesting intermediate and some of them can be debated, but these studies are very very difficult to do and therefore, it could be highly rewarding, but perhaps still it is not 100% clear how these things must be happening in the enzyme and this is where synthetic studies can be quite useful if one can have the crystal structure of such compounds then in the matching of the data with that of the cryo crystallographic technique of the of the enzyme can be extremely useful.

So, let us look back the mechanism once again. So, what has been proposed that this iron III+ is getting reduced to iron II+ first still it is not part of the complete porphyrin plane upon oxygen binding and electron transfer, it forms the iron III superoxo. It gets into the cavity of the porphyrin and then electron transfer protonation gives the iron III hydroperoxo species.

Now these oxygen binding or superoxo intermediate structure or the peroxo intermediate structure these intermediates remain quite exciting to crystallographically characterized, but overall this intermediate is suggested to be crystallized, but again although crystal structure is quite definitive usually, but in these cases there is still debate whether this is really the intermediate that is been trapped, but nonetheless this mechanism has been

supported by many different groups and evidences in support of these intermediates has been collected, although crystallographic characterization remain bit controversial.

We will come back some of these iron oxygen chemistry pretty iron hydrogen peroxo chemistry pretty showed just to remind you that the cytochrome P450 once again is quite exciting enzyme. In the next part of today's lecture we will discuss peroxidase and catalase ok. So, you know these are once again the heme iron center and these makes quite an exciting story for themselves, because they can convert also intermediates into quite fascinating examples.

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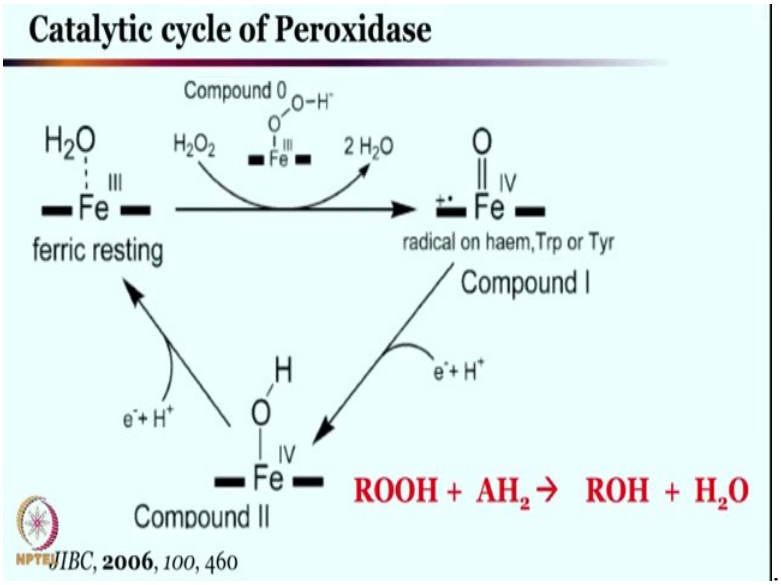

So, for example for instance you can start just like cytochrome P450 peroxidases are starting with iron III aqua complex, it is in ferric resting state ok. As you can see of course, it would be out of the plane and aqua molecule is over there.

So, this is in resting state in presence of hydrogen peroxide just to remind you as we have discussed, iron III can react with hydrogen peroxide to give the iron III hydroperoxo species right that is the compound 0 we have discussed it in the peroxide shunt mechanism. So, this is the peroxidase chemistry 1 equivalent of hydrogen peroxide gives rise to of course, displaces one water molecule and gives rise to the iron III hydroperoxo species. This hydro peroxide species upon protonation of this hydroxide you need terminal hydroxide you need gives another equivalent of water and it forms the iron IV radical cation oxo intermediate ok.

So, this is the same chemistry as you have seen in case of cytochrome P450, but these are completely different enzymes or in other words cytochrome P450 in absence of suitable oxygen and the reductant these species can be formed. So, these are the completely different class of enzymes, which are fascinating, which converts hydrogen peroxide into corresponding corresponding water right. It can be instead of hydrogen peroxide it can take an alkyl peroxide in presence of the hydrogen atom donor it can converts into the alcohol, if it is let us say alkyl peroxide corresponding alkyl alcohol, alcohol will be formed along with the formation of water molecule.

So, once again we start from ferric resting state react with we will we have a water binding, this hydrogen peroxo reaction with iron III gives rise to the iron III hydroperoxo intermediate. This displaced water is one of these water and then the protonation on this hydroxo terminal hydroxo gives rise to the gives rise to the another water molecule that proton source can be one of the terminal proton from the hydrogen peroxide, right. Overall it forms this iron IV oxo radical cation intermediate which is nothing, but as we discussed iron V oxo as you know that this iron III hydroperoxo it can be called as compound 0, this iron high valent oxo intermediate can be called or it is usually called in the literature as compound 1.

So, radical as we have discussed in the last place and seen that radical will be on the heme site on the heme usually or it would be a tryptophan or in tyrosine. Overall it would require still 1 electron and 1 proton; that means, hydrogen atom to convert these high valent iron oxo into the iron IV hydroxo intermediate ok. We have seen this before in the same way in cytochrome P450 this compound iron 4 hydroxo without the radical cation on the porphyrin is known as the compound 2 ok. So, compound 0, compound 1 and compound 2 from there on another equivalent of the hydrogen atom which is the combination of electron and proton will gives rise to the generation of the ferric resting state.

So, overall as we discussed that if it is hydrogen peroxide then it will end up giving 2 equivalent of water, if it is alkyl hydro peroxide it will end up giving one equivalent of alcohol and another equivalent of the water molecule. So, these peroxidase chemistry comes into the picture when there is let us say even for cytochrome P450 when there is no oxygen available or reductant available these sort of chemistry start kicks in, but these peroxidase enzyme is quite effective in converting the hydrogen peroxo into water and

this is useful because hydrogen peroxide can do quite a lot of other site reaction in absence of such mechanism of the peroxidase chemistry.

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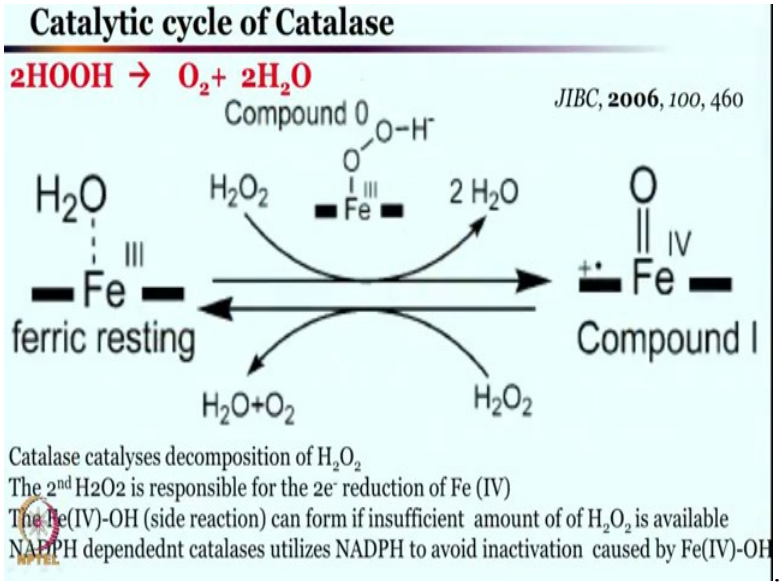

The next let us look at the catalase chemistry, catalase this is the catalytic cycle of cartilage once again we start from the ferric resting state. This is the iron III + water molecule the ferric k resting state and in this particular case we will convert once again similar to what we have seen we have taken hydrogen peroxide and converted into 2 equivalent of water just via this 3 hydroperoxo intermediate as we have discussed in the peroxidase cases.

It forms this high valent iron oxo intermediate iron IV oxo with the radical cation intermediate, now this radical cation iron IV oxo intermediate can then go back to the resting state directly without having those 2 hydrogen atom if enough hydrogen peroxide is present right. If enough hydrogen peroxide is present then this hydrogen peroxide can give another equivalent can give water and oxygen to degenerate the iron III hydroxo aqua species.

So, catalase catalyzes decomposition of hydrogen peroxide the second H2O2 is responsible for the 2 electron reduction of iron IV. So, this is well of course, this is essentially iron V because this is a radical cation is there. So, the second H2O2; that means, this one is responsible for a reduction of 2 reduction by 2 electron to form these high valent iron intermediate to iron III intermediate. The iron IV hydroxo that is getting

generated in the peroxidase chemistry that can still form in here if insufficient amount of hydrogen peroxide is available. So, the amount of hydrogen peroxide will determine whether this species is directly going back to there or this iron hydro peroxo species is getting formed into the process.

So, this shuttling between these 2 intermediate will be clean if enough hydrogen peroxide is there, if enough hydrogen peroxide is not there still this intermediate can start again and these are in lot of cases these catalyst activity are dependent on NADPH those are called NADPH dependent catalysis which actually essentially ensures that the electron reduction or electron transfer to this site occurs without any trouble even if there is deficiency in hydrogen peroxide concentration or a amount of hydrogen peroxide.

So, what we have seen so far in the peroxidase and catalytics catalase cycle is, essentially it is a page out of the cytochrome P450 chemistry. It has in particular for peroxidase chemistry it has compound 0, compound 1 and compound 2 which is exactly same as what we have seen in cytochrome P450. But for peroxidase chemistry it is essential to have 2 hydrogen atom donation from an from a source, but in presence of such 2 hydrogen atom donation hydrogen peroxide can be converted to 2 equivalent of water, not only hydrogen peroxide it can take care of any alkyl hydro peroxide in the process. As you can see compound 0, compound 1 and compound 2 all are part of the peroxidase cycle just like what you have seen in case of cytochrome P450.

The source of hydrogen peroxide or alkyl hydro peroxo will depend what type of chemistry or what type of product is forming over there, but for catalyzed chemistry it is only usage of or use of hydrogen peroxide no alkyl hydro peroxide will be involved. Now this hydrogen peroxide will cleanly form just the oxygen and water molecule 2 equivalent of hydrogen peroxide will be converted to oxygen and water molecule. So, this is quite fascinating provided there is enough hydrogen peroxide is involved or enough hydrogen peroxide is available this catalytic cycle is quite exciting and will be able to degrade hydrogen peroxide quite efficiently to oxygen and water.

In absence of enough hydrogen peroxide there is always a possibility of forming this high valent iron 4 hydroxo intermediate, but this sort of intermediate prevents formation in the catalyst activity can still be shut down if they are NADPH dependent hydro hydro

peroxidases right or sorry catalases if it these are NADPH dependent catalysis then it still ensures that this is the catalytic cycle that is being formed.

So, to summarize what we have seen today that catalyze is the easiest enzyme to kind of follow it shuttles between the iron III aqua molecule of course, going by iron 3 hydroperoxo to form a high valent iron IV oxo radical cation and these 2 intermediate resting state and the active site shuttles among each other very quickly, but for that to happen you need to have enough hydrogen peroxide and NADPH can be also be of health.

So, in the case of the peroxidase it is just the peroxide is important in addition there is also this hydrogen donor hydrogen atom donor right that comes into handy. So, as you have seen these all these chemistry has relevance with respect to cytochrome P450 as well, cytochrome P450 mechanism let me take you there once more very briefly we can have over here.

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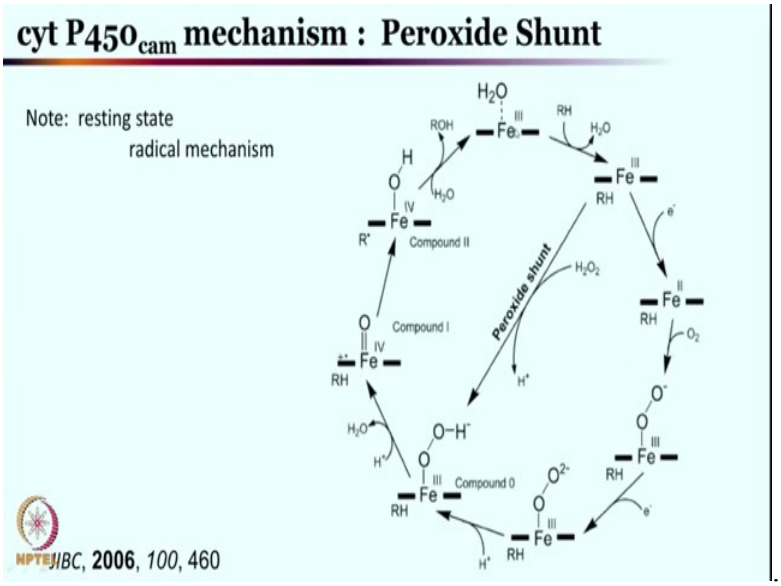

As you can see peroxidase chemistry it is shuttling here, this is the chemistry we are seeing for cytochrome P450 we can go through all these way, for in case of catalyzed chemistry it is the shuttling between this state and that state ok. Well of course, it goes via the hydroperoxo, but so, it can be this intermediate to that intermediate to that back to that ok.

So, this shuttling is quite interesting see it is part of the bigger cycle only, but it still shuttle. So, cytochrome P450 mechanism is exactly the circular 1 peroxide shunt or the peroxidase mechanism would be over here. This is the peroxidase mechanism for the catalyzed mechanism it is going to be from here to there to there and back there. So, these 3 intermediate iron III, compound 0, although it is transient and then compound 1 back to the iron III resting state right.

So, you see the bigger picture it is very simple and clear once again cytochrome P450 goes by this mechanism, peroxidase goes by this mechanism ok, hydrogen peroxo is converted into water completely to equivalent of water by this process and catalyzed mechanism will be from here to there to there and back in there ok, 1 2 3 only these 3 are involved. Well as you can see there is always a possibility of drifting little bit, but the NADPH and the concentration of hydrogen peroxide ensures that none of these are happening way too much ok.

With this I would like to conclude today our discussing cytochrome P450 and peroxidase and catalysis keep studying I hope it is getting clear and these are really interesting enzyme and as you have seen can really do quite a lot of beautiful chemistry. So, we will be back soon keep studying cytochrome P450 and other enzymes.