Bioinorganic Chemistry
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Lecture - 11
Electron Transport Proteins-VII

Hello, so we were with that extra diol aromatic one.

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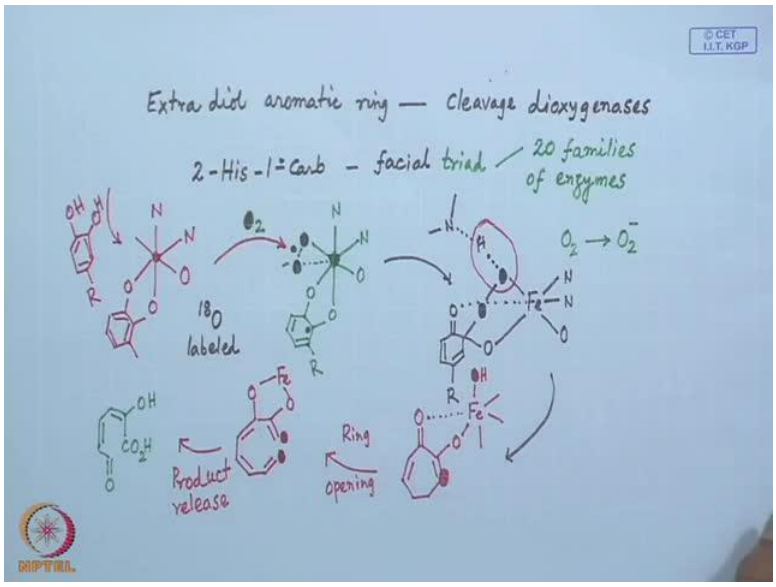

So, if we have a extra diol aromatic ring how we can clip that? So, these are the cleavage dioxygenases. So, we consider them that as extra diol aromatic ring cleaving agents. So, they are the very good reagents in that way and the ligand environment we are talking about is 2 his 1 carb type and is basically a facial one. So, we can consider it as a facial triad that means you have three donor atoms and there are large number of such enzymes available and at least 20 families have been identified so far of enzymes.

And simply we have seen that if we have that iron and immediately we can have the octahedral site, two of them will be occupied by nitrogen and one by oxygen and the facial orientation. That we get after if you have the site, the catalytic site and then the catechol is binding there. So, have the catechol there. So, it will bind these two sites. So, five positions are occupied now and the same simple strategy that we will take or we will take the help of di oxygen molecule and that di oxygen molecule will now go and bind to that particular available site. That means the sixth coordination site. So, straightway we

can write all these positions. So, you have the nitrogen, nitrogen, oxygen, then O, this is also O.

And interesting thing happens here onwards that if yours centre is getting reduced that means your bound ligands should be oxidized. So, if we have these two positions like this so we have a oxidized catechol site and if that oxidized form is there we know that there are two other forms when we go from catechol redox process. The first one is the semiquinone and the second one is the quinone form. So, single electron oxidized form is coming into the picture and you have the binding of these di oxygen. So, di oxygen is simply bound over here and this is also reduced.

That means this di oxygen is going to the corresponding super oxide species. So, the change in the corresponding status for this di oxygen and the changing the ligand environment basically, giving something where the reaction is mediated basically through this iron site. And that can be very easily identified if we just simply use some 18 oxygen that means the leveled oxygen's are used sometimes. So, 18 O labeled one is used that means we want to have a track for the di oxygen one where it is going.

So, initially it is bound to this two positions then the binding thing is changing towards the semiquinone form, R is there, this is one and this is the original oxygen. That means this position is getting activated which is the radical centre which we generate from here. So, this position is activated and this di oxygen molecule will come and attack this particular position. So, once it is like this. So, this di oxygen still bound to your iron site and this one will also a have a typical coordination from this oxygen as well as this oxygen.

So, we all the time we are living with something where we are just talking about some facial positions. So, all the three positions that means N N O are still occupied by the protein chain and we are doing something on the other three positions. And these oxygen, so these two oxygens are you're, the new di oxygens which are, can also be labeled one and this particular arrangement is not so stable arrangement in a particular organic molecule when it is attached to this particular benzene ring.

So, it has to be stabilized. So, a seconds fear of interaction is from some imidazole nitrogen which is available from the protein chain. So, this particular arrangement because you have a charge already on O 2 and that charge is getting stabilized through

the hydrogen bounding interaction with the hydrogen. Then in the next step you have the ring opening reaction and that ring opening will give you a seven membered ring with this oxygen inside. So, this was still bound to your iron.

This oxygen is there and this O, if you consider these two will be now your O H bow. So, now you will have one O H group attach to this iron. So, you are living behind the other three positions. Then at this last step you have the ring opening now. And these are your labeled oxygen's. Here leveled one is coming that means we are able to insert both the oxygen atoms which are available their and still the opened form of the catechol still it is bound to your iron site.

And lastly at the final state our product is released from iron coordination and thus we get the extra diol clipped product which is C O 2 H. So, the interesting part of the entire reaction sequence is that the particular iron site can activate the di oxygen. So, not only you have vacant site over here and these vacancy is taken off by the di oxygen molecule and that di oxygen molecule when it is attached then only we get some internal electron transfer.

And this sort of thing is very common because in most of the cases what we have seen in case of cytochromes, that you can have something where the metal centre is not getting oxidized, your ligand centre is getting oxidized and you get so many other products that means you can generate a free radical system. And that free radical mediator reactions are very common with that of your iron site which is a redox active metal centre which can settle between the plus two and plus three oxidation state. Similarly, some other type of system will find that copper can also play the same role to generate the radical site and that radical site can attract the hydride and the product is something different from their and the reactivity pattern is little bit similar to this system.

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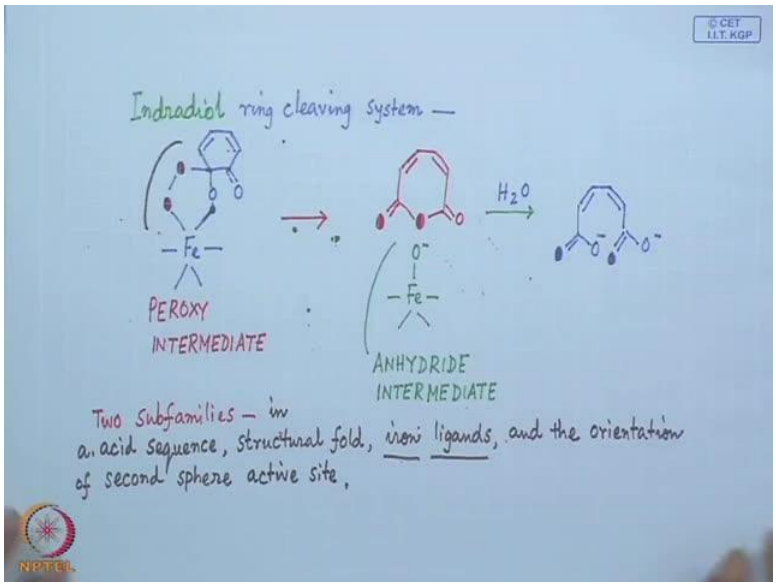

But in case of that indradiol cleaving agent. So, we will have the indradiol one. The simple difference is you have now the same type of peroxy intermediate, but in different mode of action. So, if we just write this hexa coordinated site like this you have oxygen so you have peroxy link and that peroxy link is attached to our semiquinone unit with oxygen like this. So, in this particular case you have the labeled oxygens which is coming out from outside.

So, this is our peroxy intermediate. That peroxy intermediate that means you whatever you have supplied to the system as di oxygen molecule which is getting changed to a peroxide species and that therefore, giving some different product in this case and where we get intermediate as a acid anhydride. These are the labeled one and iron still changes either a hexa coordinated form or sometime if this particular motive is not getting disturbed the protein environment is such that this particular positions are not altered.

So, you can sometime get a five coordinated intermediate on iron. So, this is O H or O minus. So, this particular part what is forming there is our anhydride intermediate. And the final step which is very interesting that now this, in this particular case water is not attacking here, water will be here to break your anhydride link. So, this is our product we all know now this for inter diol clipped product is this one. And these are the two labeled oxygens.

So, these two particular system that means these two sub families. So, there is some difference between their environment because we see that this can give you some different reactivity pattern. So, we have basically two sub families. So, one is responsible for extra diol cleavage and the other one is responsible for indradiol cleavage, but they are different. So, they are different in the sequence, the amino acid sequence, in amino acid sequence.

There structural fold is also different that means both tertiary and quaternary structures should be different and their corresponding pockets are also different around iron. You can have the nitrogen and oxygen bearing ligands, but the exact nature of these ligands are also varying because with this generalized picture we try to focus our attention on twenty families. There are large number of these groups depending upon the biological source what we have.

So, one source to another, but broadly we can classify based on these. So, in one case to another you have a different ligand around the iron centre and lastly the orientation of the secondary sphere. That means the hydrogen bonding interaction what the imidazole ring is, imidazole ring is showing through its hydrogen on the super oxide or peroxide oxygen. And the orientation of second sphere which is important, second sphere active site. So, we want to have the hydrogen bonding interactions, how we can stabilize this particular arrangement?

Otherwise, this particular arrangement the peroxide link is not so stable when it is bound to this iron. So, we need to stabilize with some hydrogen bounding donor atoms. So, with this information that your one particular system is cleaving catechol in one two fashion and another case it is cleaving in the three four fashion. So, people try to have something that means small molecule system which can show its functional activity.

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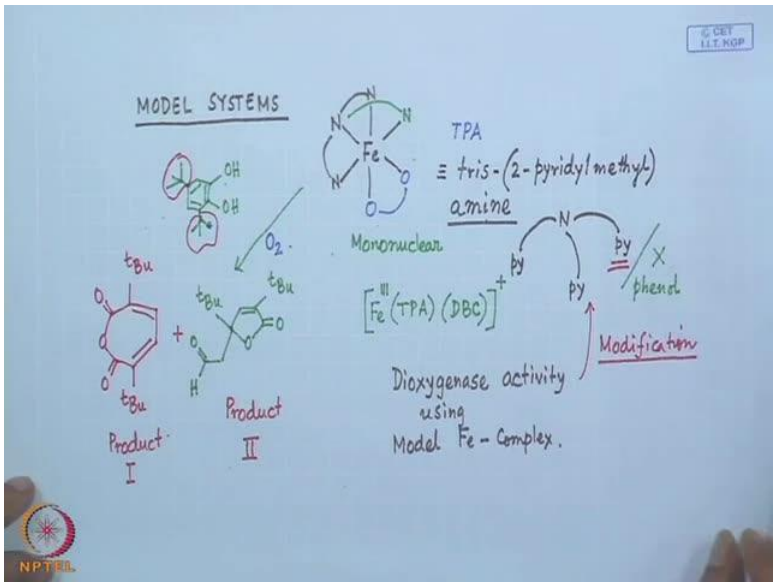

That means the model system. How we can make in the laboratory which can show this sort of reactivity that means the catechol oxidase reactivity pattern it can show. So, one such example you can find where this particular case that means you have the iron site and (( )) ligand we always try to take and we are getting? That we are getting the reactivity pattern from these three positions or sometime from the two positions. So, atleast two positions should be vacant which can be occupied by the molecule which will decleaved that means the catechol molecule.

So, at least two of the positions must be vacant around iron and you can have a particular ligand system which can bind all the four positions. So, one such example of this ligand is well known also and it can be abbreviated as TPA ligand which is tris 2 pyridyl methyl amine. So, that means you have a tris 2 pyridyl methyl amine. So, you have this nitrogen that means amine nitrogen in your hand and you just functionalized it like pyridine, pyridine, pyridine.

So, is basically a tetrapodal ligand, we call it as a tetrapodal ligand. So, this can go, so this is the nitrogen, this is non pyridine nitrogen. So, this nitrogen which can go for binding this way, this way and lastly the other one. So, you have a regular octahedral complex. So, mono nuclear octahedral complex and we just try to see whether this particular molecule is very much reactive or not. So, the compound what we can

synthesize from here is Fe TPA which is a tetradentate ligand and your substrate. Substrate is tertiary butyl.

So, it is tertiary butyl and di tertiary butyl DBC, di tertiary butyl catechol and since this catechol is their and interestingly we will find that there is no phenol type of ligand. So, all of them are nitrogen which are neutral in nature. So, we basically try to go for where it, this particular ligand system can also stabilize iron in the low oxidation state that means the plus two oxidation state, but here the synthesized complex is in the plus three oxidation state and catechol is providing two negative charge.

So, overall compound is cationic. So, when we allow this compound to react with, with the molecular oxygen we get something that means this is a typical model compound. If we find something, some product that means once we generate the semiquinone form and the radical form is sometime because of this corresponding electron donation from the tertiary butyl group compared to you're the normal catechol unit. The tertiary butyl substituted one will have higher stability in the semiquinone form.

The radical form what we generate over here that will be stabilized, so that is the reason why we are taking this substitution, the tertiary butyl substitutions on the ring. So, we get basically two products from here the anhydride one plus one cyclized product because we are looking for something but sometime we get some other transformed product as well depending upon the stability of the intermediate what we can generate through this redox process based on the iron site.

So, the iron is definitely a very good catalytic site in that way, this is the t B u substitution over here. It is a five membered ring cyclized product and people have identified that and at the end here you have the aldehyde function. So, this is one product. So, basically from this reaction after characterization of all these we get product one and product two on a model system. So, as a non biological system you can make the compound in the laboratory and you see say something where you can have the dioxygnase activity using model iron complex which you can make in the laboratory.

So, having known all this informations that this one particular iron compound can show something, so what we can do next that means the reactivity pattern for some other substrates or catechol type of other substrates or some other molecule, sometimes some amino acids having some aromatic ring or some part. That means we can go for the

modification, if we try to see more we just go for the ligand modification, you can the ligand system that means you can attach one such phenol unit to the substitute that pyridine unit or some donor groups including sulfur or other two oxygen atoms.

So, modification of this can lead to something where you can have some different type of reactivity pattern. So, this is all about your iron that electron transfer proteins based on iron. Now, we will go for something where you have the copper proteins.

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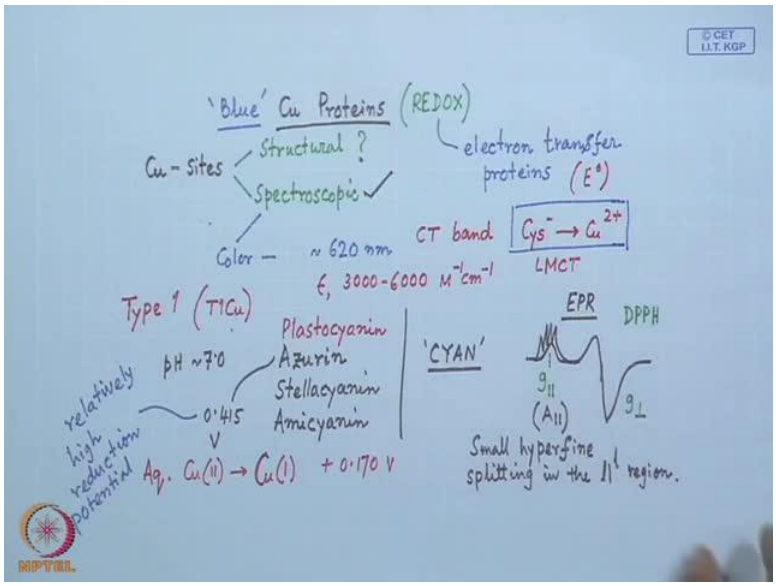

And these copper proteins they are of different types and they are present in biological system. So, they are type one, type two and type three and we can analyze those sites, so different copper redox proteins. Because, we are looking for some electron transfer behavior, so these are electron transfer protein again. So, the copper based redox proteins. So, like iron so copper can also settle between the oxidation states like plus one and plus two, the cue plus as well as the cupric.

So, spectroscopic characterization that which one is copper one site, which one is copper two site and which one is copper three site that can be known if we study in detail the spectroscopic features. So, characterizations of these copper sites are of two types, one is spectroscopic characterization and another is structural one. So, spectroscopic characterization when we talk about for this electron transfer proteins because we are talking everything on electron transfer proteins. So, spectroscopic signature is one such that what gives you the color, what is the color of this system?

So, they are all most of the cases blue in color. So, they are also known has blue copper proteins and this blue copper proteins having a corresponding band at 620 nanometer, that is why it is colored blue and its corresponding epsilon value is pretty high and it can be categorized as a charge transfer transition. And therefore, there is a charge transfer band which is coming and is basically originating from cysteine sulfur to copper 2 plus ligand to metal charge transfer. So, it is LMCT type charge transfer.

So, once we get that this strong electrical absorption. So, electronic absorption spectra immediately tells us that this particular type of species having a lambda max value of 620 nanometer and epsilon value ranging from 3000 to 6000 mol inverse centimeter inverse. So, immediately we will find that when we get this particular signature we level it as a type one blue copper protein or sometime we abbreviate as T 1 C u and there are large number of such molecules available, one is they can be from plant origin, they can be from bacterial origin, one is our well known molecule plastocyanin.

Then one is also well known is azurin, then stellacyanin and amicyanin. So, in most of the cases you see the name is ending with cyan which is a color, it is a, the level, the adjective for that is for the color the cyan, blue color. So, this particular case that means once you know the corresponding band position and its epsilon value you can say that spectroscopically you can determine that the type one copper is there. Another spectroscopic signature is your EPR because sometimes it is very difficult to identify the metal centre first and what is corresponding environment around the metal.

So, EPR is the second information which can supplement your spectroscopic characterization for this one. So, these are basically a typical type of your EPR spectra for that and sometime we get the hyperfine one for the copper centre. So, three line can be there. So, this can be splitted into four lines and we level these, it is a very well known one which are for all the IPES spectra for copper, this is the g parallel region and this is the g perpendicular region and for all these things we just report this value and this value with a reference of DPPH.

So, these values also tells us something so long we are not knowing anything related to the structure of the molecule, what type of structure it has. That means this coordination number and the geometry. So, these particular one, if we know large number of synthetic molecules and we can correlate those with that of our synthetic molecules, that the EPR

signature is this and we have the corresponding splitting. So, small hyperfine splitting is there. So, this splitting basically and this splitting are leveled as A parallel.

So, we have small hyperfine splitting in the parallel region, not in the perpendicular region. So, this color and the EPR characterization then gives us some information that since we are talking these all for electron transfer behavior, so they must have some typical E 0 values. So, you go for another level of characterization of this molecules will measure the corresponding E 0 value. So, in most of the cases E 0 is measured at pH close to 7.0 and in case of azurin say one such example I will give that some list for that.

So, in case of azurin it is 0.415 volt and if we just compare this corresponding value with that of our aqueous copper to copper one potential. So, all we know is that, what is our aqueous copper to copper one potential which is copper one, this plus 0.170 volt. So, you have a corresponding potential which is high compared to your copper centre which is aqueous copper in solution that means any copper salt in solution. So, a relatively high reduction potential.

So, it is relatively high reduction potential. So, this particular one, so that means you have something that where we need some extra potential value to reduce this copper two to copper one centre and that particular one when we give. So, step wise if we find that we initially find that you have this coordination. So, this particular information will give you that at least you have one cysteine sulfur attached to your copper site. Then you have this one is very close to tetrahedral or five coordinated geometry.

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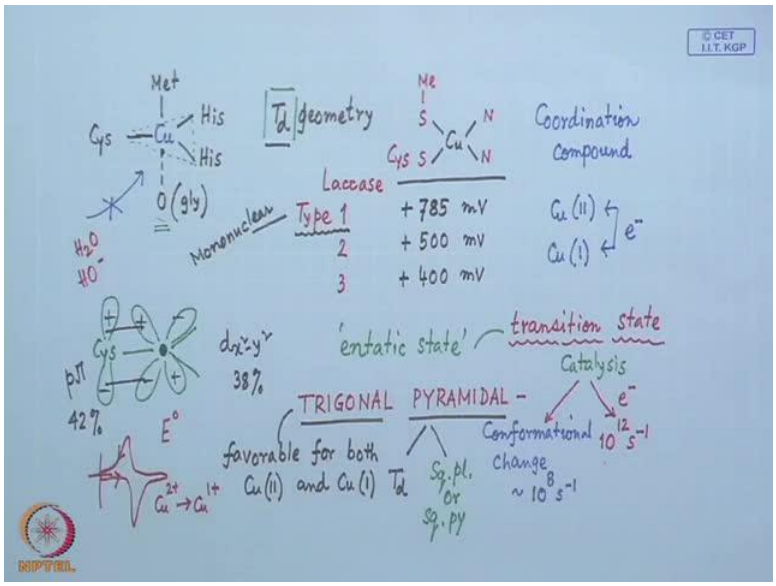

And this particular geometry, then without knowing the exact structure of this particular site gives us some idea that it has to be a copper centre attached to one cysteine sulfur, cysteine sulfur and two of them are histidine nitrogen and one is s methyl that means methionine and a weak one oxygen from some carboxy end say a glycine type. So, within this environment what we get that this one when this oxygen is not present that means this particular coordination is pretty weak; we get a weak coordination from the oxygen.

And overall you have a tetrahedral geometry, if we just consider that you have these four coordination's only that means a copper in a environment of that means simply we write sometime that the type one copper is blue copper proteins are four coordinated one, two of them are nitrogen and two them are sulfur. So, it is a four coordinated system. So, this particular four coordinated system when we go for corresponding determination of E 0 values that when with this particular species go for the catalysis that means the electron transfer catalysis. So, this is for type one basically.

Then there are some which are type two and some are type three. So, in this particular case when we have the type one case, so one such potential just now we have seen in case of azurin which is a type one also and is close to 0.4 milli volt of sorry 0.4 volt or 400 milli volt, but in other cases for system like lacease, one such example is lacease. Their potential is pretty high, that gives us some idea that how far like cytochromes, that

potential can change. So, from 0.4 it can go up to 0.7. So, which is 785 milli volt and for the same lacease is the multi copper oxidase system, lacease is nothing but there are so many centers because in sometime if the system is pretty complicated one we get type one.

So, far we have seen only the type one which is the simplest one and the type one, the simplest possible one is your mononuclear one. Then you have type two where potential is around 500 milli volt and type three which is close to azurin potential which is around 400 milli volt. So, in all these cases like azurin, lacease and all these we see that that there is a corresponding charge transfer transition and the centre that means the copper centre is their present and this particular one, the copper one is more strongly bound to these two groups that means the histidine nitrogen from here and there.

So, this trigonal plane, this is one trigonal plane. If we consider this weak bounding from oxygen we can consider it as a trigonal bi pyramid geometry. So, this is the trigonal plane. So, these bondings are pretty strong. So, that immediately tells us that because you have the cysteine sulfur and we have the charge transfer transition at that immediate charge transfer transition in this plane, the trigonal plane tells us that you have a very strong interaction between one these sulfur, that means sulfur of cysteine and that basically is responsible for your color, but your methionine sulfur is s methyl sulfur and that interaction is not so strong.

So, that is why one of the cysteine sulfur is taking that this particular trigonal plane. So, the basal plane it is occupying and one this methionine sulfur is taking the apical site and during this particular interaction if we just find that you have this cysteine sulfur, so there is a typical corresponding covalency. This covalence is generated when we talk about that from the detail calculation of all these molecules. So, if you have a typical orbital over cysteine sulfur and the corresponding one from copper.

So, you have a particular type of covalence is generated from that p pie of cysteine sulfur which is hybridizing with your copper d x square minus y square and from the calculations and all other spectroscopic parameter comparization we will find that because these two are very much interrelated so about 38 percent of this bond, this orbital is participating with that of our cysteine sulfur which is 42 percent. So, if we have some strong interaction for these two groups.

So, they are basically dominating your color and the reactivity pattern. So, when we go for this particular change that means methionine sulfur is interacting with the copper centre and when we consider as a tetrahedral geometry. That means this geometry, if we just simply consider this that you take out this oxygen and you remain with these two, but this particular geometry is not this geometry, is not a regular tetrahedral geometry. So, when this particular oxygen is removing is a highly distorted geometry and this particular in biology we will call in all these spaces they are entatic states.

So, we generate the entatic state by simply removing this nucleic coordinating oxygen atom. So, if we just simply remove this quickly coordinating oxygen atom and this particular one, so your interaction might little bit increase from the out of plane methionine sulfur and that out of methionine sulfur has some role to play because this particular one is closer to the transition state. So, for electron transfer to take place there is a corresponding transition state which is again highly distorted.

So, for a typical or a rapid electron transfer your coordination geometry or that particular molecular geometry such that initially when we talk about this one, if you remove this oxygen. So, after removing these we will end of with a geometry which is trigonal pyramidal. So, this trigonal pyramidal geometry will tell us that during the catalysis what is happening to the copper centre because why this copper is so unique and copper can function for a typical catalytic role.

So, during catalysis two things are happening there. That means electron transfer is taking place and all we know from Franck Condon principle and all that your electron transfer is pretty fast which is in the range of 10 to the power say 12 second inverse. So, during that transition all we know from typical coordination geometry knowledge that the copper two is all the time stabilized in one particular coordination geometry either square planner or square pyramidal, but copper one will be stabilized in tetrahedral geometry.

But if we want to have a very rapid electron transfer within this range that means the electron transfer rate is very high which is 10 to power 12 second inverse. We are tagging something that means we are looking for that electron transfer for a corresponding conformational change around copper. So, you will have a conformational

change and which is also taking place, but it is little bit slow or compared to your electron transfer rate which is 10 to the power 8 second inverse.

So, what is happening that during the electron transfer, so one particular confirmation is getting changed to other confirmation. So, for typical or standard coordination compound what we know, for a typical coordination compound what we know that if you have a corresponding copper two compound or a copper one compound what we find that copper one will be stabilized by low coordination number and sulfur type of donor groups can stabilize the copper one. But the copper two is stabilized by some other different compound.

So, if we see that for a typical coordination compound synthetically prepared in the laboratory and we look for some electron transfer. And if we try to isolate both the compounds it will be very difficult for the same ligand environment to isolate this copper one compound as well as the copper two compound. Because in case of copper you will be losing some donor atoms, you will be losing some ligand groups and in copper two it will attract more ligand groups.

Suppose, copper can be stabilized in a coordination number of two or three only. So, when you oxidize a copper one compound to a copper two compound, what will happen? It will try to attract because you are just oxidizing the centre, it will be electron gridy and it will attract more ligand centers and if those ligands are not available like your biological system, the biological donor atoms are not available, in synthetic molecules it is not available, the suitable ligands are not available, what it will do?

It will simply attract the water molecules or hydroxide groups. So, it will stabilize the thing in a higher coordination number, but that is not happening in all this biological system. So, you, centre should be protected such that you will not have some vacancy that when you move from copper one to copper two compound no water molecule should enter to this particular site. So, no water, no hydroxide groups can enter to this particular site. So, this copper site is highly protected.

So, we should some intimidate coordination geometry which is your transition state. So, in the transition state we will have a typical coordination geometry which is neither a tetrahedral geometry nor a square planner or a square pyramidal geometry. So, that is why all these copper one type of molecules, we have a highly distorted system and that

distorted system, one such example for that is your trigonal pyramidal coordination. If you just simply go for this trigonal pyramidal geometry which will be favorable for both copper one and copper two.

So, this is the basic idea behind doing all these studies that it is favorable for both copper two and copper one. So, if we get some intermediate geometry then from this trigonal geometry, if you go for copper two then it simply goes like that, it will attract some weak interaction from this oxygen like water. This glycin interaction, why it coming? Because, it is in the plus two oxidation state. So, when it is in plus two oxidation state it will attract some water molecule or hydroxide such that it can move from a four coordination site to a five coordination site, but when it is not there, when we want to stabilize it, so it is in the trigonal pyramidal one.

So, after that electron transfer so when you have the electron transfer because sometimes electron transfer is taking place after your conformational change or sometimes after electron transfer you have the conformational change. So, when you have a intermediate arrangement, that means it is very easy to go from this intermediate arrangement to a tetrahedral arrangement with a minute adjustment with a very small adjustment from a trigonal pyramidal arrangement, you go for a tetrahedral arrangement or in other case a square planner or square pyramidal arrangement.

So, it will attract more molecules or more groups and the simplest possible one is your water molecule. So, there are plenty of water molecules available in the system and that basically tune this particular (( )) in geometry. And when we directly measure it, in our next class we will see that when we just simply measure it for its different E 0 values by looking at their corresponding cyclic voltammogram. So, for this cyclic voltammogram the pattern of those cyclic voltammogram will tell you that whether there is a typical conformational change or not during this electron transfer.

Suppose, we are going for a copper 2 plus to a copper 1 plus reduction. So, this basically a cyclic one, and when both the geometries are inter convertible, then only you get a corresponding very symmetric cyclic voltammetric response, otherwise your response will not be symmetric in nature. So, if your measurement will tell you that some asymmetry has been generated for this cyclic voltammetric pattern, you can say that during that electron transfer your conformational change is taking place. So, one

particular geometry is changing to the other geometry, so that we will see in our next class.

Thank you.