Bioinorganic Chemistry
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Lecture - 7
Electron Transport Proteins – III

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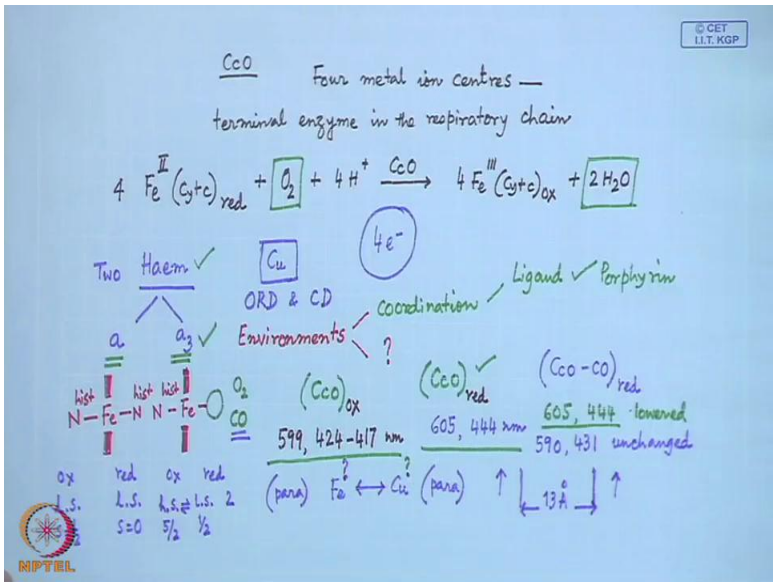

Hello. So, today we will still continuing cytochrome C oxidize, in which we have seen last time that it has four metal ion centers. So, when a system, particularly a catalytic site which has own metal ion centers, it would be definitely a complicated system and it is attached to the terminal enzyme in the respiratory chain. So, we have, if it has the iron site and the basic function of it we all know now that if it can supply the electrons from the reduced form of cytochrome C.

So, if it gives us four electrons which is very important that we are talking about that on the left hand side is if you have the reduced cytochrome C which ultimately delivering electrons to our dioxygen molecule along with the consumption of four hydrogen irons. So, from the left, the cytochrome C oxidize is basically consuming four electrons, and thereby it is oxidizing the cytochrome C site to its corresponding cytochrome CO x means cytochrome C oxidized form plus 2 H 2 O.

So, that is the basic reaction what we are talking about involving cytochrome C. That means how we can conferred this O 2, the mitochondria O 2 in the disparity chain 2 2 H

2 O and identification of these centers are also pretty much difficult and characterization of these ultimately tells us that we have the haem site, the iron per firing site, as well as we have the copper site and these haem sites are two different. One is a, another is a 3 and two copper sites are also different. Optical rotatory dispersion and circular dichroism techniques not only appear on other physical techniques, but also sometimes some other techniques are also involved there to identify the different environments, these all of them have the different environments.

So, to find out the environments are important and as we all know now, when you talk about something; that means, the environment around any metal center which may be catalytic which may not be catalytic, but to identify the exact environment, it is very difficult sometime because we have the coordination environment. That means, the ligand what we have in our hand and when you identify this haem, we know that the ligand is your porphyrin and when we differentiate these two between a and a 3, your coordination environment must be different.

How do you know that? If we get something and if we have some idea that this haem a, and haem a 3. That means, one is cytochrome a, that is Fe. This is the porphyrin plain and we have two nitrogen. That means it is coordinatively saturated. That means, it is octahedral and six coordinate. So, a typical corresponding electronic spectral pattern as well as your ORD or CD pattern which are completely different from a 3, where a 3 is nothing but the same iron attached to porphyrin with one nitrogen. That means, it is now penta coordinated. So, when we get a penta coordinated, one spectral feature will also be different and also, if we are able to identify that this particular right hand side, so this is the vacancy and this vacancy can bind O 2 molecule. So, O 2 can be a very good ligand for this site or it can bind carbon monoxide.

So, this is the right information what you can have when we are talking about the environment in terms of its coordination structure or coordination environment. Then when it binds to CO, it gives some typical characteristic feature and we all will be interested to know because they are at several things involving over these two sites. That means, one is this iron site which is nitrogen form. We all know now these are all obviously the histidine nitrogen. This is also your histidine nitrogen and the vacancy. So, this second one, what is that? That, I will come to that point, but before that if we find that in your hand the cytochrome c oxidize in its two different forms. How it is that?

That means, two different forms mean these two iron can have plus two as well as plus three oxidation state, and we all know the spectral features even that corresponding invisible spectra or the ligands spectra. These two are different for iron two as well as iron three and not only in the plus two or plus three oxidation state, but they also differ in high spin state as well as the low spin state.

So, if we are able to find out the corresponding basic lamaramavs values for the oxidized form and the reduced form and those are sometime very much characteristic because in this particular case in the visible range, it shows they band at 599 nanometer. Then two or three bands in the range of 424 to 417 nanometer. That means that high energy range. So, these two are definitely like the porphyrin band. That means what we know that as the solid band in some cases, the 450 band for some of the cytochromes. So, these are high energy band and one is low energy band at 499 nanometer.

So, in the reduced form, we get some other bands which are at 605 nanometer and 444 nanometer. So, this you can have. That means, you identify immediately looking at the spectral characteristics for the oxidized form of the cytochrome c oxidize as well as the reduced form of the cytochrome c oxidize. That means, in oxidized form, we already know that you have the two haem center and two iron copper center.

So, in the oxidized form; that means, when we talk that they are fully oxidized, so there are 4 electron transferring the system. So, these four electron if it is they are; that means, one after another that the system which is reduced by four electron. That means which is super reduced like iron sulfur proteins. So, fully reduced form have some characteristic feature and fully oxidized form have some characteristic feature. So, in between you can have one electron reduced product, two electron reduced product, three electron reduced product. So, based on these spectral characteristic, you can identify the fully oxidized form, the fully reduced form or one electron reduced, two electron reduced or three electron reduced form.

So, not only these characterizations based on invisible spectra, but some time, we can have some idea about the same cytochrome C oxidize, but this is now to this. That means, binding at this iron by carbon monoxide because we all know that when it binds to carbon monoxide, it has different spectral properties. So, in the reduced form, first we will check whether it will bind carbon monoxide or not. If it binds that carbon monoxide

that immediately tells us that this is the site. That means, your haem a 3 is involved in carbon monoxide binding and the spectral feature related to this particular iron side is getting modified and this particular one we have the spectral positions, like 605 or 444. They are lowered in intensity compared to this reduced cytochrome C oxidize.

So, they are lowered in intensity whereas, the other signatures; that means the corresponding (( )) values at 590 and 431, they remain unchanged. So, if we can have a data base like this; that means, if we can have all the spectral feature for the oxidized form, reduced form as well as the inter mediate one, so it would be very easy for us to identify these particular forms because in these two cases, if we have the oxidized form which is six coordinate and the reduced form, we know it can have the low spin form or in the reduced form, it can also have the low spin form because these are the most predominating form for ex coordinated iron side for haem a. So, you have is equal to half and reduced form s is equal to 0. Similarly, for a 3 site which is oxidized as well as reduced and interestingly, since it is five coordinated one like myoglobin and hemoglobin, this can be in equilibrium between these two spin states. That means high spin as well as low spin. That means, you can have spin value 5 by 2 or half integral, but the reduced one will have a spin value equal to 2.

So, this is making a situation very much complicated. If we cannot see one after another, it will be very much difficult to identify the actual iron site as well as the other metal ion which is also paramagnetic in nature, and it can also provide on paired electron to the system. So, this particular one; that means, if you have the iron sites and the copper site and both of them can be in the oxidized form as well as in the reduced form, and sometime if we can find that they are interacting to each other, so that identified after wards. That means one of the site which is paramagnet in nature and this is also paramagnet in nature.

So, positioning of these two sites is very important and until and unless we have the corresponding execs structures in our hand, we cannot say that how far they are. That means, whether these two sites are 13 angstrom a part or 30 angstrom a part because this biological electron transfers are all the time, they are going through a outer spear mechanism and in a long range, say 32 to 35 angstrom because you have the protein backbone the amid backbone.

So, outer spear mechanism is operative in a very long distance which is unlike for our molecular systems, where we need for a typical exchange. That means, if you have in this particular site one unpaired electron and on the right hand side, you have one unpaired electron. If they are within a molecular system within 2 to 3 angstrom, then only they can interact together. When they share a common orbital, we call them as a magnetic orbital. So, we all know that.

Then you can have the corresponding ferromagnetic or anti-ferromagnetic interaction. So, looking at these values, particularly that if you have iron because we have just started our discussion for there on sides and their two different oxidation states and the two different spin states. So, we can have some basic idea. That means, what are the number of electrons present on this iron and the number of electron present on this particular site. That means, whether some of these cases, they will be EPR active or EPR inactive and in the background, you will have the corresponding spectral characterizations.

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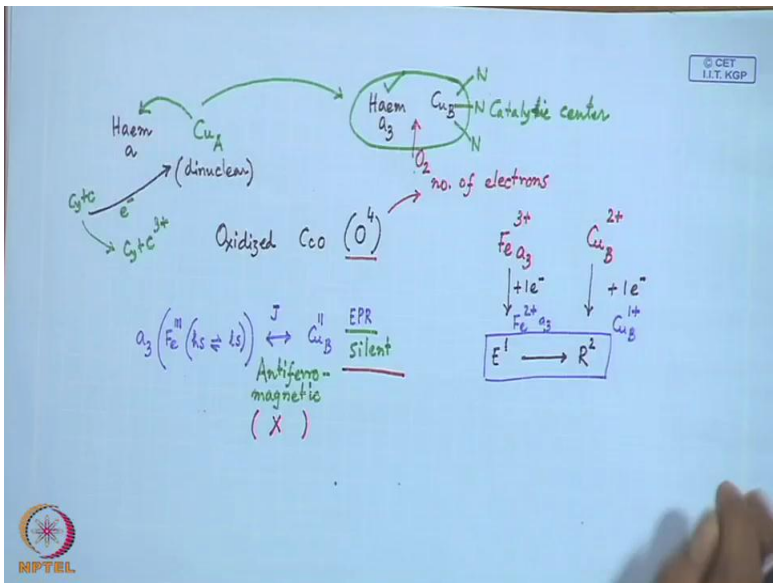

So, during this reduction process that we have seen earlier, that if you have one site and we designate that site as copper A site and the other one is our copper B site and close to that copper B site, we have haem a 3. That means, the distance between the copper center and the iron center can be determent. Similarly, this copper A site which is close to our in a site. So, you have one, two, three, four sites in your hand. Two of them are with iron and two of them are with copper and this copper A site is dinuclear making the system

much more complex. It is not a mono nuclear system. So, this particular copper is height which is a dinuclear one and if you just recall what we have on the left hand side, we are having cytochrome C, then you have the cytochrome C oxidized and on the right hand side, you have the dioxygen molecule.

So, this particular copper A site basically receiving the electron from cytochrome C. It is receiving the electron and which is therefore, responsible for the oxidation of the four molecules when it is receiving the electron from the cytochrome C and this cytochrome C will be converted to cytochrome C freight base means cytochrome C 3 plus. So, when it gives that electron and that electron if it goes to haem A site, so if you can get one after another, that means, you need not only the single electron transfer, but you need four electron transfer to the cytochrome C oxidize site. That means, the particular system is either a four electron oxidize form or a four electron reduced form, and four cytochrome C molecules are responsible for transferring electron to convert your cytochromes because the entire assembly now known as your cytochrome C oxidize and that cytochrome C oxidize is reduced by four electron reduction from the cytochrome C.

So, these two, now they are close to each other and after reducing this, center electrons are ultimately post into this particular system of haem A 3 and copper B because this is our catalytic center. Why? Because it can bind the dioxygen molecule. So, you are getting at one hand the four number of electrons. The four electrons you are getting from this particular side and you are having something, where you have the dioxygen molecule which is attached to this catalytic center and ultimately, one after another if you think that if we post those four electrons to the orbital's of O 2, then you will be able to reduce that O 2 to water molecule.

So, this reduced form we get after we have the oxidized form of cytochrome C oxidized. So, this is oxidized. That means, it is fully oxidized and this fully oxidized form sometime designated as O 4 because these are some states, basically because biology sometimes do not bother about the iron sites and iron oxidation state, but they can identify the corresponding species in terms of, it appear activity in terms of its electrons spractal activity or any other physical activity.

So, the oxidized form if we level it as O 4 and because it is four electron oxidized form, so the number of electron transferred to the catalytic site. So, it can accept four electrons.

So, the total number of electrons it can get. That means, when it remove those electrons, you get the corresponding catalytic site, both haem A 3 and copper B. That means, if you have Fe a 3 which is in the trivalent state and copper B in the bivalent state, so this is our only option we can have. That means, the ferric site of a 3 and cupric site for copper B which is your catalytic center and as just now we have seen that we can reduce these two. That means, you can have a fully oxidized form or you can have a fully reduced form and in between, you can supply one electron, two electrons or three electrons to that. So, if we can go for a reduction of one electron of this particular state and one electron of this particular state, we get one another state which is leveled as E 1 and the second electron; that means E 1 will go to R 2.

So, do not be confused with this. These are difficult level for this is not much, but it is the corresponding reductions. When we have the fully oxidized form, it is O 4. 4 is the number of electron transferred. When you reduced it by single electron, it is E 1. E 1 is the number of electron transferred to O 4. It is the corresponding number of electron. That means, two electrons has been transferred. So, it is reduced form which is R 2, but what actually happens there in our case; that means, we will see that this particular iron site will be converted to 2 plus. So, a 3 is converted to Fe 2 plus and your copper B is converted to 1 plus. So, that is very important because if we see there that these two are close to each other; that means, you have the a 3. So, another form is there. That means, you have a 3, which is Fe 3 and just now, you have seen that it can be in equilibrium between high spin and low spin and you have a nearby copper B which is copper 2.

So, now, this gives us some idea about that this particular center is paramagnetic which is also EPR active and this is also EPR active, but like your iron sulfur clusters, what we have seen, sometimes you can have two sides and individually both of them are paramagnetic, but they are strongly coupled to each other depending upon your coupling and we considered that they are corresponding j values. How strongly they are coupled? We tried to record there the corresponding EPR spectra and it is a paramagnetic one. That means, we are just nullifying some of these paramagnetic spices and sometime, we get that it is EPR silent. We do not get any signal corresponding EPR signal. That means, this is strongly coupled and the coupling is strongly anti-ferro magnetic.

So, this gives some clear picture for this reduction to this particular spices. It means already we have seen that this particular haem a 3 is 5 coordinated. So, it is 5

coordinated. So, it has some position which can bind your dioxygen molecule and also, we have seen this particular copper B side was only bonded to 3 nitrogen. So, it is not even four coordinated ones. So, both the iron side as well as your copper side is coordinately unsaturated because it can have a maximum 6 coordination sides. This can also be four coordination sides. So, we are pushing. What we are pushing? Just talking about this ligand, talking about the binding of dioxygen molecule.

So, basically you have now the dioxygen molecule in your hand and we are just simply dumping this dioxygen in the catalytic center, and you have iron, you have copper which can bind one more extra ligand which can be your O 2 molecule, which can be your oxide molecule, which can be your paroxide molecule and if they are breeze, so anti ferromagnetic interaction we find and if it is through some bridge. That means, bridge either the entire O 2 molecule or some reduced form. That means, the super oxide or paroxide or you can find it that nicely whether you can have the OO bond cleavage. So, if you have the O O bond cleavage. Then you generate the oxo. So, it can also be aironoxo copper site and if it is aironoxo copper site and if they are strongly coupled to each other, we get a system which should be EPR silent. So, these particular sides when it goes from one after another starting from our O 4, E 1 and R 2 will find that what other spices because we are not talking about all the four electrons.

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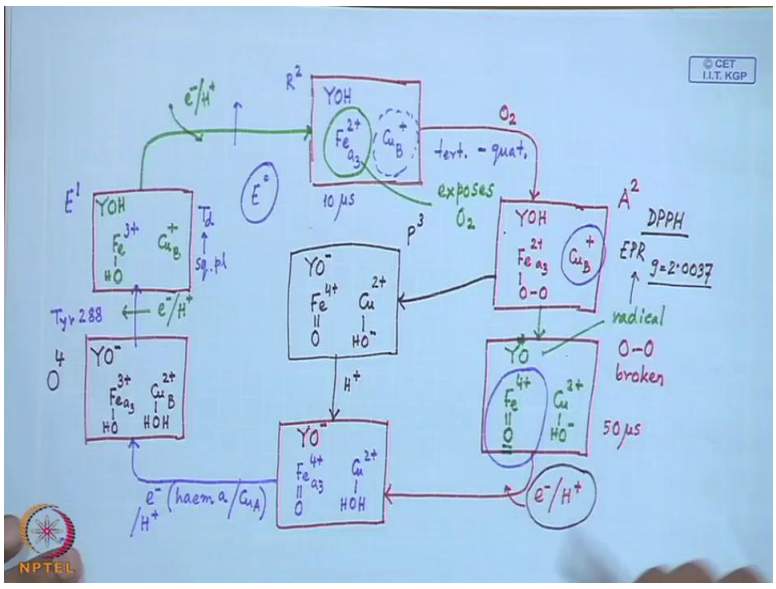

So, you can have several sides for this catalytic endeavor. These are the different steps. So, then on the right, you can have one more here and one more. So, here is nothing but our O 4. What you already have seen this particular O 4 state and O 4 state is we all know. Now, is your Fe 3 plus, then copper 2 plus. Fe is your Fe a 3 and copper is your copper B and this is bonded to a water molecule copper site and this iron site is bonded to a hydroxide group and this particular one has also nearby YO minus.

What is that YO minus? YO minus is another thing which we are bringing into the picture. It is the organic part which can give rise to some unpaired electron that is your tyrosine residue. So, this tyrosine residue which is coming from TYR tyrosine 288 because we all know that a phenol unit can be oxidized. So, phenoxyl radical, so the radical mechanism. So, it is not fully dependent on metal center redox activity, but it is also dependent on some organic redox activity. That means, the phenoxyl radical is also involved over there. So, this YO minus is present and this tyrosine residue is very interesting because it is covalently cross linked to one of the copper B ligand because already we have seen just now that this copper B is bonded to three nitrogen's. This copper B one nitrogen, second nitrogen and third nitrogen from the histidin residues and that histidin residue is then covalently linked to this tyrosine groups.

So, this tyrosine group is not going away. That means this would be close to your copper site. If it is covalently attached to your ligand site around copper; that means, if you have the copper binding from three nitrogen's of around this copper, so your tyrosine group is also nearby and that tyrosine group through oxidation as well as depot nation because it will accept proton as well as accept electron after it has been oxidized. So, this has some important role to play for the electron acceptance as well as electron donation. So, this particular O 4 group you have already seen there. That means, already we leveled that it can be reduced by a single electron to a E 1 state.

What is that this E 1 state? It will be something where we just through this particular state, we give electron and electron are not moving along because these all mitochondrial system, they are all membrane bound. We have the phospholipid groups and those phospholipid groups have the attached cytochrome C oxidize groups whenever we have a electron transfer. So, when you have the electron, either it is donated or accepted to the site. You have to have the side by proton transfer as well. So, from left hand side if your electron is post into the system, the proton will come from the opposite side. So, the

proton transfer will also take place over there and some extra proton is also pumped to the system along with these electron transfer. So, this proton, either it goes directly to the system; that means, in this particular case when proton is post over there, you have the depononated form of the dirosin which is getting protonated. So, your YO minus is going to YOH.

So, that protonation always keep in mind that this particular case is a membrane bond thing. We should also think of the corresponding ph values. So, if your ph is something which is not matching with the corresponding pk value of the dirosin residues, it will remain as its depononated form and when it is little bit acidic, it goes to its corresponding protonated form. So, this thing and these two sites you have also. So, you have Fe site as well as the copper site and now for the singular electron transfer, there will be a competition for the corresponding electron acceptance for the corresponding e 0 value for the site that whether your iron site will be reduced or the copper site will be reduced. So, as you move from one site to another; that means, you have a corresponding potential gradient.

So, in the first case, it is your copper site which will be reduced. So, cupric copper will be converted to cupra's copper and same iron, you will have which is still bonded to your OH function. So, this you get as your E 1 state and then we move for here. That means, in this particular case, you get the corresponding electron transfer again. So, E minus and H plus. So, the second electron which gives us the corresponding state which is known as we already seen as R 2, the first electron E 1 electron transfer, this is the second electron transfer and this particular case, if you go there, you go for this, not only electron transfer as well as the proton transfer, this particular water molecule sometime go away when you reduce it from cupric to cupras.

So, in this particular case, but the happening is that you loosing also this particular water molecule attached to your cupric copper site. So, that means, when you have already it is tri coordinated and one loosely bond water molecule you have and you are reducing it. The copper site is reduced to copper one and it is losing your water molecule, because all these coppers we know when we move from system that the copper one always have a coordination preference for a tetrahedral geometry.

So, if you have already a square planer one in this particular case, so it will simply move from a square planer to a tetrahedral geometry and not only the immediate coordination environment for copper as well as iron, but you have in the second level, you have the protein environment and that protein environment due to changing the coordination site for the copper is also getting little bit modified. So, when you move to your R 2 state in this particular case, you have still YOH. That means the tyrosine in the protonated form and now, the second electron goes to your iron site.

So, your Fe 3 iron is now reduced to Fe 2 iron which is based on the haem a 3 and you have copper B. So, this particular a 3 haem protein now is ready to accept because we all know now that for the myoglobin or the hemoglobin system that when you have the iron site in the reduced form and if it is penta coordinated, then it can accept the dioxygen molecule. So, this is now ready to bind this dioxygen molecule and within a very small span of time. So, 10 micro second. Within 10 micro second, it binds the dioxygen molecule to our haem a 3. So, 2 electron reduction and you have the penta coordinated iron site in the reduced form which is utilized for dioxygen binding. So, within 10 micro second, you get the binding of O 2 and this particular one means, we have not pushed any extra electron to the system, but we have the binding of dioxygen molecule.

So, R 2 is going from one state to another state which is now known as a 2 state. So, in this particular a 2 state, we have YOH, the nearby tyrosine residue. Then Fe 2 plus which is of a 3 and which binds to dioxygen molecule and we have copper B in plus one state. So, this particular dioxygen binding because after wards, it can give you something that if you have only the isolated pocket for dioxygen. That means, if you are other end, so this particular dioxygen molecule is not symmetrically bonded between the iron site and the copper site. That means, unlike your hemocynic. In case of hemocynic and you all know that you have two copper sites and in between, you have the dioxygen molecule and that dioxygen molecule is dished by two of these copper sites, but if you have a terminal dioxygen binding and that terminal dioxygen binding goes when there is the terminal dioxygen binding to any of these sites.

We all know that there is the partial charge separation. That means this will be this oxygen which is directly attached to iron site and will be delta positive and on the other end will be delta negative and if this delta negative site which has some extra electron density which can not only attract your copper site, but it also can attract some of the

sites from where it can get hydrogen bonding interactions. So, if you have some imidazole site chain from the protein chain, it can be stabilized by hydrogen bonding interactions.

So, if those hydrogen bonding interaction are there, so this particular entity that means, the O 2 is only attached to your iron site is getting stabilized and if this O is strongly engaged in hydrogen bonding to some imidazole site chain or anything, then there is a possibility for your OO bond cleavage. That means, hydrolytic O cleavage is also another possibility when you have binding for one of the oxygen to the iron site. That we all know that the phenton type of the activity, we have hydrogen peroxide. So, any hydrogen peroxide or any oxygen base system issue can have which is bonded to your iron site, it can go for a cleavage reaction and that cleavage reaction is taking place on your iron site and this iron can go for another oxidation state which is four plus.

So, when it is going for a plus four oxidation state, your OO bond cleavage has taken place. That means, this we all know, now is well known species which is known as the corresponding ferial spaces. So, you have the iron in the plus four oxidation state and this particular state, you have this O is in the oxo form and this copper which is also oxidized in the lot of changes taking place at this particular point which is attached to H O minus and the other one. That means, the other part; that means, your Y group is there. Now, it is forming a radical. So, this is your radical forms.

So, tyrosine in radical form is there and this is also a very fast process and form within 50 micro seconds. It is very difficult to identify all these spices and for this particular state, we get a corresponding O bond cleavage. That means O bond is broken for this particular system. So, basic idea behind this is that you have already the corresponding spices. That means, the four electron transfer for cytochrome site to the dioxygen site and these dioxygen when you go for the cleavage reaction, you should be able to produce the corresponding water molecule.

So, when this is attached to the next step, here again you get was already we have consumed two electron, here and here. So, now, you have another electron again and the proton to the system and in this particular case, the proton and the electron which we are pushing to the system, the electron goes to your tyrosine residue. That means, your oxidized form is moving back to your phenoxyl phenoxide ionic form. So, it is going

back to YO minus form. Iron is present as Fe 4 plus attached to a oxo form, which is your a 3 still and your copper is in 2 plus and this proton basically even we push this proton, it is forming water.

So, we just simply now complete our cycle. How we can complete our cycle? Now, because we have pushed again some electron as well as proton and some extra proton is also pumped from there and these electrons are coming from. Where these electrons are coming from? Haem A and copper A. So, these electrons basically what we are giving to the system, they are accepted from haem A. So, haem A, the function of the haem A is nothing but it can accept the electron from the left hand side from the cytochrome C and it can donate that electron at this step, similarly this particular one. That means, the formation of these corresponding radical function is important because this particular case, the enzyme which extra three electrons. So, enzyme which basically extracts three electrons. How we are getting that?

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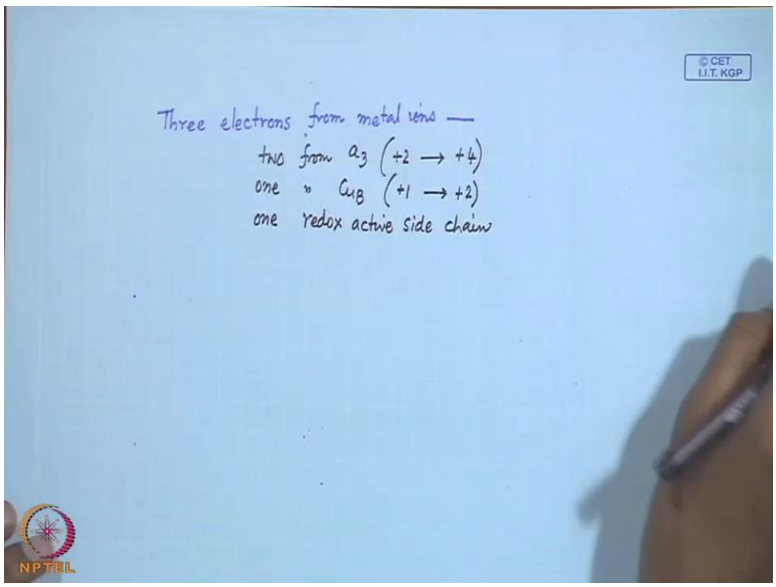

So, we are getting three electrons from metal ions. So, do not confuse that you are getting all four electrons from four metal centers because you are having all four metal centers. So, immediate answer can be that both the iron centers involved for electrons transfer and both the copper centers are involved for electron transfer, where it is not that. So, two from a 3. That means, when it is moving from a 3; that means, plus 2 to plus 4 oxidation state and one from copper b, it means when it is settling between plus 1

and plus 2 and the third one is from the redox active side chain. That means the tyrosine residue because this sort of activity based on the redox active side chain is also known for some other important molecules.

So, if we go back to the cycle again, we will see that when we have all these; that means, if we consider that known this is not forming. So, another alternative proposal was there like the permission of the corresponding oxidized ligand form because this is a nearby ligand. Though this tyrosine oxygen is not directly attached to the metal center, but it has some potential for donation to the iron side or the copper side, but it is not attached because it is in such a way it is fixed within the protein chain that it cannot come to the coordination distance to the iron site or to the copper site. So, they are the corresponding distance tyrosine residues. So, if we cannot go for the corresponding oxidized form of this tyrosine group, it can only go for some di ponototion reaction.

In this particular case, what should be our alternative proposal for that? It is if you have Fe in 4 plus oxidation state which is attached to our oxo form; that means, what we are getting from here now without going for this radical inter mediate. So, if we do not go for radical inter mediate because some of these spices where we are unable to identify the corresponding radical formation in this particular case because the radical, we all know the radical can be very easily identified by EPR spectral characterization. It has a very standard g value at 2.0037. So, the presence of radical can also be very easily identified if we can simply go for the measurement in the solution state for this particular state and if you can identify these particular one because this is closely matching to our standard for the EPR measurement di phenyl picarel hydrazine. DPPH is the standard, but we measure for electron paramagnetic resonance measurement. So, if we can go for these; that means, the radical is not forming and here ferile oxygen is present as the Fe 4 plus O 2 minus.

So, what is your alternative form? Your alternate form is that you have the cupra site. So, cupra site should be oxidized over here immediately. So, you have Cu2 plus which is attached to your HO minus and this then after accepting because this extra step for electron transfer, we are not requiring. This is not required. When you avoid this particular path, we just simply go for this path. We simply go for the proton addition to that and this proton addition will convert this cooper to two copper water spices and this particular one is known as a form which is E3. So, why we are talking so much about all

these things. It is very important because we will see in our next class that how we can generate these particular species. How easily we can generate even in the case of your Fenton (( )). From your college days, you know all these that when you have a ferric species and in the ferric species, in some little bit reducing environment, you push some hydrogen peroxide.

So, these hydrogen peroxide if they are giving you some permission of simferayl species and not only give us this particular information that this ferayls species forming and the fesail electron transfer because we are starting from a case, where you have this particular case. This was basically 3 plus. So, between 2 plus, 3 plus and 4 plus. So, iron can move from a plus 2 oxidation state to plus 3 oxidation state to a plus 4 oxidation state and if we have some amount of confusion regarding another extra electron that whether you are oxidizing a particular system by another extra electron. That means, your tyrosine residue is getting oxidized or your iron site is getting oxidized.

So, it is very difficult to identify, very difficult to establish that thing between another configurations where your iron site is in plus 5 oxidation state and this particular radical form is not present. So, this we will find in case of catalysis and peroxidases will find that since you have some nearby phenoxyl radical, and if you have some very useful E 0 value for this oxidation of this phenoxyl radical because all we know that sometime, the E 0 value we can modify when your phenol unit either in the protonated form or the unprotonated form, which is bonded to the metal center.

When it is bonded to the metal center, it can modify the corresponding E0 value or the electron potential. That means, you can oxidize that particular site at a lower oxidation potential and if you can have some another E0 value for the oxidation of the nearby 4-5 entry, so that we will find that whether in some other combination that this tyrosine residue is not getting oxidized, but in case of catalysis and peroxidases, we will find that your profilingring is getting oxidized. So, you have large number of unpaired electron over there. We have the CC double bond and that CC double bonded backbone can be oxidized. So, your pe in that particular case, p will be p plus.

So, that is very important and interestingly, also when we go for this tetrahedral structure for this copper one and this tetrahedral structure because we are pushing the electron from here and you are changing your copper site from a cubic site to a cupras site. So, if

we are able to change the environment around copper and this copper is close to that of a iron site what we are willing to get; that means we are willing to get such a situation or a such a conformational arrangement around the protein environment for this entity that this O 2 should go and bind to your Fe 2.

So, during that transformation, this particular environment around your copper 2; that means, the corresponding tertiary as well as quaternary structure, quaternary protein structures around this copper is getting modified and due to that transformation, what will be getting is a 3. That means, that environment around your a 3, this environment you have the corresponding exposer. So, you have certain positions remain vacant. So, this exposer is important. So, it exposes to our O 2. So, this exposer is important. So, you see the simple coordination chemistry. That means the movement from square planer to a tetrahedral geometry around copper and this copper is already bound to the protein structure.

So, due to this transformation, you are modifying the corresponding environment from a treasury structure as well as the quaternary structure and you have some avenue for exposer to dioxygen and that dioxygen molecule is nicely bonded to your iron center for its corresponding ferial formation. So, immediate information for all these thing is that how they are strongly coupled to each other. That means, these two sites are close to each other and how you can generate the ferial for the different re activities. So, not only for the next we will see that your peroxides activity or the catalytic activity, sometime this oxygen will be a very good reagent for transferring this oxygen to some organic substrate for hydroxylation reaction.

So, the generation of this ferial iron is an important tool to go for your hydroxylation reaction for any organic sub state, even in our drag molecules which are hydrophobic in nature, and if you have a corresponding phenol end, that phenol end will be hydroxylated by using this particular thing. That means, this particular reagent if you have iron and hydrogen protoxide and sometime iron and your simple dioxygen molecule.

Thank you.