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

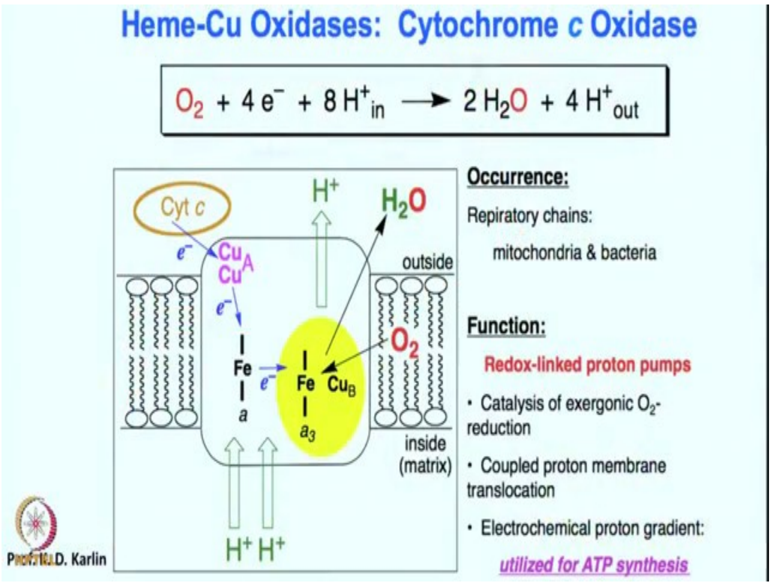
Lecture - 21
Cytochrome C oxidase

Hello, how are you all doing? Hope you are enjoying the class. Well, you need to study a little bit more I think you need to refresh your you know notes from time to time.

Today we will be discussing heme copper oxidases, as the name suggests there is one at least one heme center and a copper center. The enzyme that we are going to discuss is Cytochrome C oxidase, this is one of those enzyme which is really important; it converts oxygen molecular oxygen into water. In fact, two equivalent of water; it requires 4 electron and 4 proton to convert oxygen into water.

Now this is although a seemingly simple reaction, but you see the power of this reaction, this is one of those best reaction perhaps we can think off. In biological system it can be done effortlessly because everything is organized and designed so perfectly.

(Refer Slide Time: 00:51)



Heme-Cu Oxidases: Cytochrome *c* Oxidase

$$O_2 + 4e^- + 8H^+_{in} \longrightarrow 2H_2O + 4H^+_{out}$$

Occurrence:
Repiratory chains:
mitochondria & bacteria

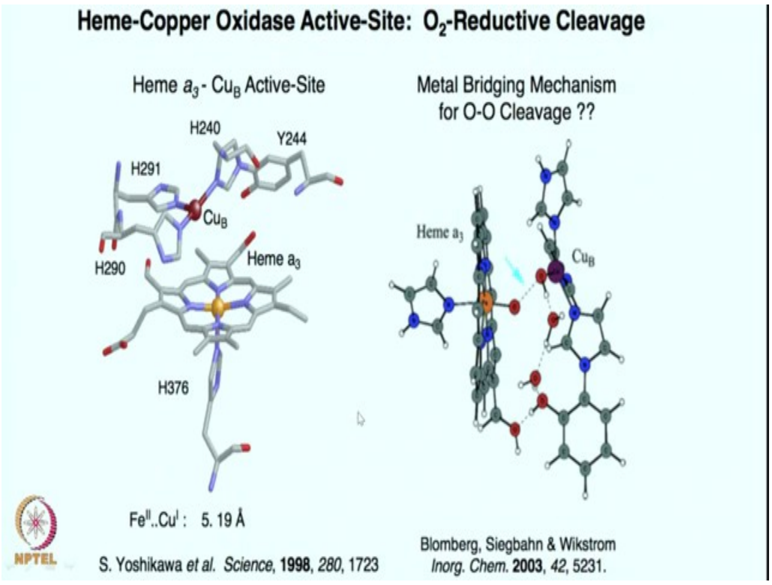
Function:
Redox-linked proton pumps
- Catalysis of exergonic O_2-reduction
- Coupled proton membrane translocation
- Electrochemical proton gradient: utilized for ATP synthesis

If you look at the active site of this metalloenzymes, cytochrome C oxidase of course, there is a heme iron center and a copper center that we will see in a moment. But there is also a cytochrome C, electron is transferring where to the copper A center and another iron center is also attached with it or tagged along with it.

Overall, it is a fascinating enzyme the relay of electron transfer happening all the way from cytochrome C to copper A to another porphyrin center and then from there finally, electron comes into the heme a_3 and the copper B site. So, this yellow colored part is the real active site of course, these are part of cytochrome C as well.

These enzyme metalloenzyme occurred in respiratory chain, in mitochondria and of course, in bacteria also it is found. It acts or its activity is redox linked proton pumps. It is involved in this redox linked proton from where this 8 proton comes in and then 4 proton goes out of it over all 4 of the protons are involved in this process. This is the catalytic generation of oxygen reduction into water and this is involved in the coupled proton membrane translocation and this creates electrochemical proton gradient which is utilized for ATP synthesis right. So, this is a membrane protein as you can see ok.

(Refer Slide Time: 03:53)



Heme-Copper Oxidase Active-Site: O_2-Reductive Cleavage

Heme a_3 - Cu_B Active-Site

Metal Bridging Mechanism for O-O Cleavage ??

$Fe^{II}..Cu^{I}$: 5. 19 Å

S. Yoshikawa *et al.* *Science*, **1998**, *280*, 1723

Blomberg, Siegbahn & Wikstrom *Inorg. Chem.* **2003**, *42*, 5231.

Now, this is when we try to zoom into here, let us look at the crystal structure of the species. So, this is going to be a heme a_3 species and this is a copper B site.

Quite interestingly it has two histidine of course, there is also a third histidine three histidine I would say bound with copper but these are normal histidine; these histidine is cross linked with a phenol moiety. So, a tyrosine moiety which is quite amazing; as you can see this phenol OH is pointed towards this core of the iron and copper centers right. So, is this by designed in nature or this phenol may have a role in this overall oxygen to water production. As you can see the heme site of course, the porphyrin the iron center and there is an axial ligand; once again histidine.

The distance between the copper and the iron is 5.19 angstrom, there are crystal structure known which is clearly showing that this is the main active site where oxygen is getting reduced to water. Of course, there are few crystal structures perhaps claiming also the intermediate, but none of those are quite reliable so far and we need to get better quality crystal structure to see the intermediate. This is the completely reduced form of the enzyme and once again the iron copper distance is 5.19 angstrom.

Perhaps just enough or little bit longer than one perhaps could expect for interaction among these two center directly through an oxygen molecular oxygen. We will come back to this again and again and which must remember this structure before we move forward. Once again, copper has three histidine with tyrosine a cross linked with the imidazole or histidine moiety pointed towards hydroxo group pointed towards this.

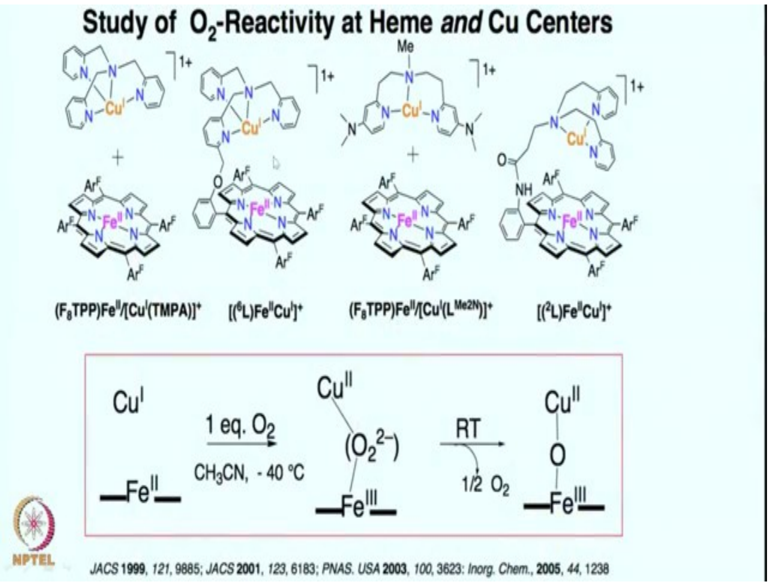
And more importantly this heme a_3 heme center is having also a histidine this will as you will see that these perhaps also have a role to play. So, the ligand which are apart far apart such as histidine and even this phenol or tyrosine perhaps will have a role in the overall oxygen to water production. We will try to understand the role of each of those ligand and each of these metal center.

Now wait a minute, let us look at very simply the transformation. This is oxygen is getting converted into water right, you need 4 proton and 4 electron of course, during this process a proton gradient is created that is fantastic. Now, if you notice carefully the oxygen-oxygen double bond like one sigma bond and another pi bond in the molecular oxygen in air has to be cleaved completely. Well that is not going to be easy right? To cleave that two bond one sigma bond and pi bond so that oxygen is converted into water.

What we will try to see is the efforts from various groups, to understand this chemistry how this might be happening at a molecular level that is always a tricky business, how things are happening stepwise because there is 4 electron and 4 proton. How stepwise things are happening? To understand that I think the synthetic studies are essential, you cannot rely on just enzymatic studies or biological studies to understand at minute details what happens when one electron is added because this process are very fast like catalytic process.

So, there is almost no way to understand each and every step in minute detail. This is where synthetic studies or inorganic chemists, bioinorganic chemists, synthetic bioinorganic chemist, computational chemists were involved in understanding in this problem. Of course, a number of spectroscopic studies are required; all of us can put together a perfect picture of what might be happening in a minute scale or in a very detailed way.

(Refer Slide Time: 08:43)



Study of O_2-Reactivity at Heme *and* Cu Centers

$(F_8TPP)Fe^{II}/[Cu^{I}(TMPA)]^+$ $[(^6L)Fe^{II}Cu^{I}]^+$ $(F_8TPP)Fe^{II}/[Cu^{I}(L^{Me2N})]^+$ $[(^2L)Fe^{II}Cu^{I}]^+$

JACS **1999**, *121*, 9885; *JACS* **2001**, *123*, 6183; *PNAS. USA* **2003**, *100*, 3623: *Inorg. Chem.*, **2005**, *44*, 1238

We will see that. So, what one perhaps can think of doing is to take 1:1 mixture of iron and copper. One of the danger is it going to react with oxygen in a 1:1 fashion or if they are going to have some coordinated movement?

For example iron can itself react with oxygen and it can if require another iron can come into a scheme. Because this is synthetic studies right, there is no restriction of having only one iron center that is possible in enzyme. But in synthetic setup when you take let us say whatever one mole of this material if you take; then you take Avogadro number of this molecule.

So, there is no dearth of having this molecule present more than one right. Similarly copper there is more than one copper available as you have seen such copper can gives rise to let us it end-on bound peroxo species right. This is known to give end-on bound peroxo species. These are known species for formation of superoxo peroxo or other intermediate based on the electron and proton available.

So, are they going to play independently while oxygen is added; even if it is added 1:1? Are they going to form or are they going to form a complex that engages both of them that is quite fascinating; specifically when they are not attached with each other. What if they are attached with each other through this porphyrin ring? Are they going to talk with each other or they can have an intermolecular reaction another molecule of this can come in and the copper of the other molecule will talk with copper and iron with another molecule will talk with iron.

These are tetradentate ligand system, one can perhaps also think of reacting or taking a copper complex. These are mind you these are all reduced form right; the crystal structures that we were previously seeing; these are reduced form. So, we are trying to mimic this sort of crystal structure or the orientation or the chemistry essentially.

So, this is a tridentate ligand; why tridentate ligand we are interested? That is mainly because this is tridentate in enzyme. So, these are histidine, but it is not necessary to take all the histidine; if it can be taken that is fine; if not the chemistry is not going to vary too much, if let us say instead of histidine pyridine is taken or an aliphatic amine is taken.

So, the chemistry is going to be similar very little or almost no difference can be observed. So, can we prepare such compound? Yes, we have seen the oxygen complex of this compound which gives it is a tridentate ligand once again, can give you side on bound peroxo; two copper center can come in just like similar to what is happening in hemocyanin right. This is this can lead to those hemocyanin like dicopper di perox dicopper peroxo intermediate.

Well if we mix them 1:1; mix the porphyrin synthetic porphyrin complex with these model complex 1:1, perhaps we can get independent chemistry from each of those core or perhaps we can think of having you know correlated compound where one of the compound that is formed first will talk with the other one.

Another important thing is what is going to happen if they are tethered, they are attached with porphyrin? Are they still going to form a dinuclear copper copper species or the iron will be forming a di iron intermediate? So, all these question we are going to look at to understand the Cytochrome C oxidase chemistry, we need to have these studies done and over the last few decades quite extensive studies are done towards this but essentially what we are trying to mimic or trying to understand is; what happens to this Cytochrome C oxidase iron and the copper center when one equivalent oxygen is added at low temperature. Are they going to form a species where iron and copper are linked with the oxygen molecule?

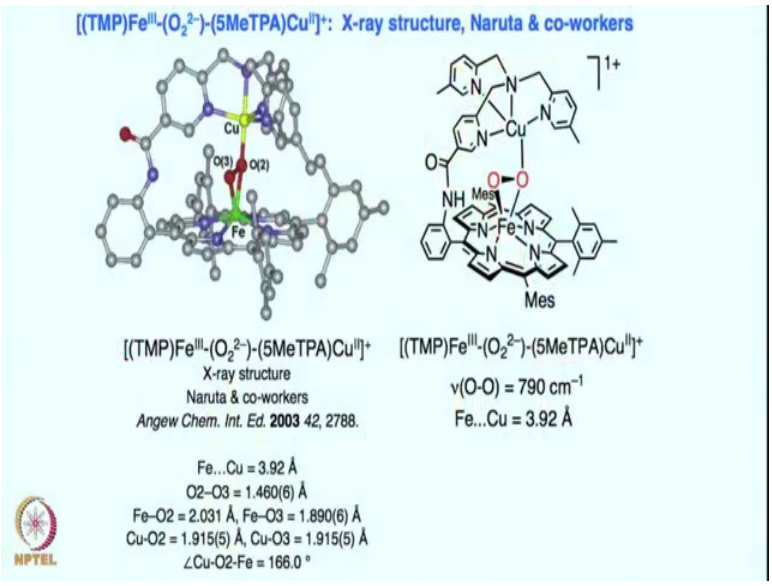
Subsequently, can what is going to happen if this is happening at room temperature is of course, some sort of other thermodynamical stable product can form. Let me tell you first thing first; that if we are attaching them like tethering them as you see over here or not tethering them as you see in there; all of them are going to form these sort of peroxo intermediate which is quite phenomenal.

So, what we are trying to say is iron II copper 1 you mix into solution 1 is to 1; they will find their partner and not a copper copper or iron iron species, but a iron copper species is going to form. That is quite phenomenal; I think that is going to be quite exciting in a way that that now we can get reliably and intermediate perhaps for the cytochrome C oxidase right.

As you can see over here and these species of course, are not very stable intermediate; it can go immediately or upon heating or upon keeping for long time; it can form these oxobridge species depending on the ligand on copper center, these can be linear or can be bend a little; we will not get too much into that. There is a series of studies, systematic studies has been done which clearly suggest that 1:1 mixture will give you this peroxo; which is I think to start with is quite exciting.

Let us move on and see what type of peroxo are these. Well a lot of studies has been done synthetically, but crystal structure are very difficult to get by.

(Refer Slide Time: 15:21)



$[(TMP)Fe^{III}\text{-}(O_2^{2-})\text{-}(5MeTPA)Cu^{II}]^+$: X-ray structure, Naruta & co-workers

$[(TMP)Fe^{III}\text{-}(O_2^{2-})\text{-}(5MeTPA)Cu^{II}]^+$
X-ray structure
Naruta & co-workers
Angew Chem. Int. Ed. **2003** *42*, 2788.

$[(TMP)Fe^{III}\text{-}(O_2^{2-})\text{-}(5MeTPA)Cu^{II}]^+$
$\nu(O\text{-}O) = 790\ cm^{-1}$
Fe...Cu = 3.92 Å

Fe...Cu = 3.92 Å
O2–O3 = 1.460(6) Å
Fe–O2 = 2.031 Å, Fe–O3 = 1.890(6) Å
Cu-O2 = 1.915(5) Å, Cu-O3 = 1.915(5) Å
∠Cu-O2-Fe = 166.0 °

This is one of those; you know prized possession where of the synthetic bioinorganic chemist where you can see the heme iron center and the copper center are bridged by the oxygen in an side-on end-on fashion eta2, eta1 fashion and this is the work by Naruta's group where they were able to get the crystal structure.

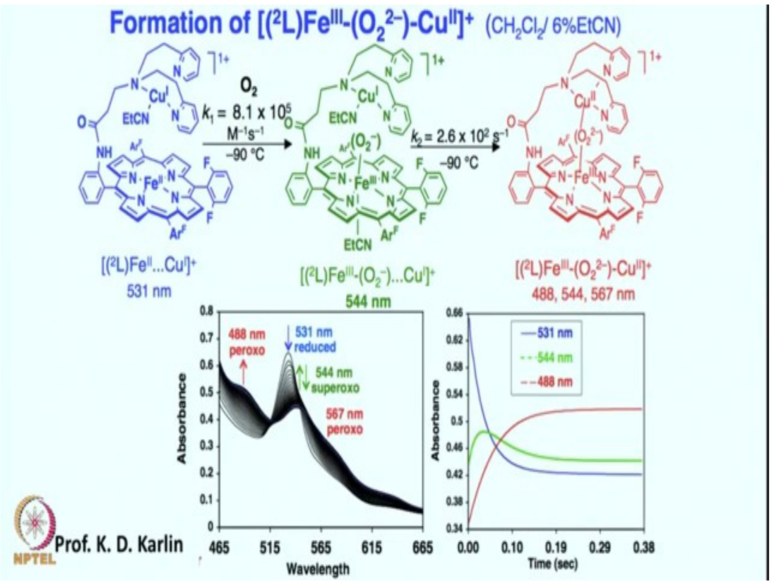
This is I think it is quite phenomenal because to get such a crystal structure, hetero bimetallic intermediate bound with oxygen is quite phenomenal. Note the iron copper distance this is 3.92 Angstrom, iron to copper distance 3.92 Angstrom, oxygen distance is 1.46 Angstrom; that is quite also great.

All these are indicative of the fact that these are peroxo; this oxygen bond length and more importantly you can have all sorts of distance calculated from your crystal structure; it gives you pretty easily and the oxygen stretch is around 790 wave number which is what we do say for a peroxo species. So, superoxo species will be around 1100 wave number peroxo which will be around 800 wave number.

Once again iron copper distance is 3.92 Angstrom; remember in the enzyme, these iron copper distance was nearly 5.19 Angstrom. Of course, so as if like if you want to imagine the enzyme and try to have some sort of similarity of enzyme with this model study; it was far from each other iron center, copper center upon oxygen binding perhaps they will come close to each other right.

Because this was 5.19 Angstrom for oxygen binding this is like 3.9 Angstrom like more than 1.1 point let us say 2 Angstrom difference is there; that is quite interesting. And also more interestingly, these species are very very unstable; one need to have really a lot of effort put to get such crystal structure.

(Refer Slide Time: 17:35)



Formation of $[(^2L)Fe^{III}\text{-}(O_2^{2-})\text{-}Cu^{II}]^+$ (CH_2Cl_2/ 6%EtCN)

Let us look at some of the seminal work done by Professor K. D. Karlin’s group and these are those synthetic studies and detailed spectroscopic studies. And these studies takes it really long time to come by and these are really an effective study to understand the cytochrome C oxidase for example, these sort of compound synthesis are not so trivial.

In any case scientists were able to do this confidently and here is the tridentate ligand for the copper center. Of course, there is a solvent bound if it is synthesized or if this reaction is done in acetonitrile or propionitrile acetonitrile it freezes at minus 40 degrees. So, it has to be propionitrile and therefore, the reaction can be done at minus 90 degree C and or in other solvent which is appropriate let us say THF, acetone depending on the compound.

So, this compound we are talking has fully reduced copper I and iron II. Once it is reacted with oxygen immediately what happens the iron superoxo species forming. Note that copper superoxo species is not forming in these cases; these are tetradentate ligand system, the oxygen reactivity is not that fast with copper with sorry tridentate ligand systems, tridentate ligand copper complex does not react with oxygen that fast has it been a tetradentate ligand; then this reaction with oxygen would have been very very fast.

In any case, this iron center forms the superoxo intermediate. So, one of the electron from the iron center is now transferred to the oxygen to make it iron III superoxide intermediate. So, iron goes to iron III+ oxygen gets reduced by 1 electron to superoxide.

You see the copper center over here; this copper center over here remains as copper I; nothing happened to it so far, no electron transfer such an intermediate can be characterized these are very very unstable intermediate. If you are following the UV visible spectra of this compound, this is the 530 nanometer band due to this complex; this goes down and a new species is forming as you can see over here right for due to this iron III superoxo.

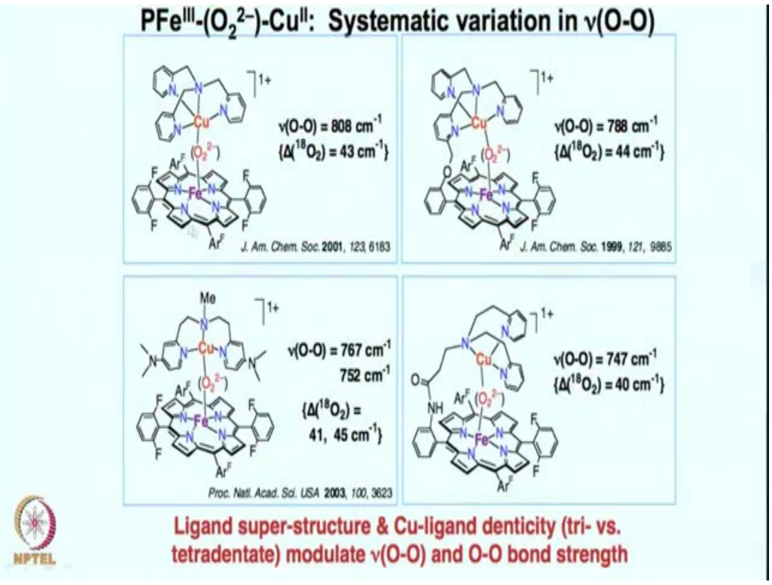
As I was telling that nowadays this spectroscopic data at low temperatures, any change of this data; if we are able to detect and then we are able to corroborate with the known literature, report this is considered as almost fingerprint identification of those compound. And these are once again very very unstable chemistry, very very sensitive chemistry need to be done at a very low temperature and the intermediate that are generated are not very stable. So, this is some of the intermediate has nanosecond, millisecond timeframe to study right.

So, 1 second would be some time 1 second would be the best perhaps you can get. So, this chemistry are so sensitive you have to do this chemistry really carefully, solvent has to be good quality and temperature has to be as low as possible for that given solvent and the complex has to be very pure and I think we have noticed that there is an axial ligand also in there right. There is no imidazole or histidine or you know pyridine attached with it; it is the acetonitrile which is coming from likely solvent. And this species as you see grows up and then goes down to gives rise to a new species that is at 488 nanometer; that means, over here as well as 567 nanometer overall giving rise to this iron III, copper II, peroxo intermediate which is quite amazing right.

You have seen the crystal structure with a tetradentate ligand system ok, but those are really a very recent phenomenon. If you see this is in 2003; these studies are going on for decades; so these are earlier studies or solution studies which also the; these are also you know contemporary studies which shows clearly what goes on getting crystal structure is one thing. But understanding and the chemistry is also very essential in addition to have the crystal structure. As you can see this species is disappearing quickly and the red species over here is forming, but before this red species form; there is another intermediate that forms and quickly decomposes.

So, these sort of spectra very clearly correlation shows that the intermediates are involved and one can characterize this intermediate to by using various spectroscopic technique and once again these are going to be very expensive studied, very sensitive studies and very careful studies. One needs to be really confident in doing these reproducing it multiple times and then draw the conclusion of about this chemistry in a given context.

(Refer Slide Time: 23:19)



PFe^{III}-(O_2^{2-})-Cu^{II}: Systematic variation in ν(O-O)

ν(O-O) = 808 cm^{-1}
{Δ($^{18}O_2$) = 43 cm^{-1}}
J. Am. Chem. Soc. 2001, 123, 6183

ν(O-O) = 788 cm^{-1}
{Δ($^{18}O_2$) = 44 cm^{-1}}
J. Am. Chem. Soc. 1999, 121, 9885

ν(O-O) = 767 cm^{-1}
752 cm^{-1}
{Δ($^{18}O_2$) =
41, 45 cm^{-1}}
Proc. Natl. Acad. Sci. USA 2003, 100, 3623

ν(O-O) = 747 cm^{-1}
{Δ($^{18}O_2$) = 40 cm^{-1}}

Ligand super-structure & Cu-ligand denticity (tri- vs. tetradentate) modulate ν(O-O) and O-O bond strength

Well, here is the summary of some of the studies that has been done as well we did not go through each of them in detail but to summarize these sort of porphyrin iron complex where we have started from iron II and copper I; while it is reacted with oxygen, each of the iron and copper gave one electron to oxygen making it peroxo. So, one electron reduced species is called superoxo, two electron reduced species is called peroxo.

So iron III, copper II peroxo intermediate is generated even if they are added 1:1. So, this copper I complex was added 1 equivalent and this iron porphyrin iron was added also 1 equivalent. Despite having no linkage or despite having the possibility of forming a di copper peroxo intermediate which you have seen end-on bound peroxo intermediate.

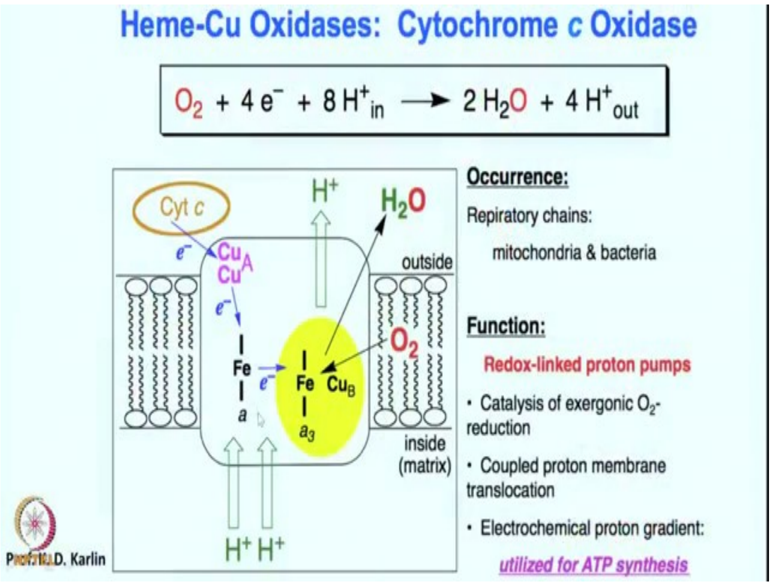
Still the mixture went for or thermodynamically went for this peroxo species formation. As you have seen the first form intermediate would be a superoxo intermediate right. You can get these species, characterize it UV visible spectroscopy different other spectroscopic technique including the resonance Raman which is very very characteristic of such species formation. It is giving 808 wave number for this sort of species, which is clearly indicating that this is peroxo.

We do not know the mode of binding; but as you have seen it is going to be eta2 binding over here and eta1 binding with the copper. So, single bond with copper kind of double bond or like I mean you know equidistance from the oxygen. Both the oxygens are equidistance from iron and one of the oxygen is attached with the copper.

More importantly as we you we have we have noticed that these sort of oxygen stretch can be shifted further; if O^{18} is used. See if each of these oxygens are leveled with 18 and 18, then this 808 wave number band will be shifted further to down by 808 minus 43; that would be for O^{18} leveling. This sort of shift delta $^{18}O_2$ as well as the oxygen stretch quite clearly indicates that this is the species right.

So, so far let me tell you what we understood in this class is this fascinating enzyme known as cytochrome C oxidase. These are heme copper centers having heme copper center and of course, this is this is not all of the metal center. There is also another heme center and another di copper center which is attached or which is which is correlated or which is part of the part of the cytochrome C oxidase, but those are not involved let me get back to those.

(Refer Slide Time: 26:36)



Heme-Cu Oxidases: Cytochrome *c* Oxidase

$O_2 + 4e^- + 8H^+_{in} \longrightarrow 2H_2O + 4H^+_{out}$

Occurrence:

Repiratory chains:

mitochondria & bacteria

Function:

Redox-linked proton pumps

- Catalysis of exergonic O_2-reduction
- Coupled proton membrane translocation
- Electrochemical proton gradient:

utilized for ATP synthesis

Those are not involved into the oxygen activation chemistry. So, this is the heme and copper that is what activate the oxygen right over here, but the other centers this copper; this iron center they are just participating in the overall process by delivering the electron that is required for this process right.

So, this is although their part of part of the cytochrome C oxidase, but they are not involved in the oxygen activation right. And as you have seen this is having a clear cut heme iron and copper structure, it is a trinucli or tridantate structure for copper tridantete ligation for copper. This is a porphyrin center appended with the histidine and more importantly there is this phenol.

Of course, if you look at the model studies that we have shown, we do not have yet any axial ligand except in one of those cases propionitrile that is not going to be a great ligand anyway um. So, we also have this phenol appended that sort of study or that sort of compound we did not discuss yet.

But, but the take home message I; I would say from these this class would be very simply; even if you mix iron and copper together with or without tethering still they are going to be the best friend of each other. They are going to come closer and shake hand through oxygen, this is quite phenomenal right. So, and it is going to form a complex which excites us because this is how perhaps our oxygen activation is happening to convert it into water molecule ok.

In the next class, we will continue on that, but more importantly do not forget this crystal structure that is reported which is I would say really priceless, where iron and copper is bridged with this peroxo in a way that is shown over here. Will, we have seen these iron chemistry with the tethering copper and we will we will come back to this chemistry in the next class.

Thank you keep studying, we will see you soon.