Metals in Biology
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Lecture – 15
Copper - Oxygen chemistry - Part II Cu – O2 complexes

Hello welcome back to Metals in Biology and the world of Copper Oxygen chemistry. In the last class we were trying to discuss the relevance of copper oxygen chemistry in biology of course, these species can be synthesized in the laboratory pretty easily, but many problem exist in synthesizing these complexes in laboratory. One of the problem that we were trying to discuss while attempting to synthesize these complexes in laboratory is the thermodynamic favored dinuclear or multi nuclear species formation.

Of course, in enzyme such as PHM and DBM only a particular species is forming and that is reacting with organic substrate to give the substrate hydroxylation product but to be sure and to learn about this chemistry in greater detail one must understand the inorganic chemistry from a research perspective from labs perspective. Of course, the chemistry is not going to be different compared to what is known or what can be done in laboratory versus the enzyme because that is the beauty of doing chemistry that irrespective of biological setup or in synthetic setup the chemistry is going to be the same.

But understanding too much in biological setup is also little problematic because this is biological system in dealing with enzymes and getting the information in that possible minute possible details are not that easy in case of the biologist. This is where the chemist perhaps come into the picture quite importantly they can study these intermediate at laboratory and try to see if any conclusion that can be drawn from the synthetic laboratory can also be applied for the biological system. Let us look at the copper oxygen chemistry from an inorganic perspective and then try to understand what might be going on in biology.

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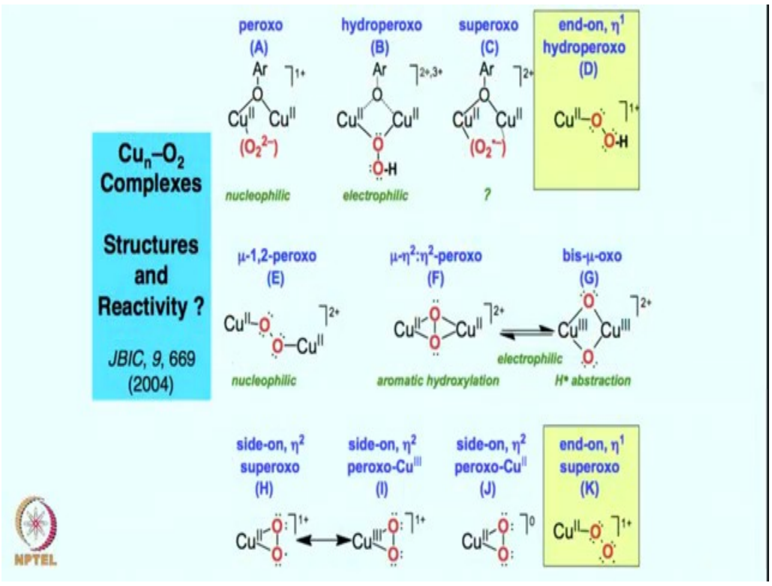

Cu_n–O_2 Complexes

Structures and Reactivity ?

JBIC, 9, 669 (2004)

Well let us go back to this slide as we were discussing in the last class, if anyone is trying to react in laboratory a ligand copper I complex with oxygen this is one of the preferred intermediate that can form it could be end on superoxo or it could be a side on superoxo. Well of course, there are other mono nuclear species can also be formed, but one of these mononuclear intermediate will be formed first let us say this one is forming and this intermediate can then usually be reacting with the second copper I center that copper I center will transfer one more electron to the superoxo unit to give a dinuclear copper II in this case endon peroxo intermediate.

So, what we have just tried to say is ligand copper I reacts with oxygen to give a ligand copper II superoxo intermediate with a suitable ligand it could be end on superoxo or it could be a side on superoxo. Once again the ligand will control the geometry around this copper center and how these copper superoxo might will look like. In addition to the ligand of course, solvent temperature and other affect can also come into the picture in providing one of those intermediate in clean and clear for pattern.

But once this intermediate is formed immediately these are very very fast reaction as you will see in subsequent slide immediately this copper intermediate can react with another ligand copper complex which is there in solution in synthetic set up to give the peroxo intermediates such as these or the bismuoxo over here. Such a problem of reacting one copper center with yet another one is not really there in biology because biological sense you see that, even there are two copper centers they are so much separated from each other they cannot react with each other, right.

So, in biology everything is perfected and everything is well placed, but in synthetic setup if you want to study it’s going to be much more difficult than the biological system and this is where not only mononuclear copper superoxo chemistry we need to worry about because those are in PHM and DBM once needs to really understand what happens or what are the all possibilities out there.

So, this intermediate then can react with another copper to give an end on peroxo intermediate or yes of course, end on peroxo intermediate for the ligand which are usually tetradentate in nature. So, if this is a four coordination on copper center this is likely going to be an end on peroxo intermediate. As you have seen earlier that if this copper center is a tridentate ligand containing one this will give rise to the side on peroxo intermediate.

And well these are the usual trend this is a tetradentate ligand system, this is a tridented or bidented ligand system this is also bidented and tridentate ligand system these two species are in equilibrium with each other. If this oxygen oxygen bond is completely broken, then it is a copper III; copper III peroxo intermediate this is bismuoxo sorry this is not a peroxo this is a bismuoxo intermediate this is copper III plus this is a copper III plus in both these cases these are copper II plus and copper II plus after cleaving the oxygen oxygen bond one can get these intermediate.

These species can also be in equilibrium with each other. Of course, there could be a number of other species that is forming that includes these different other species, but more importantly there is also a possibility of formation of copper II hydroperoxo upon abstracting hydrogen atom by this copper superoxo intermediate. So, one proton one electron that is hydrogen atom or hydrogen radical H dot can gives rise to the copper II hydro peroxo species, these are the two species we are going to mainly look at.

This reactivity pattern of these dinuclear species are quite well understood, it is turning out to be the nucleophilic in nature this is turning out to be the electrophilic in nature both of them are quite interesting and their properties are quite; quite; quite similar and these both these intermediates are found or implicated in number of other enzymes where substrate hydroxylation or related oxy oxidation or oxygenation chemistry are involved, we will come to that on a different context.

So, today we are trying to see how these copper superoxo is forming in synthetic setup and what is the reactivity pattern of this intermediate. Also you are trying to see how this intermediate is forming and what is the reactivity pattern of this intermediate both these highlighted intermediate are the one which has been suggested in PHM and DBM cases. The two very important mononuclear active copper oxygen intermediate containing enzyme which is really kind of catalyzing the very important substrate hydroxylation chemistry.

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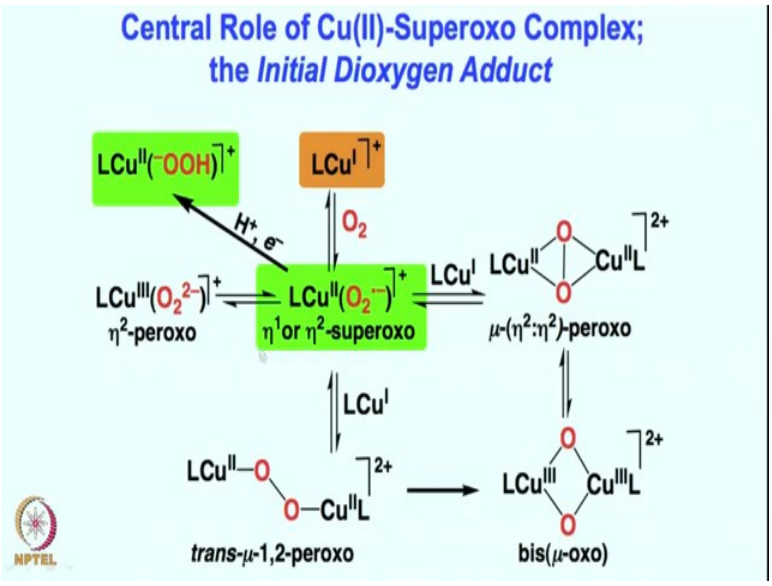

Central Role of Cu(II)-Superoxo Complex; the *Initial Dioxygen Adduct*

Well, let us give an overview what we were trying to discuss in the earlier slide that ligand copper I will react with oxygen to give a fast form intermediate which is very very reactive intermediate that is ligand copper II peroxo intermediate sorry ligand copper II superoxo intermediate.

Now, this what has happened in the process that this copper I transfer one electron to the oxygen to give you ligand copper II and superoxo intermediate one electron comes from copper I it gets its it reduces the; it reduces the overall moiety to give overall oxygen moiety to give the superoxo species. Upon transferring one proton and one electron it is possible to form the copper II hydroperoxo intermediate from this copper superoxo species. Of course, one can think of also a inequilibrium A peroxo species can also be formed, if this superoxo intermediate is reacting with another copper I it can form ligand copper II peroxo intermediate.

So, dicopper peroxo intermediate also known as trans mu-1-2 peroxo intermediate it is forming. So, what is happening is one more electron from copper I is going into the system copper I becomes copper II superoxo becomes peroxo. Similarly, this is usually for tetradentate ligand of course, other ligand can also form, but predominantly tetradentate ligand is forming this short of intermediate. Now, this copper superoxo intermediate if the ligand is tridentate or bidentate then it can react with another bidentate or tridentate ligand to give a peroxo intermediate which is now side on bound.

So, as you see one of the electron from this copper will also be or can also be donated to this intermediate to form a copper II copper II peroxo intermediate, copper I gets oxidized to copper II superoxo once again gets reduced to peroxo. These are the binding motive as you can see both the copper centers are equidistant from this oxygen moiety, this oxygen moiety is peroxo just like what you have seen in here, but the ligand which is attached with the copper actually controls the geometry around the copper center, so that it is now peroxo in a side on fashion or peroxo in an end on fashion.

From this intermediate we can immediately generate or there could be an equilibrium where this oxygen oxygen bond can be cleaved and it can be called one more electron from each of the copper can go into the system to break this bond and then copper III copper III peroxo bismuoxo species can be generated. Well, it is also possible to generate bismuoxo species from there.

So, essentially what we are seeing that in synthetic setup if anyone is trying to do the ligand copper oxygen chemistry there is possibility of forming mean copper oxygen intermediate. Which copper oxygen intermediate will be generated will majorly depend on the dentisity of the ligand whether it is a bidentate, tridentate or tetradentate ligand.

Now, despite having a well defined ligand metal complex one still has to worry about whether it is the mono nuclear species or dinuclear species forming of course, all these intermediate are very reactive these are not truly stable intermediate these can only be stabilized at very very low temperature right. These intermediate can also then go on to react further to give the let us say for example, copper hydroxo intermediate, these all of these are reactive intermediate all of them are very reactive towards organic substrates.

But as we were discussing the major focus of our discussion today is the mononuclear copper oxygen species ok. Let’s try to generate them from this possibilities or sea of many compounds that can form, let us try to take one example where we can take a tetradentate ligand and try to see the copper oxygen chemistry.

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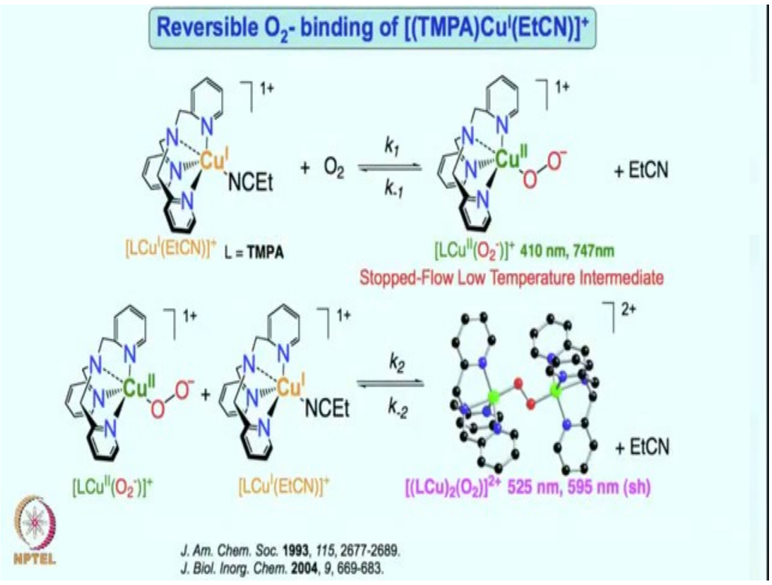

Reversible O_2- binding of $[(TMPA)Cu^I(EtCN)]^+$

J. Am. Chem. Soc. **1993**, *115*, 2677-2689.
J. Biol. Inorg. Chem. **2004**, *9*, 669-683.

Now, in order to understand this chemistry in synthetic set up these many different compounds been synthesized, this is one famous ligand copper complex known as copper TPA or copper TMPA ligand system, it can react as you can see this is a tetradentate ligand system copper is ligated it is a very stable and nice complex and very sensitive complex air and moisture sensitive, it can react with oxygen to give fast and intermediate which was not known what exactly it is at the beginning, we will come to that.

Now, this intermediate can immediately react with another equivalent of this to give an end on bound peroxo intermediate which was the first ever crystallographically characterized copper oxygen intermediate, so two copper center bound by this peroxo. Now, this is a thermodynamically prefer intermediate, but the first firm intermediate is this one for which not much was known what exactly it would be.

So, if one is reacting this one with oxygen at a very low temperature such as - 80 degree centigrade one can immediately see a very nice purple colour solution and that is this end on peroxo species where both the copper is in +2 oxidation state and the oxygen moiety is reduced doubly to form the peroxo and this is an end on peroxo as you can see one copper is bound with one oxygen only and the other oxygen is not bound with the same copper, but in this case another copper. So, this is end on peroxo species it has a very characteristic feature I mean very intense features around 525 nanometer of course.

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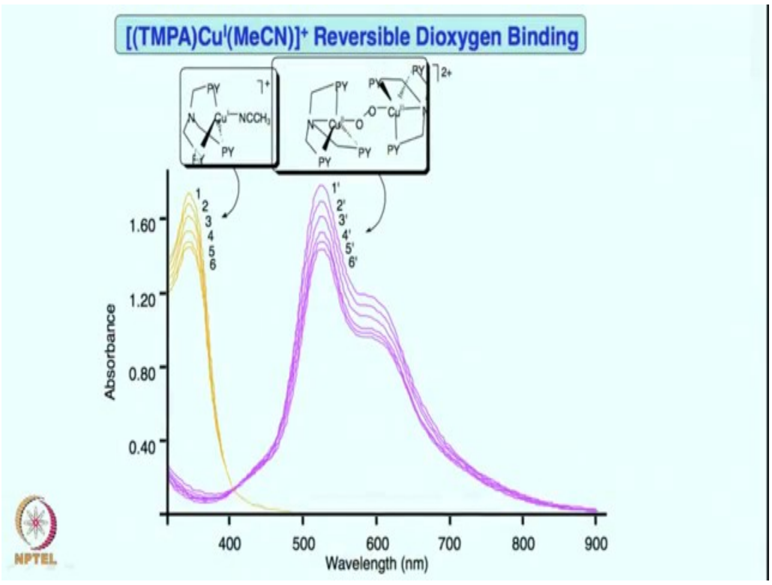

$[(TMPA)Cu^{I}(MeCN)]^{+}$ Reversible Dioxygen Binding

Now, if we are following this reaction by simple low temperature UV visible spectra what we all see is that disappearance of this ligand copper come one complex the first complex and that is disappearing and this band over here around 525 nanometer which is responsible for purple colour is forming. So, this is disappearing and this is forming at a very low temperature such as -80 or -40 degree centigrade, this can systematically be recorded, this is decomposition and this is the formation of these species.

Now, well this is not going to be that very simple to sees an intermediate in between this because this is already very cold temperature you can stabilize or slow down the reaction as much as possible. In order to study the first form intermediate that we were saying that there is an intermediate like this, but not much information was there what has been done is the they have carried out a stop flow kinetics measurement.

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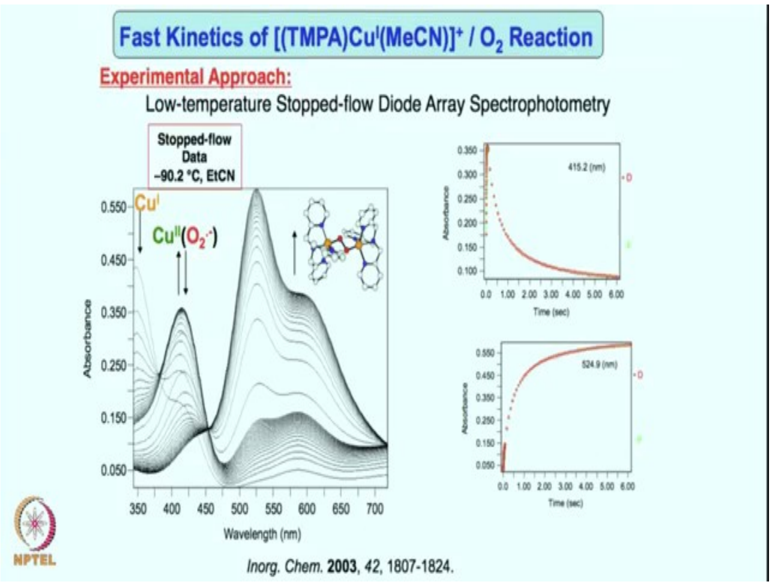

Fast Kinetics of $[(TMPA)Cu^I(MeCN)]^+ / O_2$ Reaction

Experimental Approach:

Low-temperature Stopped-flow Diode Array Spectrophotometry

Stopped-flow Data
−90.2 °C, EtCN

Inorg. Chem. **2003**, *42*, 1807-1824.

What it does is essentially it allows you to record the spectra at low temperature and immense within very short timeframe as you can see within you know within these 5 to 6 second these many spectra are recorded what that allowed you is to see the disappearance of copper I complex and formation of a new species which was not there at the low temperature UV vis but this top flow data clearly shows that there is a new intermediate forming immediately and then decomposing at the same time to give you subsequently this peroxo intermediate. So, a new intermediate appears all of a sudden when a very fast spectroscopic technique such as stop flow kinetics study with UV visible spectra was recorded.

So, this clearly indicates that there is an intermediate. So, these intermediates are so unstable and so reactive there is no chance of seeing them in naked eyes at room temperature. You do this reaction none of the superoxo or peroxo at room temperature one can see, but all these temperature studies low temperature studies and fancy spectroscopic studies allows us to really look into what is happening at a molecular level and at very fast timescale. As you have seen there for then there is an intermediate whether this is a really a copper superoxo intermediate or not that is not really known.

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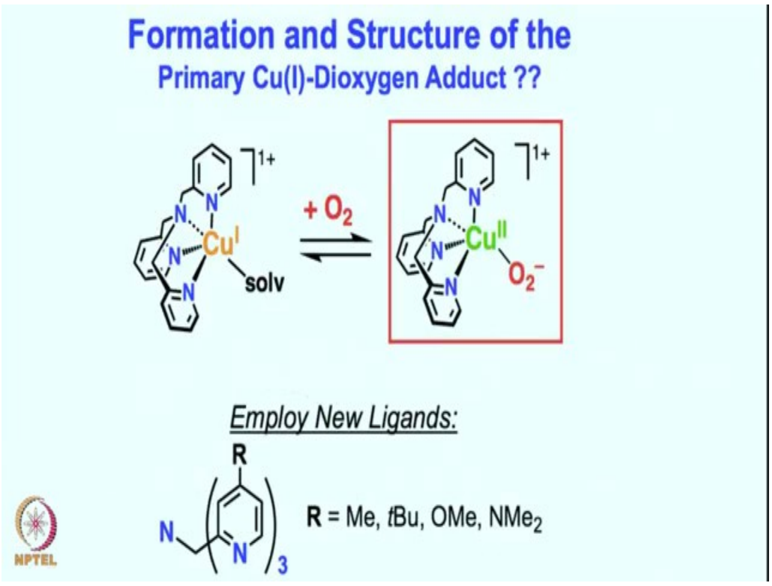

Formation and Structure of the Primary Cu(I)-Dioxygen Adduct ??

Employ New Ligands:

R = Me, *t*Bu, OMe, NMe$_2$

Well what was required is a quite an extensive study and suitable ligand designing temperature, solvent and optimization of different reaction condition and more importantly understanding of what goes on behind the scene when nothing can be seen.

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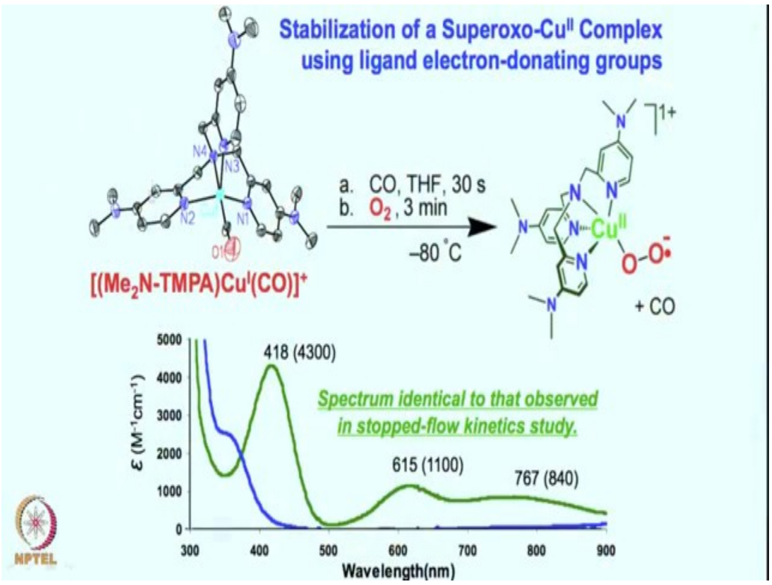

Stabilization of a Superoxo-Cu^{II} Complex using ligand electron-donating groups

[(Me$_2$N-TMPA)Cu^I(CO)]$^+$

a. CO, THF, 30 s
b. O$_2$, 3 min
−80 °C

Spectrum identical to that observed in stopped-flow kinetics study.

After subsequent many studies what is found that with a suitable ligand now, a derivative of the previous ligand one can stabilize somewhat at a very low temperature for some period this copper superoxo intermediate which is having a spectra at 420 or 410 nanometers region which is exactly what is found in the stop flow kinetics for another ligand. So, this is a different ligand, it allows you to see this intermediate rather clearly.

So, it was possible by using a suitable electron donating ligand to prevent the peroxo formation along with the suitable solvent, concentration and a strategy of cleaving CO in-situ was successful in giving such a intermediate.

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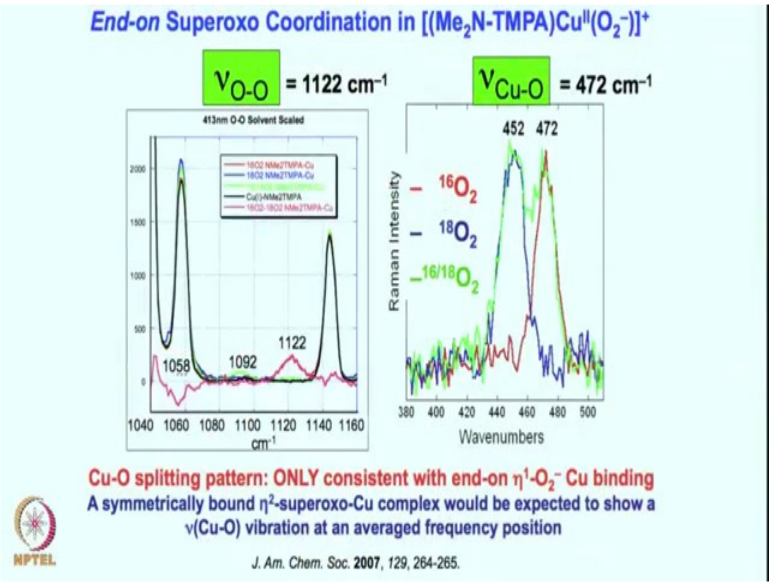

End-on Superoxo Coordination in $[(Me_2N\text{-}TMPA)Cu^{II}(O_2^-)]^+$

$\nu_{O\text{-}O} = 1122\ cm^{-1}$ $\nu_{Cu\text{-}O} = 472\ cm^{-1}$

Cu-O splitting pattern: ONLY consistent with end-on η^1-O_2^- Cu binding

A symmetrically bound η^2-superoxo-Cu complex would be expected to show a ν(Cu-O) vibration at an averaged frequency position

J. Am. Chem. Soc. **2007**, 129, 264-265.

Now, this was very clearly shown to be an end on superoxo intermediate where the oxygen oxygen stretch by resonance Raman is what is found to be exactly what is expected here 1122 wave number is shifted to 1058 wave number upon O18 labeling, copper oxygen’s band shifted from these 472 wave number to 452 wave number upon O 18 labeling.

Now, both these resonance Raman data are also consistent with the fact that there is superoxo further it was confirmed by a mixed labeled 16 18 oxygen where both copper oxygen peak are coming due to both 16 and 18 and more importantly the average out oxygen oxygen spec peak is coming around 1092 just in between 1058 and 1122 this is further suggesting that this is an end on bound species well.

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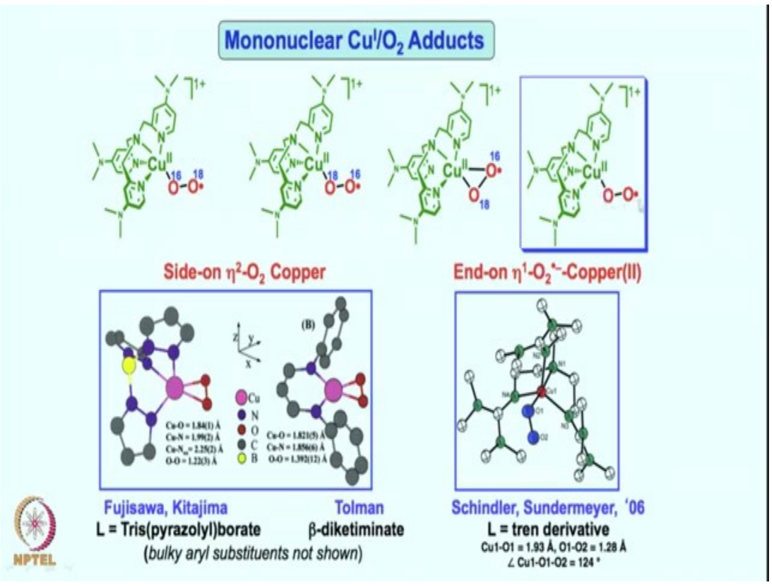

Mononuclear Cu^I/O_2 Adducts

Side-on η^2-O_2 Copper

End-on η^1-$O_2^{\bullet -}$-Copper(II)

Fujisawa, Kitajima

L = Tris(pyrazolyl)borate

Tolman

β-diketiminate

(*bulky aryl substituents not shown*)

Schindler, Sundermeyer, '06

L = tren derivative

Cu1-O1 = 1.93 Å, O1-O2 = 1.28 Å

∠ Cu1-O1-O2 = 124 °

Overall then what we have seen so far that this tetradentate ligand when bound with copper it forming an endon superoxo species this is now clearly shown by resonance Raman structure UV Visible studies that has been consistent with a lot of other studies that has been earlier done. With a bidentate ligand such as this beta diketiminate or tris pyrazolyl borate ligand these mononuclear copper oxygen species has also been crystallographically characterized and is standing out to be quite interesting.

And with another ligand such as this TMG 3 train which is a highly electron rich yet quite efficient ligand for such a mononuclear species formation it has also most importantly crystallographically characterized. Both this and that are the two really well characterized intermediate for copper oxygen inter copper oxygen studies, let us look at what happens to such mononuclear copper oxygen species.

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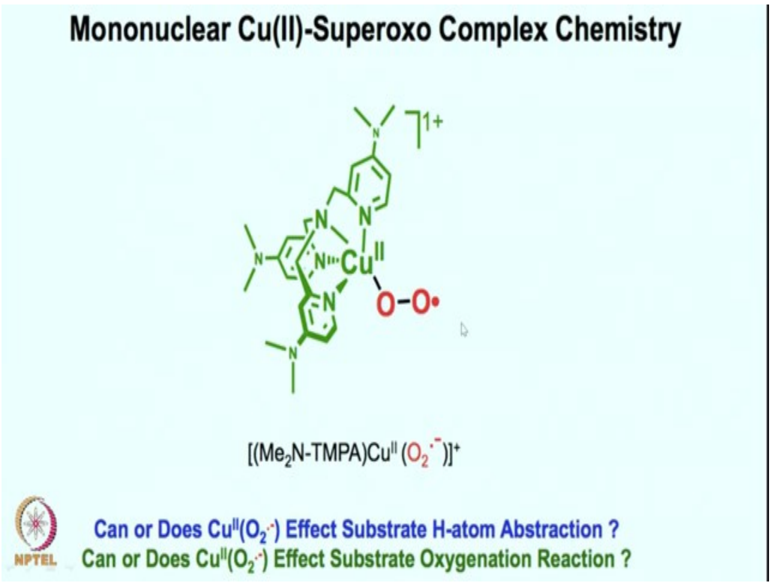

Mononuclear Cu(II)-Superoxo Complex Chemistry

$[(Me_2N\text{-}TMPA)Cu^{II}(O_2^{\cdot -})]^+$

Can or Does $Cu^{II}(O_2^{\cdot -})$ Effect Substrate H-atom Abstraction ?

Can or Does $Cu^{II}(O_2^{\cdot -})$ Effect Substrate Oxygenation Reaction ?

Well, what it is turning out to be the case that, this sort of copper superoxo species once generated it can abstract hydrogen atom from the suitable organic substrate and it can also promote the substrate oxygenation chemistry that is quite interesting. So, that has direct relevance to what happens in enzyme; in enzyme it has been proposed although there is crystal structure still it is not sure definitely not sure a reaction mechanism can always have its twist.

So, there can never be a definitive mechanism of any reaction, but quite interestingly what is turning out to be the case that it is possible to synthesize this copper superoxo reactive intermediate which are super super reactive intermediate in laboratory one can synthesize it. Once one can synthesize it can study the reactivity pattern of such intermediate and it is turning out to be the case that it is indeed capable of abstracting hydrogen atom from organic substrate. Of course, the organic substrate has to be the suitable one not every organic substrate it can abstract.

But more importantly it can also oxygenate the substrate just like what is seen in PHM and DBM right that is quite interestingly interesting. So, this studies with the synthetic laboratory studies shows that perhaps a copper II superoxo intermediate is the real intermediate in the PHM and DBM enzyme to carry out the copper oxygen oxygenation chemistry that is going on in the substrate. But still of course, there is debate will discuss that.

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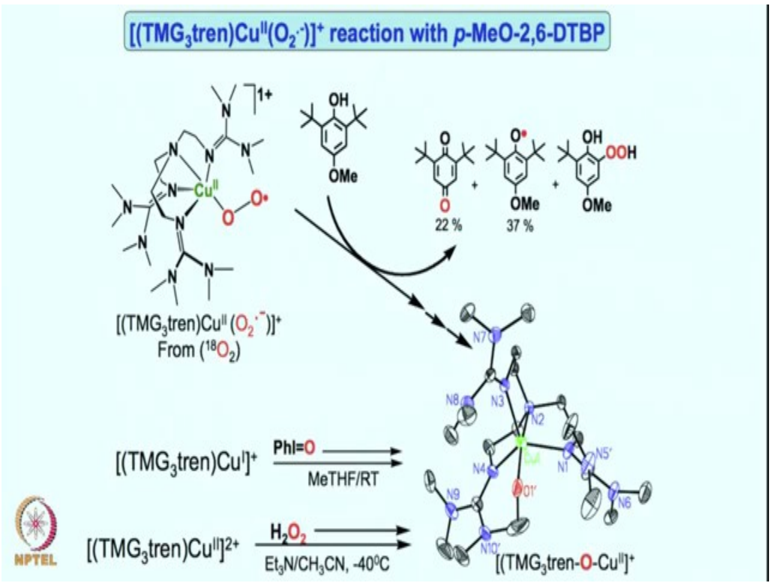

$[(TMG_3tren)Cu^{II}(O_2^{\cdot-})]^+$ reaction with *p*-MeO-2,6-DTBP

Let us look at one of the system little bit more carefully what has been done this is the other copper oxygen intermediate mononuclear one, a superoxo intermediate crystallographically characterized intermediate. Now, this superoxo abstract hydrogen atom from phenol to give the phenoxy radical of course, very other interesting oxygenation chemistry also occurs will not get into the too much detail of that, but more importantly what we also find that this ligand can be oxygenated.

So, this ligand can also be a substrate and can oxygen get oxygenated in the process, quite interestingly same intermediate can be formed by other method let us not discuss too much into those.

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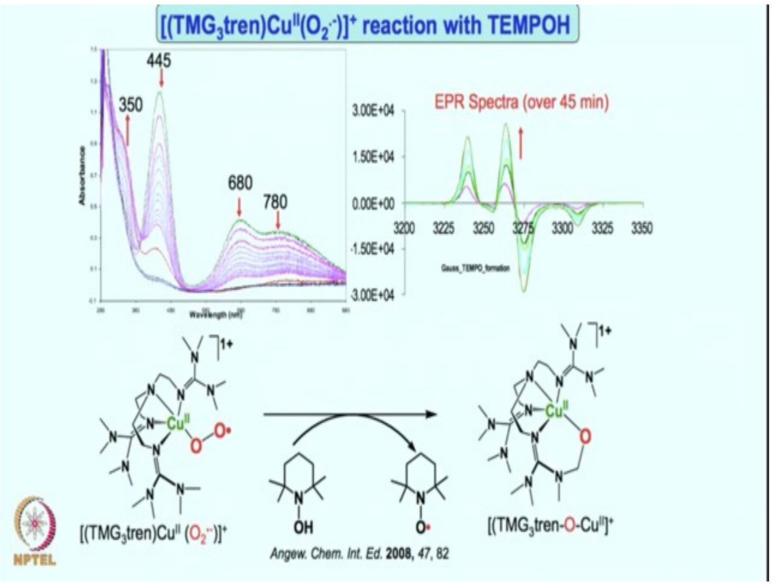

[(TMG$_3$tren)Cu^{II}(O$_2^{\cdot-}$)]$^+$ reaction with TEMPOH

Angew. Chem. Int. Ed. **2008**, 47, 82

This copper superoxo intermediate which is again not that many are known these are really price projection and these superoxo intermediate can react with something like Tempol very easily to abstract the hydrogen atom from it to give the Tempo radical as well as the; as well as the substrate hydroxylated product right these are quite interesting. One can follow the formation of these radical intermediate from Tempol to Tempo formation can be followed by EPR spectroscopy, it is it can also be formed it can also be followed by the disappearance of these species as well as appearance of some other a new intermediate over there.

So, what essentially we are trying to say is this copper superoxo intermediate now is capable of abstracting hydrogen atom. So, stabilizing this intermediate or synthesizing this intermediate, so that we can study at least for few minutes in some cases later really it can be stabilized further, but overall this reactive intermediate formation and ability to study has given an opportunity to react with organic substrate to give such an opportunity to have such an opportunity and can then compare the reactivity pattern of the synthetic intermediate with the enzymes intermediate are quite interesting.

This study shows that it is indeed perhaps possible that this copper superoxo intermediate that has been suggested earlier in the enzyme may be the true active species of course, the debate is still on. Let us look at how this is forming in the enzyme or in case of this organic substrate

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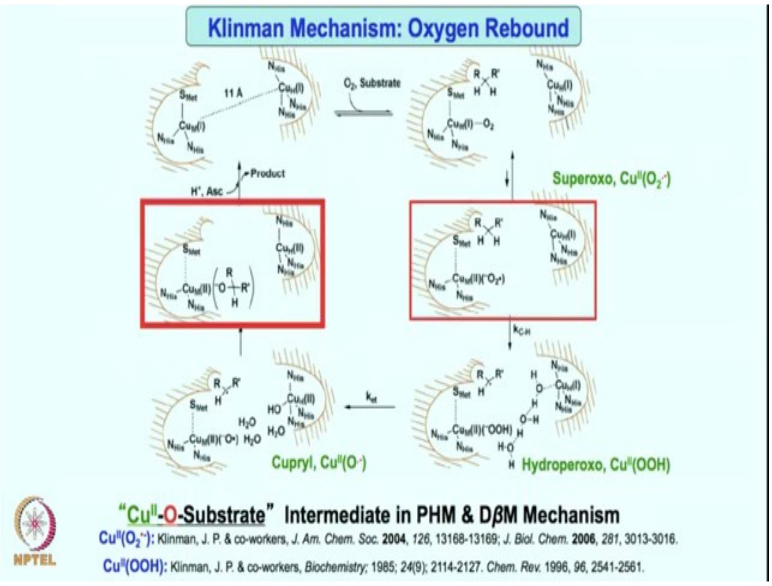
Klinman Mechanism: Oxygen Rebound

“Cu^{II}-O-Substrate” Intermediate in PHM & DβM Mechanism

$Cu^{II}(O_2^{\cdot-})$: Klinman, J. P. & co-workers, *J. Am. Chem. Soc.* **2004**, *126*, 13168-13169; *J. Biol. Chem.* **2006**, *281*, 3013-3016.

$Cu^{II}(OOH)$: Klinman, J. P. & co-workers, *Biochemistry*; 1985; *24*(9); 2114-2127. *Chem. Rev.* 1996, *96*, 2541-2561.

As you would remember we were discussing there is a copper alkoxo intermediate, so in these cases as you can see this copper alkoxo intermediate is looking something exactly similar to what is found in the enzyme. Let us come back to this in the subsequent class, so keep studying and we will see you soon.

Thank you.