Metals in Biology

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

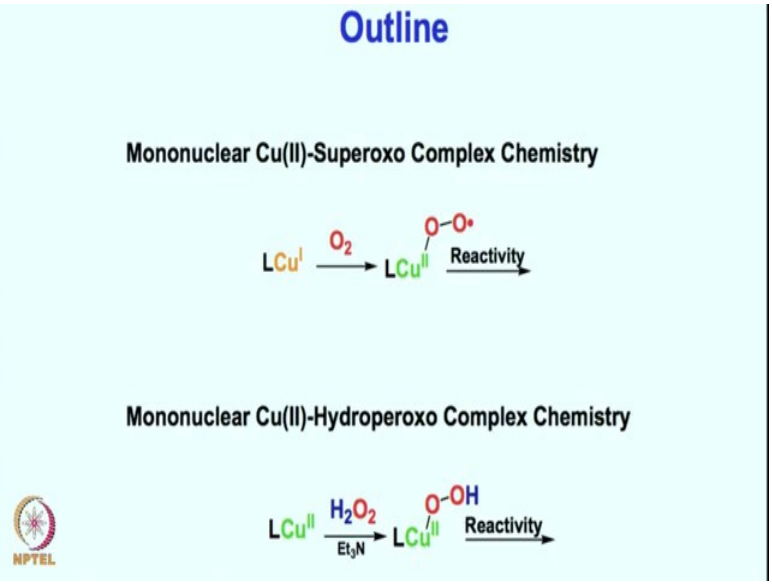
Lecture – 14
Copper - Oxygen chemistry - Part I Mononuclear copper - oxygen

Hello, welcome back to Metals in Biology. We have seen how efficiently metals in biology can control the reactivity pattern of the reactive intermediates. By utilizing these reactive intermediates, we have seen tremendous amount of interesting synthetic transformation can be carried out by these organometallic or the bio inorganic complexes, which are having the metal center at the core. Today we will see the copper oxygen chemistry more precisely mononuclear copper oxygen chemistry. How when you have one copper center and one oxygen molecule is reacting with it the species formed, and then the reactivity pattern of such species.

As you have seen earlier that reacting copper or any other metals such as iron manganese reacting them with oxygen often leads to the dinuclear, trinuclear or even tetra nuclear species. Stabilizing a mono nuclear intermediate by reacting with small molecule oxygen is really challenging because, usually the first formed intermediate will always react with another equivalent of metal center. So, essentially no matter what happens when you have a ligand copper I complex or ligand iron II complex; these complexes will react with oxygen and these reactions are extremely fast.

Right after reacting with oxygen the first firm intermediate will be so, much reactive that it will react with another equivalent of the reduced metal. Therefore, usually we end up getting a binuclear complex, but stabilizing a mono nuclear complex is always challenging we will try to discuss that today let us say.

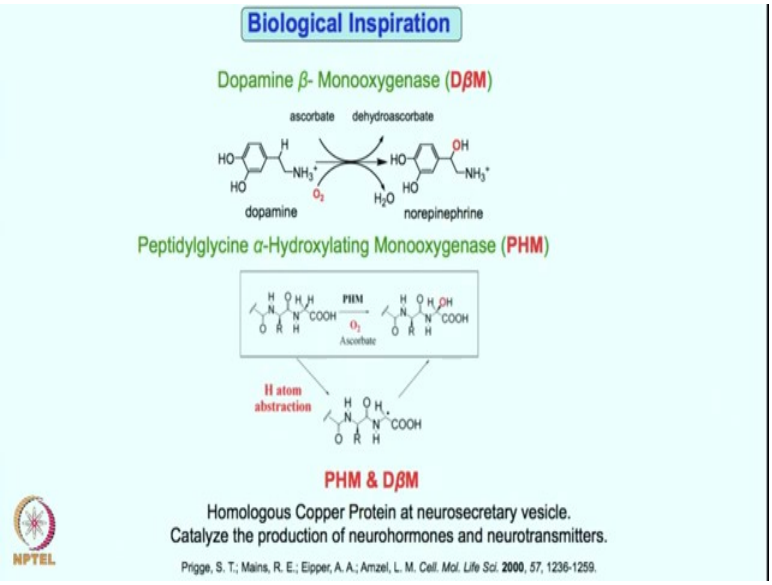
(Refer Slide Time: 02:45)



So, the outline of today's lecture is going to be on mono nuclear copper superoxo complex chemistry, where one ligand copper complex is reacting with one oxygen to give ligand copper superoxo species and how these superoxo species will react with organic substrate. The second topic would be the reaction of a ligand copper II complex with hydrogen peroxide in presence of base to give the ligand copper hydroperoxo species, and what the reactivity pattern of such hydroperoxo species be. Well why we are interested in such mononuclear copper oxygen intermediate.

This is precisely because of the fact that there are number of enzyme where such mononuclear copper oxygen species have been implicated and the key intermediate for doing the or carrying out the enzyme activity. So, in nature in biological system, we have both propositions for a copper superoxo as well as copper hydro peroxo intermediate for doing substrate hydroxylation chemistry, let us try to look at the you know enzymatic relevance of such species.

(Refer Slide Time: 04:13)



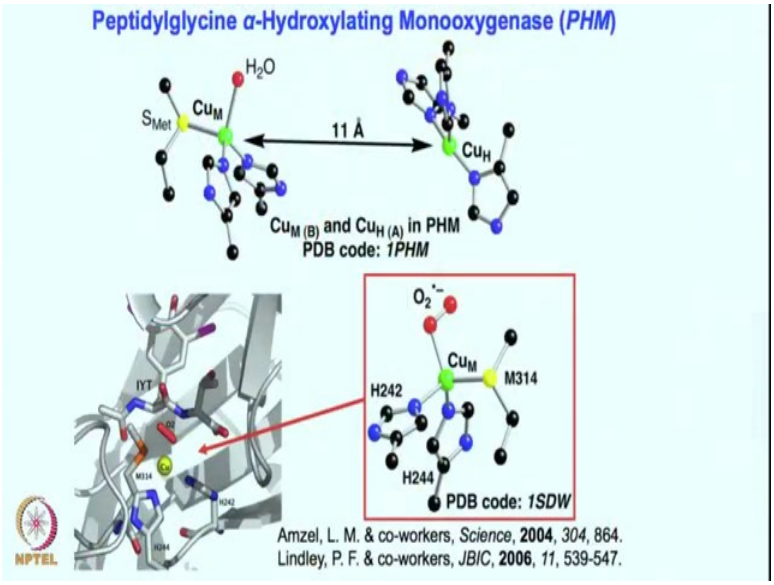
So, the biological inspiration for studying such mononuclear, copper oxygen species comes from the fact that very important enzymes such as dopamine beta monooxygenase and peptidylglycine alpha hydroxylating monooxygenase PHM both DBM and PHM are having a mononuclear copper oxygen intermediate which can react with its corresponding substrate to give the substrate hydroxylation product.

For the case of DBM it is the dopamine as the substrate that is reacting with the copper oxygen species to give the substrate hydroxylation product in the in the overall process. The dopamine is called converted into norepinephrine. In case of PHM it is the C terminus backbone of the protein residue which is getting hydroxylated selectively by using the active copper oxygen species. Now these both the PHM and DBM are homologous copper protein. So, what is true for one enzyme is going to be true for the other one and they are resided in the neurosecretory vesicle.

By catalyzing this hydroxylation process they actually help in production of neurohormone and neurotransmitter. So, if these processes are not happening or if this hydroxylation chemistry are not happening, there is going to be physiological consequences for this non activity. So, we wanted of course, you know it is a very very important procedure for converting substrate into substrate hydroxylated product by using copper oxygen chemistry and therefore, it is natural to try to understand this

method in greater detail to gain into the to gain the insights into these processes a lot of studies has been done so far.

(Refer Slide Time: 06:35)



This is the crystal structure of the peptidyl alpha hydroxylating monooxygenase; all as I was saying that there is a mononuclear copper oxygen intermediate, you might be surprised to learn that it is not a mono nuclear active site it is having two copper centers. But quite interesting with these two copper centers are separated from each other by 11 angstrom. Indeed this copper H side which is a t shaped copper center does not participate in to the oxygen activation; it is acting just as the electron transfer center. So, it can provide one electron during the copper oxygen chemistry. The main chemistry that is actually happening at the copper M center where it is ligated with 2 histidine and 1 methylene unit of course, a water molecule is there which is a labile center.

Now, this copper B or also known as copper M, this is the same center copper B and or copper M this center will react with oxygen to give a reactive copper oxygen intermediate. Now, from this copper center this copper H is once again separated by 11 angstrom they are not coupled with each other during copper oxygen reactivity. So, this is just going to be a spectator almost a spectator for the copper oxygen chemistry, but as I mentioned this is going to participate indirectly by providing one electron during the complete catalytic cycle of this PHM activity.

So, similar structure is true for DBM once again there is 2 copper center copper M and copper H also known as copper B and copper A this is the site which is going to be the active site or the copper oxygen reaction center. Quite interestingly you see that there is a methionine binding one can assume that this sulfur is going to be very reactive towards the reactive copper oxygen species and therefore, may also get oxidized to something like sulfoxide or sulfone.

But nature has designed in such a way so, that still the selective hydroxylation of the substrate can be carried out by this copper center. A number of attempts has been made to get the crystal structure of such species, this is the reduced form of the species which is also interesting, but most interesting is the one where oxygen is reacted with the copper center. After long deliberation and decades of effort researchers were able to crystallize this intermediate, where clearly this copper M center is bound with oxygen.

Now, this oxygen is turning out to be a super oxide species; that means, this copper center is now a copper II and oxygen unit is reduced by 1 electron to give the superoxo species. Just to remind you in the reduced form both the copper centers are in + 1 oxidation state. While, it reacted with oxygen then the oxygen gets reduced to superoxo and copper gets oxidized to copper II + therefore, this is a copper 2 superoxo species. As you see that only one of the oxygen atom of this copper oxygen moiety is bound with copper the other oxygen atom really is not bound with copper.

So, this sort of geometry is called the end on geometry, only one of the end is bound with one of the metal the other oxygen atom is not bound with this metal. So, this is an end on copper II to superoxo species end on copper to superoxo species is being formed and it is clearly demonstrated by the X-ray crystallography. Now does it mean then this copper superoxo species is the reactive species in the PHM and DBM substrate hydra hydroxylation chemistry?

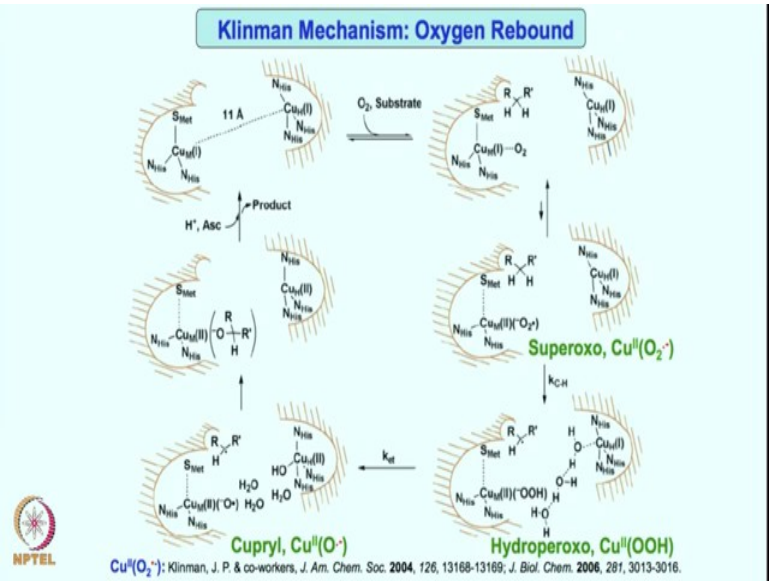
Well that could only be you know concluded after much deliberation, but the debate is still on whether it is really the copper oxygen species, which we are showing over here the end on copper superoxo species is the active species for such chemistry. Now, therefore, let us try to understand the long standing debate here, let us go back to the PHM and DBM here we see that substrate is getting hydroxylated both the cases substrate is getting hydroxylated right.

So, its been over decades, there has been deliberation, there has been you know controversies among the scientist or in the literature that what is the real active species that is doing the chemistry. Some believes that it is the copper superoxo species if we go back at the last slide. So, this is the copper superoxo species that is the species we are trying to discuss some other believes that this is the copper hydro peroxo species which can be formed upon hydrogen atom abstraction from the organic substrate by this copper superoxo species to form this intermediate.

So, the debate is essentially focused on whether this copper superoxo species is the reactive intermediate or copper hydro peroxo intermediate is the reactive intermediate because both are likely to be formed in the PHM and DBM. Although crystal structure now, is known for these enzymes with copper oxygen bound, clearly showing that it is a copper superoxo species that is doing that is forming. But, still it cannot rule out clearly the possibility of formation of a copper hydroperoxo species originating from the copper superoxo species.

Therefore, I will come back to this debate in a moment once more let us go on. So, this is the copper superoxo species and there has been proposal by various research group, that this is the species which is responsible for the substrate hydroxylation chemistry. Let us look at the mechanism by which this copper superoxo species perhaps can react with the organic substrate to give the substrate hydroxylation chemistry.

(Refer Slide Time: 13:34)



Well this is a mechanism proposed by Judith Klinmans group, where and from university of California Berkeley, where Klinmans group suggested although they have previously suggested a copper hydroperoxo species in this work and subsequent work they have suggested that this is the copper superoxo species that is responsible for the substrate hydroxylation chemistry in both PHM and DBM. Now let us look at very simplified manner. So, this is 2 copper center that we have say seen in the last slide if just to remind you once again, here is the copper M here is the copper H or copper B sorry copper M or copper B copper H and copper A is the same site. So, copper M and copper H copper B and copper A these are the nomenclature in the literature we need to get familiarized with ok.

So, now, you have copper M and copper H separated by 11 angstrom 2 histidine 1 methylene is ligated with copper and this is the copper H which is having a T shaped geometry 2 histidine over here sorry 2 histidine over here another histidine over here. So, overall this is the active site it is a dinuclear copper centers which are in the or dicopper center not the di dinuclear say dicopper centers which are present this is the reactive site as you see that oxygen will come in and will reside very close to this copper M center and will bind with or bind with this copper center the substrate is residing right next to the active site.

Which is quite interesting as you see the substrate binding site is right over here extremely close to this copper M site. This substrate binding and this copper center is not very very close to each other, this also once again indicates that this is the real active site where the chemistry is going to happen in any case. So, upon binding with or binding with copper oxygen copper ox copper with oxygen so, we subsequently will get a copper superoxo intermediate so, this is a still not a great reaction the you know the intermediate is really in equilibrium, where equilibrium is mostly towards the copper oxygen in the it copper oxygen bound intermediate.

So, here what is happening from copper M to oxygen, this is a copper 2 superoxo species is forming one of the electron for copper M is getting transferred on the oxygen to form the copper 2 superoxo species. Still the substrate is sitting very close to this active site 2 histidine 1 methionine and you have a copper superoxo species so, right over here.

Now, from there on what happens the superoxo species can abstract hydrogen atom from this organic substrate to give the hydroperoxo species wow. So, as you can see that copper superoxo is right over there and copper hydroperoxo is right over there. As you have drawn over here by this mechanism this is the copper superoxo species which is abstracting the hydrogen atom which is undoubtedly the key step for the substrate hydroxylation chemistry.

As you can see the substrate is now forming a radical and the copper II superoxo species has been converted to copper hydro peroxo. There is a extended hydrogen bonding network with water where this you will see that it over all this hydroxo can be transferred back to the copper H as in the form of hydro copper II hydroxide ok. Now, as you can see this radical and copper 2 hydroperoxo sitting very close to each other there is going to be an electron transfer in the next step where this copper I + oxidation state of the copper H will provide one electron to this intermediate overall will help you break the oxygen oxygen bond of this copper II hydro peroxide species.

The hydroxo radical that generates over there gets converted to hydroxide by accepting this 1 electron from the second copper center, this hydroxide gets relayed to the copper II center now at the copper H. So, copper it was copper I plus center gives up 1 electron. So, it becomes copper II + that 1 electron is picked up by the hydroxy radical from the copper II hydro peroxo species, this hydroxy radical is now converted to hydroxide and they bind with each other to form the copper II hydro hydroxy intermediate.

Now, as you see what it has happened in the process also that we are now able to generate yet another interesting intermediate, which remained elusive so far in the literature in terms of full characterization and that is that cupryl intermediate. So, called copper II O dot or copper III oxo species now that is quite exciting ok. Now, this copper III oxo is copper II O dot or copper III oxo is then going to react with the substrate radical to give the copper II alkoxy intermediate ok. Overall then we are going to get copper II alkoxy intermediate which is now you can see upon protonation it can give the substrate hydroxylation product and rest of the catalytic cycle can be completed upon reduction from ascorbate to give the copper I and copper I cycle.

So, what you have seen over here essentially the 2 copper centers separated by 11 angstrom from each other and only one of them is reactive the other one is almost a

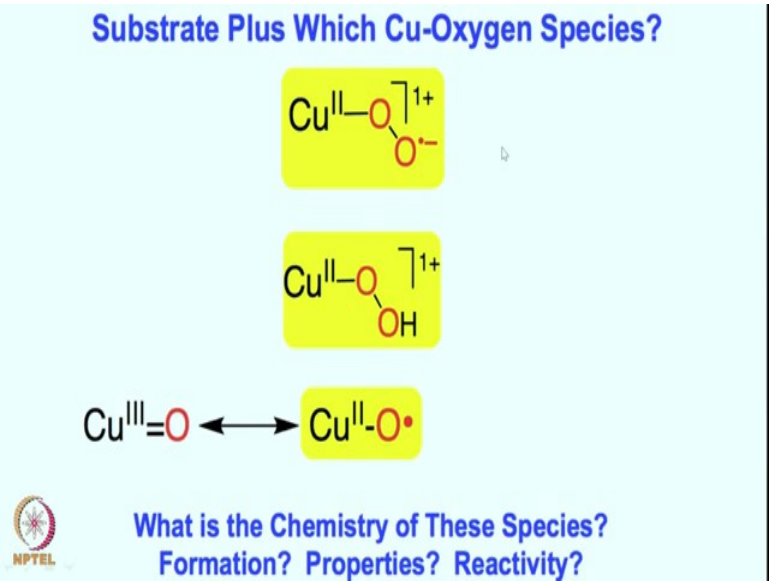
spectator of course, not truly a spectator, but almost a spectator where it participates in electron transfer process during the oxygen oxygen bond cleavage of the copper hydro peroxo intermediate. Now, in principle over this mechanism we can see that the copper M center which is also known as copper B center will react with oxygen to give first the copper oxygen bound addict subsequently it gives you the copper superoxo intermediate.

And then it abstract hydrogen atom to give the copper II hydro peroxo intermediate, which then undergo a homolytic copper oxygen-oxygen bond cleavage to give the hydroxy radical and then that hydroxyl radical upon accepting 1 electron from the copper gets transferred to the copper II center copper II center to give the copper hydroxy, intermediate and this oxo radical actually gets transferred to the substrate radical intermediate which were generated by hydrogen atom abstraction of the substrate. Now this recombination or rebound overall happens to give the copper II alkoxide intermediate reach upon protonation can give the product.

This is quite fascinating and quite interesting intermediate but the only problem here is, one can draw a mechanism almost by changing a very little thing which I will come in subsequent slides, where you will see that even a copper hydroperoxo can be formed without abstracting hydrogen atom by copper superoxo. That means, one electron transfer from the copper H center and a protonation can also give this hydroperoxo intermediate and it has also been proposed by quite a few group, that this copper hydroperoxo is that real intermediate that is doing the chemistry.

Let us not get into too much of that we will come back to that in a in subsequent slides let us move on ok. So, I hope what I am trying to tell you over here is, although crystal structure of a copper superoxo intermediate is reported or known now still the debate is on, in case of this very important PHM and DBM enzyme what is exactly the real intermediate, that is responsible for the substrate hydroxylation chemistry.

(Refer Slide Time: 22:56)



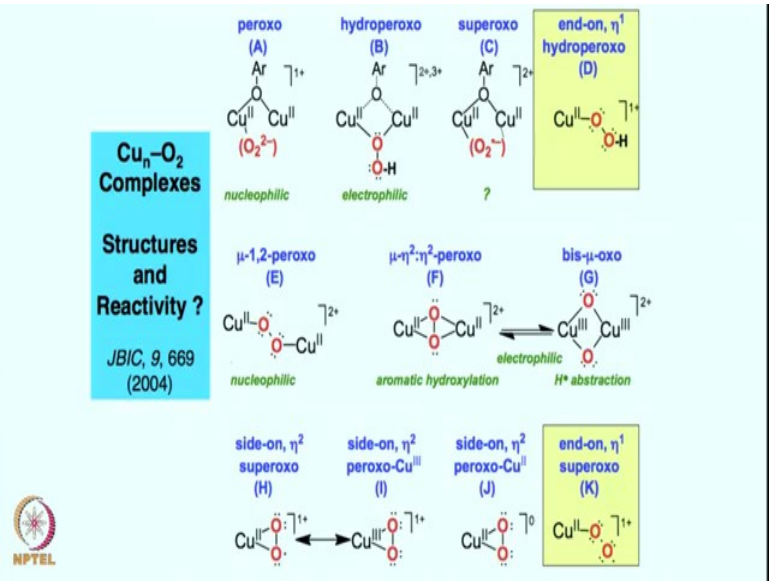
For instance as I have discussed very briefly that copper superoxo species can be the active species, we have seen the mechanism proposed by few groups. Now, it is the same group which were previously proposing that copper superoxo is the real active intermediate, they were like Klinman groups were previously proposing also that the copper hydro peroxo is the real intermediate for these chemistry we will come back to that again.

Well, yet a series of studies also suggested that it is neither of the copper superoxo or copper hydro peroxo that is doing the chemistry, but it is the copper superoxo and hydro peroxo generated intermediate which can be a cupryl is really the true intermediate and therefore, responsible for substrate hydroxylation chemistry. Or one of the thing is really this intermediate so far even in synthetic setup is not crystallographically characterized or the characterization of such intermediate so, far is not that great, and since no synthetic chemistry is known so, far to give reliably these species and the reactivity pattern of these.

So, our studies or our discussion will mainly focus on these two. Once again this is not too much known even in synthetically I mean of course, not too much known in terms of enzyme also. But, that does not rule out these as a reactive intermediate this still pretty much stay as the reactive intermediate, and we will mainly try to focus on the first two intermediate and that is copper superoxo and copper hydroperoxo.

Well we would like to see briefly how we can synthesize these complexes and what is their reactivity pattern of course, what are their properties and so on briefly we will try to mention. So, today for the remaining part of the lecture we will try to see these copper superoxo species formation and how it is reacting ok. Copper superoxo, copper hydroperoxo, cupryl or CO2 O dot.

(Refer Slide Time: 25:05)



Well before getting into this I think we must understand that this chemistry is going to be quite sensitive. As you have seen briefly earlier that whenever a ligand copper complex is reacted with oxygen, there is this thermodynamically prefer down nuclear intermediate formation. So, it is very difficult to stabilize a mononuclear although this is the kinetically first formed intermediate.

But still a mononuclear intermediate such as these are usually very difficult to stabilize in the mono nuclear form, because they tend to dimerize. So, this is the species you have seen that crystallographically characterized in enzyme, but getting such intermediate in synthetic setup, where many copper centers are available is very difficult. Because once such intermediate is forming, another copper I center can react with this to give any of dinuclear intermediate over there. Well, it is a great challenge to stabilize such mono nuclear copper oxygen intermediate. Ok.

Now, one thing that can of course, influence this intermediate is the ligand for this copper center right. Now this ligand for this copper center and in combination with the

strategy, solvent temperature all of these will have effect in stabilizing such a mononuclear intermediate where this thermodynamically prefer dinuclear intermediate formation can be prevented ok. So, those chemistry I will be discussing in the next class, let me sum off by saying that today we have discussed although not in great detail so, far will come again and that is this debate ongoing debate in the literature that whether copper superoxo, copper hydro peroxo or cupryl is the reactive intermediate.

You have seen these are the three species that are in debate. We have discussed the reaction mechanism involving copper superoxo as the real active species for both PHM and DBM enzyme. It perhaps can be thought of that this copper superoxo is the real intermediate, but the debate is still on a lot of groups are proposing that either this cupryl or copper hydro peroxo can also be the reactive species, which is responsible for the substrate hydroxylation chemistry that we have seen in PHM and DBM.

Will we discuss on how to synthesize these mono nuclear species and their reactivity pattern in the next class; the challenge essentially is the stabilizing such mono nuclear intermediate, how one can think of stabilizing such super reactive mono nuclear intermediate where really thermodynamically preferred product is the di nuclear one. Will come back to that, keep studying some of the literature references are cited you know feel free to read from any of the book or any of the literature that we have cited.

See you soon in the next class discussing about the reactivity pattern of the copper superoxo and hydroperoxo species most importantly how to prevent the dinuclear species formation and form the this mono nuclear species with that.

Thank you very much, see you soon.