Biological Inorganic Chemistry
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Lecture – 36
Oxygen transport and SOD activity

Hello, good morning everybody, so today we will start from your new module, which is your module number eight, and where we just go from iron to the copper ions. So please try to recapitulate whatever you have learned so far about the chemistry of copper ions because we have to take that copper ion and we have to put it that in the biochemical environment.

And then we will study or extract the properties of the different copper ions in the different sites. So this lecture is devoted to the oxygen transport like your haemoglobin and myoglobin, how we find it in hemocyanin and one part will be for the SOD or Superoxide Dismutase activity.

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So the concepts basically will just see that the biological systems if you have the copper ions, what are its implications and how the copper ions can show or can function differently. Then like myoglobin and haemoglobin will have that oxygen binding as well as will go for the activation because side by side if we read little bit about the activation of the dioxygen molecule also it will be interesting because when we go for again the copper based oxygenases and oxydases.

Then the last part of this lecture will be for your SOD, SOD1, 2, 3 et cetera. Then what are the mechanism of action when you find that is catalyst for your dismutation reaction involving your superoxide and ion and which is basically functioning as some antioxidant activity because this super oxide is under the class of ROS molecules or ROS systems, which is your reactive oxygen species. Then finally, we should see also that how these all are related to the human health and human diseases.

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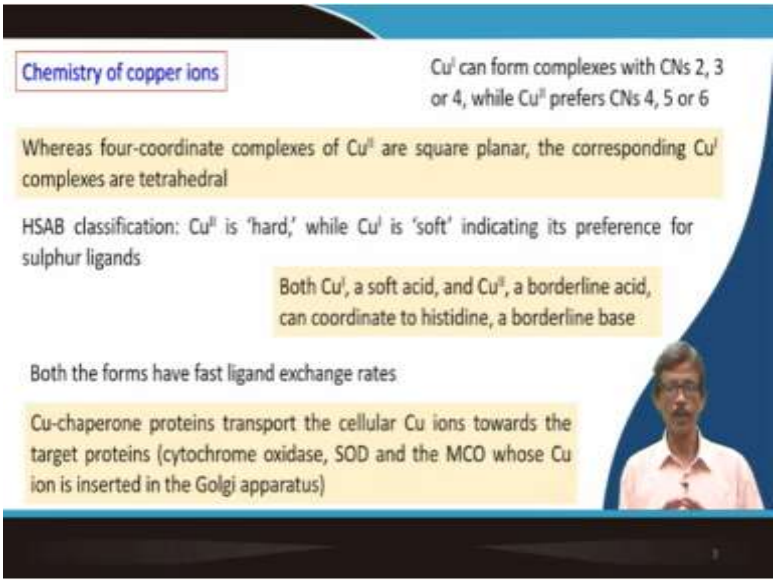

So just let us see something related to your copper ions what we are studying from our school days. Again, and again I am repeating all these things because we are not going any far away from that particular chemistry what we have learnt so far. So when we have a two oxidation states because we know that if you want to see the chemistry of copper ions you try to dissolve some salt, if you have some salt like copper sulphate which we first know the corresponding metal ion salt in our lifetime that copper sulphate, or we can have the copper acetate in our hand.

And if we know the corresponding structure, the crystal structure of copper sulphate pentahydrate is the hydrated form because the solid state structures of all these species will be different if you go from a hydra species to an unhydra species. But when you dissolve these two salts in two different test tubes say, test tube number one and test tube number two.

What you see that when you are dissolving copper sulphate in water medium or when you are dissolving copper acetate in water medium will find that only everything will be dissociated because the polar solvent water will try to break the crystal lattice or the corresponding salt or

the compound then organic compound of copper sulphate or copper acetate. So we will only leaving behind with the copper ions.

The way our system or the living system or the biological system can take up those copper ions what we call as the Homeostasis of the copper from the environment they are trying to gather it. So when it is binding to many number of water molecules when we are trying to dissolve it in water will find that in case of copper two the coordination numbers the most preferred coordination number is always five or six and sometimes it can go down to four.

So these three basically the upper limit, if we consider that the limit is from two to six, the upper three that means the coordination number four, five and six can be reserved for the cupric state. Similarly, the lower coordination number you will be reserved for your cuprous state as we all know that the when the oxidation state is less it will have the less demand for your negatively charged or any other polar molecule to surround itself.

So when we have the four coordinate complex of copper two which is the lower limit of the coordination number limit, we can have a square planar arrangement, so we can call it as a tetragonal coordination environment. So when you have the square planar one it can increase its coordination number to go for an apical coordination to a coordination number of five which will be square pyramidal one.

And if you go from the bottom part also like your myoglobin, we go for an octahedral geometry to a coordination number of six. But in case of copper one it will not be preferring the corresponding square planar geometry because the crystal field is such that it will only try to remain in a coordination geometry which is very much similar to that of its electronic configuration of 3D, then like your zinc 2 plus.

So, it will try to remain in a tetrahedral geometry. So the corresponding hard soft and acid base theory is telling us that copper two is hard because is the higher oxidation state while copper one is soft so it will have the corresponding preference for copper one will be for the softer ligands and copper two and one both will be preferring a borderline other donor group which is your nitrogen that will see with time. So copper one as it is soft acid and copper two is a borderline acid can therefore bind to the histidine nitrogen.

Histidine has two different types of nitrogen one is the tertiary nitrogen only and another is the nh nitrogen, so which one is binding to the copper so definitely you can have some little

bit structural difference electronic property and the hardness difference very slight difference in the hardness is there. But in terms of its corresponding nature of the donor atom it is the borderline base so you can have the corresponding binding for these two cases.

So, in that way the histidine nitrogen is a unique one to coordinate both copper two and copper one is not like that if you go for the oxidized form or the reduced form one of the donor atom will be just removing from that coordination side and can remain as the dangling. And they both can have very high ligand exchange rates, so water molecule is coming and it can also go and it can exchange with other incoming ligands also.

Then the transport proteins, in case of copper which is chaperone, so copper chaperone is basically we can have and this particular case you can have the corresponding cellular copper ions towards the target proteins for the synthesis or biosynthesis of those protein molecules. So if we want to synthesize the SOD and other related molecules we have to go for the cellular copper ions which is your store and some other molecules.

The copper chaperone will be available to transport all these ions to the site of synthesis and if you have a multi copper oxidase thing that means MCO so maybe in the next class will just talk about the multi copper oxidases, where many number of copper ions are there and but the individual copper ions are getting inserted within the Golgi apparatus of our cell.

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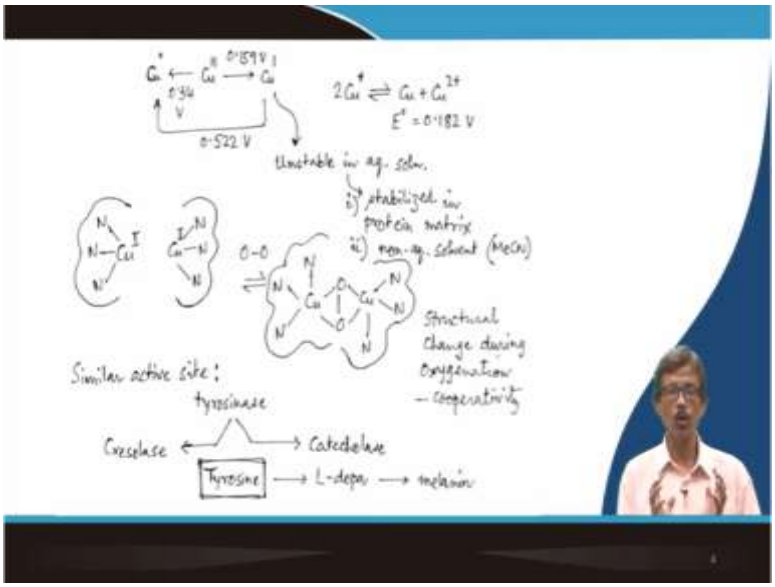

So if we just quickly correlate it will try to correlate it the copper in two different not two also the other elemental state also which is your copper zero, so from the top if you see that

you can have the corresponding one as your copper zero state and that copper zero state is basically is a metallic state, then you can have the copper two.

So if you go for the reduction of copper two in two cases that one is your copper two at the center, so you can go for the two electron reduction to the left to copper zero which is a metallic copper and its corresponding electrode potential is given which is 0.34 volt, but when you go for another reduction to the cuprous state it is only 0.159 volt.

So you see the differences in all these electrode potentials and finally, also if you move from copper one to copper zero your potential is typically different, which is 0.522. So in a sense what you can think that your copper one is very difficult to stabilize in aqueous solution that is why we do not get the copper ions in aqueous medium, all the cases you have the oxygen and copper one will be oxidized to copper two.

But when as the intermediate species if you are able to prepare at some point copper one in the solution or say if you take cuprous oxide, Cu2O and if you try to solubilize that in the medium of any aqueous solution, what will you find that immediately when the copper one is coming out from the corresponding salt of copper oxide, the cuprous oxide it will try to disproportionates and disproportionate will have a positive potential that means your negative free energy change for that particular process of disproportionation reactions.

So this copper one basically will disproportionate into copper zero and copper two plus, so how to stabilize this particular cuprous state that will see, since it is unstable so you can it can be stabilized by protein matrix such that your all these reactions in water medium is hindered. The water molecules cannot have the access and it cannot go for all these disproportionation reactions, sometimes we can go for the copper two also, but it can be stabilized in the protein matrix or some non-aqueous solvent.

If you just try to remove the water medium and go for non-aqueous solvent like acetonitrile MeCN or Ch3CN, a very good starting material in our laboratory we can prepare from your simple cuprous oxide if you take it in acetonitrile, dry acetonitrile definitely neither water nor oxygen should be there because water is basically go for this dispersion reaction and the molecular oxygen is also responsible for the oxidation.

And your very interesting starting material you can have with the copper one as Cu MeCN whole four and outside you can have the typical an ion like perchlorate or pf six. So that

copper center is the tetrahedral copper in the solid state also you will get it with the coordination from the four nitrogen donors of the acetonitrile molecule, so which will be very much similar to that of your histidine coordination.

So if you are able to bring three such donor groups, what we see in case of your hemocyanin also this is the reaction what we will study for the hemocyanin. So three donor centers one, two, three from one phase and another three from the other phase, so if the protein is coming like this and these three are allowed to grab copper number one and these three is also allowed to grab the copper number two you get some by nuclear entity.

Even if you are acetonitrile because in case of acetonitrile, it will just directly going for coordination to the four acetonitrile molecules not three, it will be very difficult to remove the fourth acetonitrile molecule otherwise you can get this sort of thing. So some designing of the ligand system is required if you try to model this particular environment this geometry.

And we will also see when this dioxygen is binding we get a very interesting type of binding and there will be a typical structural changes during oxygenation reactions which is much more than that of your myoglobin and haemoglobin structural change. So we all know that when you have the structural change which can facilitate the binding of the dioxygen for the other molecules also we find the cooperativity.

And similar active sites we will also see for the binuclear copper oxydases which are tyrosinase system and tyrosinase can show cresolase activity and catecholase activity and when we have the substrate the tyrosinase the amino acid or some substituted tyrosine amino acid you can go for the L-dopa and the melanin our skin pigment formation also we can see so you need the copper enzymes for the formation of the melanin.

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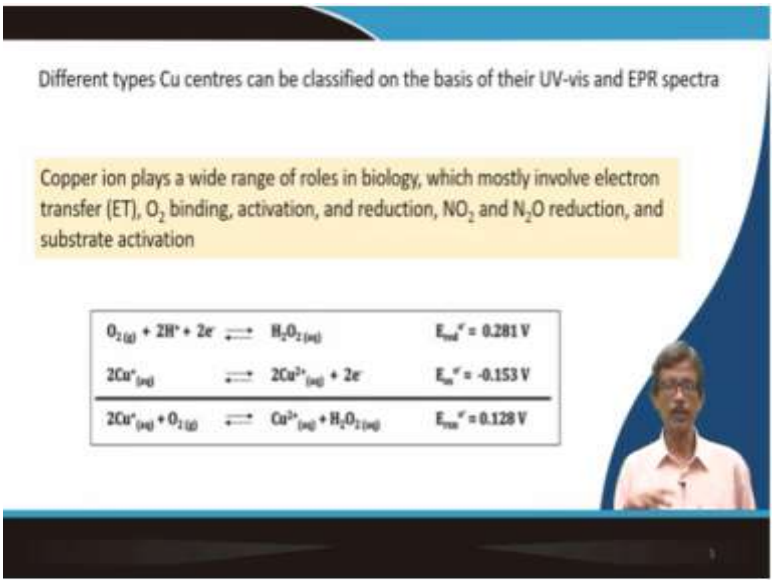

So what we can have if you have the different copper centers and if they are in the solution so what you find that how to identify the nature of this particular copper center. So first thing is that first challenge is that whether you are talking about the cuprous copper or you are talking about the cupric copper. So we will rely on the solutions and the solutions of the protein origin or the enzyme origin.

But will basically rely on two particular spectroscopic technique one is simple invisible spectroscopy or electronic spectroscopy and another is your electron paramagnetic resonance spectroscopy. So, why we study these things? So if once you are trying to locate the nature of the copper center in this particular biological environment or even in the solution of copper sulphate or copper acetate we should be able to identify these things.

But the first thing what we will study today is your involvement in typical electron transfer reactions like your rubidoxin and ferridoxine molecules, then before that we definitely will talk about the oxygen binding like Myoglobin and haemoglobin because side by side, we can compare already we have learned in our previous module what about about the corresponding iron chemistry, iron coordination chemistry and iron biological chemistry.

So where we have seen that Myoglobin and haemoglobin molecules are responsible for O2 binding then we like cytochromes will see about the ET processes then the activation how we can go for the activation of the O2 molecule and its reduction to superoxide or peroxide and

the reduction of nitrogen dioxide as well as nitrous oxide that is why we have nitric oxide, reductases and nitrous oxide reductases are available.

And finally the substrate activation like any kind of coordination from that particular molecule like catechol, we will have the catechol oxidase, so your catechol will be the good substrate. So like the equilibrium what we have seen for the copper ion, similarly what we can correlate now the oxygen because we will be talking about oxygen binding, oxygen activation as well as the substrate activation.

So, when we see that when O2 is converted into hydrogen peroxide we have some potential value, when copper is oxidizing to copper two we have another potential value what we have seen just now. So, now if we correlate these two so the copper one also can be driven towards right hand side for the state of oxidation to the cupric state is driven by the corresponding use of O2 molecule producing hydrogen peroxide.

So, if it is a very simple reaction of this type what you see that during that particular catalytic process or enzymatic process say catechol oxidase and we all know many things when you open the bottle in air, the oxygen present in air can oxidize it or oxidize that substrate, similarly the browning of your the potato skin, the potato skin is getting brown basically and that is due to the presence of catechol oxidase.

So copper is present and copper basically activating your dioxygen molecule such that you can oxidize some other substrate while it is settling between these two because this is in equilibrium between copper two and hydrogen peroxide and between copper one and oxygen.

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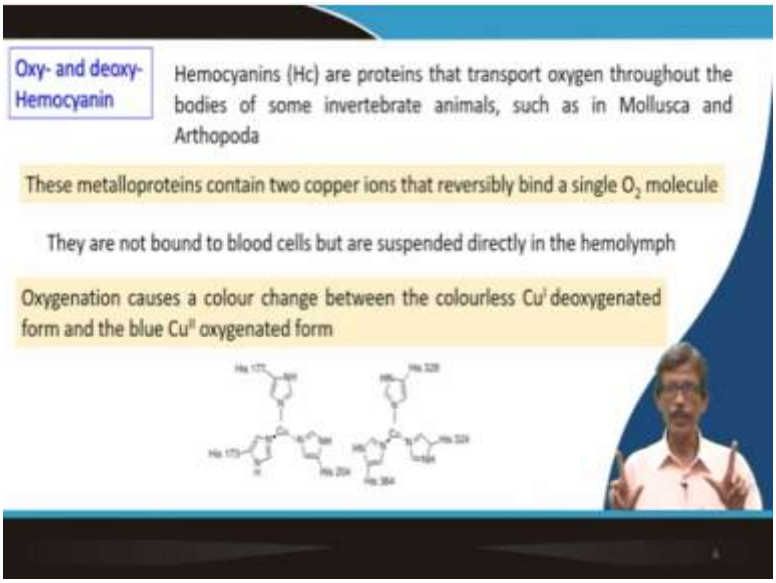

So what we have seen just now that we can have one species as the deoxy-Hemocyanin and another is the oxy-Hemocyanin. So what are these? These we get basically from invertebrates like Mollusca and Arthropoda, like your all these crabs and all lobsters and all. So they are basically of these categories, they basically have these Hemocyanin molecules to transfer or transport oxygen.

So in this particular proteins we have two copper ions which can reversibly bind the single dioxygen molecule, just now what we have seen So they are not bound to the blood cell because here no direct blood cell is required for the transfer of the oxygen but are suspended directly in the hemolymph. So within the hemolymph they are suspended and by looking at the colour change only like the way we can talk about the use of UV visible spectroscopy.

So, invisible spectrum is there and that spectrum if you have a absorption at a particular lambda max value you know the corresponding lambda max value and its corresponding amount of absorption that means the lambda max and the epsilon max are the characteristic one for the coloured one. But if we see that we have only the copper one which is blue in colour like your copper sulphate and copper acetate in solution of water.

So in the oxygenated form will be able to monitor that, but if it is a deoxygenated one will not be able to monitor it because it is colourless like your zinc two plus ion. So you will bring three of these histidine residues, so you have now numbered, we can also number these as in

the fashion on the left hand side and on the right hand side because the number on the left hand side is less which is starting from 173, 177 and 204.

And on the right hand side is 324, 328 and 364, you see two of the amino acids are nearby, always try to remember why these numberings are also important to understand. So, your histidine 173 on the left and 177 at the top left also, it is basically functioning as some extended bidented part of the ligands thing, which is therefore your this histidine type, so this histidine 173 and 117 is functioning as a bidented ligand and binding your ion center.

And the other one with after some relaxation or some flexibility, because the chain length is moving from 177 to 204, is going for that particular coordination and is binding this copper. But the way we have written over here any one of this histidine residue is not utilized to bridge these two copper center, so that is also can be another possibility, why either your histidine 204 at the left and 364 at the right is not utilizing for bridge to metal ion.

Because histidine after deprotonation you can have a charged histidine, so imidazole residue having a charge of minus one can bind through these two nitrogens, is a very useful breathing thing or bridging entity but it is not showing that entity because your protein structure is completely different so that will encounter only in case of your superoxide dismutases.

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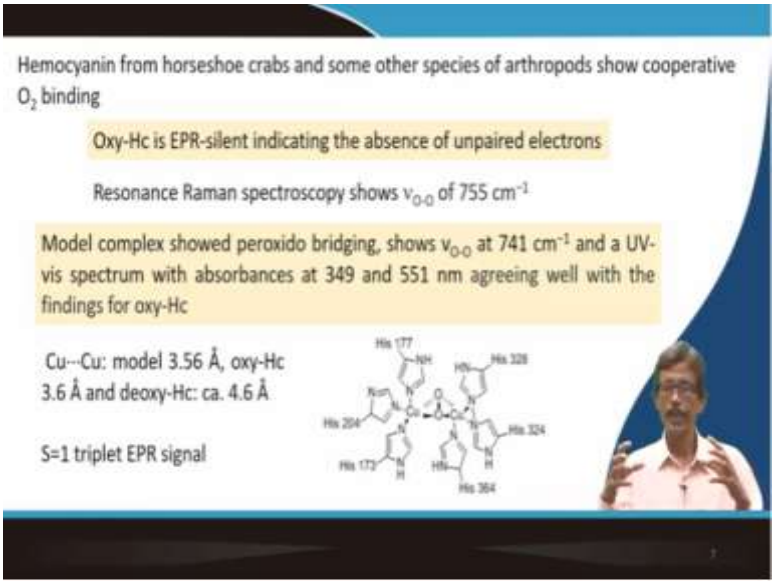

So these Hemocyanins from horseshoe crabs where we find where you determine a hurry experimentally found the structures of this category of earth arthropods so cooperative

binding what we have already seen that you can have the corresponding hills coefficient also. So oxy-Hemocyanin, when it is oxy-Hemocyanin it is blue in colour but it is EPR-silent.

But what we know now that you have individually the copper two as well as the another one is also copper two but is not showing any EPR response. So what is the corresponding explanation for that? Why it is not showing your EPR spectrum? But before that we should know about the character of the O2 which is getting inserted in between the corresponding copper two sides.

So, you basically record the resonance Raman, FTIR is not active because you have a symmetric o-o thing no permanent dipole is there so that is why 755 centimetre inverse stretching for this bond stretching of o bond stretching is important and which can give rise to very important thing and which can also be compared with the laboratory prepared model compound of any types which is the best possible thing around 40 years back or 38 years back it was published in 1992.

The first paper by Kitajima group in journal of American chemical society; that the model complex or the model compound of di-nuclear copper system is basically first identified and first predicted this sort of binding, very interesting binding. But the two things basically the EPR silence your highest FTIR or Resonance Ram staging frequency and the invisible spectrums are all your characterization data.

So now everything is remaining in the side because your histidine 204 is going back little bit and your histidine 263 is also going little bit back when your O2 molecule is coming and binding in this particular fashion, but that was not the usual binding of the peroxido molecule to two copper centers that has been established also afterwards.

So when people prepared the Cu model compound your separation between these two states are 3.5 angstrom which is very much similar to that of your oxy-Hemocyanin. So people started thinking ok since your model complex your copper copper separation is very much close to that value what we can found in oxy-hemocyanin.

So oxy-hemocyanin O2 binding which be very much similar to that offer this sort of binding which is completely different from that of the separation of 4.6 angstrom for deoxycase because we have seen that in the deoxycase it is quite separated between these two metal and center. So S is equal to one which is a triple EPR signal, so s is equal to ones so you can have

the corresponding triplet EPR signal for these two copper side but if they are strongly coupled between these two sides and if Your S is equal to zero state it can be EPR silent.

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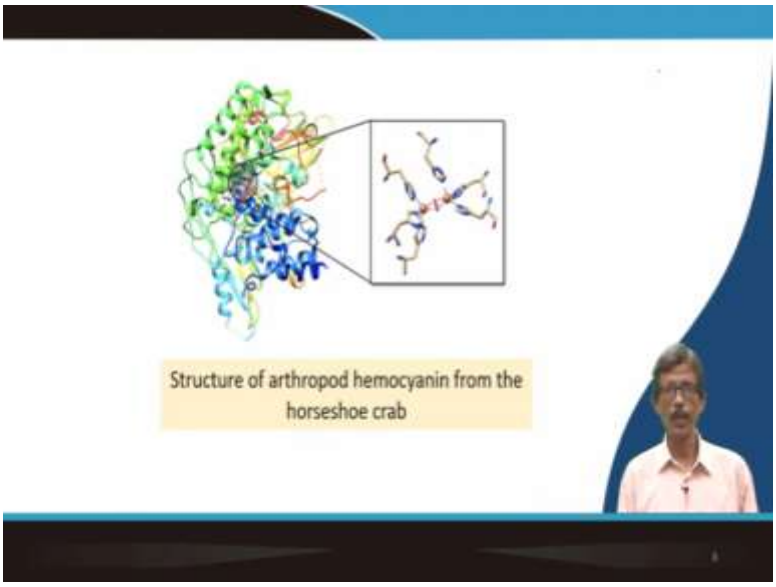

So these two states basically S is equal to one and S is equal to zero is important depending upon the corresponding interaction between these two sides so once excess structure is determined for the horseshoe crab variety of hemocyanin and which is the structure of arthropod hemocyanin that gives the very basic one which is the core basically the circle part.

So circle part has been enlarged and we find that you can identify these two copper sides is the corresponding one as the golden yellow colour of these two spheres, so those are the two copper sites and you have the red is your the oxygen molecule, so red bar is basically your dioxygen molecule. So the three dimensional structure is in your hand.

So, we can rotate this whole structure and you can find out this typical binding which is bridging between these, this is your copper center and this is your copper center and this top is your oxygen and this, so this diamond motive is forming, so this diamond motive is important, this is copper oxygen copper oxygen motive is important because this binding is very important because the peroxide oxygen is forming 4 new copper oxygen bonds and the binding is mu eta 2 eta 2 binding, eta 2 eta 2 o 2 2 minus binding of that peroxide ion.

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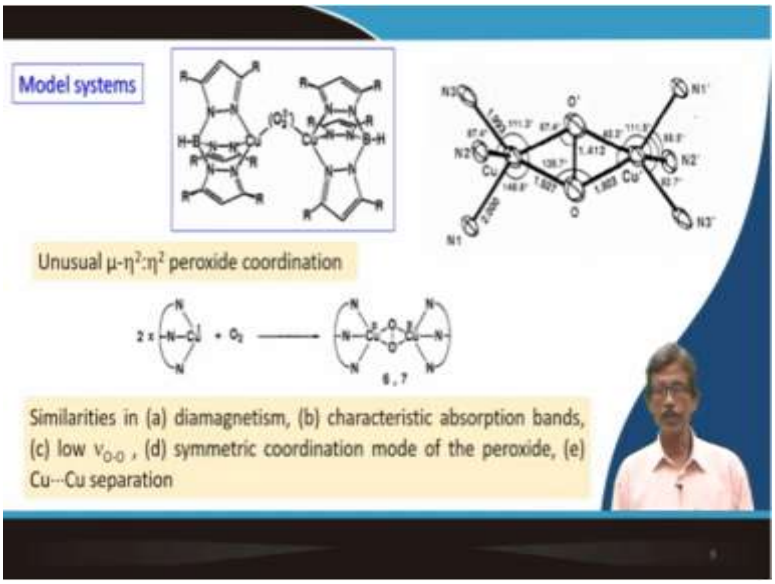

So how people have identified that and how people have established it through the studies from the model systems people used some laboratory prepared trispirazole boret is a boret ligand so you see the three part three nitrogens of the pyrazole second nitrogen of the pirajeole ring which is coordinating to the copper centers from, but it is anchored basically at the vertex of your boron center.

So is trispirazole oil borate which is a very useful and still we study using this sort of ligand for a facially capping ligand, so one phase basically you can cap for copper and another phase you can call from the other side, so basically that brings two copper centers together and when it is there no other (())(24:27) donor atoms are available like carbon monoxide and if you allow it or if you bubble the oxygen molecule it immediately go and bind those copper centers to giving you this particular molecule.

So, the metric parameters the bond distances and bond angles if you can have, it has been taken directly from that paper of Kitajima of Jacks 92, so that structure basically gives us the orientation. So, you see that three bonds basically so coming from this side and the other three from the other side so in between you have the O. So you can have the corresponding O distance, so O linkage you see is 1.412 angstrom. So, what is that?

So, you should know from your school days again you are starting the O distance in your oxygen molecule, O distance in your superoxide molecule what we have studied in case of

myoglobin and haemoglobin and O distance in your peroxide, the typical hydrogen peroxide or other barium peroxide or sodium peroxide we can have some good idea.

That also now established that, since the binding is different and the stretching frequency is also very less it is mu eta 2 eta 1 type of peroxide coordination and that is why directly it is forming, so two such system of this copper one complex in O2 medium at some low temperature say at very low temperature of 77k which is liquid nitrogen temperature we can see. So when you have the model systems and that model system can be compared from diamagnetism to the copper copper separation also all these things we can compare.

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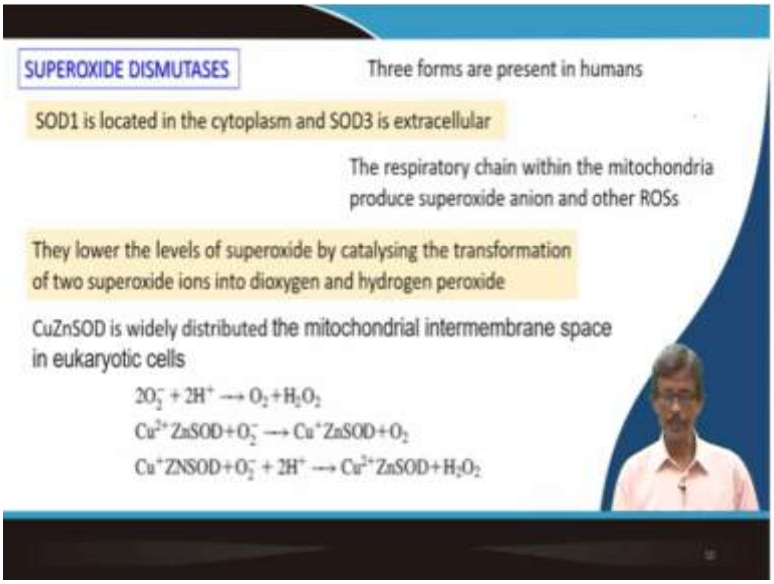

So next we just quickly see the superoxide dismutase very much similar to that system where you can have the copper copper hemocyanin system. But this is a super oxide one system and which is available in cytoplasm and another can be from superoxide three system which is from the extracellular origin. So basically we can have also three, one, two, three. So these are the three varieties we can have and which is there for the respiratory chain where we produce large number of these reactive oxygen species.

So when we have these the lower we have already seen that how we can go for the superoxide dismutation reaction between oxygen and hydrogen peroxide, so the copper zinc one is very important and which is distributed between these two reactions, so you see the follow the reaction in a sequence manner in a sequence manner when your super oxide having an intermediate oxidation state can be used for oxidation as well as can be used for reduction reaction for this particular case.

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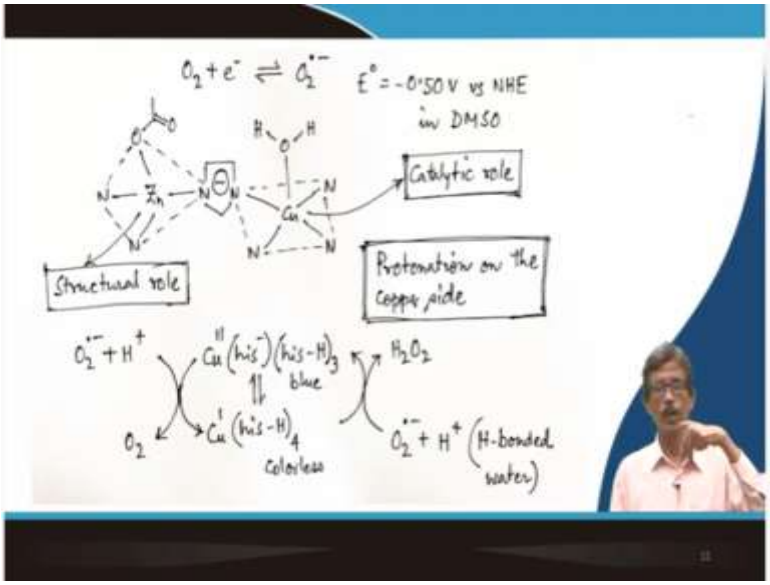

So you have this environment now you see unlike your hemocyanin case your immediate ring or imidazole ring from the histidine residue will come and be these two copper centers but zinc will play only the structural role and copper will play the catalytic role for a very simple reaction of the super oxide formation as well as the scathetic cycle. So at the left, from top left you have the superoxide and on the right you have the hydrogen peroxide and two such superoxide is basically collapsing and on that particular catalytic site producing hydrogen peroxide from the consumption of O2 molecule.

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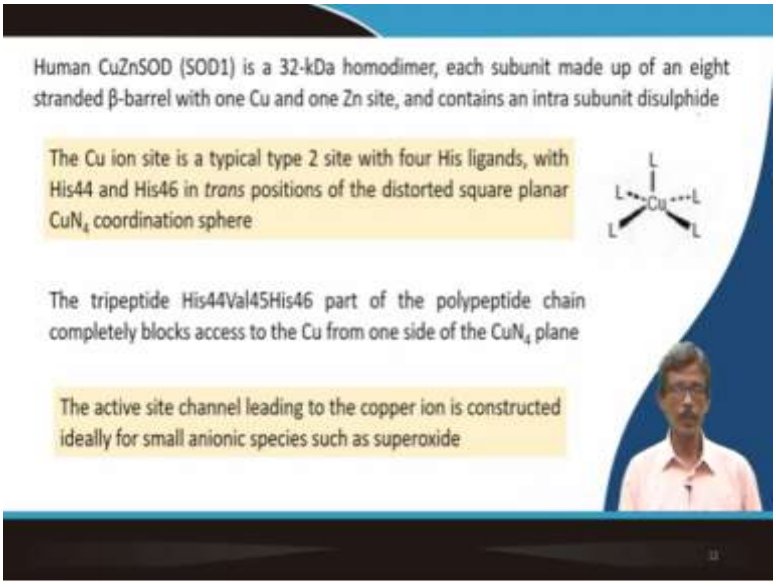

So human copper zinc superoxide which is your SOD1 and the molecular weight is also identified your structure is also identified and 2 sub units of disulphide linkage has also been identified and the corresponding numbers of the residues histidine 44 and 46 is important where you can have the corresponding binding in this particular square pyramidal environment, because in most of the time you are here is the type 2.

We have moved to a type 2 only because in our next class we will be talking about the type 1, type 2 and type 3 variety of these copper center, we will find how we can assemble this Cu and 4 system and then we can have the fifth coordination. So, this active channel basically leading to the copper ion which is constructed ideally for the species which such as sub superoxide.

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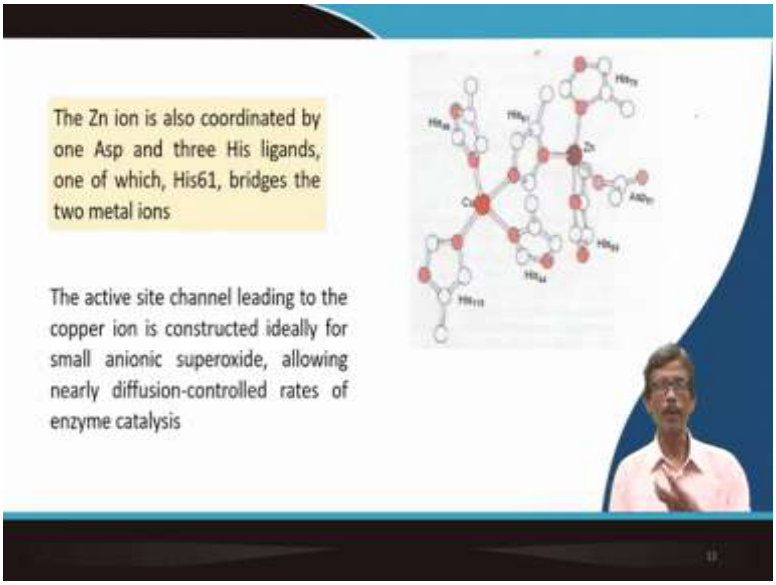

So super oxide can go and enter there and you can have all these, so that particular thing what we find actually in the structure is basically this one. So you should know about the structure you should know about the bridging and you can have the corresponding copper environment in that way, so what you can have for the copper and the zinc environment.

And you can have the corresponding passage of the superoxide molecule which is diffusion control and you can have a particular rate of that diffusion and which is very much close to that of your current corresponding rate of the catalysis.

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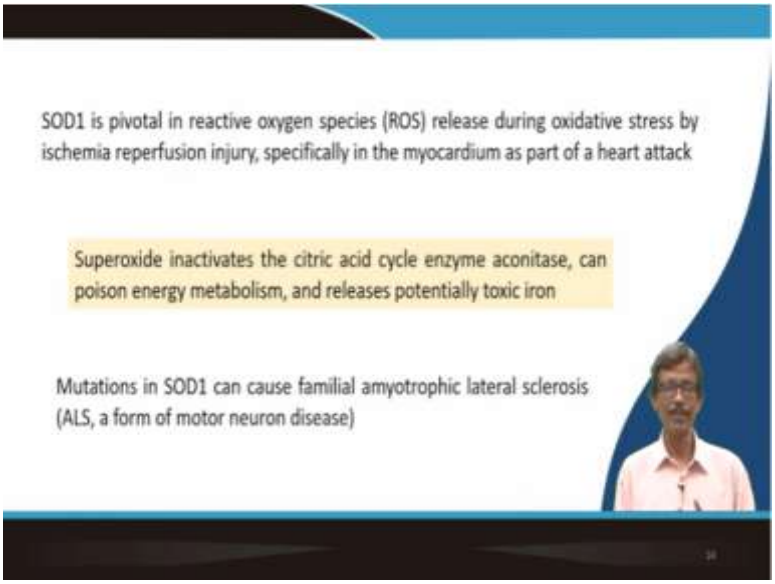

So you can have many diseases you can have the heart attack related to the excessive accumulation of the ROS molecules and which can also inactivate the citric acid cycle in our body and also a very typical disease the motor neuron disease you know the very famous people like Stephen hawking was suffering from this ALS disease which is due to the genetic defect from SOD1 which is producing that SOD1 in our system.

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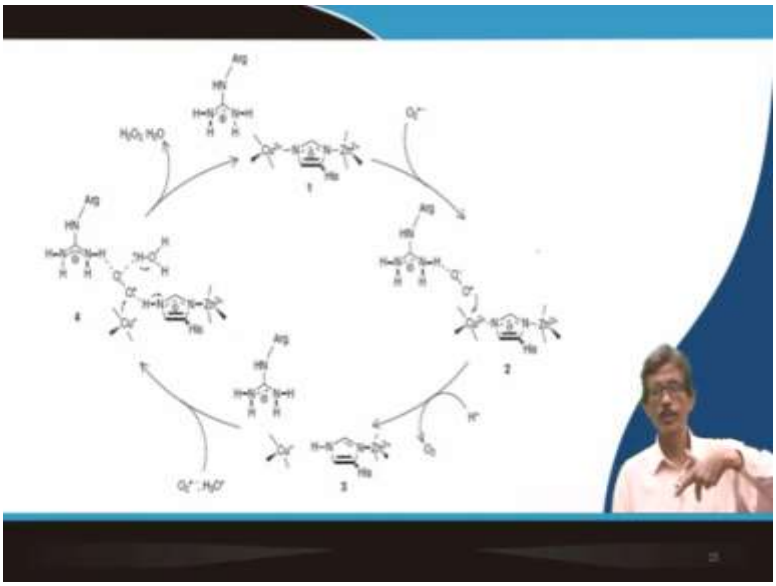

So if you see in detail how it is happening, so you have the copper two side and the copper two side is basically converting to a copper one side that is the reduced form and you can have the other groups, the arginine function is coming from the top which can show some

hydrogen bonding Interactions which are nothing but your secondary coordination sphere interactions.

So not only the primary coordinations around the copper and the zinc center but the secondary coordination from the arginine residues from the top which is showing some hydrogen bonding interactions with the superoxide anion which is a charged one for the production of hydrogen peroxide and it is a control of water molecule or the water cluster and arginine residue for the production of your hydrogen peroxide.

So you take your time you give time to just basically understand this whole cycle is a very simple cycle what we have shown earlier also that how the redox is basically required, for your redox between the two super oxide molecule to producing o2 and hydrogen peroxide back.

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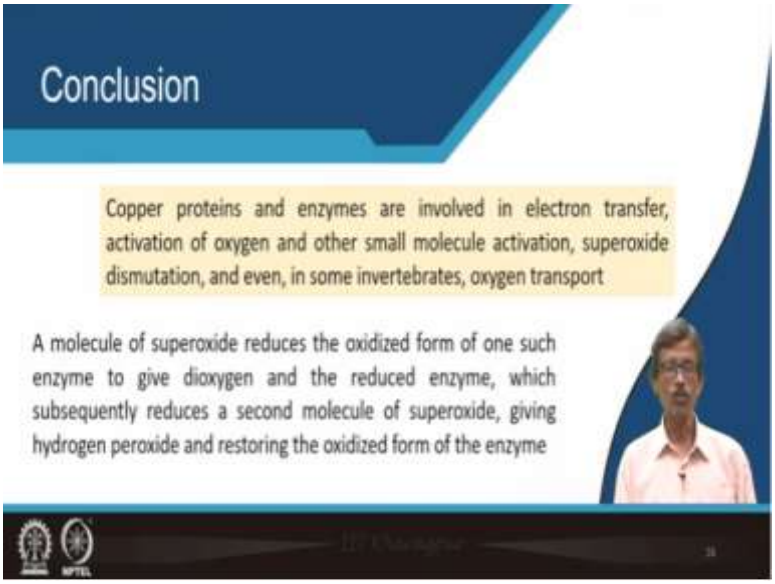

So all these copper proteins and enzymes why we have studied is a required for small molecule activation dismutation reaction in some invertebrates also like hemocyanin or deoxy-hemocyanin is required for oxygen transport. And in the case of superoxide basically we are producing the hydrogen peroxide and the O2 back but we are getting back the corresponding oxidized form of the enzyme which you require at the starting point.

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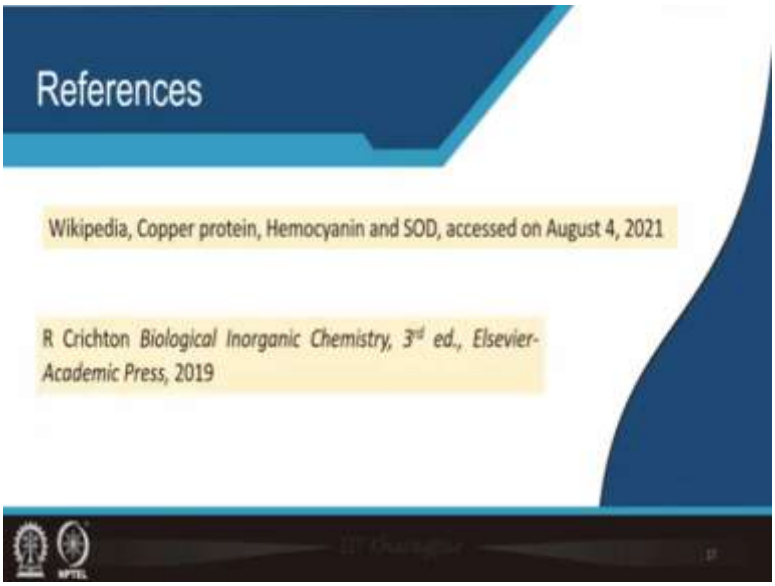

So the references for copper protein page of Wikipedia the Hemocyanin page as well as the SOD page will be useful for you and the book of Crichton. Thank you very much.