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

Lecture - 36
Summary of Dioxygen reactivity in copper

Hello everyone. How are you doing? Today, we will be summarizing for you the Dioxygen reactivity in copper.

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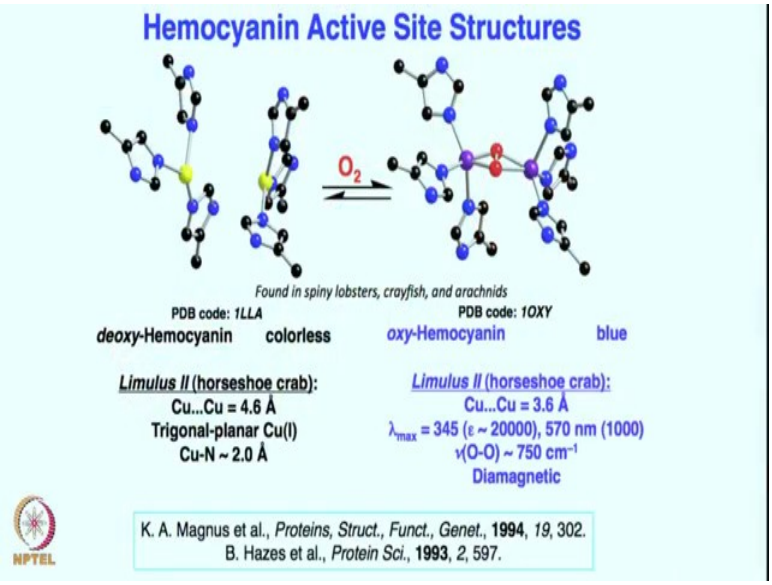

You have seen so far many different copper enzymes of course, the first one perhaps comes to your mind is the hemocyanin. Hemocyanin is having 2 copper centers with 3 histidine on each of them, and this is a colourless compound upon binding with the oxygen, it gives you very nice blue colour compound this one. Where 3 histidine are on each copper centers and oxygen is reduced by 2 electrons; one from each copper centers and these coppers are now plus 2 oxidation state.

So, this is a simple and beautiful reaction, but the best thing about this reaction is it is just acting as a oxygen transporting agent, it does not react with any organic substrate. Although these species these active species are capable of reacting with organic substrate. That is exactly what we see in another enzyme as you might will remember Tyrosinase.

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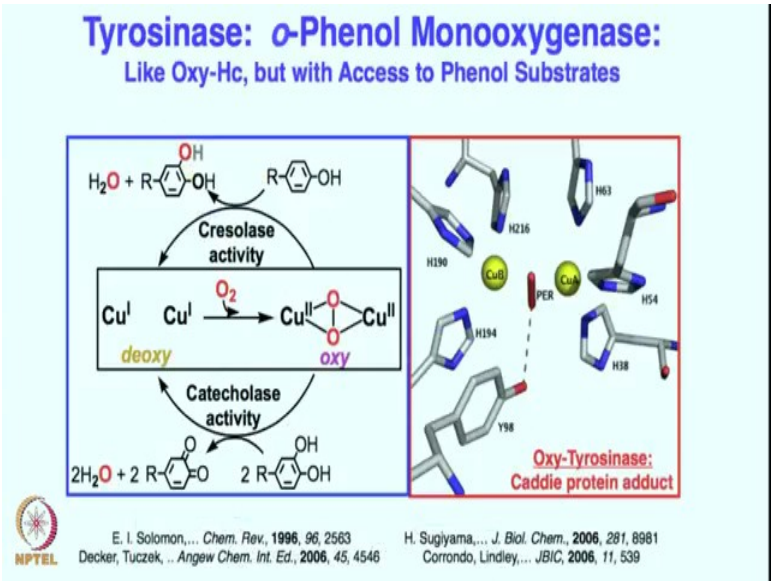

So, the tyrosinase utilizes exactly same active species that is 2 copper one centers each supported by 3 histidine are reacting with the oxygen to give the dinuclear these copper II side on bound peroxo species.

So, once again this oxygen is reduced by 2 electron, but only difference between hemocyanin and tyrosinase is now you have a phenol appended to this active site. What will happen as you know this side on peroxo are electrophilic in nature and therefore, an electrophilic aromatic substitution reaction happens; giving rise from phenyl or tyrosine to catechol, and water molecule on the other hand, if you take this catechol product into the reaction you get Quinone. So, cresolase activity and catecholase activity you can see in it with respect to the tyrosinase, I think that is really fascinating right.

So, what we essentially see here is nature's design or desire to carry out these reactions in an efficient manner, but in a 100 percent controlled manner, in a hemocyanin exactly same species as that of tyrosinase, but in hemocyanin we have only oxygen carrying properties in tyrosinase the same active species is utilized to do the substrate oxygenation chemistry. I think that is really really important to understand that nature did not put anything just for fun.

Once again it has the desired motive or desired intention by which it carries out a certain reactivity ok. So, the reactive elements or reactive species nature has chosen are limited,

but the design is such that those activities are controlled 100 percent. Sometimes very reactive intermediates are generated, but without using them they can do their activity.

Sometimes these reactive intermediates are used or utilized for their reactivity purpose such as hemocyanin versus tyrosinase. All these are the dinuclear species and you have seen synthetically in laboratory if you are want to synthesize these compound you run into problem I would say.

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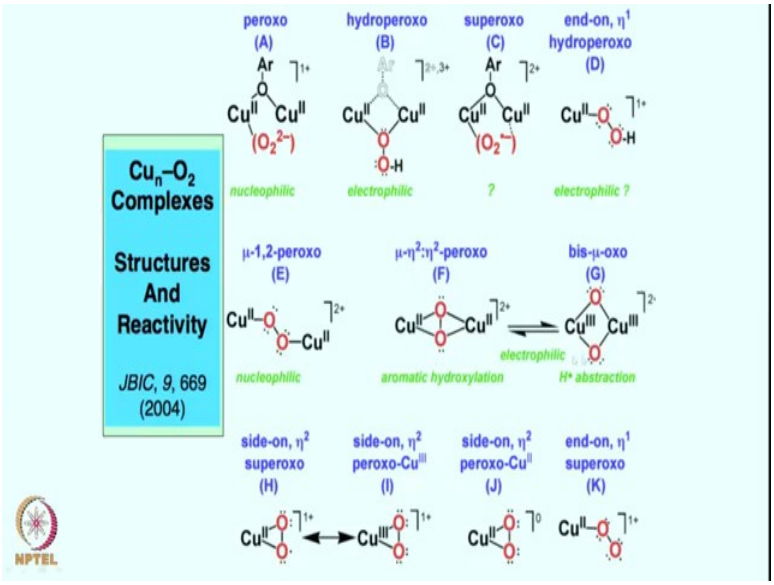

Many different types of dinuclear species not to mention these one, but let us say just focus on this one to keep it simple these different types of dinuclear species can be generated, if the ligand in synthetic setup. No, this is now not in enzyme right. This is just let us say nothing to do with enzyme at this point.

If you take in ligand tetradentate ligand, then you can get an end on peroxo, quite interestingly this is nucleophilic in nature, as we have mentioned this is going to be delta minus, if you are taking a tridentate ligand most often you end up getting the side on bounds peroxo species, these are electrophilic in nature, as you have seen aromatic hydroxylation reaction is possible by electrophilic aromatic substitution mode.

If you have often a bidentate ligand, then you end up going to get this bismuoxo species where copper is now plus 3 oxidation state. In these cases coppers are in plus 2 oxidation state, only this case copper is in plus 3 oxidation state. Essentially from oxygen

molecule, dioxygen molecule, you have broken all the bonds between the oxygen oxygen to get into this bismuoxo species. This species it is also believed to be the active species in tyrosinase in addition to this side on peroxo species.

There is enough literature report now, which suggests that this although forms fast, but during phenol interaction this is the species which is being generated. And therefore, this is the true active species as opposed to perhaps this one. Just to give you a quick hint, over here we were discussing that this is the final product, or this is the real active species, the recent studies, very recent studies suggest that it is the peroxo to start with, but then during phenol interaction specially with phenol. It is the bis-mu-oxo these species that generate.

As you have noted that these species are in equilibrium with each other therefore, it is very difficult to maintain 100 percent purity between one versus, another although both of them are capable of doing the chemistry. It is also to be noted that these are completely ligand control mode of reaction. All not necessarily tetradentate ligand has to be always giving these there are exceptions, which violates the usual understanding that it is tetradentate, tridentate, and bidentate, ligand system gives all these species, there are enough sterically crowded or electronically monitored ligand, which can which can cross the barrier and can give one versus another.

But, perhaps most importantly these species these dinuclear species are forming through the mononuclear intermediate. So, one of these mononuclear intermediate is involved during these dye nuclear species formation we will come back to this soon ok.

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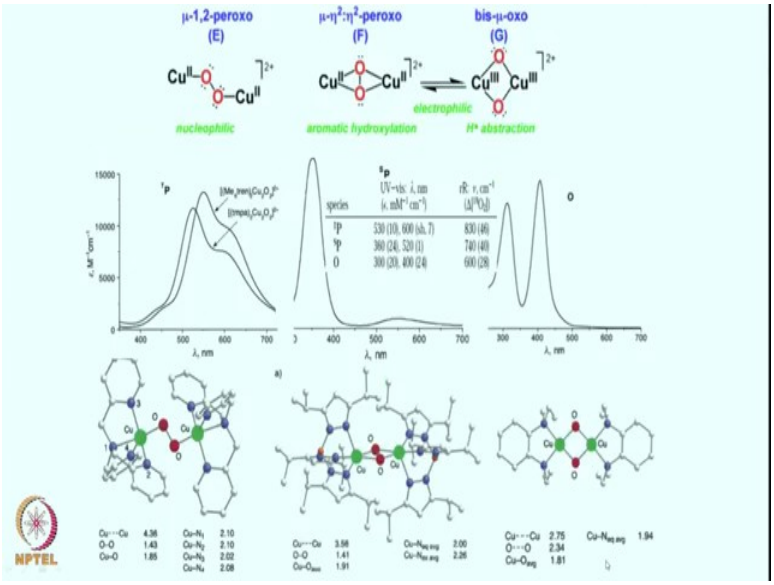

So, these species these dye nuclear species as you have seen earlier will have very characteristic spectroscopic features including UV visible resonance Raman of course, accepts whatever spectroscopic features you want to characterize them with they are completely distinct and identifiable. These are very very low temperatures intermediate, these are not that very stable intermediate, these are extremely reactive intermediate, all of them will at room temperature, or at higher temperature, or even at low temperature will undergo further reaction to give you the decomposition product hydroxylated product, you know dimeric hydroxylated product different product from these can be generated ok.

These are so, reactive even the ligand for the metal center can also get affected depending on it is design ok. All these chemistry, if you are trying to study in your laboratory, you have to do under the inert atmosphere like under nitrogen atmosphere, or under argon atmosphere, some of the handling has to be done at glovebox condition or under glovebox condition.

You can store these compound the starting material, let us say ligand copper one complexes, you have to store it under the inert atmosphere often it is stored in the glovebox right. So, these temperature, these reactions are also extremely temperature sensitive, if you are doing at a high temperature, such as room temperature, which is considered very high for these species.

You will not able to see any intermediate formation. You have to slow down the reaction by lowering the temperature to let us say minus 80 degree centigrade, minus 120 degree centigrade and so on.

So, therefore, you have to choose the right solvent also, because solvents should not freeze at those particular temperature. So, we have discussed those earlier in any case I think what we are trying to tell you that these such can sensitive chemistries are of course, very powerful right. They can do the reaction, they can do the synthetic transformation, the type of transformation a synthetic organic chemist perhaps could never think of doing, that is that is the beauty of these species right.

So, you have different species completely crystallographically characterized; these species has direct relevance in himocyanin, this is also had direct relevance in tyrosinase, but, but perhaps both of these species are having both of these copper cores are having relevance in tyrosinase, that we have seen earlier ok.

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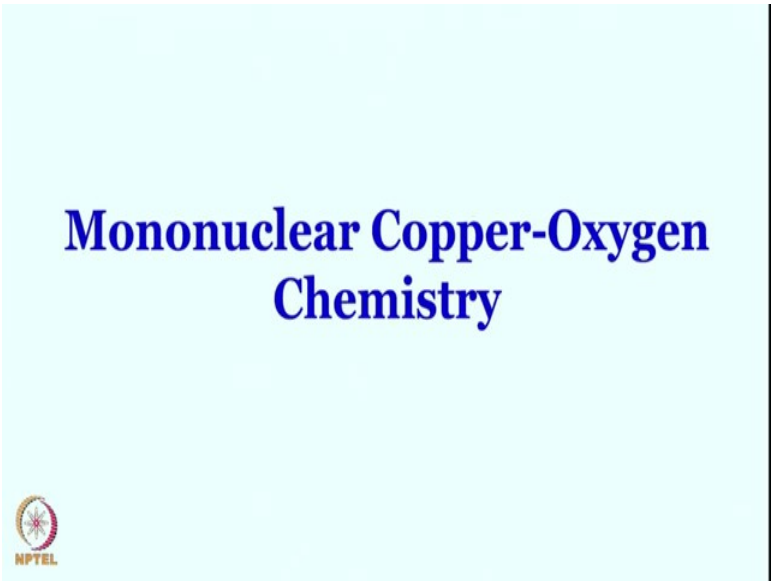

Let us move on, let us try to discuss mononuclear copper oxygen chemistry. So, of course, dinuclear copper oxygen chemistry is interesting, but mononuclear copper oxygen chemistry even is even much more sensitive. And, therefore, it has to be dealt with extreme care not that dioxygen, di copper chemistry is not sensitive, but modern nuclear chemistry is super sensitive right.

Because, these are the intermediate which forms, prior to your dinuclear species formation these are kinetically first formed product right. These are the first step and therefore, and this first step leads to the second step which is the peroxo species formation. Let us say for example, those dinuclear, but stabilizing them is extremely difficult. We have seen many enzymes where we have these mononuclear copper oxygen species two of them we discussed, one of them is PHM, the other in DBM these are homologous copper protein meaning that, what is true for one is also going to be true for the other one ok.

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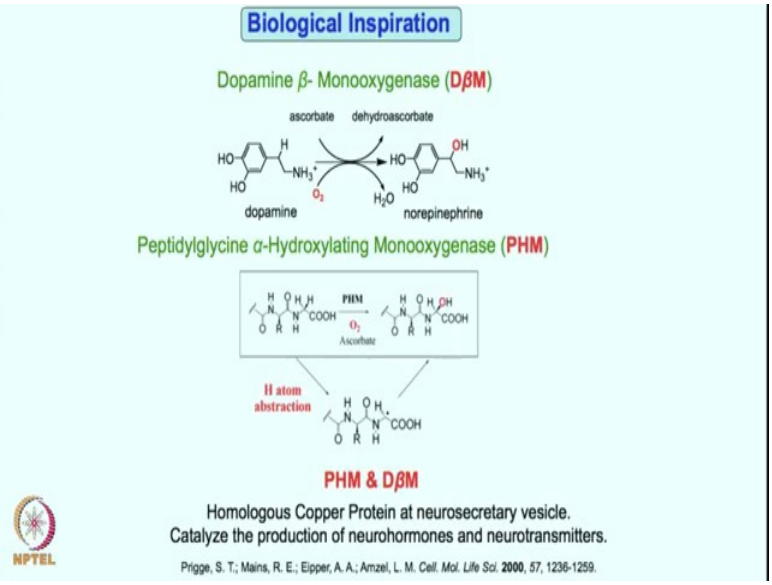

Both PHM and DBM can do the substrate hydroxylation ok. As you can see the substrate is getting hydroxylated by copper oxygen species, but these copper oxygen species are not the binuclear one, this is the mononuclear one. Quite interestingly though if you look at this active site there is this dinuclear or 2 copper center, yet only one copper center is doing the chemistry ok.

So, they catalyze both of them catalyzed production of neurohormones and neurotransmitters which are quite extensively studied and quite important so far we have understood that, right.

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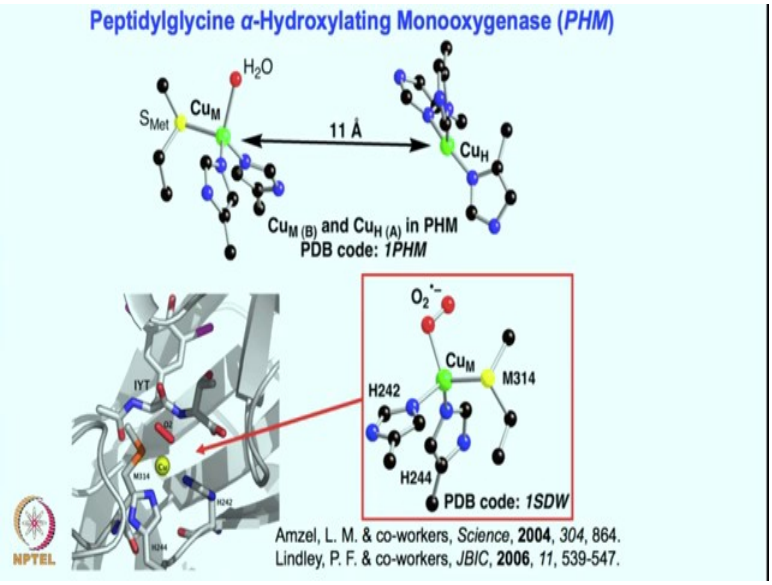

So, here is the 2 copper center although once again. Although these are mono nuclear copper oxygen species that is going to be formed over here, nothing happened over here in terms of the copper oxygen chemistry. As you see this beautiful crystal structure of the starting material this is the reduced from, this whole structure is further reduced from, if you react it with oxygen, it gives rise to this mononuclear copper superoxo species.

We have discussed the debate in this field and that is, even if this crystal structure is known people believe that just like tyrosinase peroxo and bismuoxo here people believe that not the superoxo perhaps other species such as hydro peroxide or the copper oxo. Mononuclear copper oxo is the real active intermediate, but there is also enough proof in favour of these mononuclear copper superoxo species. So, that is forming over there nothing happens over there and is the real active species ok. Let us look at that mechanism that we have tried to look earlier.

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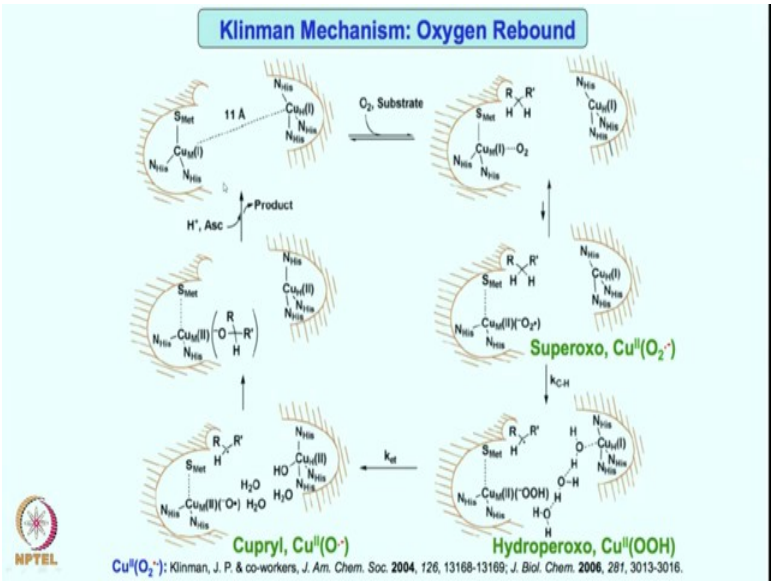

So, this is the 2 copper centers separated by 11 angstrom, no oxygen activity happens here, this is the site where oxygen activation occurs, oxygen binds, substrate binds, electron transfer occurs. So, copper superoxo species formed, superoxo abstract hydrogen atom from the substrate, saying that this is the active species ok. In this form of the mechanistic hypothesis, it is forming copper to hydroperoxo species which is giving rise to the radical formation, then this oxygen bond cleavage gives rise the hydroxy radical and that transfer over there, and leaving out copper to O dot, which is nothing, but Cu III plus double bond O right Cu2O dot is C3 double bond O the cupryl species ok.

Now, this cupryl and the radical will combine to give the copper alkoxide and then the product goes out and ascorbate comes into the picture, for reducing the copper center and overall catalytic cycle goes on. The similar mechanism one can draw for copper hydro peroxide at the active species; that means that this substrate will not be reacting with this one at this step, in that alternative mechanism, but this electron transfer will be over here.

So, it forms peroxo and a protonation gives the hydroperoxo, this substrate will be sitting without this hydrogen atom abstraction, because if we are saying that hydroperoxo is the active species hydroperoxo will abstract the hydrogen atom.

So, in that of mechanism you have seen that almost everything not almost everything everything falls into place, but the active species changes, little bit change of these

relative steps like electron transfer over here and proton transfer over here and the C-H activation over there that takes place.

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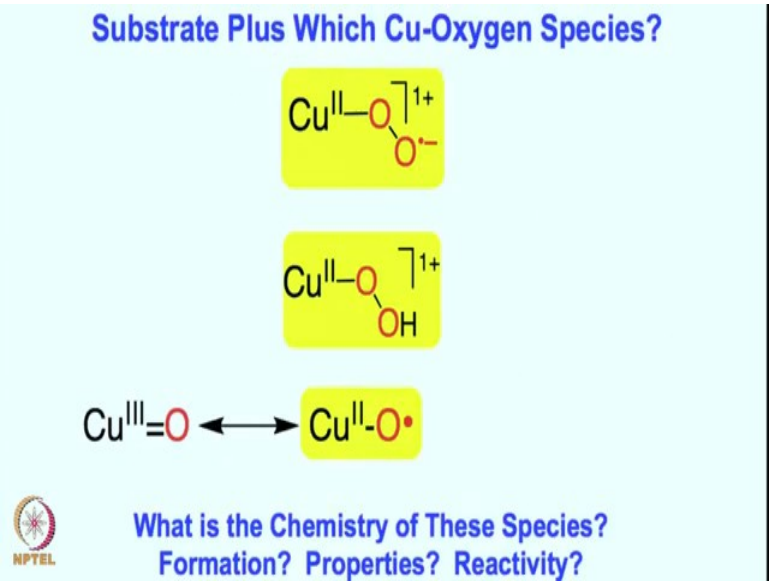

So, all these alteration can go on, but we should know that still this debate exists and this is pretty much alive if you are excited, you can contribute perhaps towards this. Because, this is really important enzyme these are a really important enzyme and that is PHM and DBM, which is you know; which is quite interesting from the perspective of the neurohormones and neurotransmitter biosynthesis.

So, it is one one really important enzyme to understand, but state of the art understanding is none of them you can rule out at present. Maybe decades and century of research will rule in favour of one of these enzyme. So, this species people are able to synthesize and see that it is reactive, this species also people are able to synthesize and found that this is also reactive for hydroxylation. Of course, the substrate has to be judiciously chosen, but nonetheless this species is always believed to be much better than these 2 species, but synthetically no direct examples or crystallographically characterized example exists.

So, this is an elusive intermediate, but then there are enough proposal so far in the literature saying that these are the species, which is doing the chemistry this debate we have discussed earlier. So, you can refer to our earlier classes, but these remains the plausible intermediate for doing the chemistry ok.

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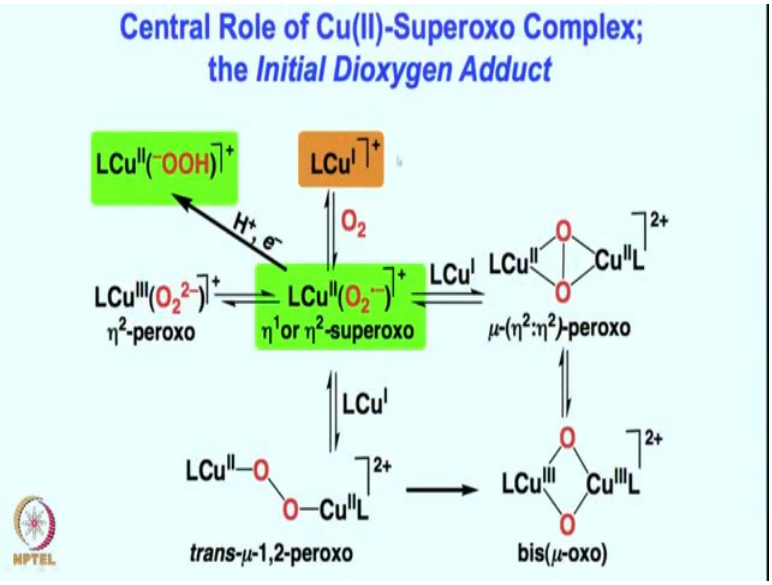

Moving on as we have seen earlier the first formed species, the super sensitive ligand copper 1, if you have ligand and associated with copper 1 and these species are going to be very very reactive ok.

Usually perchlorate PF6 bars or you know BF4 minus or this sort of this sort of counter an dions are huge with this ligand copper 1, it reacts with oxygen to give the superoxo intermediate, subsequently a proton and electron transfer to this intermediate gives rise to the hydroperoxo species. It is the combination of H dot and alternatively this can react with another equivalent of copper 1 to give an end on bound peroxo, if the ligand is that tetradentate ligand. If ligand is the tridentate one these superoxo species can react with this tridentate bound copper one to give you the side on bound peroxo species.

If, these ligands are tridentate also then also there could be this equilibrium usually with the bidentate ligand you see this sort of species formation, but as you can see these are all in equilibrium. So, depending on the ligand whether it is an aliphatic donor or an aromatic donor, whether it is a nitrogen donor, or oxygen donor, or sulphur donor depending on that these chemistry can get even more interesting ok. Even this end on peroxo can be also in equilibrium or can be converted to these bismuoxo species. This is pictures showing that everything is linked or integrally related.

So, there is an opportunity for shuttling between or among these species in the reaction solution. So, this makes the ligand copper chemistry or synthetic chemistry very very

sensitive as well as difficult to face out for it is reactivity, but as we mentioned these are nucleophilic in nature, these are electrophilic in nature, these are electrophilic in nature. So, therefore, enough chemistry can be done with this chemistry.

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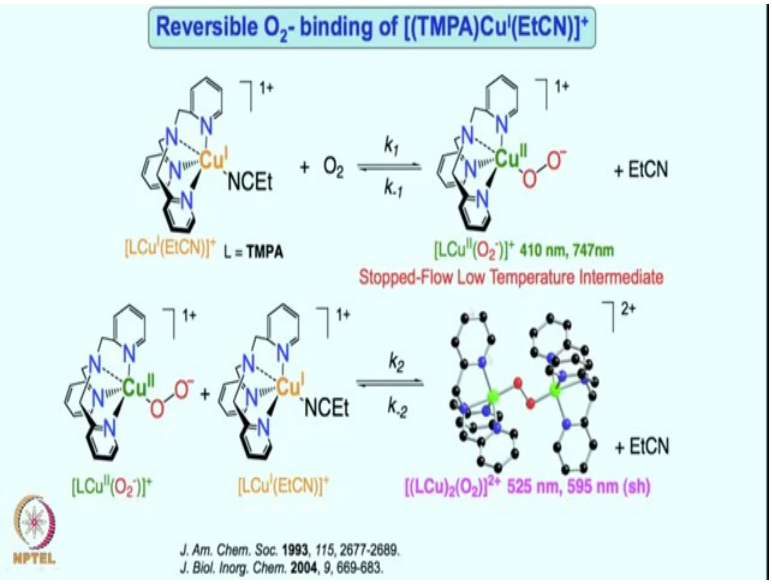

And, with these species right; so, we have seen that these species are completely well understood I would say at this point where the ligand be tetradentate tridentate or bidentate, let us say the study is done with the tetradentate, which can react with oxygen to give the superoxo species. Now, it is characterized and been confidently can be said that, this is an end on bound superoxo nor the side on bound on this end on bound superoxo where one of the oxygen atom is bound with the metal center, another oxygen is far away from the copper center or not really bound just like this.

So, if both of the oxygens are bound with this copper center it would be called side on bound superoxo species. This is an end on bound superoxo species, it can react with another equivalent of this ligand copper one complex to give you the end on bound peroxo species, which is quite clean and simple to understand. You can follow these reactions by UV visible spectrum, even you can follow this intermediate is disappearing towards formation of this.

So, there is no doubt this is the intermediate for this type of species formation. We have seen those spectra and these studies need to be done once again at very low temperature and if you want to see something like this intermediate you have to really utilize the best

spectroscopic technique. The fastest possible spectroscopic technique we have seen the stop flow kinetics data how within a second hundreds of data point can be collected, and those data point can build up to the story and can tell very clearly that this intermediate is present in those cases.

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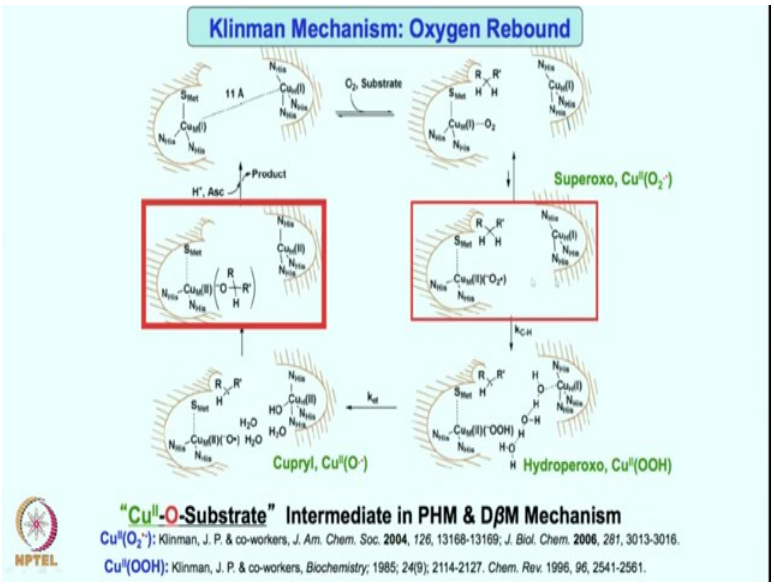

Now, moving on and as we have discussed that there are relevance for this intermediate; thought for this intermediate where both the superoxo and alcoxo species can be generated for the mono nuclear one. So, mono nuclear superoxo species can be synthetically achieved, characterized and also it can be sure that this is perhaps the pathway followed and we can also get the crystal structure of such intermediate. So, there is enough proof so, far in the literature that suggests that the mono nuclear superoxo can be generated and can do the chemistry right.

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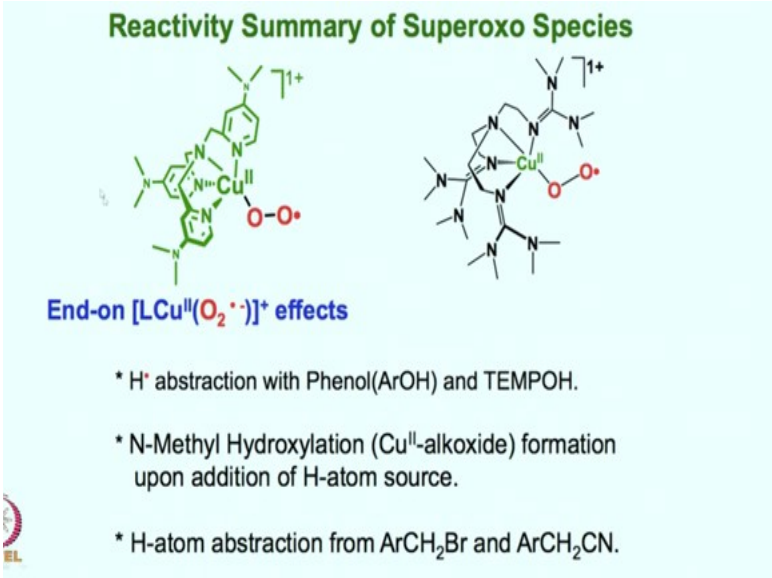

Moving on there are many different compounds that is now characterized not too many, but this one enough both the or all the end on bound superoxo so far shows that they are also capable of abstracting hydrogen atom from organic substrate, just what is seen in the PHM and DBM cases. These enzyme once again are involved in neuro hormone and neurotransmitter biosynthesis. And therefore, the implication of the synthetic studies saying that, these are involved into the hydrogen atom abstraction is quite phenomenal right.

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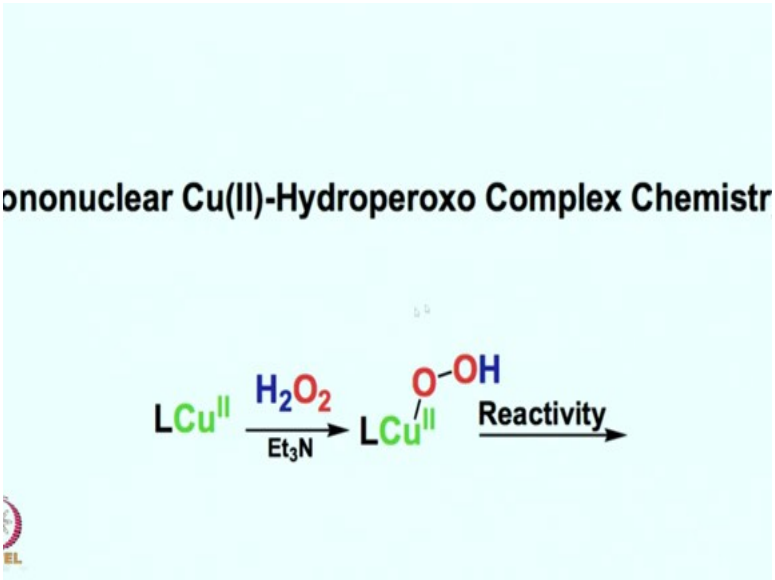

And, we also have seen that scientists were able to synthesize, this chart of copper hydro peroxides by appending the substrate.

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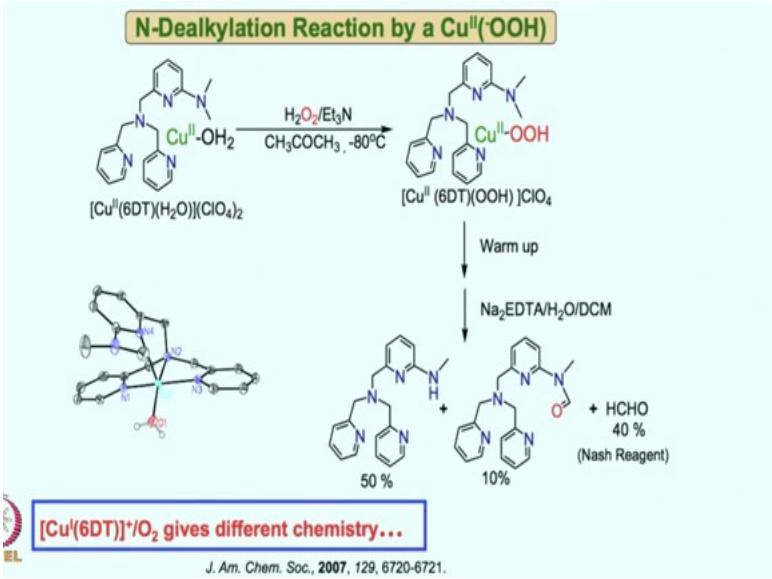

Within the ligand moiety it is also able to show that our scientists who were able to show that, this N-Dealkylation or substrate oxygenation and subsequent rearrangement chemistry or subsequent follow up chemistry can gives rise to the N-dealkylation chemistry. So, suggesting that these not only copper super oxo, but copper hydro peroxide are also capable of doing these chemistry right.

So, in the perspective of the engine I we therefore, I think we understand that both superoxo and hydroperoxo are capable in forming the desired substrate hydroxylation chemistry.

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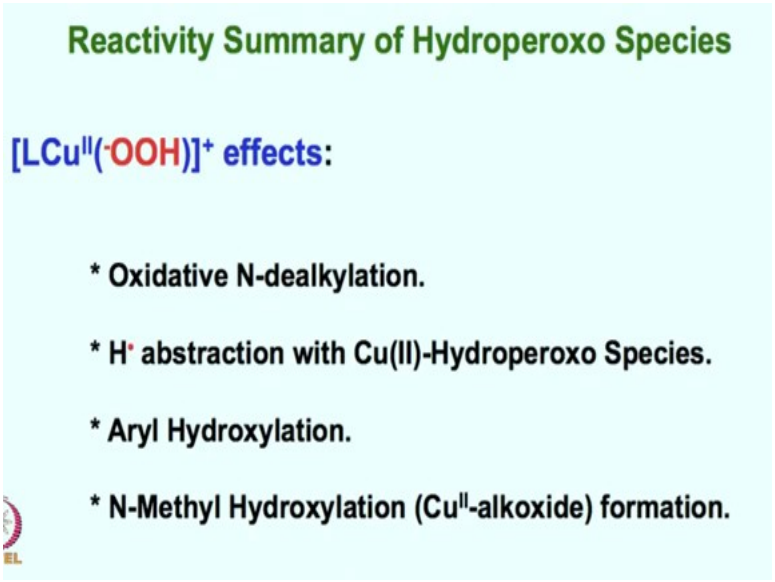

Well for the hydroperoxo species summary then, we know that it is possible to do this hydroperoxo synthesis, these hydroperoxo species. These hydroperoxo species are capable of cleaving the carbon carbon bond or in the carbon nitrogen bond or of different type of bond, hydroxylation process can be possible to then put this whole thing in the perspective both the enzyme. PHM and DBM are still very much in the picture in terms of understanding them in greater detail.

Synthetic studied so, far suggests that it is perhaps both superoxo and hydroperoxo are capable of doing the substrate hydroxylation chemistry in PHM and DBM, but nonetheless I think more and more people started believing that, it is the copper superoxo species. Just like what we see in the iron cases these copper superoxo species, sorry copper high valent oxo species is going to be the real active site ok.

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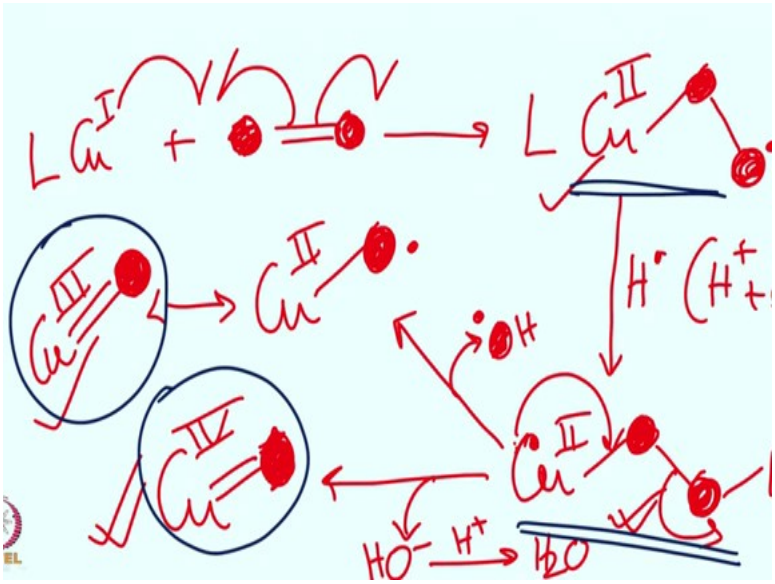

So, in case of copper I one can think of reacting it with oxygen that is what we have seen right. So, it can be reacting of course, there is a ligand phrase and depending upon the ligand geometry will change, but the chemistry will remain similar. So, you will get ligand copper II O dot right. So, this is ligand copper II O dot.

So, that is how the homolytic cleavage if you are following and then from there on you can you can add an H dot which is nothing, but H plus plus electron to give you copper II OOH right. Now, this copper II OOH can be further transformed into giving you copper III O H or copper IV OH.

So, for example, if this is the mode so you can form copper IV oxo. So, far of course, this also has been proposed as one of the reactive intermediate, but never really I have any proof so far in the literature, but what is also possible that you can have a homolytic cleavage to give you Cu II O dot right giving rise to along with O H dot formation right.

So, that is possible in this case what you are having is HO minus out. So, which can be protonated to give you water species. So, that is putative, but of course, no direct evidence exists for this. Now, this Cu 2 O dot is nothing, but your Cu III O this is the cupryl these people believes that that one of the important intermediate in the whole process right.

So, overall as you can see this Cu 2 O dot or Cu 2 OO dot can react with H atom come over here. Of course, this hydroperoxo then can react to give you give you the species alternatively, it can form copper II O dot to give you copper III oxyle or cupryl species both of them are high valent cupryl species and these are quite phenomenal species people believe that this is what exactly is happening, in in case of the PHM and DBM. This still remain debate, which is the active species, whether this is the species or this is the species or even this is the species.

Even some people end of suggesting these at the species right. All these species can be further debated, but again in absence of the suitable you know experimental studies, we can keep thinking that these are the all possible intermediate that is responsible for the chemistry right.

Also, you should know that there are similarities between these species and that is these can form quite exciting intermediate themselves for instance, if you are looking at the iron chemistry, iron chemistry and copper chemistry can also go parallel, that I will discussed in the next class.

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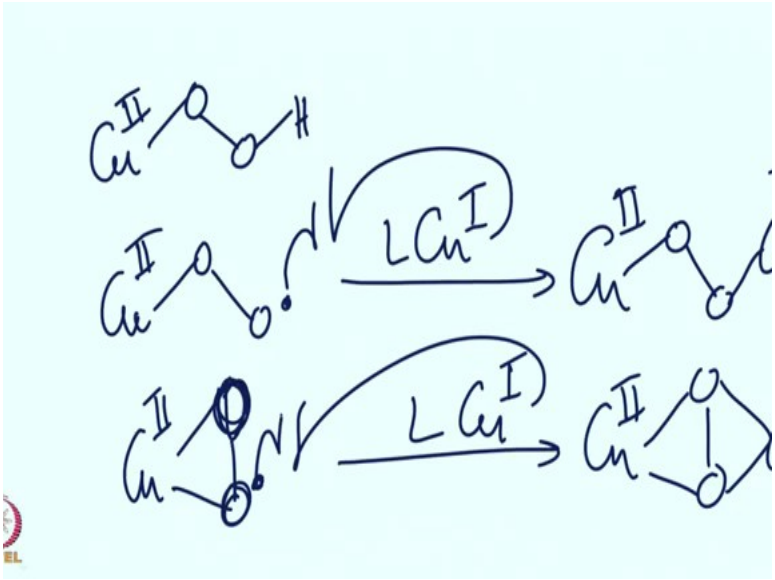

But, let me let me try to say that this copper II OO H the species that we were discussing earlier or even copper II OO superoxo species, these are then can react with copper I, ligand copper I to give you these copper II OO. So, this can give you one electron and that can give you another electron to give you the peroxo, if this superoxo is side on

bound in nature. So, once again is the same, this is one oxygen sorry for the drawing. And this again can give another electron into the or into the mixture to give you copper II OO whole thing copper II right. That is how the chemistry is happening and overall this chemistry can go on.

Now, I hope you have seen that copper oxygen chemistry is really exciting right. These are mono nuclear chemistry, these are dinuclear chemistry. And, one thing we did not discuss there are multi nucleus chemistry as well we have discussed earlier right. There are multicopper oxidases, which can even convert your oxygen to water much like what you have seen in the case of cytochrome C oxidase right.

So, there are mono nuclear chemistry, di nuclear chemistry, and multinuclear copper chemistry, which can you know kind of parallel to what we know in the iron chemistry ok. We will see the summary of the iron chemistry soon keep studying and good luck with the exam which will be soon.

Thank you very much.