Biological Inorganic Chemistry
Professor Debashis Ray
Department of Chemistry
Indian Institute of Technology Kharagpur
Lecture – 37
Type 1 blue copper proteins

Hello, everybody. So welcome back to our class of Biological Inorganic Chemistry where in this particular class we will be talking about type 1 blue copper proteins. So, as we discussed in our last class, you can have type 1, we can have type 2 and we can have type 3 and all of them are coloured in blue.

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So these are basically useful in knowing the corresponding typical electron transfer reaction what we have seen in case of iron proteins of Cytochromes, then what are the different types, as I told you just now that is type one, type two and type three and two immediate categories for this type one is azurin and plastocyanin from plant origin and which is studied in detail and we know all of these very much for these two.

And then little bit complicated one which is having different number of copper center that means your multi-copper centers are Laccase and Ascorbate Oxidase.

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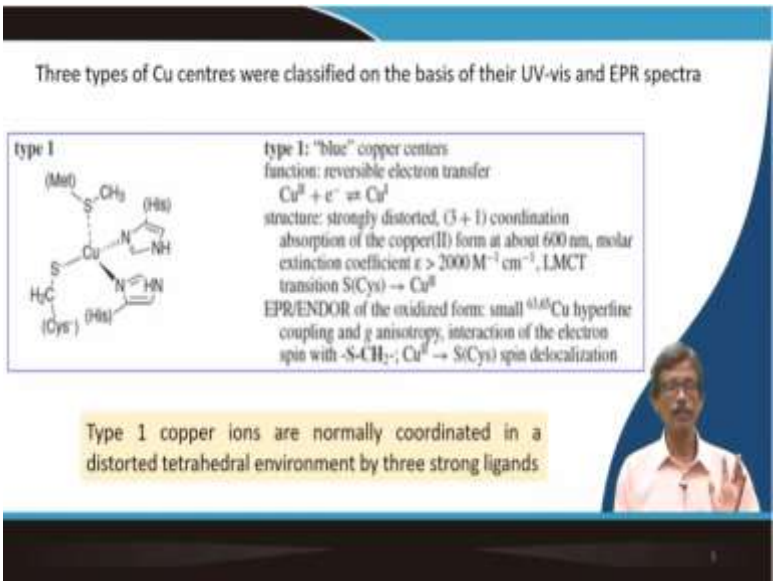

So if we have three different types of copper centers, so not only identification of copper in hemocyanin, but also in the different types of copper centers. We can also find out with the help of only the electronic spectra and the EPR spectra. So what are they are for type 1, so we are talking about the blue copper proteins and we have already seen since they are used for electron transfer. So there will be a very fast electron transfer between the cupric copper and the cuprous copper and a coordination environment of not four type.

We are writing as three plus one, so three will have very strong bonding, which is a trigonal plane, not a tetragonal plane like perfirin, is a trigonal plane which is strongly bond, and then one loosely bond one is therefore, is three plus one category, which is trigonal pyramidal, not tetrahedral. And in terms of its corresponding UV-visible spectroscopy, you can have a band at around 580 or 600 nanometer and epsilon of 2000 mole inverse centimeter inverse, which is due to the cysteinate sulphur to copper transition.

Then other complicated EPR or the electron double resonance, we can also find out for seeing the hyper fine coupling, the g anisotropy in the detail EPR characterizations, so is normally coordinated in a distorted tetrahedral environment, but here the distortion you see that structure has been already shown, now instead of three histidine residues. Now we can have two histidine residues and two sulphur residues of different type. One is cysteine sulphur and another is methionine sulphur.

So if you have the trigonal plane, two of them is histidine nitrogen and one is your cysteine sulphur. So we all know the nitrogen have a strong coordination to both copper one and copper two. Similarly, the cysteine sulphur having charge will also have a strong bond with copper, but the methionine sulphur, which is a thiolate sulphur, which is coming from the top, which is the weaker one. So that will have a long bond distance.

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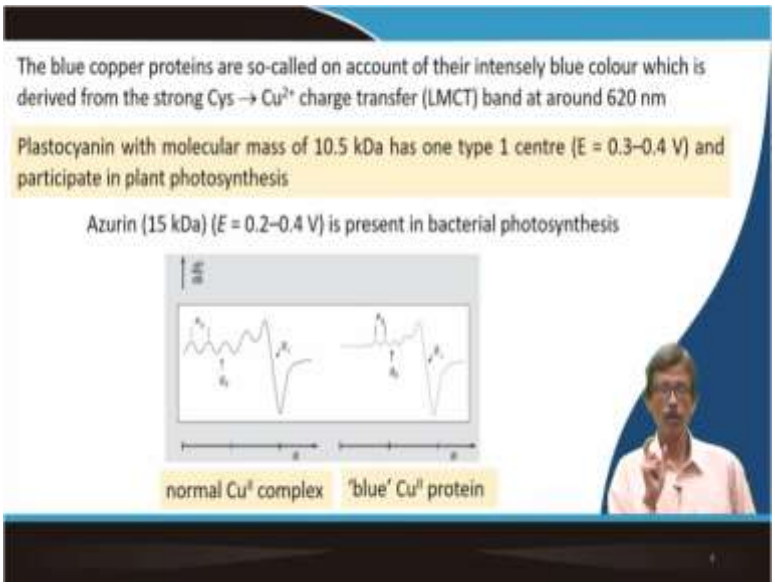

So that basically gives us the situation where you can have this particular three plus one coordination environment. So 620 nanometer, so it can basically vary from 580 to 620 nanometer, which is blue in colour, but looking at not that the corresponding Lambda max value, but you should see the corresponding epsilon max value.

That means your molar absorptivity, so the molar absorptivity is basically will tell you whether you have a charge, transfer transition or not so several thousand. It can be 2000, it can be 4000 or it can be 6000 of that is due to the charge, is transferred from cysteine net ion to copper, two plus, because the copper is electron greedy, it will accept the charge density from the cysteine and ion.

So the Plastocyanin, what is available for plant photosynthesis, because we know for photosynthesis, we require to transfer more number of electrons, which we do not get from the similar type what we have in our body, like your rubidoxin and ferrydoxine molecules, but here the available molecules are your blue copper proteins, having a molecular weight has been identified and one type of copper center and the redox potential, not very much only 0.3 to 0.4 volt is important.

Similarly, another variety is azurin variety which is available in bacterial photosynthesis, so the plant photosynthesis and the bacterial photosynthesis molecules, which are required for copper, electron transfer proteins, has well studied during the last say for 40 or 50 Years, and people are now knowing all these things very nicely.

But if you now go for apart from your LMCT transitions, the ligand to metal charge transfer transitions, we see the corresponding EPR spectrum. So two types of EPR spectrum, I am saying one is like that what we have seen in our previous class, the dissolution of copper sulphate in a test tube of water or the dissolution of copper acetate in any other organic solvent. We just put it in the cavity of the EPR spectrometer and run the spectrum.

What will you get? You will get a normal copper, complex spectrum. So is something like that, but on the right hand, side what you get it will be for your blue, copper protein. So what are the differences? Now you have to find out the g parallel, g perpendicular, what are those values, and how much they are different. So in case of normal, you see, the difference is more for your g parallel and g perpendicular.

But in case of your copper protein, it is smaller between the separation between these two and the a, the hyper fine splitting you have the four lines in this particular g parallel region, which is due to the hyperfine splitting of the nuclear spin of both copper 63 and copper 65. The nuclear number having i value equal to 3 by 2.

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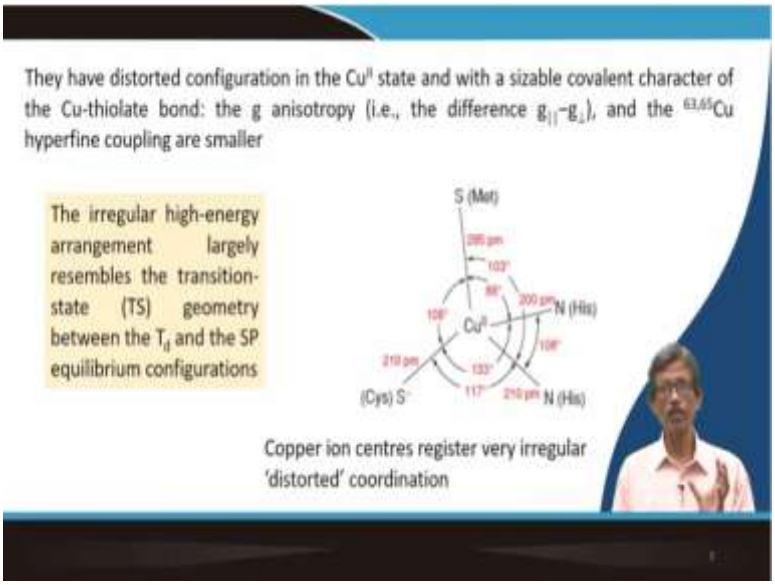

So you have a corresponding tetrahedral structure and the tetrahedral structure is talking about the copper thiolate bond and that is why you have the corresponding charge transfer transition and that charge transfer transition is basically telling us that you can have certain amount of covalency, so percent covalency is also important.

If you find out the corresponding molecular orbitals involving the copper as well as the sulphur of the thiolate ion, will find that corresponding overlap of all these charge densities and certain percentage of this charge is residing on the orbitals, the molecular battery centered on copper and equally amount of, equal amount of that corresponding charge is also deciding on the sulphur molecular, sulphur based molecular orbitals.

Then the g anisotropy the difference basically the g anisotropy is also changing whether it is decreasing or increasing compared to your reference material your reference material is your copper sulphate and the hyperfine coupling is of smaller type, so the separation is smaller and the hyperfine coupling is also smaller we are of the same direction is not that one is decreasing and another is increasing.

So we have to have a very irregular distorted octahedron and truly speaking it is a three plus one category not a coordination number of four of tetrahedral type or square planar types, it is neither a square plane or nor a regular tetrahedron. So it will be of immediate one, intermediate one which is of distortion of d to d type, so if you find the entire geometry, which we consider that can be a tetrahedral type so what you can have.

A tetrahedron, when you get the tetrahedron, the copper, you put the tetrahedron within a cube and alternate corners you occupy them by two nitrogen and two sulphur center. So what you can have you can have 6 bond angles and 4 bond distances, so these are they are basically the magnitude of all these bond distances are given and the corresponding angles are also given and you can have very good idea.

I am not asking you to memorize all these values, but you try to understand which sort of geometry it is. Definitely is not a square planar geometry and also it is not a tetrahedral geometry, because the tetrahedral geometries always you can have a 107.5 degree, but only one of them can be close to 108 degree, which is your sulphur copper sulphur angle. So, you have the sulphur copper sulphur angle is only the tetrahedral angle but the remaining are either compact or more and some case is intermediate.

And the bond distances are also, what we have seen earlier also and again and again will see that you can have a very long bond for the methionine sulphur and solder bonds for all other three so is a high energy arrangement is distorted one and distortion makes the molecule little bit unstable and that is why it is a high energy transition state and which is in between your tetrahedral geometry and the square planar geometry.

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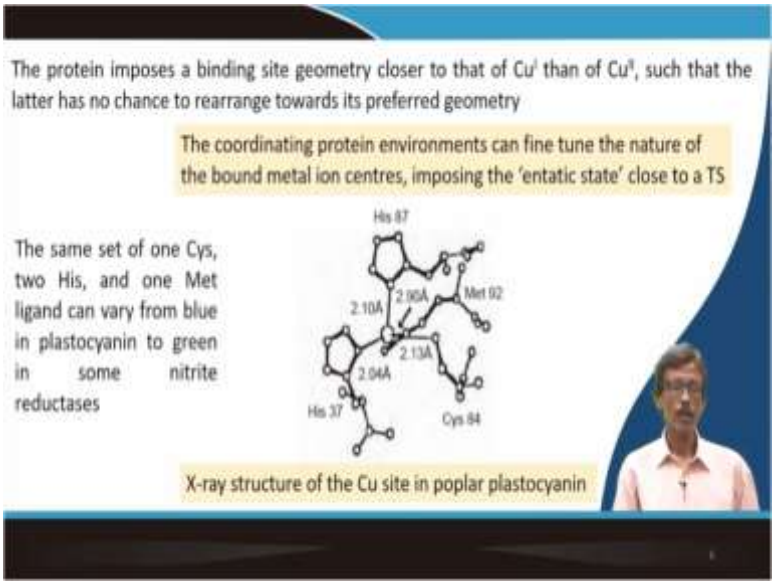

So what we find now that the protein basically is responsible for the imposition of a side geometry because the protein donor centers are important and how the protein donor centers are available and how much we can drag towards the metal ion center is important. If the interaction is strong as per your availability and the flexibility of the protein backbone you can have a good bond distance around the copper center.

And once it is stabilized at equilibrium distances, we can have the regular structure that we basically try to crystallize and get the final excess structure. So the side geometry is closer to that of copper one than that of your copper two because the copper two we have levelled it at it will have a tetragonal symmetry that means the square planer symmetry, but the copper one is the tetrahedral, so more and more it will be in a three dimensional geometry.

So the structure is not of the planar type, it will be a three dimensional tetrahedral type, and therefore the latter part, that means the later ion that means the copper two ion will not get much chance to rearrange towards his preferred geometry, which is a square planar geometry or square pyramidal geometry because before you go for the change, the Frank Condone principle will tell us that how much time is required for a change in the geometry.

The structural change, before you see the corresponding electron transfer change which is very fast which is within 10 to the minus 12 second inverse compared to your corresponding structural reorganization. So the system will not see will not be able to understand that electron transfer is taking place whether you are in a copper one state or whether you are in a copper two state, so it is so fast…

The electron transfer rate is so fast that within some second, fraction of seconds time your electron transfer is taking place for all these biological electron transfers. And your copper will not get time to change its corresponding coordination geometry depending upon its nature that is why it is frozen in some distorted intermediate coordination geometry.

So you can have therefore a fine tuning and the nature of the bound metal ion centers which we call as a transition state or a 'entatic state', which is close to that of your transition state. So in poplar plastocyanin, so poplar the plant origin of plastocyanin and azurin you have seen that you can have which is close to that of your previous structure because the different varieties of these structures plastocyanin from different origins basically.

But the previous one the methionine distance was 2.85 now it is 2.90. So what do you find? That methionine now it is shown in the in the right hand side basically but if you look at it the three and the one it will be at the top which is the longer one. So you orient yourself in your preferred direction and you can think of what the coordinations are, so we can have the other varieties also where the same set of N2 S2 coordinate sphere is preserved. N2 of same type but S are of different type, one is a thiolate sulphur another is the thioether sulphur.

So you gets at some point the blue plastocyanin but if you go for a typical distortion your charge transfer energy or charge transfer band as well as the colour will change so coloured transition will be such that you can have a particular point and you can have the corresponding region which is different which is blue to green it will be moving from blue to green sometime it can move to red region also depending upon its distortion. So you can have another variety which is your green variety of these copper proteins.

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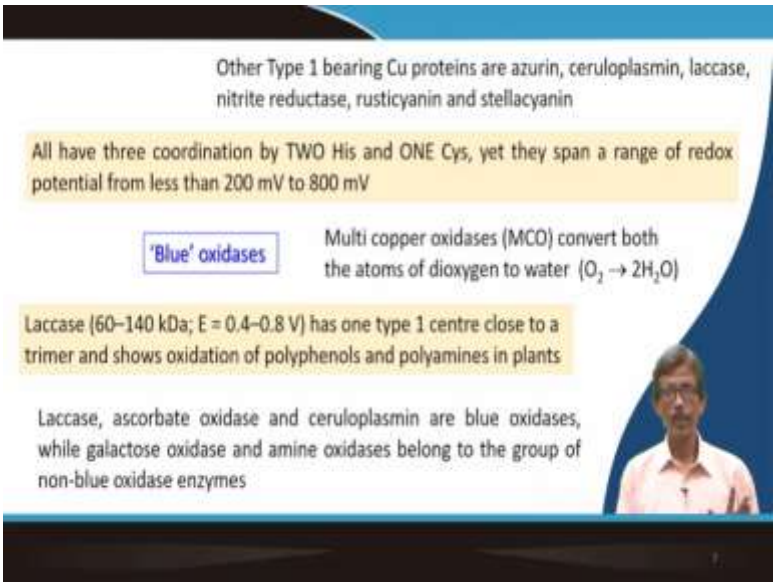

So, not only azurin you can have the ceruloplasmin, laccase, nitrite reductase, rusticyanin and stellacyanin. So all are of different varieties basically but all have the three coordination, the strong coordinations from the histidine and the cysteine and they basically span a wide range of electrode potential, so structurally they are different similarly the electrode potential is also different the colour wise they are also different.

So, that is why our typical biological inorganic chemistry is so interesting that you can extract so many properties and so many functions out of the same copper ion, same copper centers only thing that you have to understand the nature of these copper within the biological environment. Now from the typical electron transfer properties or electron transfer enzymes or blue copper proteins because the blue name is labelled for those particular proteins which are responsible for very fast electron transfer reactions.

But the blue type because the colour is blue, if it is only the cupric copper because you cannot go away from the colour. So the oxidized form of those oxidases, again they are blue in colour, but for a complicated one also if you have more number of copper centers which are not of type one, but only one is of copper one type, so that type can show or pertain the corresponding colour to the system as blue.

So, different MCO's or multi-copper oxidases you can have which can convert both the atoms of dioxygen to the water molecule, so O2 will be converted to your water molecule. So one such very good example is laccase, not a very huge molecular weight at the lower side

which is starting from 60, from one origin to 140 kilo dalton and a range of redox potential for this particular copper one site if that is required for your electron transfer reaction is 0.4 to 0.8 volt.

And one type of on copper center close to a trimer and shows oxidation of polyphenols and polyamines in plant. So we know that we can consider that in plant origin, we consider them as the polyphenol oxidases, so you can have a trimer. So, three copper centers you can have so timer in your hand and one monomer and your hand so that is basically the total arrangement we can consider as MCO.

So examples are we only study will give the examples for laccase and ascorbate oxidase only because they are very important and are well studied also and also when we talk about the other variety that means the type 2 will talk about the galactose oxidase and the amine oxidases, if time permits and but they belong to non-blue oxidase system so that we are not bringing into the picture we will talk in some other day about this non-blue oxidases, so you can have the blue oxidases, you can have the non-blue oxidases.

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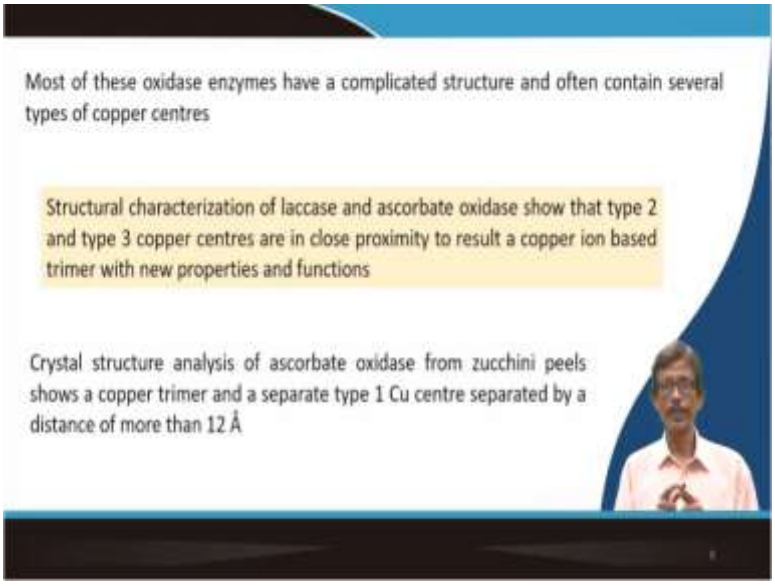

So they are having very complicated structure therefore and often contains several types of copper centers, so in one particular multi-copper oxidase you can have copper type 1 copper protein, type 2 as well as copper protein type 3. You have a tetra nuclear system because the copper type 1 is mononuclear type 2 is also mononuclear only type 3 is a dinuclear system like hemocyanin.

So this particular structural characterization of all these molecules of laccase and ascorbate oxidase is capital AO, we write and laccase we simply write lac or LC basically, so the type 2 and type 3 copper centers are in very close proximity so if you have a type 3 dimer is there and a type 2 copper is there so is basically a trimer.

So these 3 copper centers where they are close to each other we can get a trimeric arrangement and a long distance you can have a type one copper center but why we are discussing all these over here because we are trying to understand everything from the light of type one copper center we have identified type one in your blue copper proteins the plastocyanin and azurin.

But if it is present in your multi-copper oxidase also because separately we again again I am telling you that if time permits we can again go back to your multi-copper oxidase in one of the class such that you can bring the whole picture in such a way because we can have a very good example already we have seen in case of iron enzymes that cytochrome C oxidases, where you have the copper.

So when we are learning copper nicely then we will bring these two together about the copper as well as the iron center the way we have seen in case of our previous classes that you have the ascorbate not the superoxide dismutase thing where we are bringing zinc and copper but we have not studied yet about the zinc because the next module of our classes will be devoted to the zinc. So these three we should be mastered enough not only in terms of coordination but also the biological coordination chemistry. So they have the separate and the copper one is well separated at a twelve angstrom distance.

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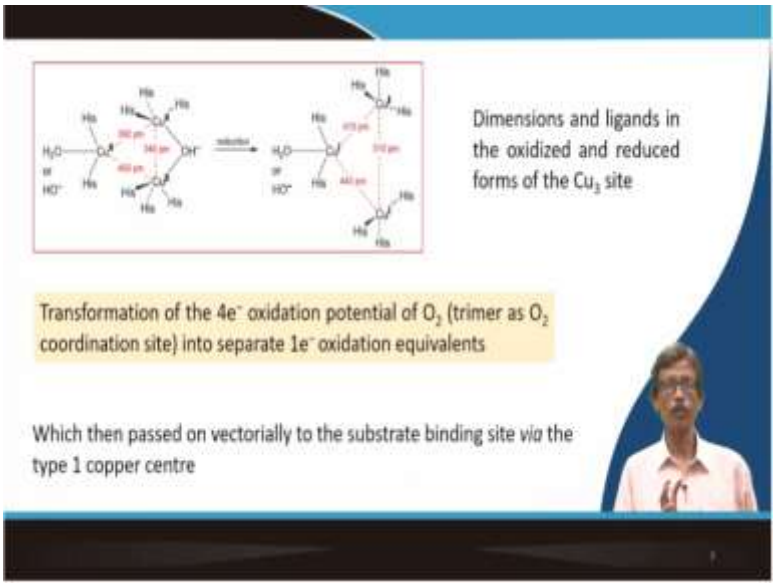

So, how far they are and what are the dimensions of the ligands in the oxidized and the reduced form of this copper three side. So, bring the copper two side, so that is the copper two side which is having a different coordination environment one water molecule is bound but the type three is very much similar to that of your hemocyanin what we have studied the facial capping one side and another facial capping from the top.

So one copper side and another copper side, so these are the thing, so this is the thing that's why you have this particular right hand side this copper copper system, so this copper copper system is therefore you have and that by nuclear copper site as well as the mononuclear which one are not very far, which is only up to 3.9 angstrom distance or 4 angstrom distance from these individual coppers of this type three copper centers.

So this basically the trimeric unit the Cu three site we can have in laccase (())(19:11), then if we go for the reduction, when you go for the electron transfer reaction interestingly what you find that this coordination environment is important such that you will be able to transfer one electron each to each individual copper centers and the copper centers which were in the plus two state can be reduced to the plus one state.

That means all three will be your cupric state cuprous state and you see now the distance the inter metallic distances are also increasing and you can have the copper copper distance in your type three, the hemocyanin type of system which was 3.4 angstrom is going beyond four

which is 5.1 angstrom what we have seen earlier already in case of your deoxy-hemocyanin case. So is nothing is unusual.

We can correlate it and we can compare it not only with the model system but also the biologically obtained structures what we know from many years about your hemocyanin in these two cases that means your oxy-hemocyanin and the deoxy-hemocyanin. So not that three electron transport, if we can have a fourth electron transfer, why fourth electron transfer, because we are talking about the electron transfer to two oxygen atoms of the dioxygen molecule.

We all know that if you are able to dump or if you are able to feed four electrons to the antibonding orbital sequentially on the O2 molecule you will have the O bond cleavage will be able to cut the o bond, so will have the separate oxygen centers when you have the peroxide you break it then further and then you can further transfer electrons such that your individual oxygen centers will be O two minus the oxide ion.

So, this four electron transfer is therefore can be in one sort can take place between this four copper site and your O2 molecule and we can have therefore, one separate oxidation equivalent involving your type one copper center which is very much similar to that of your electron transfer copper proteins or blue copper proteins.

So the redox equivalent what you call accumulating which will be passed then factorially to the substrate binding site via type one copper center, so type one copper center because this co3 center is responsible for binding of your o2 molecule. And your type one center will be reserved for your substrate binding that is why we require elaborate multi copper oxidase system.

Why you require so many copper ion systems or centers for this particular type of reaction? Because it is involving multi-electron transfer reactions, so always try to remember if you have a multi-electron transfer reaction it will definitely attract some system, some biological system or some biological molecule where you can have more than redox active different metal ion centers like copper one, copper two, copper three and copper four.

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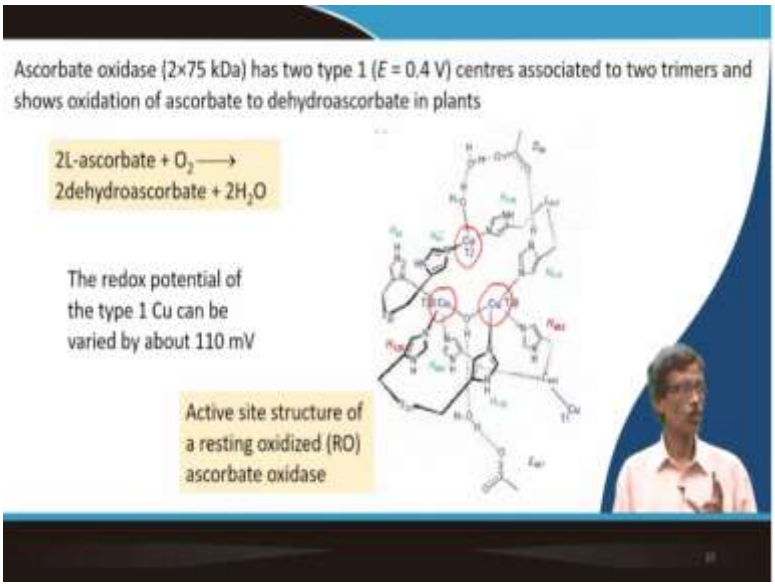

Similarly a corollary basically will draw a corollary over here, with regard to your laccase and that of your ascorbate oxidase, only the E zero value will be different because your environment is different we all know if your ligand environment is different your electrode potential is completely different. We all know very simple example of bipyridine which is binding to your iron center and orthophenanthrin you can have different corresponding E zero value for your simple ferrospheric redux couple.

So you can have then again not only a trimer but you can have two trimers and two mononuclear centers therefore associated or two type one centers are associated showing a corresponding electrode potential of .4 volt, to give you the dehydroascorbate based and ion and which is there in plenty number again from the plants.

So L variety of ascorbate and ion because you must go for the depotension first and then you can go for the electron transfer reaction and presence of your o2 molecule we are not able to produce the hydrogen peroxide in some cases what we see that o2 is required for hydrogen peroxide production for two electron reduction.

But here the reduction is four electron and will straight away go to for the production of water molecule but at the same time you should also be able to tell the oxidized form of the substrate of L ascorbate and ion which is your dehydroascorbic and ion you should know the structure I am, I do not have enough time for that to knowing about the structure but you should know about the structure of ascorbate and ion and the dehydroascorbate and ion.

The redox potential of this type one copper sun can be varied between a range very small range of 110 millivolt range and what about this form if you go for the oxidized form that means the cupric form where you can consider as the resting oxidized ascorbate oxidase form. Resting means you can have all these sites available to you that basically tells us that all the copper sites are available to you and at the resting state is not, the resting states are not active states.

So the resting and the oxidized form, so when you have the oxidized form you see you minutely are trying to locate all these sides what you can have is basically the corresponding resting oxidized as carbon oxidase, so you see that you can have the copper type 3 and the copper type 3 also, so it is labelled as copper t3 alpha and t3 beta. Then copper as your type 2 copper, so how the different networking so networking of the different numbers of hydrogen bonds, so networking of all these hydrogen bonds are important.

So once we get and the positioning of all these are very important from a particular seat or the helical structures, what you can have, how the histidines around this t3 alpha case the t3 alpha case what we get basically about this particular case, what we see about this particular copper side and this is your another copper side and this is your t two copper side.

So, what you see that this particular ones the t3 alpha side and the t3 alpha side if you can get that so you bring the three histidine residues, so how they are coming, so they are also level they are also numbered like your hemocyanin. So, these three are coming and in the other side also the t3 beta also the t3 beta sides that you can have this thing, so again you have the three histidine residues.

So, on the right hand side also you have the three but to further stabilize it you need some bridging from either water molecule or hydroxide group or some other hydrogen bonded hydroxide group, so you see the networking for that stabilization it is not that only separate hydroxide ion is there so the biological world you can have the intricate hydrogen bonding interactions over there and those intricate hydrogen bonding are so important that it can not only give you a network of all these things but also it can stabilize these things.

So the stabilization what we can have the stabilization what we can have is that you have not water molecule but you can have OH. So one proton is going out from there and that proton can be utilized for the protonation of some other point point but what you see that it is basically tracking or it is basically grabbing one other water molecule, so it is hydrogen

bonded first with the water molecule and that water molecule is again hydrogen bonded to any carboxylate end.

So, carboxylate end basically what you have that the bottom basically the bottom part E four eight seven so that carboxylate the E four eight seven so that oxygen is involved in hydrogen bonding interaction but that is the part of the protein chain; so you see that all these donor groups so some are giving you the coordinate bonds that means the immediate residues and some are providing hydrogen bond.

So hydrogen bond donors are also coming they are accepted in terms of two hydrogen bonding but they are providing that particular bond they are providing hydrogen bond to the assembly to the aggregate. Similarly, the top one is also stabilized by two histidine residues and one hydroxide group so that particular arrangement and the stabilization is also very important which is not very usual in common or the naturally under that is what is laboratory available coordination chemistry.

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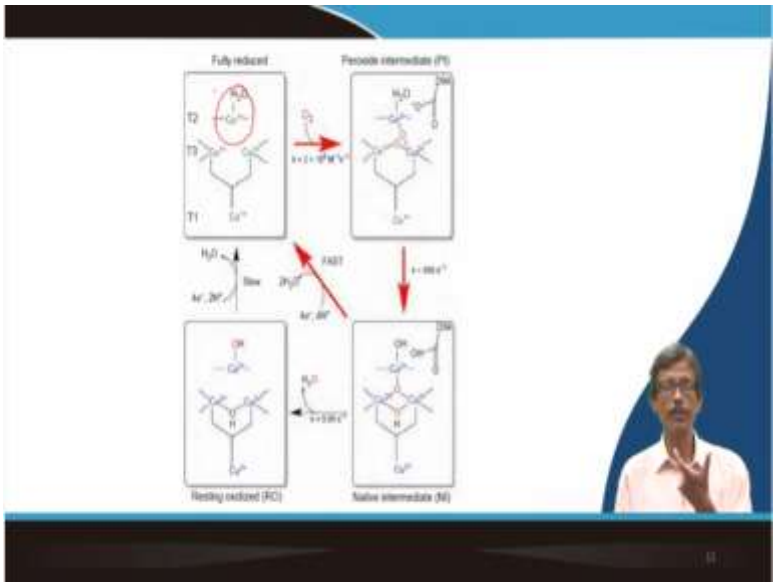

So finally, what we see now that what is happening in your resting oxidized form at the left bottom part that you can have copper copper bound to your hydroxide group and you can have another copper center is there also and you can have the another copper as the type one copper. So this type one copper basically when it is basically accepting these electrons and all these electrons basically what we can have…

This basically what we get that this is a different one what we find over here so this particular one what you see look at that is the three plus one so try the trimeric copper is there you can have an as another one and where you can have the binding site the binding site is the type three copper site O2 binding side. So you can have the t3 t2 and the t1, t1 is at the bottom so bottom is this result for your substrate binding, so again it is a simplified cartoon type of drawing to locate or to show the positioning of all these copper centers.

Then you have the corresponding electron transfer once you have the electron transfer you have the removal of the water molecules and water molecules are going out from there and is due to the protonation of the hydroxide bridge. Then you have the activation of the O2 molecule O2 is coming and start interacting with the copper centers in a similar fashion like that of your hemocyanin coordination.

So, two copper centers are binding in a the mu eta two eta two type of binding and then is a very fast reaction which is the 560 rate is 560 centimeter inverse and you have the native intermediate. So peroxide to intermediate then native intermediate and a very fast movement for your water movement or the fully reduced form.

So resting oxidized state is a different state which is not involved in the catalytic cycle involving these three form so the red arrows are important which are involving these three states but if you go for something some reductant if you use, so reductant is basically reducing the resting oxidized state to the fully reduced form, so the fully reduced formula the active form and that active form is taking part in your whole catalytic cycle.

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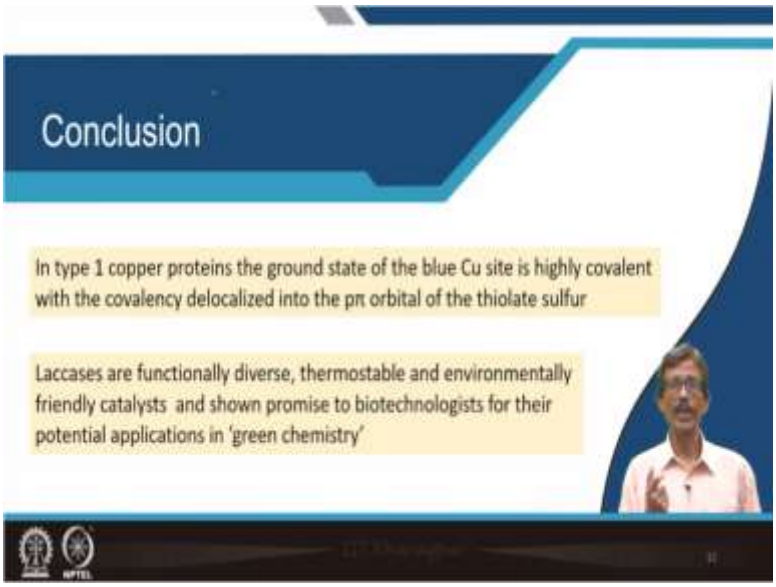

So what we have seen so far is that the type one copper proteins in the ground state of the blue copper site is highly covalent in nature and the covalent connectivity is due to the delocalization huge amount of delocalization with the p pi orbital of the thiolate sulphur. Thiolate we know that is a is a bigger one is oxygen down to sulphur in the periodic table and you can have the bigger atomic orbitals and the pi orbitals and the p pi orbitals are available for its interaction with the copper center.

So your delocalization is possible but in case of laccases which can be of similar type that of your ascorbate oxidase which are thermally very stable and environment friendly also because everything in is of bio origin and whether we can use them as good catalyst because the biotechnologists are very interesting interested with this thing for their potential application in 'green chemistry' because what we can see in a very simple reaction condition in native condition or the biologically accessible condition you can go for multiple electron transfer reaction as well as the substrate oxidation.

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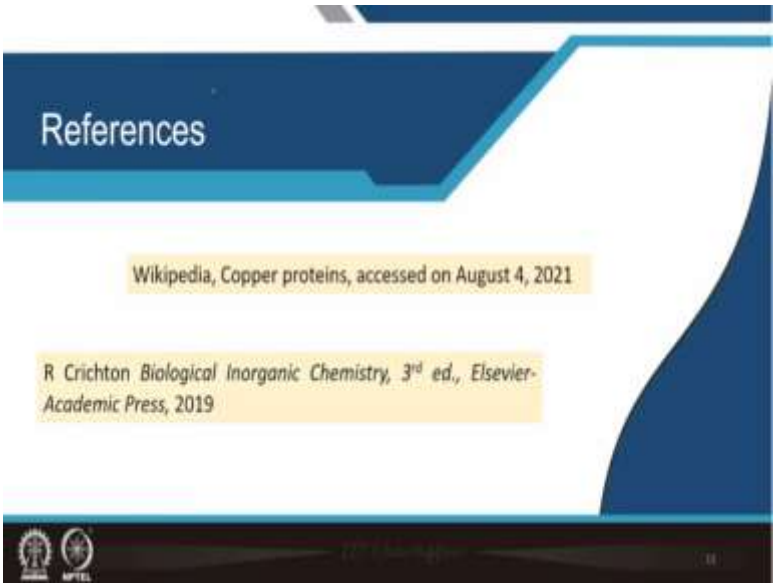

So we should go for the copper proteins page of the Wikipedia as well as you to consult the whole book of Crichton. Thank you very much for your kind attention.