Metals in Biology
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Lecture - 22
Systematic variations in O-O stretch in Iron - oxo - copper ligand complex

Hello, welcome back. So, you in the last class you have seen cytochrome C oxidase right that is quite a fascinating enzyme this heme copper oxidase.

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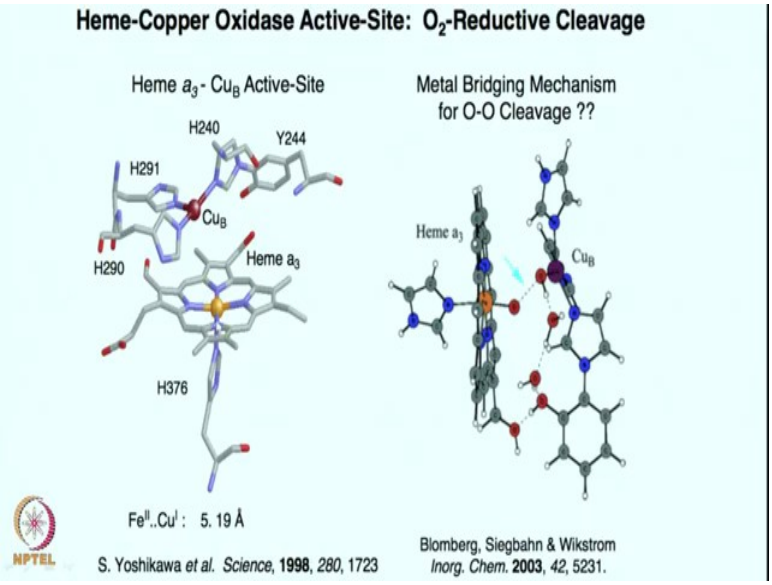

You have seen this heme and how copper center is oriented right in front of this heme, but you have 5.19 Angstrom distance between iron and the copper center. We have seen how synthetic chemist has tried to understand the mechanism of this reaction. I think one of the major query that need to be answered is how these 4 electron and 4 proton process is taking place so that a oxygen can be converted to water in a perfectly catalytic manner. Well, we started seeing how synthetic chemist has approached this problem as in enzyme one heme and one copper center and they are planned perfectly or placed perfectly right.

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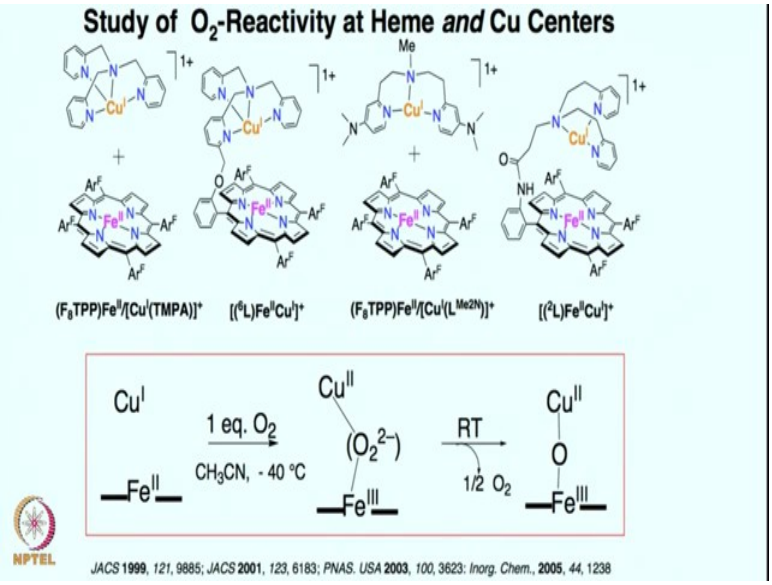

Synthetic chemist tried to do that by various ways; one of the ways that we have discussed as we have discussed this is the 1:1 mixture of this iron and copper complex. And then we also can have a tethering the same thing with the tether; these are tetradentate ligand there is tridentate ligand and the porphyrin center.

Of course, we can have a porphyrin center attached with a tridentate ligand. Overall no matter what we you do; I think the exciting part was irrespective of the cases in all these cases, we have reduction of the oxygen. We are going to create water from oxygen, we have reduction, doubled reduction one each from electron each from iron and copper to form the peroxo species right.

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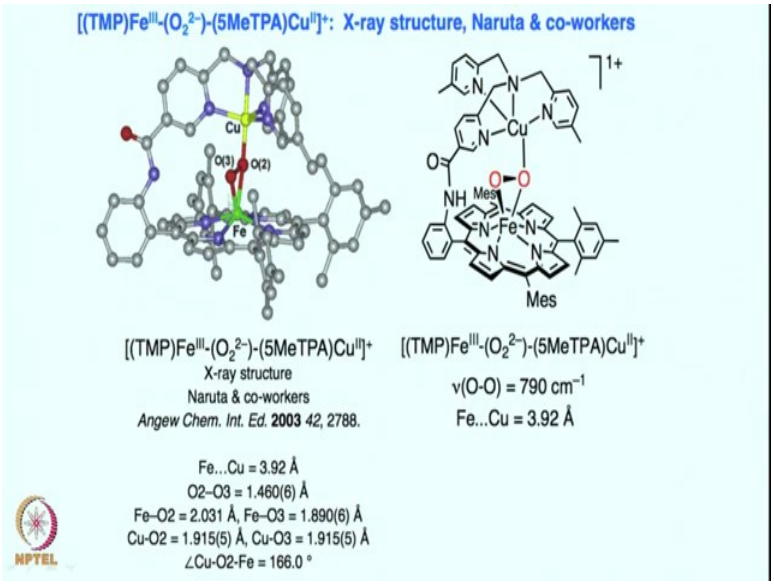

We have seen that; this has been crystallographically characterized in one instance where we see that heme center iron center is bound with oxygen or peroxide in an side-on bound geometry and the copper is bound in an end-on bound geometry, as you can see the parameter from the crystal structure; this is a very very interesting crystal structure.

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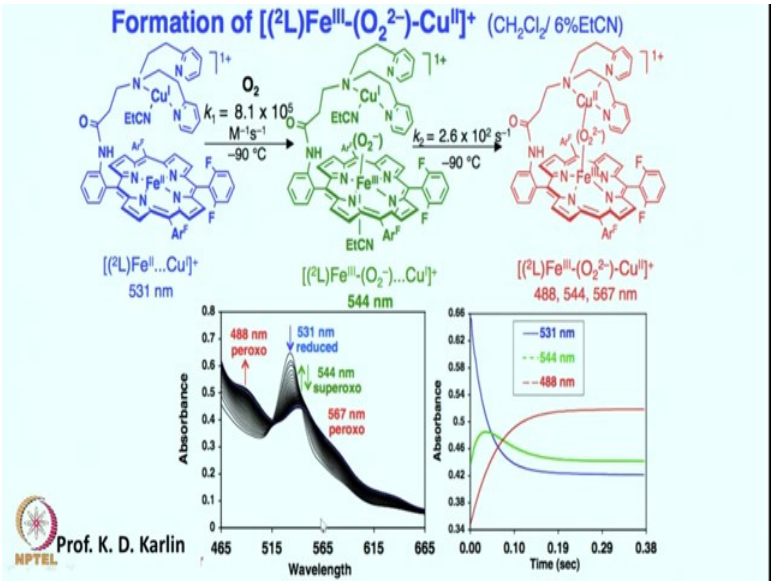

Well the solution study of course, many studies are done and these are very very you know sensitive study you have to do it really perfectly and lifetime of these species intermediates are not that great; so, you have to take care of those issues as well.

So, when heme is appended with this tridentate ligand, this is a little bit better model that is mainly because of the fact that copper B in the cytochrome C oxidase has this tridentate ligand system of course, not exactly this ligand. But, the three nitrogen centers are there right; so what it has been found that immediately it forms upon reacting with oxygen a iron III superoxide species which can then subsequently be reduced further to peroxo species.

So, see what it allows us to do essentially is we can have stepwise understanding of how oxygen is getting converted to water. Oxygen is getting reduced stepwise and the intermediate forming during this process can be followed smoothly right. Of course, we need to have the spectroscopic technique and a experimental skill set right.

But as you can see from the spectra this blue compound over here is decomposing a new green species is forming and disappearing and overall this new iron III; copper II peroxo species is forming.

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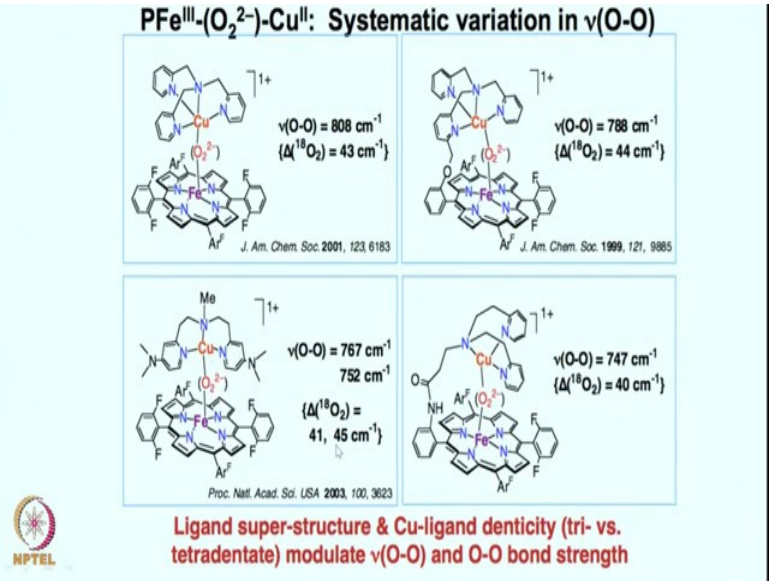

This can also be followed of course, by UV Vis and other spectroscopic technique. But as I was telling you that in the last class irrespective of what we do in all the cases it is a iron copper hetero dinuclear peroxo species. There is no homo nuclear peroxo species forming between the two copper centers, because you can have another copper coming in irrespective of whether it is tethered or not tethered; you have a peroxo species forming in between iron and copper.

This is for a tetradentate ligand system, you have seen the crystal structure how this is side-on, on iron and eta 1 on copper and in case of tethered also as you see these values are almost same right; this further emphasizes that these are the exactly same compound. Of course, these changes are due to this you know tethering slight changes, but these are completely characteristic of you know absolutely characteristic of the peroxo species oxygen-oxygen stretch at that you have seen in many other; many other classes where oxygen-oxygen stretching in case of resonance Raman is quite diagnostic of this peroxo species being present over there right.

We can do the O^{18} leveling so that can show that it has been shifted to let us say 744 wave number in this case and therefore, not 744; 788 minus 44; yes that would be 744 and then delta $^{18}O_2$ is 44 wave number right; so as you see over here this is completely consistent with this compound as well. Well for with a tetradentate ligand system as you have seen Naruto got a crystal structure that is clear, but for a tridentate ligand system; there is no crystal structure so far neither in enzyme nor in synthetic setup.

But what scientists were able to show very clearly that it is a peroxo species is forming between the iron and the copper center and each of the centers are giving a one electron to the oxygen moiety to make it peroxo just like what we see over here. Interestingly, I think most interestingly that these oxygen-oxygen stretch in resonance Raman is coming 767 and these are consistent with the fact that there is a peroxo species forming.

Similarly, one can see that there is a peroxo species formation between the iron and copper if it is tethered ok; that means, attached together. So, without attaching with the porphyrin moiety or with attaching with the porphyrin moiety; the resonance Raman data remains similar also the delta $^{18}O_2$ remain similar. All these are consistent with the fact that stepwise reduction is happening, as you have seen in this case first iron gives one electron; then copper gives another electron right.

So, ligand superstructure and copper ligand denticity tridentate versus tetradentate modulate the oxygen-oxygen frequency, as well as their bond strength right. That is very very here very very simple I would say these can be followed by resonance Raman and UV visible in some cases EXAFS and other spectroscopic technique, these are all done at very low temperature so that these species have some live time or it can be followed little bit better ok.

At room temperature these studies are invalid because you know you will end up getting a iron oxo copper 1 oxygen atom iron oxo copper, iron oxo copper or some other side reaction might will happen.

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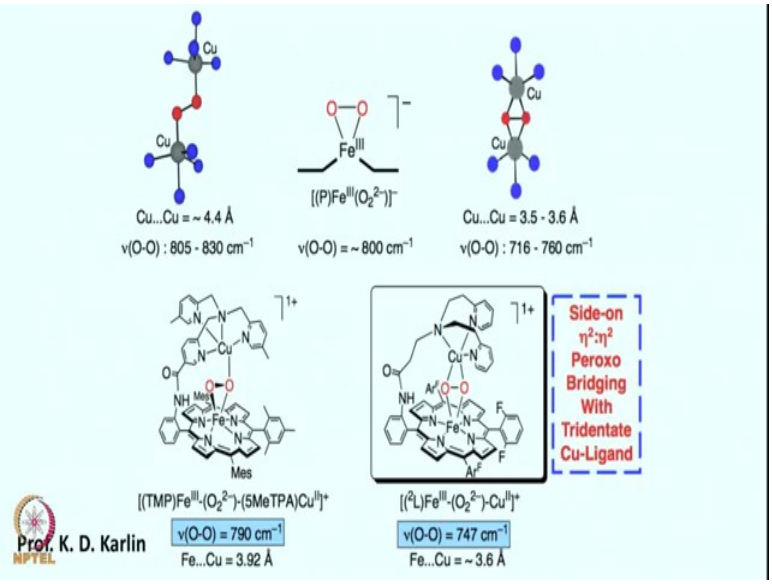

In any case what you have seen so far in copper chemistry that; if you take these copper ligand complex similar to what we were taking earlier; these copper ligand complex itself can react with each other to give the copper end on bound peroxo species right.

So, these are tetradentate ligand 4 nitrogen center, 4 nitrogen centers will give you end on bound geometry right. If you are taking a tridentate ligand for copper and reacting these are copper one complex to start with then reacting with oxygen. So, each of those copper will give 1 electron on the oxygen moiety or to the oxygen moiety to reduce it by 2 electron and it would be peroxo same over here as you have seen earlier.

But you know that this is also electrophilic in nature; these oxygens and these are nucleophilic in nature. We have discussed this earlier, but these sort of species are very very well established and you know in number of enzyme you have seen which has such structures such as hemocyanin which just carries oxygen in crab and crayfish and so on. So, this is the species forms in crab and keep them alive; their copper copper distance was nearly 3.5 Angstrom to 3.6 Angstrom between these two copper and oxygen-oxygen stretch is around the 730-740 region.

So, this is the species which is responsible also for tyrosinase activity; remember the phenol or tyrosine is getting hydroxylated to catechol and can then also be further oxidized to quinone benzoquinone right. So, this is the species without substrate; it just acts as a oxygen carrier in enzyme like site such as hemocyanin. But with a substrate it oxygenase right of course, in many other reaction we have seen that it can carry out right oxygenase the substrate. So, none of these or that one is forming when iron is in the mix; when iron porphyrin is in the mix none of these are forming.

Another interesting thing is for the porphyrin system, you can first form a superoxo species then you can reduce it further to form the peroxo. If you are taking starting with iron II reacting with oxygen, you will get a iron III superoxo species first where iron gives up 1 electron to oxygen and then further 1 electron reduction by adding a reducing agent one can get the iron III peroxo species as its shown in here. But none of these species are actually you know forming there when iron and copper are mixed together, instead what they decide is they decide to share the responsibility.

They try to be friend with each other and they try to reduce the oxygen together of course, one of them acts first and the other one acts then subsequently right. In this case as you have seen iron donates the electron first to oxygen and then copper donates ok. See this sort of clarity is quite interesting and it has come over the studies; studies over the decades right. So, as you have seen this is crystallographically characterized; oxygen-oxygen stretch is 790 wave number; iron copper distance is 3.92 Angstrom.

Once again remember the iron copper distance was 5.19 Angstrom in cytochrome C oxidase, but here this is 3.92; of course, that iron copper distance was from for the reduced form. So, it is expected that perhaps also in cytochrome C oxidase iron and copper distance will be around 3.9 Angstrom, where both iron and copper has to move closer to each other if they have to communicate through these oxygen centers right. So, without oxygen this we believe that or it is believed that iron copper will be far from each other; a little bit far like 5 Angstrom far and with oxygen it would be closer.

Now, well one of the thing is so far we did not show what would be the iron copper oxygen orientation or the geometry around the copper and iron for the tridentate case. Because remember this is a tetradentate ligand system as you can see also and in enzyme in cytochrome C oxidase; it is essentially a tridentate ligand right. So, this may not be the

case in although you know electronics or I mean the reduction will be similar reduction of oxygen will be similar but then the geometry should be as you can expect; this should be side-on or eta 2 as you see over here, but as you see for tridentate ligand, this is side on both.

So, therefore, the copper is expected to be that; actually now there are some evidences which suggest that this is perhaps or this is likely to be the correct orientation. So, you see the oxygen oxygen stretching and iron copper distance; these are now can be measured by different spectroscopic technique snd although the crystal structure does not exist, but this side-on peroxo bridging with tridentate copper ligand is likely the scenario; most likely I would say I think you know this is the beauty of the bio inorganic or synthetic bio inorganic chemistry coupled with spectroscopic and computational studies.

Now, we as a community synthetic community and the bio inorganic community as are quite sure that this is the case in enzyme. So, this is how iron copper will be bound with oxygen where oxygen is reduced by 2 electrons. Let us go back one time to the enzyme here you can see this iron copper will bind the way we were showing perhaps over there. This is a crystal structure of the reduced form, there is no oxygen binding dioxygen binding or the superoxo or peroxo binding right over here; that crystal structure is not really known.

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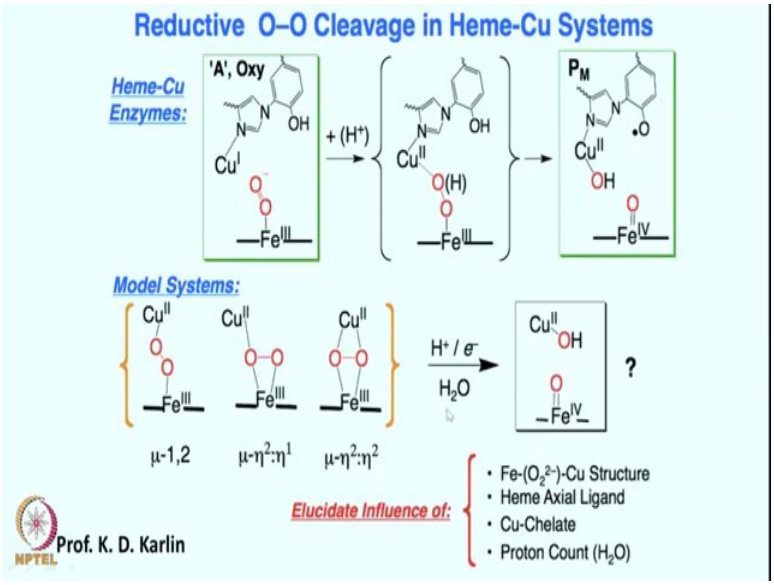


And what we see next is how these species are then further reacted to form water we did not see that yet right. Well let us go back to the enzyme one more time this is a schematic presentation. So, how the reduction of oxygen to water is happening ok?

If you have followed, so iron will give one electron to the oxygen first to form iron III superoxide. Then copper will come into the picture it would be copper II iron III peroxo speciesa nd from there on a hydrogen atom transfer likely happening from this phenol which is cysteine and sorry tyrosine and histidine a cross linked phenol.

This phenol OH dot will be provided to these peroxo species to cleave the oxygen-oxygen bond; that part we did not discuss yet in synthetic study. This is completely speculated mechanism you know based on some of the synthetic studies been done, but we will see how people have or synthetic chemists have approached this problem right.

So far we have seen superoxo formation and then peroxo formation and we know the geometry right what would be the peroxo like this would be iron III copper II peroxo most likely this is the case in a eta 2; eta 2 or side-on bound geometry. What we now missing is proton and electron that can be coming from phenol you need to give you iron oxo and copper II hydroxo. If it is shown that this is what is forming from these peroxo species because these peroxo species are now well characterized; very well characterized right these two are very well characterized.

And one thing we need to really get then these iron IV and iron IV oxo and copper II hydroxo formation which is nothing, but water this is also nothing, but water upon protons and both of them are water. So, essentially what you have seen if this is forming then it would be 2 equivalent of water here and water here upon protonation.

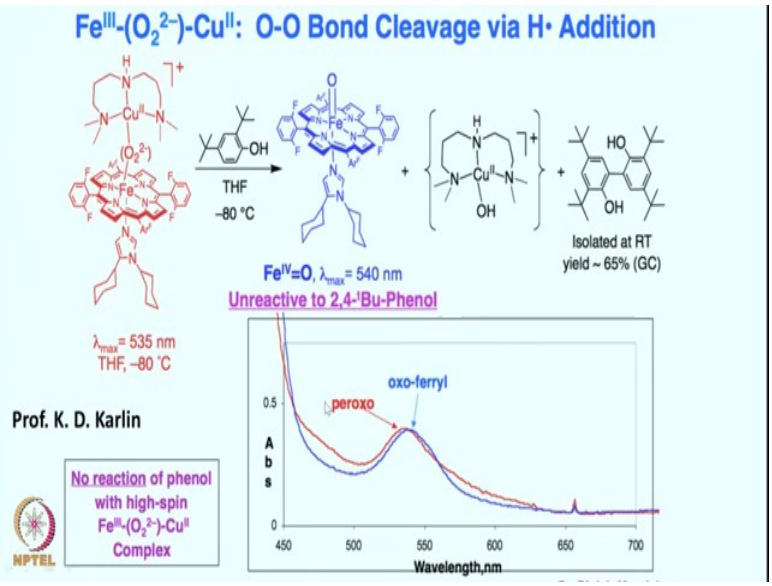
So, this is what we would like to discuss; what we have not seen synthetically that there is no axial ligand added; will it have a role to play ok? And you know the proton count ok; for the water molecule formation you need two proton over here another proton over here.

So, as you can see two proton over here; one proton is already there third proton another proton will be required that is fourth proton electron count is also absolutely perfectly matching; to regenerate the catalytic cycle, to undergo all these iron IV copper II species.

And then subsequently come back or bring back to iron II copper I species; overall you need 4 electrons.

Let us look at what will happen to these peroxo species? Because these peroxo species are not forming this yet right; nothing is happening which is closer to this. So, something need to be done to these peroxo species, if we are to see that these species are forming ok. Of course, obviously, it is happening in enzyme, but we do not know how it is happening and that is once again is that you know beauty of the synthetic studies or these biomimetic studies. The bioinorganic fraternity thrives on such sort of problem, where almost nothing is known in terms of the enzyme and it is really kind of a black box and the light is shown or shaded right you know by the synthetic bioinorganic chemist.

And this is what we now we will see; we will try to start with this and try to see what is the role of an axial ligand? Is it necessary the role of phenol, is it necessary? Is it what it should be? Although we have proposed already, but this is what its most likely happening, but we need to see that these are really required or not right or without them these things still would go on or not this oxygen-oxygen cleavage will go on or not. So, the big question now we would like to answer how the oxygen-oxygen bond that is still present between these two atoms are going to be broken or this bond is going to be cleaved. (Refer Slide Time: 21:07)



So, here is first; so this is a fully characterized system by now; as good as it gets I guess. Most interestingly as you have seen now an axial ligand is added; without this axial ligand things are not happening it is not able to cleave that oxygen-oxygen bond.

As you can see this oxygen-oxygen bond without that axial ligand it is not breaking ok. So, you need this dicyclohexyl imidazole or imidazole basically that is what you have in the enzyme histidine is appended or attached with this iron center right without these things are definitely not happening.

So, let us add that one this complex is also characterized, this complex is completely synthesized and characterized. Well, even after that also things are not happening, what is turning out to be the case that if you add phenol such as this one; 2,4-di tertiary butyl phenol; well bingo, I guess that is fantastic.

Now you have this iron IV oxo species formation which has a very characteristic band in UV visible and it has been previously synthesized independently by other method. So, to be sure these; these are really really the species happening, but it is essential; absolutely essential to have this phenol, without phenol things are not happening.

Now, also these copper II hydroxide is forming, this phenoxy radical or O dot that is forming over there of course, this is not going to be stable; it is going to dimerise to give the give this carbon carbon adduct. If you are blocking this phenol with another tertiary butyl group over here that would be 2,4,6-tritertiary butyl phenol, then this sort of coupling will not happen and you will end up getting phenoxy radical, which can be characterized by other spectroscopic technique including UV visible EPR and so on.

So, what we are trying to tell you here is; it was absolutely necessary to have this phenol, as well as this axial ligand and that is why perhaps in nature we see that both the axial ligand is there and the tyrosine tyrosine histidine crosslinking is there. I think that is quite phenomenal to be able to really show that that these all these components are absolutely required.

You need iron, you need copper of course, oxygen is required, axial ligand is required, phenol is required that is why tyrosine histidine cross link is there for the oxygen-oxygen bond cleavage in molecular oxygen to give water; that is I think quite exciting ok.

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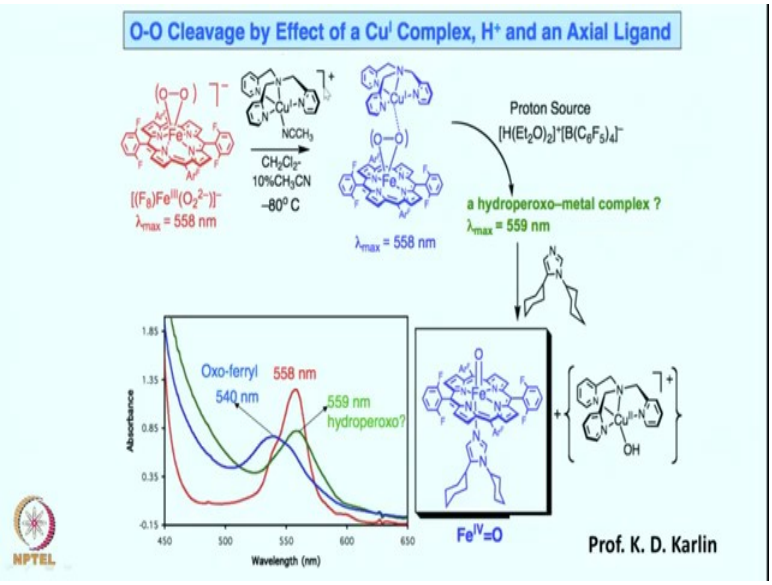

In other word you can do the similar studies; even with a tetradentate ligand system where you can start with a peroxo species. Iron III peroxo species independently generated superoxo reduced by one electron iron III peroxo and then add copper ok.

This can form an adduct as it is shown over here and then still you need a proton source which can give another alternative pathway of forming hydroperoxo intermediate. Here you see the proton and an extra electron in the form of synthesizing the peroxo is already given. So, H dot equivalent is given which was coming from phenol in the enzyme.

Now, it is given in the form of H^+ and another electron on the superoxo to form the peroxo. So, you started with iron II, reacted with oxygen to give iron superoxo and then you give another electron to make it iron III peroxo. This electron extra electron and this proton is the H dot; overall then you add an axial ligand once again you end up forming iron oxo copper II hydroxo.

So, this is I think is really getting clear where each of the iron and copper will give one electron each; iron gives first then copper gives the electron and then you have a still a requirement for a proton and electron; that is coming from your tyrosine moiety and the role of axial ligand is also very very clear.

Overall, starting from oxygen molecular oxygen you have iron IV oxo which requires protonation to give you water. This copper II hydroxo another equivalent of water is

right over there upon protonation. Just to summarize then what you have seen; well it is a very very simple, yet complicated, yet interesting process right you started with oxygen got 2 order.

And you now know how most likely things are happening how stepwise things are happening in the role of each and every component in this in this cytochrome C oxidase or heme copper oxidases. Well, I think nature did not put anything for fun everything has a role right whether we understand or not; it may take decades in it may take centuries or we may never understand, but nature has deliberately decisively put each and everything right over where it is absolutely required.

Nothing is placed just for fun, everything has a role to play, everyone is a part of a bigger game, everything is synchronized. I think that is what we really need to appreciate nature that everything is where it is supposed to be, everything has a purpose. I think we begin to understand with the scientific journey, we begin to understand with scientific community how things are happening how things are; so perfectly designed.

To understand this, it is very very difficult always to for scientists, for researchers to do the experiments on enzyme itself or in the biological system and this is where precisely the role of a synthetic bio inorganic chemist or biochemist, biomimetic chemist comes into the picture and they really are crucial in putting the puzzles, links, species together.

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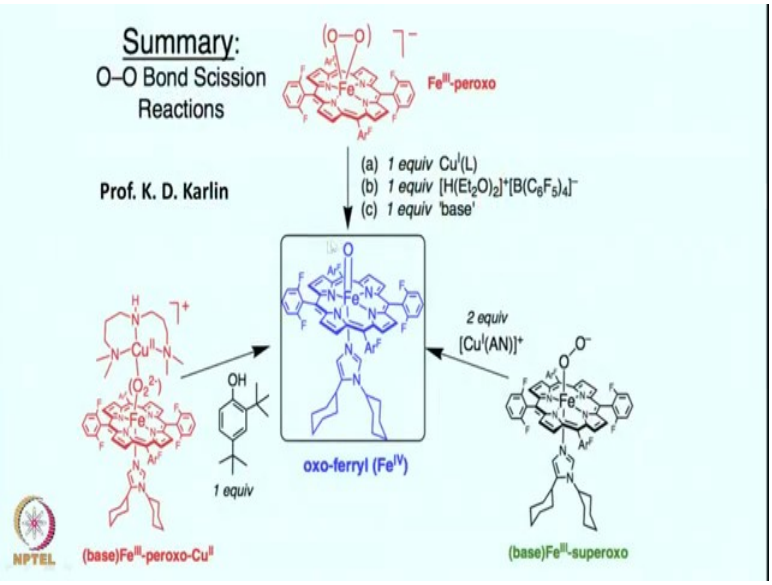

As you have seen the key of this process was iron IV oxo formation and the copper II hydroxo formation for 2 water molecule from oxygen and many different pathway can give you the same result starting with a peroxo ligated with a imidazole and coupled in with a phenol gives you there. You can start with a peroxo upon adding one more electron from the outside and then 1 equivalent of copper, 1 equivalent of proton and 1 equivalent of base.

Overall all the paths lead to the same product; so different routes are lead to one target. And that stage oxygen oxygen bond cleavage to give water and that is phenomenal I would say; by different path it is actually possible to show that exactly same thing is happening. And the ligand has a key role to play tridentate versus tetradentate as you have seen. But all of these species together kind of giving a larger than life picture or clearer picture that this is what is happening ok. I hope you understood the chemistry behind this oxygen to water formation.

Now, from for cytochrome C oxidase we will come back; I think next class we will be discussing the non heme iron enzymes right. Keep studying.

Thank you very much.