Bioinorganic Chemistry
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Lecture - 13
Electron Transport Proteins – IX

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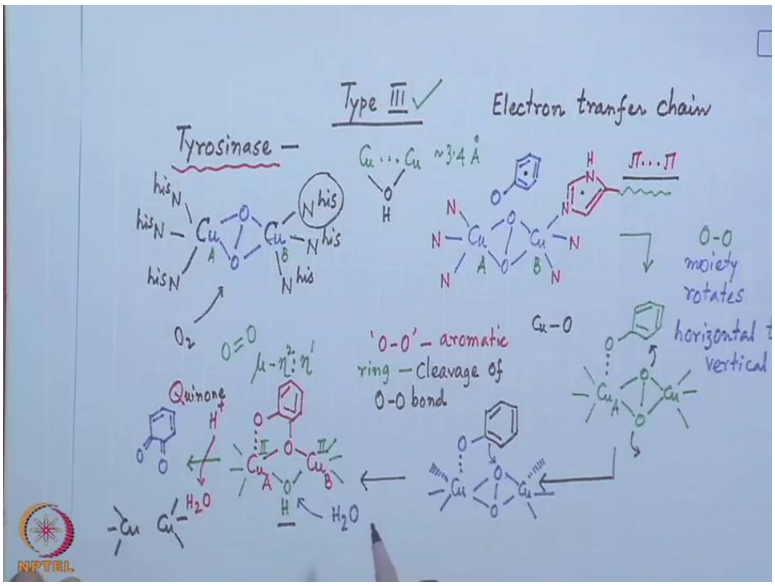

Hello. So, we were still with that type three copper proteins, so which we know already that they are electron transfer and sometime they function as some particular part in a typical electron transfer chain. So, if we have an electron transfer chain and type three copper site can be involved there, but when we talk about the enzymatic activity like that of tyrosinase activity; that means we want to oxidize the tyrosine function, and in our last class what we have seen that the typical alignment of the dioxygen molecule between the two copper centers.

So, this is definitely a dinuclear system, and sometimes if you have the close proximity of two copper centers, we will find that they are close to each other, but if you have the typical dioxygen binding that you all know from the knowledge of hemocyanin chemistry and we have three other binding positions from the nitrogen residues of the protein chain. That means they are all of nitrogen from the histidine chain, from histidine origin, all histidine origin. So, this we all know from in the case of hemocyanin also, but what is the basic difference if we talk about in terms of its tyrosine as activity; that means the question also will come to us that whether certain assembly where you have a

binucleating system and that binucleating system can take up two of the metal centers such that the two copper centers and you get a binuclear copper complex.

And that binuclear copper complex is utilized for activation of these dioxygen and the nature of these dioxygen molecule, because this is typically coming from the O 2 the typical dioxygen molecule; it can be your O 2 from the air or it can be your O 2 from some other source and around these; that means when both the copper centers are pentacoordinated in type and this particular histidine nitrogen play some important role that we have seen that if we have this two copper, straightaway we write this function and we bring the tyrosine function; that means the phenol unit and that phenol unit when it is coming close to one of the copper center; it is not that it is symmetrically positioning between the two copper center, but it is close to one copper center and the alignment of this is important, this imidazole ring and the alignment of this imidazole ring will tell us that how it can be helpful for positioning of your substrate molecule.

So, the tyrosine end is coming and there we have seen that it can show when they are close to each other they can show pi-pi interactions. So, this pi-pi interaction is therefore typically important because you see that this is some kind of secondary interaction; like sometime we find in case of hydrogen bonding interactions also that if you have a metal center which is bound to some water molecule or any other function which can donate your hydrogen bond. So, that water molecule can show some hydrogen bonding interactions to something else which is not bound to the metal center. Here also these pi-pi interactions why we want to know all this information because for the supramolecular interactions what we call beyond the molecule when we have this ligand system; that means you have the three donor groups here and three donor groups here and we know that this in the hemocyanin case when three of these; that means the apoenzyme when copper centers were not there and initially both the copper attached to these two sites are in cupra state it is copper one.

Then it goes to cupric state with the transfer of electrons to dioxygen molecules slowly it is becoming peroxide part but when this particular part. So, it is not that this is one part of the long chain the protein chain. So, this particular part when interacting and its typical positioning of this will give you some pi-pi interaction and it brings the substrate near to one of the copper center which is your copper A, this is also copper A and this is also copper B. Then what happens this particular positioning will tell us something that

this particular substrate molecule the tyrosine molecule with this one oxygen which is close to this copper one will start interacting with this copper center; that means it will start interacting with copper one and due to that what you have is still these groups are there.

So, this dioxygen molecule this particular moiety which is already bound if it is a typical dioxygen molecule you will have a dumbbell shape structure; that means you have O-O this sort of thing and this positioning of these O-O; that means the typical dumbbell shape this particular type of binding is unique. So, not that all peroxo linkage bind in this fashion; sometimes it binds. So, this copper and this oxygen and so both the oxygen's on one side. So, this is a typical binding. So, if there is any such interactions coming from these because the ultimate goal what we are just looking for is that these oxygen is our reactive reagent now which will attack your ring only this tyrosine ring for the hydroxylation reaction. So, this entire this dioxygen group basically; that means this O-O moiety then rotates. So, the rotation of these two; that means your O-O group; that means O-O function or O-O moiety we basically right rotates.

Why it is rotating? Because we have started something; that means this oxygen is starting its interaction with this copper and copper will have always a typical preference for a five coordinated site and we are still expanding to go to six coordinated site, but that is also a favorable one, but your bonding pattern will change because this there will be steric congestion. So, this dioxygen moiety basically rotates from some orientation we call it as from horizontal to a vertical orientation; that means orientation basically changes. And we sometime calls it that the movement is going from a horizontal position to a vertical position because these are all three dimensional structures. It is very difficult to visualization on a plane of paper that how it is moving, but it is moving in above the plane and below the plane.

So, some orientation is taking place and why this orientation and we sometime we put two arrow for that that there will be some orientation; that means the reorganization of the dioxygen molecule which is still bound to your dioxygen center and that is so happened that your phenyl ring; that means this part basically is coming close to this oxygen. Initially you have pi-pi interaction for suitable positioning of this tyrosine residue to that of your imidazole ring and then once that particular positioning is established that you know that for a typical pi-pi interactions, we always have some

distance the centroid to centroid distance and the pi stacking also we call. So, if this particular distance which is also within 3.5 to 4 angstrom and pi-pi stacking is favorable; that means your substrate is getting positioned.

So, you see that it is very interesting thing that your this particular part; that means the ligand part from the long protein chain which is the histidine residue which is attached to your copper center, then it is basically bringing the substrate molecule. So, through this pi-pi interaction your substrate molecule is coming close to this particular entity. It is not that this particular phenol ring is coming and directly attaching to the copper system; what we see in our laboratory experience that what you get that if you react with something; that means if you want to have something where you can have some ligand exchange reaction; that means if the phenol is strongly bound to the copper center your one of the bound for this will go. So, either your entire dioxygen molecule will go or the peroxide linkage will go and if it is directly bound to this particular one; that is why in biology all these weak interactions are so important and weak interactions are predominating for their catalytic activity.

So, this pi-pi interaction first, then the typical orientation because of this interaction also when we write in this fashion; that means this bond is bigger than copper oxygen bond. This bond what we know the typically for a apical coordination if you find that it is the vessel is there and little bit of apical coordination is a long one and you have some gentler distortion also you expect through that you get something that you have a copper oxygen long bond. So, this is also a weak interaction like that of your pi-pi interaction. So, this movement basically bringing your this particular moiety; that means this phenol group to a suitable position such that your oxygen will be attached to that ring. Still we will consider as the dotted line because that distance can change little bit. Initially this distance is bigger and this distance is still bigger; it is not within the bond distance which should be very much similar to that of other known or reported copper oxygen bonds.

So, still you have the three other bonds from the nitrogen's and these oxygen in the same way we just have with that copper one bond, one bond and the third bond. So, this one then in the next step this will react with this oxygen. So, you reagent is ready. So, your reagent will transfer this oxygen to this phenyl ring. So, electrophilic attack of this O-O moiety the entire O-O moiety, this O-O moiety is attacking on the aromatic ring. So, you have an electrophilic attack on the aromatic ring. So, this particular unit though it is

attacking your aromatic ring and something else is also happening at the same time; it is the cleavage of your O-O bond which is also important. So, attack of this O-O moiety to the aromatic ring and cleavage of the O-O bond. So, that basically gives us something which is also very interesting to know there that once it is cleaved you have the first copper which is copper A and you have copper B and this one sometimes you can write the solid line bond or you still can write it as dotted one.

This oxygen is now transferred to your aromatic ring; that means your phenyl ring and this will remain as O and if you have this thing; that means you have this C-H bond here. So, this proton from here will transfer to this oxygen. So, basically what people have characterized because you never know which particular species you are going to characterize for all these biological systems and identification of this particular part; that means this copper copper part as a type three unit is very important; that means you can control over here; that means you know that what should be your copper copper distance whether it is 3.3 or 3.4 or 3.2 angstrom. So, it is close to, say 3.4 angstrong and that particular one is also not only dependent on the other groups which are bound to these two copper sites, but also the main binding group the main bridging group here this is a main bridging group also this what you have generated the hydroxido bridging.

So, this particular entity; that means the code structure you can call it is the code structure of type three copper proteins where you know that it as a copper copper distance of this much and this hydroxido function is attaching to one copper and not only this hydroxido function, we also find something that this phenol unit you have and this phenol group we all know when it is there if it is not a terminal one, this phenol unit can bridge two copper sites, but this is not a typical phenol one but it is a catechol. So, binding of catechol of this sort is very important. So, just from the tyrosine we are producing this catechol and this catechol is ultimately bound to this dicopper center in a fashion. So, binding more is a bridging mode and one of the oxygen is utilized to bind both the copper sides. So it is eta 2 and next one is only one copper. So, it is a mu eta 2 eta 1 binding mode of this catechol unit.

So, that is why we get that the studies of this sort of system is very important; that means whenever we get that corresponding models studies or small molecule modeling in the laboratory, immediately we are always tempted to get some species where the phenol is utilized as your ligand system and the only phenol if it is a terminal one it cannot bridge

the two copper sites. So, if you have certain groups over here like this catechol unit and another group on that site, it would be a very good ligand system which can bind both the two copper sites and this particular hydroxido function which we have generated from this dioxygen. So, your dioxygen is your source for this hydroxido function but sometime when we go for some laboratory experiments, when we do something in the laboratory, we occasionally find that this is not coming if it is not going through some redox reaction. It is not coming from O 2, but the major source for this hydroxido bridges dicopper complexes are water molecules.

Because immediately if you see that water is bond, sometime water is bridging the copper system and when both the two copper centers are bridging this or sharing the lone pair of electrons on the water molecule, you have immediate deprotonation by changing the p k of the water molecule and you get hydroxido bridging. So, once it is happening; that means you have these two copper sites which is hydroxido bridging and ultimately what we get, we just then release this product in a form which is not the catechol bond form; what is released from there is the oxidized version of the catechol. So, it is the quinone which will be released from there.

So, that quinone it is oxidized from and because what is happening? The everything is happening simultaneously together; that means you have the hydroxo bound system and this particular catechol unit is bound to the dicopper unit as mu eta 2 eta 1 fashion and that eta 2 eta 1 group when it is there and this two copper is bound because initially when we started from here, it is that that we get that thing that you have one, then electrons are transferred to this dioxygen molecule you have the copper two. So, these are copper two before the release of the product. So, immediately what you find that the catechol unit is then responsible for the reduction of the copper centers and your catechol unit is getting oxidized to the quinone product. So, you have the quinone product which is liberated. So, it is not for any one such quinone product. So, all sorts of reactions having anything; that means having a tyrosine part because we are talking about something which is amino acid based thing.

So, any of this type where you have some pendent tyrosine unit, any protein chain having a substrate unit; that means tyr is there; that means one part is tyrosine and the pendent tyrosine O-H is involved or the O group is involved for copper coordination, there will be a possibility for going for this corresponding oxidation in that particular part. So, for

this all hormonal molecule and all these things where you have the catechol part is involved. Initially if you do not have any such oxygen you get it from the hydroxilation reaction followed by the tyrosinase activity. So, the tyrosinase activity is responsible for that and these O-H group will be taken care by the H plus available in the system and it will be released as water. So, you will end up with the system where you have the corresponding dioxy form of these copper. So, these dioxy form of these copper centers basically then it will be released as one copper and another copper without any bound dioxygen unit over there.

So, both these dioxy forms; that means, this particular dioxy form you get and your quinone product. So, quinone product is liberated or released. So, that gives us some clear idea about all these groups what is being involved for this dicopper chemistry basically; at the same time we are learning so much thing about the dicopper chemistry and if it is O H group which is either forming from the dioxygen molecule or it is coming from the water molecule, we can go for something; that means how we can spectroscopic ally characterize; that means we are little bit going back after knowing the mechanistic path for this transfer.

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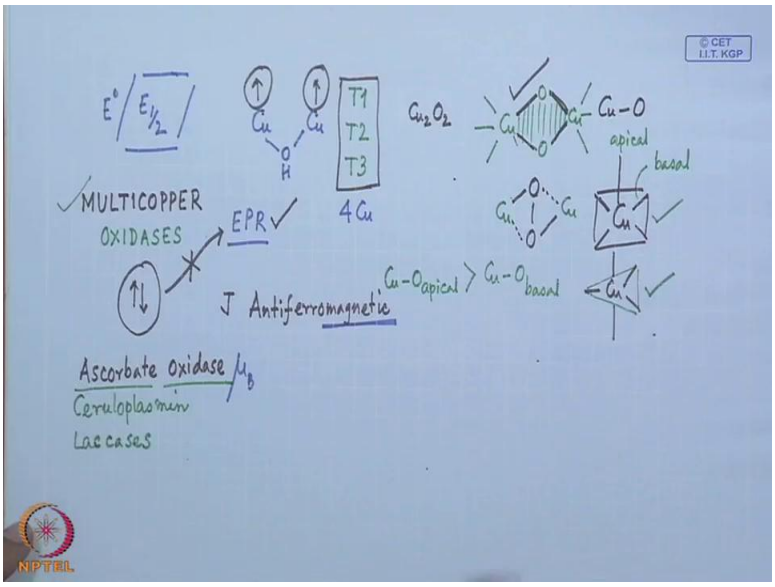

So, if we have any such system where you have the copper-copper one which is bound by O H group, this is important. So, in that particular case we get also another bridging from the phenol unit. Basically what we get; we get a unit which is known as a Cu 2 O 2

unit or a Cu 2 O 2 code and when this Cu this is 1oxygen of, say, phenol and this is oxygen from hydroxido unit.

So, you get a typical diamond-like unit. So, that is Cu 2O 2 unit this is diamond like unit because this is very important to know because structurally we can know so many things out of this. Because if you just compare this particular part that if you have this why people have very easily identified this basic unit of that instead of having a type of interaction like this from a dioxygen molecule; this is one of the possibility. So, when this dioxygen the both the oxygen's are not attached to the copper. So, one of the mode of peroxide binding is this binding and three dimensionally when they are basically rotating in three dimension what we find that this sort of oxygen bridging; these are the bridging units.

So, bridging oxygen atoms you have and if we just can determine this is a very simple unit, but structurally if you can see that there are four copper oxygen bonds and we know the regular bond distance depending up on the nature of the oxygen whether it is water oxygen or hydroxide oxygen or OXO-oxygen or phenol oxygen and copper in the corresponding oxidation states; that means it is a cuprous copper or cupric copper, you can have this copper oxygen bonds. Then if you know this corresponding bonds and then you try to compare this, immediately you will find that if this oxygen when they are making bonds with this copper this particular copper oxygen bond and this particular copper oxygen may not be equal to each other; that means, one of this copper oxygen bond can be short and another one is long.

Similar thing also happens to this oxygen and this copper; that means it immediately tells you that whatever we are drawing on this plane of paper or the piece of paper and we are talking that this is a diamond code, but what we are drawing is in a plane of paper; that means we are restricting our drawing on the plane and we are thinking that all the four atoms are on the plane of the paper but it not that. So, if this oxygen is going up or going down, one is going up another is going down. So, what basically we get? We get when we think of that you have a symmetrical binding from these oxygen's either it is from the hydroxide oxygen or phenol oxygen; that means if they are all in plane it can be a symmetric one; otherwise you can have a long bond and you can have a short bond. Why this is so? Because if you just think that copper this particular copper is in square pyramidal geometry or the other alternative is your trigonal bipyramidal geometry.

So, now think of it that you have a three dimensional structure and your life is much more complicated now. So, this two copper this particular basic code whatever we are talking about we are thinking that it is in the plane of the paper, but once you draw the other three bonds this bond this bond and this bond. Now is a complicated one, whether you have a copper center of this or a copper center of that and there also we get something that you have these four bonds when you have a square pyramidal geometry, this four bonds are short compared to this apical bond. So you we are talking something, these bonds we call as the basal bonds and these we call as apical bonds and all these copper oxygen; that means the copper oxygen apical bond systems will be greater than copper oxygen basal. Similarly in this particular case also this is trigonal bipyramidal also you have one basal, one apical.

So, initially because mechanistically also you can think of you can just think for while for these that whenever this oxygen is coming into the picture starting slow interaction with the copper center and then it is forming the bonds not that all the bonds are forming together. So, one is approaching first then it goes for the second bond. Initially you can have this sort of interaction. This immediately tells you if you draw instead of drawing these, if you draw in this fashion that immediately will tell you that this solid line is the shortest line and these are the long one. So, all the time you get that. So, you have some asymmetric geometry. So, this asymmetric geometry will tell you and if we want to characterize this because particularly this type three copper is a basic component from some other particular proteins which we call as multicopper proteins or multicopper oxidases. These are multicopper proteins and they are multicopper oxidases; we sometimes we call them such that this ascorbic acid oxidase, ascorbate oxidase and all.

So, when you level it as a multicopper system; that means you can have more than one copper center. So, the minimum one, the binucleating one, how you characterize it first? So, already we have seen the type one, the T 1 center already we have seen, then T 2 center type two proteins and now we are in T 3. These multicopper oxidases are sometimes is such that you can have all the three types together. You see the situation is getting much more and more complicated. So, individually if you are able to characterize the copper one site, all the typical spectroscopic properties and all other characterization detail if you know, you can find out T 1, you can find out T 2 or you can find out T 3 and while characterizing this thing; that means they are showing these oxidase activities. So,

you have a system where you have more than one copper center as there. Sometimes if you have a system; that means all three are present together, the type one copper, type two copper and type three copper; that means you are having a system where you have four copper centers which will be a tetranuclear one.

But in the biological system what people have tried for that and we were succeed to characterize it there that if it is not a different one; that means it also contains a T 1; that means the typical signature is for the T 1 is present for these, T 2 is there and T 3 is there and if you sum up all three types it will be one, two and plus two. So, four copper centers will be there and when this particular multicopper oxidases is showing the oxidase activity and initially when we try to know the oxidation state; that means whether these two sites are copper two plus or one of them is copper two plus, one is copper one plus or both of them are in copper one plus. That is also important because every time we are looking for something where electron transfer is taking place and they are basically reversible one. What you can have from a reduced species to an oxidized species? You can get several E 0 or E half values which will be characteristic for a single electron transfer; that means one electron you are putting, the second electron you are putting and one electron you are taking out or the second electron you are taking out.

So, one of the characterization values for all this entire is your determination of the redox potential value. So, redox potential you determine first and then the corresponding distance that how far these two copper sites are if they are bound by a hydroxido group, then the resonance spectroscopic technique; that means due to unpaired electron; that means we can again utilize the EPR spectra. So, that EPR spectrum is related to that you have a corresponding unpaired electron. Then if you have a unpaired electron system and if you have one unpaired electron when both of the copper centers are plus two, one unpaired electron one first copper; that means the copper A and the second is copper two. And if they are showing some signature that the center; that means the type three centers is EPR active; that means you have some unpaired electrons present over there.

So, some amount of unpaired electron density is there; that means your both the two unpaired electrons on copper A and copper B are not interacting very strongly such that your ground state is S equal to zero. When it is S is equal to 0; that means it is paired of, you will not find any EPR activities. Otherwise you can have the corresponding determination for the J value the coupling constant. When your magnetic moment is less

than that of your sum of the individual centers, we call the J is there and they are engaged in some antiferromagnetic interaction. Why we are determining this magnetic property? We are talking somewhere the catalytic behavior; now we are talking the antiferromagnetic or magnetic part. Because this magnetic characterization is very important whether in all sorts of things whether it is a copper based system or a nickel based system or a covalent based system, those who are the metal centers are providing unpaired electron; whether there is some relationship between this magnetic properties because it is a physically measurable quantity

So, you have now some very sophisticated magnetic instrument or magnetic technique is available. In olden days, say, 50 or 60 or 70 years back, we used to get this magnetic moment the simple mu B by using a balance. Balance you have, then you take the two different weights in the presence of a magnetic field and in absence of a magnetic field by a typical Gouy balance technique. So, this particular mu B the magnetic movement when you get; that means how much your electron density is residing on these two copper centers; that means how they are interacting to each other. So, the individual orbital's the atomic orbital's which were present on the two copper sites and the unpaired electron on these; that means the d x square minus y square or d z square, how they are interacting is depending on this picture; that means what three dimensional structure you have; that means either you have a square pyramidal structure or a trigonal bipyramidal structure.

That structure will tell you which orbital will be available for your unpaired electron and then how close they are; that means this distance this copper-copper distance which is also important. How close they are for their suitable interaction because everything what we are getting over here is not that based on the carbon geometry which can go up to attaching to four some species on the carbon center, but this when it is attaching to five such species and these orbital's are very important and these interactions. So, magnetic moment you can correlate with those groups because in these multicopper oxidases when we have more than those groups, say, for ascorbate oxidase; one is ascrobate oxidase and sometimes this we can have also the ceruloplasmin or sometimes laccases.

So, this particular part that means how the same copper site which is being utilized for tyrosinous activity which can be utilized for ascorbic acid oxidation also. So, we know that this ascorbic acid group is a very good reducing agent and which can be utilized for

your ascorbate oxidation. So, when it is in the deprotonated form we call it as ascorbate oxidase; otherwise we typically call it as a corresponding oxidation reaction for your ascorbic acid.

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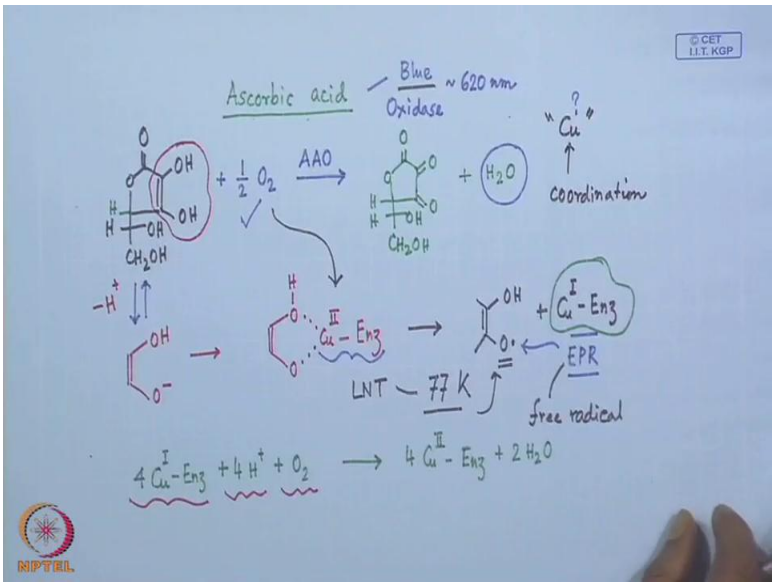

So, it is not a very simple system because it is a multicopper oxidase molecule. So, you have more than two, three or four copper centers present over there and when we characterize; that means we will find that it has a color which is very much similar to the one type, but we have characterized earlier which is our type one type. So, type one copper center is present. So, if we get something; that means the band is close to 600 nanometer or, say, 620 nanometer. So, that is the corresponding signature from the electronic spectroscopy. So, that electronic spectroscopy immediately tells you that these multicopper oxidases can have a blue color also because you have a corresponding band at this particular nanometer and it catalyses the oxidation of ascorbic acid. So, it is the corresponding oxidation of the ascorbic acid. So, this is our ascorbic acid.

You have so many O H groups and during deprotonation also, we should know that which particular O H group is getting deprotonated such that it is producing the corresponding ascorbate anion and this basically is reacting with half of this O 2 molecule and your ascorbate oxidase or ascorbic acid oxidase sometimes we abbreviate quickly as AAO which is ascorbic acid oxidase. So, ascorbic acid oxidase is there. So, when the color is there; that means you have at least one type one copper center is

present and we call it as also as a blue oxidase due to its color. So, it is a multicopper oxidase which is also a blue copper oxidase, because it has color and it has a type one copper center present. So, it is getting oxidized simply like your catechol to quionone conversion. So, catechol to quinone conversion is taking place over here again. So, if you know nicely that tyrosinase activity or catechol oxidase activity, you should be able to tell what should be the product for this reaction.

So, this is also one H is also we can show because all four bonds we are showing CH 2 OH, this is H OH. So, this is our ascorbic acid and this is our dehydroascorbic acid and which is producing also one molecule of water. So, whatever oxygen we are supplying to the system; that means this half O 2 other half O 2 which is getting utilized for H 2 O. So, it is a typical oxidation reaction what we get like your catechol to quinone conversion and similarity why we need that copper there. So, you have two successive one electron steps. So, how you get that is; that means how this oxidation step is taking place because we are thinking that copper is there, so the involvement of the copper. So, if you have a phenol unit, if you have a tyrosinase unit, we know that that particular phenol unit or tyrosinase unit is responsible for copper coordination or copper binding.

So, this coordination is important. So, you have to establish first the coordination to the copper center, then you think of any change in the corresponding oxidation state on this copper either it is a cuprous copper or a cupric copper, then you bring your reagent the dioxygen molecule. So, how all these things are taking place there. So this particular unit, when we talk in terms of that ascorbic acid oxidase or ascrobate oxidase; that means it can go for deprotonation nicely. So, this deprotonation will give sole now onwards basically because getting this particular part, because this is the part which we are interested to know because this particular part is the catechol part. So, the cis dihydroxo groups are there and those groups are basically utilized for copper binding. So, this particular hydrogen is going away. So, this one once if it formed there will start interacting.

SO-One in O H form, another is O minus form will start interacting with the copper and that particular copper what is present over there is your copper center and this is the copper 2 plus two center and it belongs to the enzymatic part. So, your entire enzymatic part is a very complex system where you have more than one copper center and this particular one is bound and thus copper is going over there. And then if you think that

this dioxygen is coming into the picture; that means what we have, we have this copper and then copper is responsible for this dioxygen binding what we know already. So, this particular part is behaving as your bidentate ligand part and that bidentate ligand part is attaching to the copper center which is already bound to the enzyme part and that particular copper center or the second one, the other copper center is responsible for dioxygen binding basically giving rise to something where you get a radical product in the corresponding oxidized form and copper is reduced to copper one and still bond to your enzymatic form.

So, this we can study. So, how this copper bound to this enzyme; that means this copper metalloenzyme is attacking on the ascorbic acid or the ascorbate anion and if we can look at something that what is our product because in some cases we will find that this products because the fate of this dioxygen molecule can either be water molecule or it can go to produce hydrogen peroxide. So, depending upon that and in this entire reaction what is there and this formation of this free radical can be proved if we just record this particular technique, is well known to us now, the galactose oxidase and all these things. Whenever you have the suspect that, no, I am not getting something where the copper oxidation state is typical changing your substrate or the particular part is getting oxidized.

So, if you in a transient form also because lifetime of this free radicals if it is very less, sometime some solvents are available and those solvents basically we get because the d p p H type of thing which is the standard reference molecule for recording your EPR spectra. So some solvent mixture is there, acetonitrile or dichloromethane or some mixed solvent like acetonitrile benzene or acetonitrile toluene. Those solvents can be frozen at a temperature of 77 K which is our liquid nitrogen temperature. So at this temperature, you can freeze the entire system and the frozen system can be inserted within the EPR spectrometer probe and we record; that means whatever lifetime you have is the transient lifetime it will have. But you can freeze the free radical and that free radical can give you the corresponding signal which is due to your presence of free radical.

So, this multicopper system what is present and the entire reaction; that means how many electron transfer basically you can expect if you think that in this particular case you have generated the copper one system. So, all together when you reoxidize the system this is the reduced form of the copper enzyme which is copper one bound to the enzyme

part. When handling our O 2 we know that what we can have; we can have four electron transfers like cytochrome c oxidase, still the same thing we are doing. We are handling on the dioxygen molecule and that dioxygen molecule with four protons on it can go for four copper center in plus 2; that means when you regenerate the enzyme system in the cupric form plus 2 H 2 O. So, the dioxygen there; that means this particular case when the enzymatic function, the dioxygen can be utilized to generate the copper in the plus 2 oxidation state; that means you are just segregating the part; that means your substrate is here, your copper two is there and enzymatic function is there.

So, if you just think of the corresponding electron transfer between your ligand and the metal center, your ligand and the metal center you get the oxidized ligand version and the reduced metal center, but we are not talking anything related to the electron transfer to the dioxygen. Now the reduced form of the enzyme, the reduced form of the enzyme is then finally reacting with the dioxygen molecule to regenerate the cupric form. So, how we can think of this; that means this is basically resistant where you have one particular species is this, this is another and this is another; that means the involvement of the proton and when you have this copper as one part, enzyme as one part and the dioxygen molecule is one part. So, you have a three component system. So, when we go to the laboratory to study this system on simple model compound.

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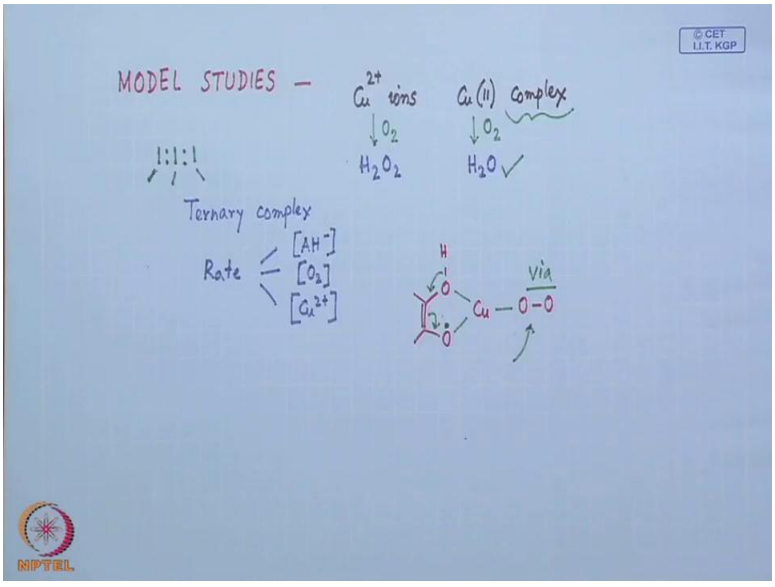

So, when we think something on the model compounds that the model systems for model study and that model study we can make with simple copper two ions or any copper two complex. That will immediately tell us that what is the difference between the simple copper ion and a copper present within the complex because this particular part that complex part what we are getting we are getting this particular part giving you the ligand system. So, it is the enzymatic part which is basically modulating or controlling the corresponding electronic behavior of the copper to be reacting with the substrate molecule.

So, if these two; that means the copper two plus and the copper two complex are reacting with this dioxygen molecule and if we are able to monitor the product of these reactions; that is one very simple clue to know a typical mechanistic pathway for any reaction, forget about for this ascorbate oxidase or anything. Whenever we talk about something related to a metalloenzyme, how you know the corresponding enzymatic function, what we want to establish that it has some role; that means copper is playing some important role and what is the role of the ligand part.

So, you just differentiate these two; that means the copper two ions and in most of the cases, if this particular reaction is producing immediately the hydrogen peroxide and this reaction is producing water molecule, definitely you will tell that the course of the reactions are different and incase of this ascorbate oxidase, your product is not hydrogen peroxide; your product is your water molecule. So, this particular one; that means the copper, you have one copper center, then one of the substrate center and the dioxygen center. So, you have the ascorbic anion, then copper and then dioxygen. So, it is basically a system what we commonly call as a ternary complex.

So when you have the ternary complex, we get some rate expression for the rate of the reaction and rate expression we see that basically if we write the rate expression, the dependence; that means it is dependent on the concentration of say AH minus; that means the ascorbate anion, it is also dependent on the O 2 concentration and it is also dependent on the copper 2 plus concentration. So, what we get therefore that whenever we have certain this reaction; that means based on the ligand part which is our substrate. Now this is basically reacting with your copper we have the copper and then the dioxygen. This is the system; it is a very simple system that you have substrate which is functioning as a ligand.

So, this particular entity you can consider as a metal complex, the substrate plus metal center, then it is reacting with your dioxygen molecule. And this particular one when it is copper two plus and this one when it is interacting with this copper and this dioxygen and we just go for this and this is going for that corresponding dehydroascorbic acid formation and you can judge the radical formation and ultimately you can see that how the electron flow is taking place through this oxygen. So, ultimately you are moving from here to there. So, that is the thing that how this electron flows is taking place via this oxygen.

Thank you.