Biological Inorganic Chemistry
Professor Debashis Ray
Department of Chemistry
Indian Institute of Technology, Kharagpur
Lecture 40
Multicopper and Mixed-Copper Enzymes

Hello, a very good morning to everybody. So, we have reached to the last lecture of module eight. Where we will be talking about the placement of more than one copper centers, that we have seen into examples also, that how we can put at least three or four copper centers and sometimes along with copper, some other metal ions.

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What are the benefits we can get or achieve. So, you can have multiple copper ions in these systems or in these aggregates. And then how we can develop a little bit side by side I will tell you about the synthetic systems in companies into the natural systems. Because the natural systems is our, all is our inspiration.

Then if you have all the copper centers of same type or similar type, then we can have the homonuclear aggregates in terms of the nature of the metal ion. But if we can have two other or one other different metal ions, we can have the heteronuclear assemblies that we will see also the last examples in the cytochrome c oxidase. So, to begin with, we will just simply see the most simple one and most well studied one is your cellular plasmin one.

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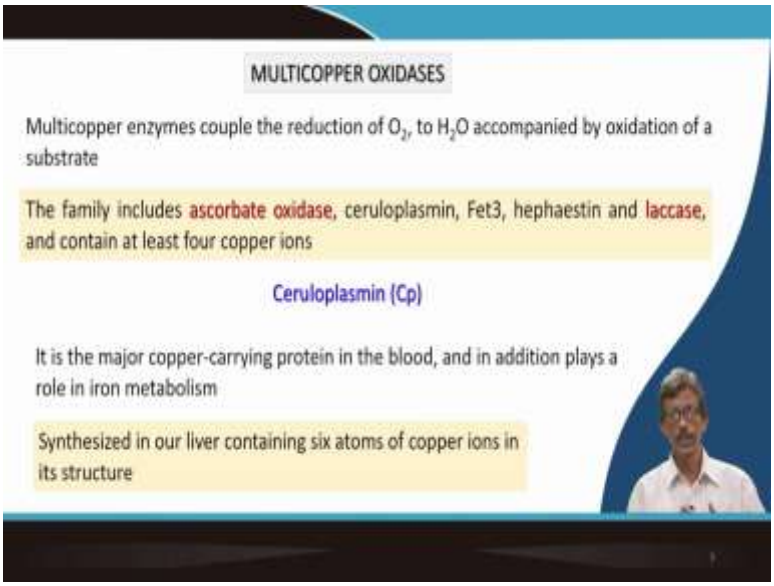

So, what does it mean basically by knowing the name actually? So, you should always try to understand in that way that, what are the oxidases? We know the definition of oxidases. In comparison to your oxygenase and you bring multi copper things. That means, it can be a binuclear, it can be a trinuclear or it can be a tetanuclear system because we will just simply go to a tetranuclear spieces.

But we will give some example for many other copper ions, where it can be trapped by a polypeptide chain such that you can have that particular assembly or aggregate as your storage of copper ions. So, they basically couple already we have seen in many cases, many examples that how we can reduce that oxygen molecule to produce the water molecule. And along with that we have to go for the substrate oxidation.

So, for all these examples, within this family of multicopper oxidases to which is red in colored. So, already we have discussed about ascorbate oxidase and laccase but with regard to some other thing, that means with regard to the type one copper centers or type one copper proteins. But here we will not discuss again, because we do not have that much time to spare about talking again about ascorbate oxidase or laccase, but we will rather talk about the cellular plasmids.

So, new examples and the most recently developed one is Fet3, or hephestin. So, they all contain basically at least four copper ions. So, basically our life will remain on a tetranuclear system.

And these tetra nuclear systems are very simple to understand also, if you take the example of the model compounds. So, if we have the model compounds and those model compounds can tell us something related to that of your hemocyanin modeling.

We can have better idea about how these aggregates are forming. So, by definition, always try to remember in this fashion is the textbook definition of cellular plasmin, what is that? So, is basically the major copper canning protein in our blood, in many other biological world also. And it also plays a role in iron metabolism. So, you see why we are studying these two different metal ions or the heteronuclear system because one is dependent on the other.

That means, the assimilation of one particular metal on will definitely be dependent on the other. That means, if we talk about the iron simulation or iron metabolism, we have to go for the typical iron center oxidation. That means, if you have any ferrous ion, you have to go immediately for its oxidation to the ferric centers. So, that is why if you go for this conversion and if the copper is responsible for that oxidation then, that particular iron metabolism process will be dependent on the presence of the copper and its redox activity.

So, what can be synthesized? That means it is biosynthesized in our liver. We know that in our some major organs or the vital organs we have, where we produce blood we produce all these groups. But where we can go for the synthesis of these cellular plasmin molecules and to us to a coordinate in chemist or to a bioinorganic model chemist, what is the particular definition of synthesis? We always bother about the incorporation or the mutilation of the metal ion.

We do not care about the huge biological world is behind. That means, the synthesis of the polypeptide, the proteins, the DNAs and RNAs, no. We are thinking something at the end only, where the copper ions should be available. And those copper ions, how they are being trapped, what are the functions we can derive out of those copper presents and its structure, function and other thing.

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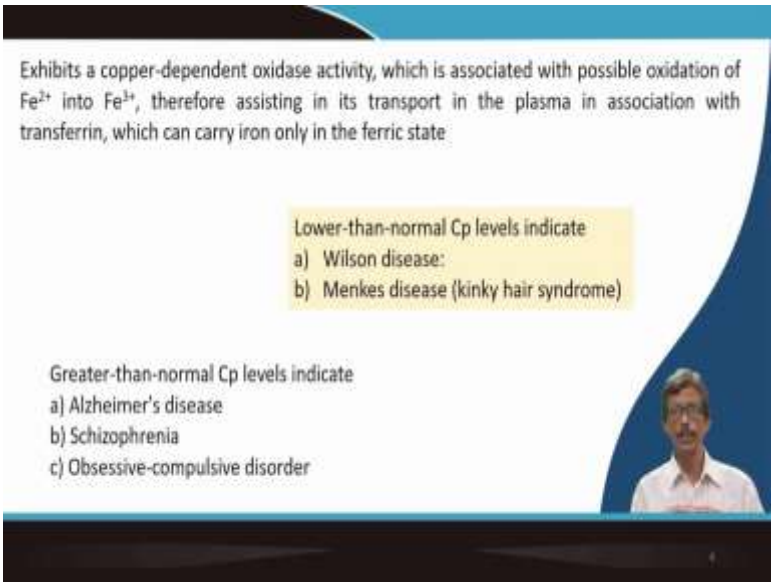

So, when we see that if the assimilation or the proper absorption of iron in our body is copper dependent, and as I told you just now, that if some redox thing is there and if we want to oxidize the feras to the ferry, so definitely we can have some oxidase activity. So, we already heard it and we discussed it also, the ferrous oxidase activity, so, the copper which is nothing but your oxidizing agent in this particular case.

And already we have seen from our very beginning of this module, that you can have the electron transfer proteins like type one and type two proteins. And there we have seen what? There we have seen, that the difference is 0 values as well as the corresponding kinetics, the rate of electron transport is very important. And depending upon your different, of all these different all these molecules like azuine to plastocynin and all this.

You can have different e 0 values. Now, if we find that okay, iron is trying to go for the incorporation in a particular polypeptide chain or the protein chain. But the binding of that particular iron will be stronger if you are able to go for a hard metal ion center which is your ferric center not the ferrous center. And if the oxidizing agent is available, we can go for the oxidation of that particular ferric ion to go for the ferric state.

And once you go for the ferric state, it can go and immediately go for strong binding to the protein and vellum. But sometimes it can so happen that the coordination can also help your

oxidation. Because we all know, that the metal ion coordination some time increase the e 0 value or some time decrease the e 0 value.

But the protein envelop or most of the time we know that when protein envelop is there, and if the iron is remaining not at the bare iron or the eco island species, but it is bound iron species to that polypeptide chain. But if you are able to decrease the e0 value in that particular fashion, so copper proteins which is your oxidizing electron transfer proteins, so those oxidizing electron transfer proteins will be available for this particular type of oxidation.

And once it is oxidized, not only it is incorporation in hemoglobin, myoglobin or cytochromes. But there are something, where we can go for the transport of iron centers. We have not discussed all in detail about the transfer in molecules, but we have seen these are iron carrier molecules. So, big proteins are there, carbonate is there and you can have the iron center. So, but that iron center is again is not your ferrous iron center, but it is also your ferric center.

And already we have seen that if your iron center is bound to the haem iron site that means they are, haem iron that means the porphyrin is there. It is nicely assimilated in our body or in our system. So, these particular only the ceruloplasmin levels are important, people can track all these things and what we can find out that if you have.

Because we can have always that problem is very important that the proper management of the concentration level of these metal ions, these essential metal anion some not so, essential metal ions. We always talk about the corresponding concentration. So, if your concentration for these ceruloplasmin level we can monitor basically, the biophysical or the biochemical studies or analysis can help you in determining the corresponding concentration of the free ceruloplasmin available for metal ion binding.

And after metal ion binding, the metal ion bound ceruloplasmin level also. But if your concentration is lower that, not that due to the corresponding availability of your copper, but proper assimilation of that copper. If it is not there, we may face the Wilson disease or Menkes disease. Menkes disease is nothing but the kinky hair syndrome, because these first manifested, first we want to know the doctor can know also, if they find that you have a kinky hair problem.

So, why it is coming? If suddenly it is not coming, not from your beginning of your birth. Then the proper balance is that why important. If it is lower than some value, and if it is that greater than that value, so abnormal that is also another kind of abnormality. But the other diseases can be manifested or can be shown which are Alzheimer's disease, Schizophrenia and what we all know nowadays is OCD, which is very important thing is the obsessive compulsive disorder.

So, that can be again due to improper adjustment of the copper concentration and the related proteins and its assimilation.

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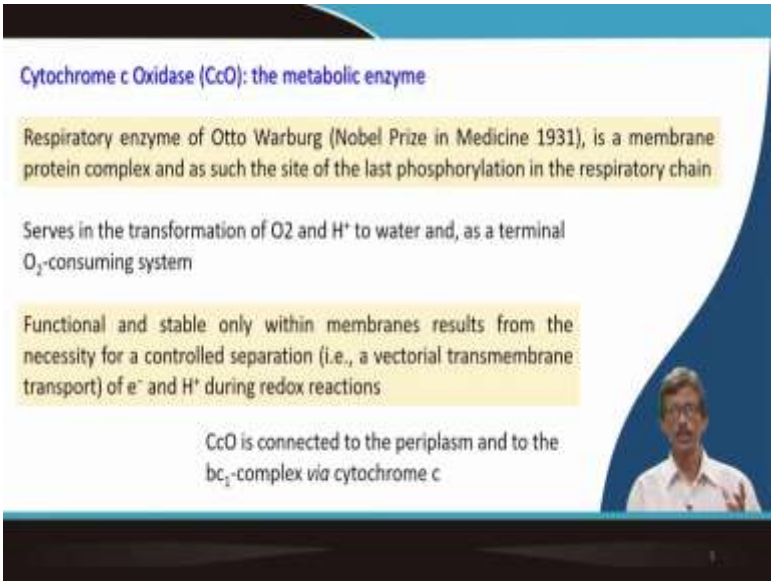

Then we just quickly go to the cytochrome c oxidase. And once, many times I gave you the examples that what is the cytochrome c oxidase, it is the terminal oxidase enzyme, which is responsible for your food chain producing many number of ATP molecules, your energy currency. And during this metabolic process of this simple glucose molecule, we have to go for the corresponding 4 electron transport to the O2 molecule.

So, that is why it is the most important and most interesting metabolic enzyme what we can know. And you see the long back almost 90 years back, Otto Warburg got the Nobel Prize on this particular molecule. But nothing was known at that time, whether copper is present or iron is present nothing was known. But at that particular time, it was isolated. It is a membrane protein complex. Because this one part, another part, another part, another it is a very complex one.

That is why it is not a coordination complex, it is protein complex. So, he gave the name at that particular time, that it is your respiratory enzyme because this is the most important respiratory enzyme which we can tackle as your metalloenzyme because it is the last step of phosphorylation in the respiratory chain and this phosphorylation and dephosphorylation reactions.

That means, the production of right number of ATP molecules and its hydrolysis basically is responsible for many important reactions within this electron transfer chain, which is your typical respiratory chain and that is why it is named as your respiratory enzyme. So, it basically what it is doing? It is basically O, O2 is superscript, not O2, O2 is super subscript, and H plus. So, the transformation of O2 and H plus to water and as the terminal O2 consuming system.

So, we have to use that particular O2 and we consume that O2, we accept that electron as well as the proton that is why you are producing water molecule. Because you are accepting H plus and electrons 2 O2. That means, the dioxygen molecule to O to minus the oxide ion. So, it can have some functional and stable thing is within the membrane, results from the necessity of a controlled separation.

That means, you can have a vectorial transmembrane transport or potential development due to the transport of two very important ingredients, for all these potentials and all these sustenance is your electron as well as the proton. So, you cannot stop something, where only electron transport is taking place, the proton transport will definitely take place in the opposite direction. So, CcO, we abbreviate in this particular fashion, when it is connected to the periplasm.

And to the bc 1 complex via cytochrome c, where it is connected, the linker where it is sitting over there. So, you can have the cytochrome c, as well as cytochrome bc 1 and as well as its complex 1 as this complex.

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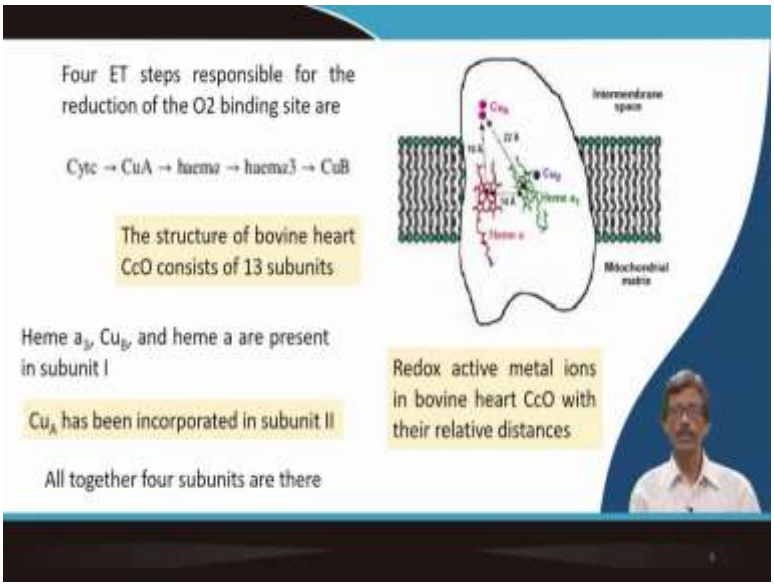

So, if you have four subunits or four complexes, like protein complex 1, protein complex 2, protein complex 3 and protein complex 2, 4 sorry. So, what do you see then that you can have for electron transfer steps. So, if you can, have 4 specie 1, 2, 3 this is not, one more. Either it is accepting or it is donating. So, if you can have four electron transfer steps for the reduction of again the O2 molecule O subscript two binding site are is the thing.

You can have cytochrome c, which is there. That means, not only this four, the four electron transfer involving five species. So, cytochrome c, cytochrome a in a, in a 3 and cytochrome B, so these are basically the chain of electron transfer. And you ultimately giving the electron, that means you are reducing, this is the electron flow, from cytochrome c to copper B center. So, you see the involvement, the nature is managing all these things so beautifully.

That at one point you have copper at the left at another point copper at the right. So, copper a is highly oxidizing, or reducing, that you should think of. What should be the potential difference? The e0 values for the cuprous, cupric oxidation between these copper one A and copper B. Because on the right hand side because after bu, copper B which is your O2. So, auto is mostly oxidizing species.

So, let us see copper B and further less is copper A. So, you have to understand in that way how the chain is going, it is basically a necklace of electron transfer. So, all these buttons are there in

between and all these buttons are your cytochrome c or haem a. Haem a is nothing but against cytochrome related a, a type of cytochrome and is a3 type of cytochrome. And when the structure is determined, which is bovine heart cytochrome c oxidase is not only the 3, it will have 13 subunits.

But here, we do not have that much time to understand all these things, but out of all these 13, some will not have the at metal ions. We are interested to see those subunits, where you have the metal ions. So, you are, here at the haem a 3, copper B and haem are present in subunits. So, if you now consider that where, what are they are in subunit 1? Then, what are there within this four. So, 4 species copper a haem a 3 and copper B. So, you require four such species.

Then four species as well as you can have the four subunits, because you have altogether four subunits. But copper A is different, copper A has been incorporated in subunit 2. That means, immediately by looking at these statements, all these informations, you immediately can understand that copper A is sitting somewhere else, is at longer distance, compared to your a3 cuB and A. So, that is the thing.

So, you have the corresponding intermembrane space and you have the huge complex machinery. If we consider that no, it is not that simple tetranuclear species or two binuclear space. One is copper binuclear another is iron binuclear, you can think of. In the laboratory we make occasionally the binuclear and complex and the binuclear copper complex. But they are not coming and giving you that assembly of cu2 fe2.

We are all of them are different, you have individually the copper A, copper B haem a and haem a3. You see the distance is also, is almost 19 angstrom, 14 angstrom, 22 angstroms, So, it is a huge distance but still you can have the electron transfer. And all these metal ions are redox active, that means they can substitute in cupric, cuprous as well as ferrous, ferric sites. And when it is determined we can find out the relative distances which are also important because how fast you will have the electron transfer rate?

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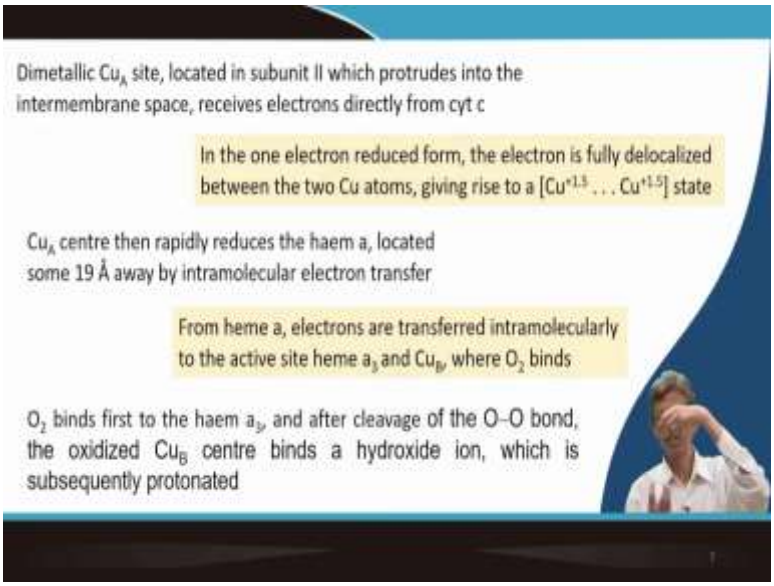

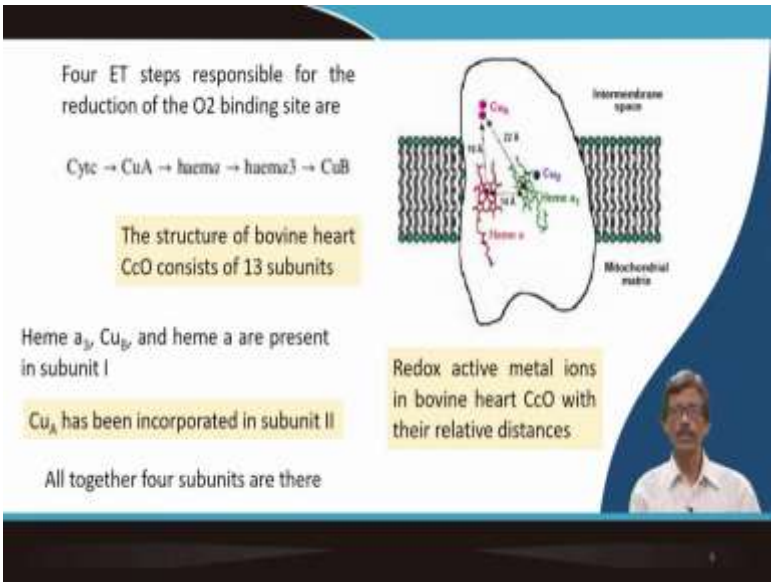

So, you see that the copper A site, what is there. The copper A we are showing two, metal ion center two spheres, pink in color, copper B is blue. So, this is your visibility only, to increase your visibility. So, it is basically a dimetallic site and dimetallic sides which basically, sitting within the intermembrane space and basically receives the electron from cytochrome c. So, you have the cytochrome c first, which is not within the cytochrome c oxidase.

But next is your binuclear copper A site which will accept the electron from the cytochrome c and will pass on the right hand site. But if the two sites are there and we can get only one

electron transfer, that means you have both the two copper centers are highly delocalized. And you cannot have a copper 2 copper 1 system or copper 1 copper 2 system instead is the, if they are highly localized, we can consider a oxidation state of 1.5 and 1.5 on each copper center.

We do not know, because the electronic site or electronic property of all these copper centers after electron transfer and before electron transfer will not be changing. So, it is better to consider this binuclear site as a single unit and highly delocalized like your ferric oxide system. High potential ferrid oxin or a low potential ferrid oxin where we have seen that you can take only one electron out or you can only put one electron in.

So, is rapidly reduces the haem a, then once it accepts it immediately transfer that electron to the haem a. But it is located 19 angstrom away. So, that is why you see so interesting thing all these things are there. Then once, it is transferring to a, then that haem a electrons are transferred intramolecularly to the active site of haem a3 and copper B.

So, immediately as a reducing agent you will get haem a, so haem a is nothing but a cytochrome type of thing, only one iron center is present within a porphyrin ring. But that is immediately go for your reduction reaction of your haem a3 and copper B and where O2 is also binding. So, if you see that involvement of all these metal ions are there, but ultimately what you can do?

You just either reduce O2 partly or reduce the metal ion center. So, what is happening then that the O O bond you have to clip them. So, if the O2 binding is taking place between these heteronuclear, haem a3 and copper B site, if they are close by your O2 is binding. So, O2 is sitting, it can be like this. You can have the different types of rotations of these, but you have iron, you have copper.

The hetero dinuclear system is a very interesting better dinuclear system. So, dinuclear, so, it will start interacting. And during that interaction, we all know that if you already push two electrons to the antibonding orbitals of the O2 molecule, already while lengthening the O O bond. That means, it has converted to peroxide. Then if you dump further electron, you can clip the thing. Such that, you can have one O2 minus, and another O2 minus. That means, you can having two O2 minus which are your material for the production of water molecule, which is your h2 O.

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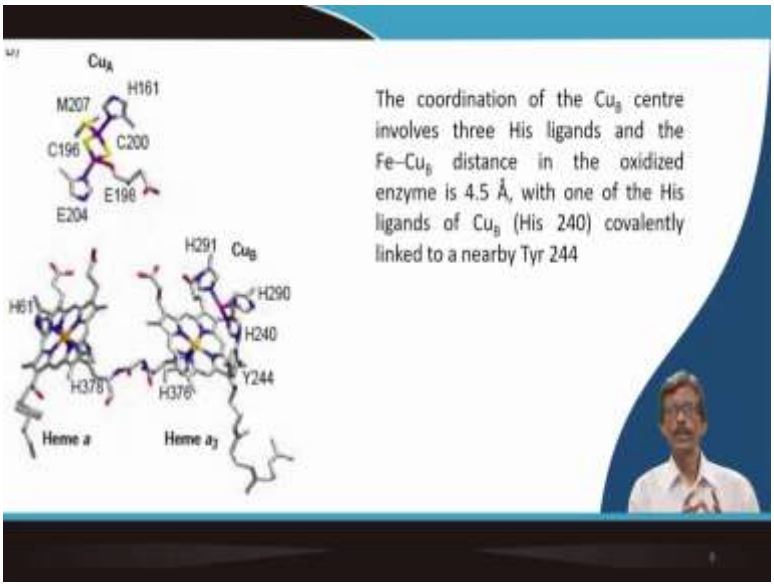

So, you see the look wise, the bigger one. The bigger, the the closer look basically for these, you have the copper A at the top, the dinuclear entity and all the amino acid side chains have now been leveled. And the structural determination only tell us, the only the clear and the final picture in our hand. So, that particular clear and the final picture, when we have we see that the iron centers are sitting nicely within the porphyrin rings only.

So, when they are sitting in the porphyrin rings and in one case it is basically transferring electrons. So, it is basically something where you can have no other choice for some binding of the substrate molecule like your O2 molecule. But if you go for the haem a3 on the right hand side, so this is the haem a3, this is the your haem a3.

So, on the right hand side if you see the thaem a3, what we find that if haem a3 is there and nearby if you have another copper center and it is a plane. So, it is flat molecule and if you bring another copper center, that we all knows is not a umbrella, but we all know that the three center coordination of copper which is very useful like that of a hemocyanin coordination.

So, if your umbrella is available, so, that is there. So, you put the umbrella on copper and that umbrella will be on this direction facing the hand, the handle of the umbrella is like this. So, it is facing on this direction. So, you have the flat thing and you are covering that. And if you have

some channels available from the surroundings, it will direct the entry of your O2 or the partially reduced O2 molecule.

Already it has accepted some electrons say superoxide or peroxide. So, those are also charged. So, they can go and enter within this particular pocket. Such that, you can have the total control of that particular molecule, between these two centers. That means the Fe and the copper B center. Fe of haem a3, sometimes we write as the Fe a3 also within bracket also because you have to locate this thing.

Because the your site is that, you have the A, A site, one is capital A and there is small a. And you have the Bb site but not the Bb site, haem a3 is also because that nomenclature is reserved for your nature of the porphyrin, is the corresponding porphyrin is of similar type, which is A type of cytochrome. So, then you see that if you bring that distance, the oxidized enzyme is giving a distance of 4.5 angstrom.

So, try to recall back the intermetallic distances what we have studied earlier in terms of your deoxy hemocyanin and oxy hemocyanin. How those were changing? So, in case of deoxy hemocyanin the copper, copper distances were 4.6 angstrom when O2 is not bound over there, but when O2 is binding, we are bringing down that particular distance to up to 3.5 or 3.6.

But here you see, after binding also your distance is pretty long because your coordination is something different. You have a flat iron center, the flat bound to your porphyrin center and you have a facial copper center. But if you have two such facial distances or the copper centers like your hemocyanin you can have a copper copper sort copper copper distance.

But since it is not copper copper it is iron copper distance, you find that your distance is only 4.5 angstrom, which is pretty longer. But still you can have your peroxide linkage but that peroxide linkage is not that of your hemocyanin in oxygen hemocyanin. So, the copper B is basically getting the histidine ligand covenant link to the nearby tyrosine 244 and the importance of that tyrosine 4, 244 is important.

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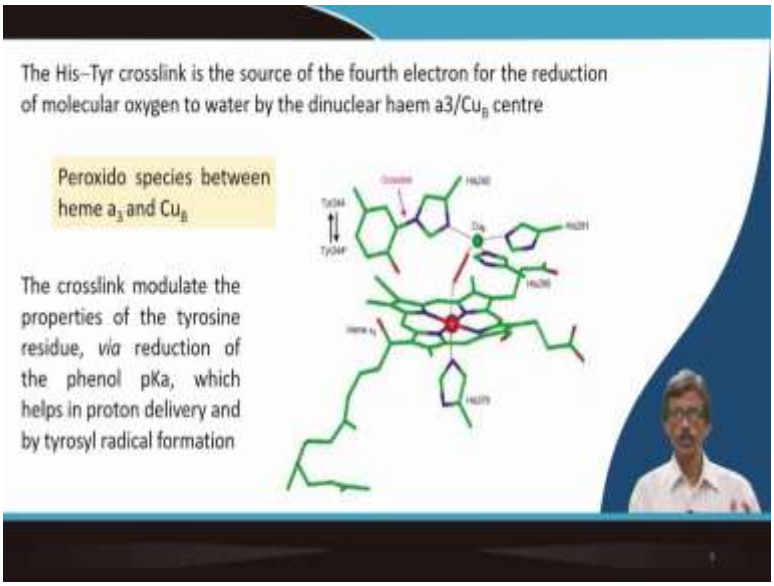

Because now already we are seeing the cysteine tyrosine linkage, the cross link or the covalent bond formation, now you see histidine also, or the histidine in the deprotonated form we all know that histidine is also charged in deprotonated form, the nitrogen can be charged one. So, like cysteine, or cysteinate ion, the histidinate ion also, the n minus if you have that can be a very good nucleophile. So, which can attack the corresponding phenol ring of the tyrosine linkage.

So, there you get that source for the fourth electron of the reduction. So, that is why if the metal ion is not available, the tyrosine linkage if it is there nearby, that organic radical can supply that particular extra electron what you needed for your production of water within the dinuclear, in a three copper B center. So, if you see that the whole structure, whole structure is like this, so, you have the binding and you have the copper center.

But one of the copper B site, you see that the imidazole binding from the histidine 240. But that histidine 240 is a special type of histidine. Because that special type of histidine is crosslink with tyrosine such that you are able to produce the radical, radical form the ligand backbone, which is your source of electron. So, that all, not only modulate this electron transfer, but that also change the pKa value of that phenol of the tyrosine residues.

And which helps in proton delivery and by tyrosyl radical formation. So, not only electron transfer, but also it can go for the proton transfer nicely, whether to and fro that you have to

understand, whether it will donate proton or accept proton, that you have to see nicely. But you can modulate through this cross linking.

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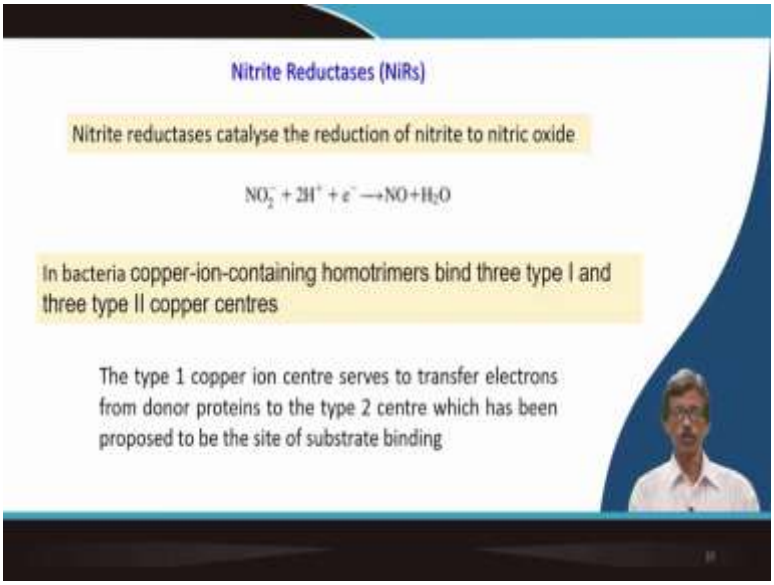

So, now quickly we see other two examples, one is the nitrite reductases, the name itself tells you we have to go for the reduction of the nitrate and I am taking the help of again the copper center. So, copper redox is nicely utilized again and again for the production of nite, NO the nitric oxide molecule from the nitride and iron.

So, it has this very useful implication, because if you are using some copper nitride salt and the nitrite is bound to the copper centre and if you are going for a redox change on that particular copper, you will end up with some production of NO and H2O. So, that is why we call, if that reaction survives or we can do in the laboratory, we can say that you can model or the modeling or biomimetic modeling of the nitrite reductases you can have.

Here you have the type one and type two centers only. And serve as a transfer of electrons from the donor proteins to the type two centers is not that much more complicated one what we have discussed just now, is your tetranuclear 1 but it is less than that.

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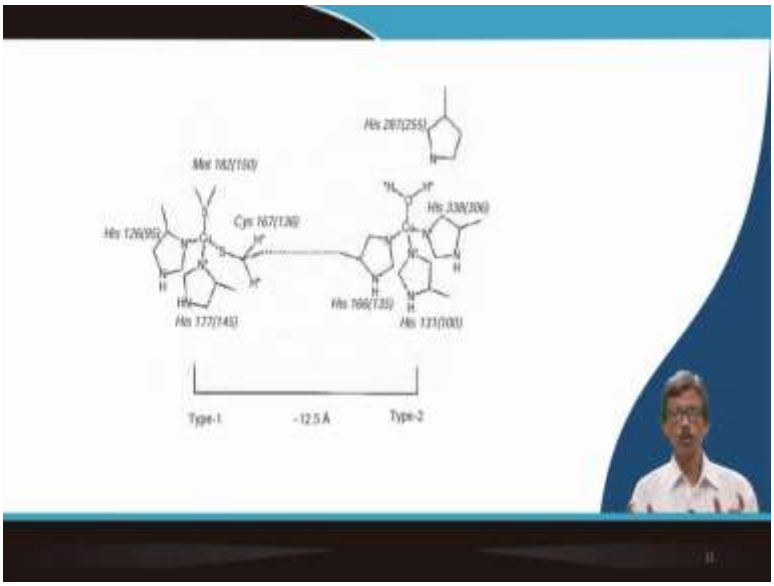

What is that? It is basically a dinuclear system, but it is a long distance dinuclear system. So, type one is there and type two already know immediately, what are the environments and we know now even if you only write type one and type two. Because in the biochemistry books and the biochemist are basically love to write as type one and type two, they do not care about the coordination environment, the geometry, the structure and all these things.

They only know about the type one and type two, because they are funs. Ultimately they are looking for their functions, then the other one, which is closely related by name only.

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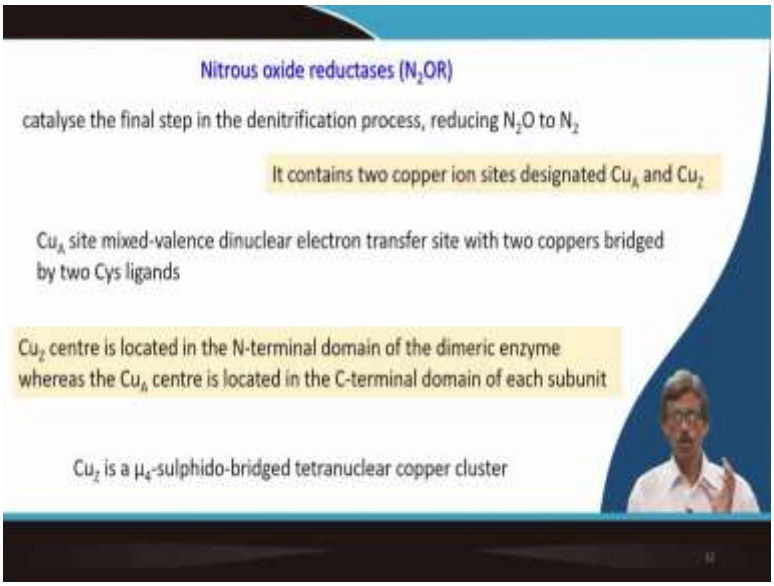

So, sodium nitride gives you the nitrate and ion, but is only the neutral nitrous oxide N2O, whether you can go for the reduction. So, what is that? If you reduce it, you will be able to take out that oxygen from the nitrite oxide. So, you can have some good understanding about the reversible reaction between dinitrogen and nitrite oxide.

If you add up simple one oxygen for your school level understanding, that we know that dinitrogen is triply bonded very inert molecule. But if you are able to add up on oxygen or attach it on it, it will give you not NON, it will give you N NO. So, terminally you will be able to attach one of the oxygen atoms to the dinitrogen molecule. So, the attachment of that dinitrogen molecule will give you the nitrite oxide.

But if you look at the reverse reaction, that means the reduction reaction. So, the reduction reaction you have to take out that oxygen, what we have attached on the dinitrogen molecule and you will end up with that dinitrogen molecule or you will be able to produce the dinitrogen molecule in good quantity. So, it contains two copper ion sites designated as copper A and copper Z. So, again already we know copper is copper A.

But we do not know about the copper Z. So, again copper A like that of your, just now cytochrome C oxidases we have seen, that the copper A is a binuclear system, (())(29:39) are

there. So, you can have the diamond code, bound by two cysteine sulfur residues, of two these copper ions, but what about the copper Z?

So, these copper Z center is completely a different center. And is located at some terminal domain, which n terminal domain of the dimeric enzyme, where the copper A is residing and is located in the C terminal domain of each subunit. So, copper A is in C terminal and copper Z is in N terminal.

So, if you have a long polypeptide chain we all know, you will have the C terminal and you have the N terminal end. So, you basically separate out these two sides of the metal ions from one side to the other, but where it is? It is close to N and it is close to C. But what is that copper Z? Copper Z, is, it is easy to remember also, Z is for that is a different one.

We have not seen earlier, is a mu 4 sulphido bridged tetra nuclear copper ion cluster. So, you have four copper ions, you have to four the tetranuclear system, unlike your Cu2 Fe2 in cytochrome c oxidases. But it is purely tetranuclear 1. So, it is a homo tetra nuclear copper species of involvement of four metal ion centers.

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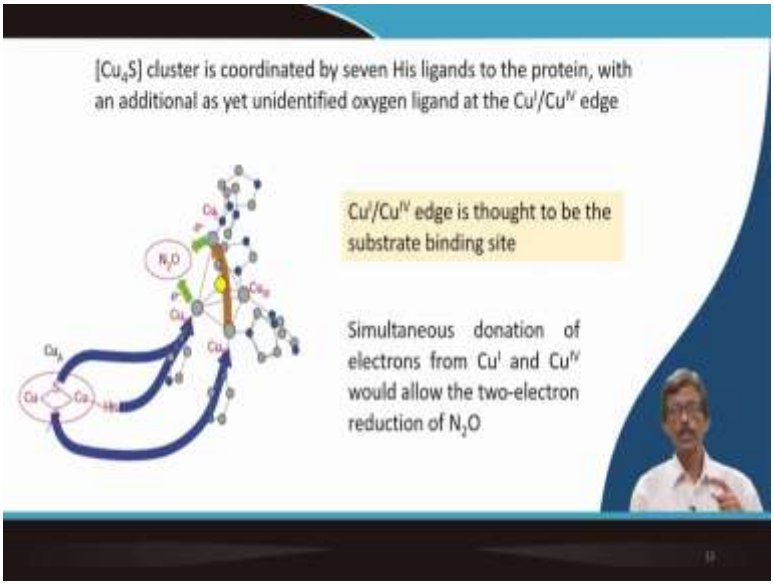

So, this particular cluster is coordinated by seven histidine residues. So, you try to remember how you can form a Cu 4 S assembly. So, as we all know like your oxide ion, it can form four bonds to the sulfur. And because initially people were confused, people were thinking at one

time that you can have the oxide center within this particular Cu 4 core. But it is not that, it is a sulfide which is the bigger one.

So, sulfide and iron as S to minor which is ubiquitous in our biological world, like your ferodoxine molecules and your 4 iron ferodixine molecules also. So, you quickly look at now the whole structure. So, at one end on the left basically you have the copper A. And you can have at another point you have the copper Z site. But where the you N2O will reach? So, you have the bigger all these arrows, because these are the paths for your electron transfer.

So, electron transfer paths are showing by these big arrows, blue arrows basically. So, the long distance electron transfers are there because there are some organic other molecules, the protein chain and all these things is not in the vacuum. Electron transfer not in the vacuum. So, the biological centers are available. So, these biological centers are basically in such a way that is so good that you have the protein envelop.

One part is reserved for binding the Cu and another part is reserved for binding Cu Z. So, 1, 2, 3, 4 you now quickly level the four copper center as 1, 2, 3, 4 and you can have the different edges if you place them in a tetrahedron. So, one of the edge basically can be responsible for that N2O binding. So, the simultaneous donation of electrons from copper A and copper 4, so 1 and 4 will allow the two electron reduction of the N2O.

So, in this particular case you required only to electron reduction, not the 4 electron reduction of that we have seen earlier for your O2 molecule.

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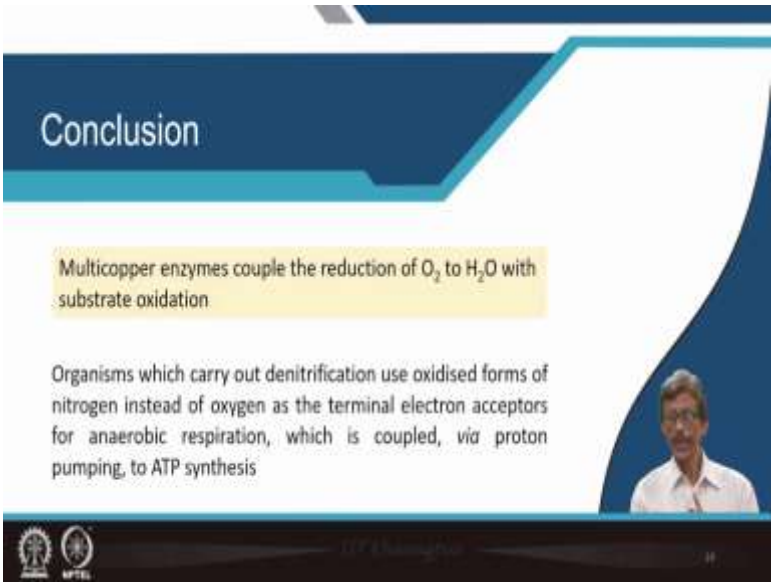

So, in essence what we have seen that, we have seen that how important the, your multi copper enzymes are. And how we can tackle not only for a system like deduction of your O2 to water molecule as well as the substrate oxidation, but in some cases more complex one what we see that for the D nitrification, we use the oxidized form of the nitrogen instead of oxygen. So, there are some organisms, which basically take up oxygen from nitrogen oxide.

We know during the lightening process or dinitrogen from the air is getting converted to nitrite oxide. So, that nitrite oxide can be a useful source of nitrogen to some other species. So, the terminal oxygen and electron acceptor for an aerobic respiration. They are not surviving on O2 but they can survive on N2O. That is why the information you can have is that okay, I am dependent on N2O, not on O2, so it is a very good thing.

So, which is coupled by a proton pumping and ATP synthesis, all the important biological thing can happen there and it can get all these things.

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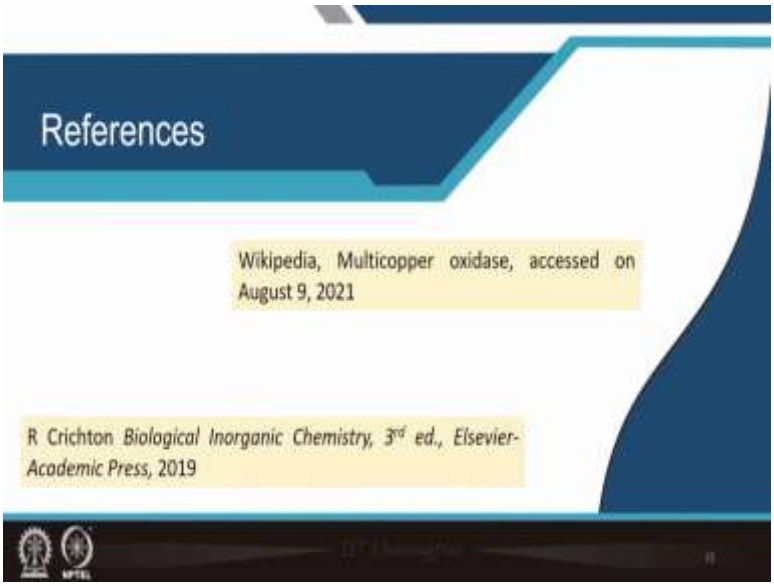

So, to start with, go for the page of wiki, Wikipedia page of multi copper oxidase and then the book of Crichton. So, thank you very much for your kind attention.