Bioinorganic Chemistry
Prof. Debashis Ray
Department of Chemistry
Indian Institute of Technology, Kharagpur

Lecture - 12
Electron Transport Proteins-VIII

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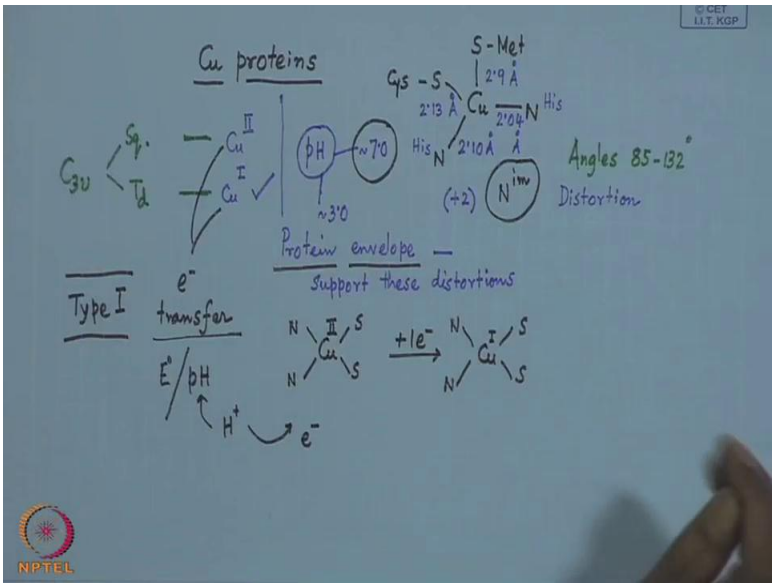

Hello. So, here with the trigonal distorted pyramidal system for the copper protein. So, we are talking about copper proteins which is severely distorted. So, we have seen that the sphere was N 2 H 2 and if we have a environment which is trigonal in nature, which can be both favorable to copper one and copper two, that we have seen, our last class; but if we just simply compare the corresponding distances which is sulphur from the methionine, sulphur from the cysteine and the nitrogen from histidine. So, this is s methionine. So here, for this two histidine and we were talking for both copper two and copper one. And in case of copper two, the distances were such that, this is the longest one, this was the distorted one, the methionine one, which is around 2.9 Armstrong, it is pretty high, the distance compared to the nitrogen distances, the copper nitrogen distances which we know that all are close to two; that means, two Armstrong, which is 2.10 Armstrong and other one is 2.04 Armstrong; and cysteine sulphur is also close to that nitrogen one which is 2.13 Armstrong.

So, when we see all the three distances and at the same time, when we know the variation in the angles. So, all these angles that means, the sulphur-copper-nitrogen,

nitrogen-copper-nitrogen or sulphur-copper. So, all these things, so how do we know that they are preferring some geometry, where you all know that copper one will be preferring a tetrahedral one, which should be highly distorted and this one should be a square based one.

So, in between either a square planer or a square pyramidal for copper two; but in between what we are getting; that means, we are getting some intermediate geometry which has a symmetry level of C 3 V, which is like that of our pyramidal one. So, these two basically support both the environment. So, angles also do vary within a wide range of 85 to 132 degree; you see, how distorted they are.

So, these values basically, this angle values will immediately tell us the amount of distortion. So, when we talk about these distances and this is for copper two. So, this is for the plus two species. So obviously, if we can make a table for the other bond distances; that means, the corresponding bond distances of copper one, we can find out that how these distances will change, and not only this change which is reflected for the oxidation state change form copper to copper one, means when you move from plus 2 to plus 1; obviously, all the distances will change, but some of these distances will change more.

So, in this particular case, you will have certain arrangement; that means, your protein envelope should support these distortions. So, you have plus 1 and plus 2, and you have for plus 1, we are just giving this distance, for plus one you have the corresponding these distance, four distances, two copper nitrogen and two copper sulphur distances; and one more interesting thing is also there that if you bring something over there; that means, the P H, we can determine the structure also at two different P H, one is close to seven and another say close to three.

So, at these two values that means, if you just consider that your medium is little bit acidic close to 3.0, we will see that there are certain positions including your imidazole ring, and the cystine sulphur, and the protein environment. So, there will be some positions which can be immediately protonated. So, basically what we get. So, if we just consider the distances, we will have two distances for the two different oxidation states and there are other also, set of distances for two different P H values. So, depending on this that means, when we talk that if you just simply change, that means these two

change, what we are getting for this copper two and copper one, this basically is dependent on electron transfer; so during that electron transfer, our structural change is also taking place, that means distortion is taking place during electron transfer, as well as, when you change the P H, your structure is again changing. So, at which particular P H, you are measuring this electron transfer because we are, what we are looking for? We are basically looking for, you have two sulphur two nitrogen that reduction. So, if we see that there is some chance that means, in the deprotonated form that means, when your medium is neutral or slightly basic, your electron transfer will be facilitated.

So, four structures as well as you will have that different E 0 values. So, whenever we report these E 0 values for those electron transfer, you should also report the corresponding P H. So, there will be something that means, you have basically this proton which supplying the electron, and that electron will control your electron transfer; either it will be facilitated at basic P H or in the acidic p h, but basically it will be facilitated in the basic P H, when you have more charge on the copper center. So, these two things that means, these two are very much inter related that means, we should not neglect like our simple molecules that, what is the corresponding P H medium that means, this can also certain positions which can be protonated, if you have some distant group also that means, nitrogen at distant position also, which is nearby that means, this particular nitrogen imidazolidin ring which is nearby, but not directly attached to the copper center, but it protonation level will do contribute to the electron transfer as potential as well as electron transfer rate.

So, these are the very simple thing for type one electron transfer. So, when you have a type one site, you have a mononuclear system, but these mononuclear system can transfer that electron which is dependent on the P H value of the medium.

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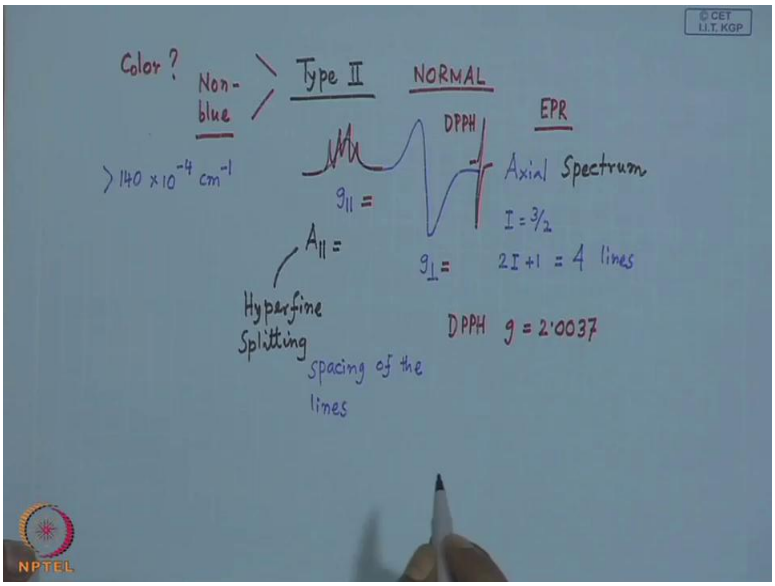

So, next after type one, we should just have some interesting information's about that type two copper proteins. So, first when we see that this is the type two copper protein, sometimes they are also known as a normal type, and what immediately we can do is that whether that particular one is colored or not, what is the color of the system? Because the previous one, we started our characterization from the color that means, you have a strong charge transfer band due to the cystine in sulphur to the cupric copper, but in this particular case this one is very frontly colored that means, we can consider that they are non-blue system.

And also for its EPR characterization. So, electronic spectra can differentiate in the liquid state in the solution state, whether you are handling a type one system or a type two system, by looking at the color only. So, there is no need to find out the corresponding lambda value or epsilon value; similarly, the electron paramagnetic resonance also can give some information about the nature of the corresponding protein center. So, we have seen that the typical spectral pattern is this, which is an axial type of spectrum and when you have the copper 63 I value which is 3 by 2. So, the splitting is two I plus one, we get four lines; and four lines, this is the g parallel region, this is the g perpendicular region. So, this particular one will be splitted into 24 lines. So, average ones. So, out of these four signals, average one is your g parallel value and the next one is g perpendicular value and somewhere at these positions, you will have the reference one, which is D P P H, which has a G value of 2.0037.

So, if we see that it is sometime, it would be little bit confusing that the copper type one site, it means the type one site also has this sort of axial spectrum and copper two also has this axial spectrum; but these values, if there is also, because sometimes we find that there is also splitting in the perpendicular region also. So, that if there is splitting. So, in this particular region, we find that these values, this is a hyperfine constant, hyperfine splitting constant due to the copper center. So, it is a metal centered hyperfine splitting; because sometime, we see also that when copper, when here is also directly bound to the nitrogen site and nitrogen has also some I value. So, if there is some interactions with the electron spin of the metal center and the nuclear spin of the nitrogen, then there is another type of splitting, but would with this very small, which is known as we put some super, that is super hyperfine splitting and we designate that as small a value. So, these values, so since we have the splitting in this region. So, a values, if we just simply compare these a values. So, in the previous case a values were less is 0.95 into 10 to the power minus four centimeter inverse, but in this particular case it is bigger compared to those values, which is minus 10 to the power minus 4 centimeter inverse.

For type 1 it is 0.95 and compared to 0.95, you have 140 into 10 to the power minus 4 centimeter inverse. So, the G value will tell you that the spacing of the lines. So, these a perpendicular hyperfine splitting is nothing but the spacing of the lines. So, we get something that means, type two, we get and it is responsible for large number of reactions.

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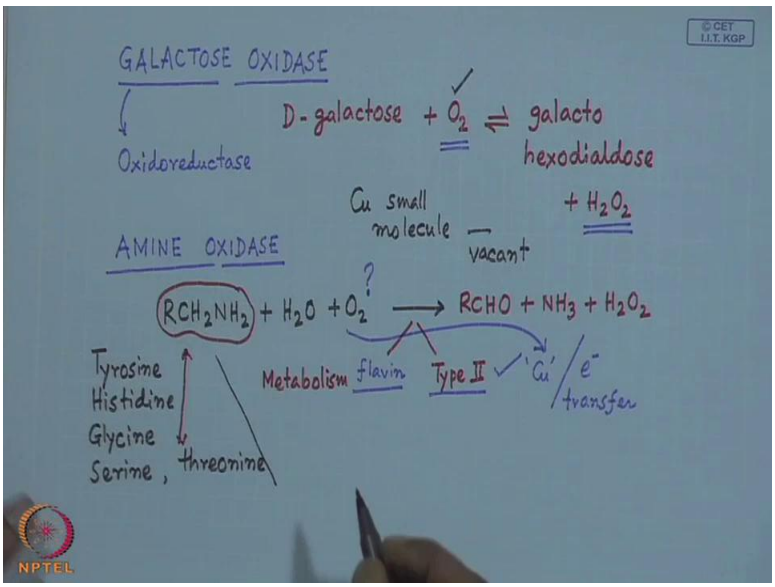

So, one such reaction is the simple oxidation reaction and that is responsible for the reaction where the galactose is involved. So, which is known as galactose oxidase. So, substrate is nothing but your degalactose. So, degalactose in your hand and in presence of O 2, it is giving the oxidized product which is galacto hexodialdoses, hexo dialdose plus hydrogen peroxide. So, this particular case, this dioxygen is consumed, we are producing hydrogen peroxide. So, this particular reaction is performing by your galactose oxidase. So, the general category of these molecules is still… Therefore, they are also oxido reductase and there are some other molecules also, which is very much useful for producing some very important bio molecules in our body, which is amine oxidase.

So, we take the help of the copper center which is bound to the protein site and activating the dioxygen molecule. So, little bit you can think of that what we know from the understanding of the hemocyanin, the dioxygen molecule you have which is getting activated by the copper center. So, here also, the copper center which is getting activated by these, and interestingly this copper center is a small molecule complex, which is a small molecule that some positions is available that means, some vacant position is there and that vacant position is utilized for dioxygen binding; once the dioxygen is bound to it, then you should decide that what particular type of reagent is required for this conversion from the left hand side to the right hand side, whether you need a super oxide or a peroxide or something related to that of our cytchrome oxidases that means, the oxotransfer reagent, that the conversion reaction and the product formation of our all these things will tell you that which particular reagent is required for galactose oxidation reaction, or the amine oxidation reaction, which is nothing but your amine in with some water plus O 2 giving rise to basically it is some sort of deamination reaction R C H O plus N H 3 plus H 2 O 2.

So, in this particular case what we have that means, you have, you are utilizing type two copper protein for this conversion and some flavin containing molecules are also required. So, that for the reaction that means, whether it is a catalytic reaction or not, then you know that the pre catalyst and some of the co catalyst that means, what are the reagent. So, this we know that this is the metal center, type two is you have the copper site. So, on copper site, you can bring these dioxygen followed by electron transfer, go for electron transfer.

So, the nature of these is getting changed. So these then attacking your molecule. So, in this particular case, when you have so, amine oxidases is. So, there are large number of molecules available that means, it can be utilized for tyrosine metabolism, which we all need. So, for tyrosine metabolism, then histidine metabolism. So, all sorts of amine acid metabolism that means, the protein metabolism.

Then glycine or serine or threonine. So, they play active role in all the metabolism of all these species. So, during that reaction that means, these are what? These are your, all these groups are your substrates. So, this is your substrate for your amine oxidation reaction or the galacto oxidation reaction. So, how we go for that? So, like your type one site, this can also be identified that this is also a mononuclear copper center.

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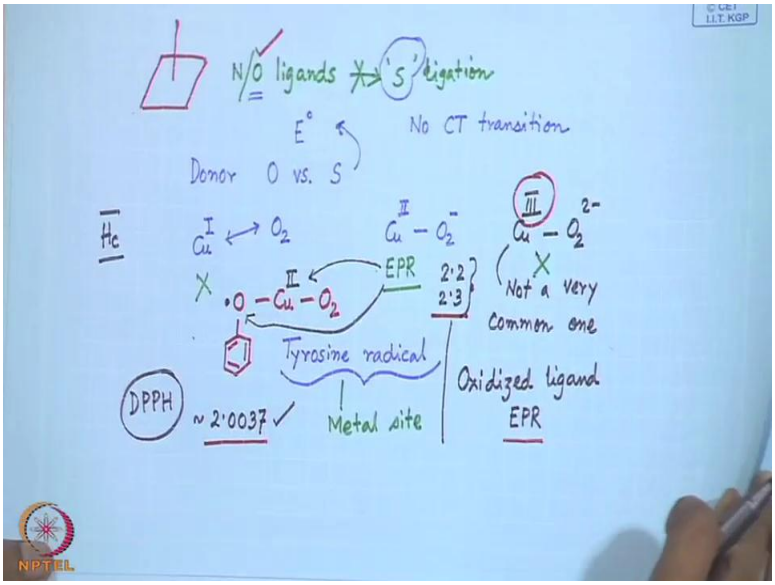

So, that is very easy to identify that whether you are handling a mononuclear system or a binuclear sytem. So, these is a mononuclear system and here now, you have less distortion and it is almost close to a square pyramidal geometry, and that square pyramidal geometry is fulfilled by nitrogen and oxygen donor ligands, but no sulphur ligation, no sulphur ligation.

That immediately tells you that why you do not have the strong blue color, charge transfer transaction is not there; once you identify that you do not have any sulphur environment, particularly the cystine sulphur. So, neither you have the metheonine sulphur or the cystine sulphur. So, the strong charge transfer transition is also absent, no

charge transfer transition. So, in this particular case, what is cared that, that once you do not have the sulphur one and we are not getting the any contribution from the sulphur, and sulphur in two forms in the type one copper chemistry, that sulphur is sometime for the synthetic molecule is not so good, because most of the time, when we get the sulphur, sulphur will try to reduce the copper center. So, you have, when you have the sulphur environment, your E 0 value for those environment is expected to be less, the sulphur compared to oxygen, because what you have now, in type one case, you have nitrogen and sulphur. So, nitrogen sulphur. So, without having sulphur, now you have oxygen. So, what you can compare, the donor property of oxygen verses sulphur. So, how that donation from oxygen and sulphur? Depending upon your charged density as well as the size, because this is smaller and this is bigger; how you can modulate the corresponding E 0 value? Whether you have a typical nitrogen oxygen environment and a typical nitrogen sulphur environment, where you get the corresponding center having less E 0 value; that means, you need some reducing agent like your flavin, or ascorbic acid, or something, that a low potential containing reducing agent can reduce the center to copper one. In this particular case here, E 0 will be little bit high. So, higher value of E 0 will control something; that means, it will activate your di-oxygen molecule. So, you have the galactose which is giving you the aldehyde and hydrogen peroxide, and you have one copper center and some group is available, which is responsible for reducing this copper two to copper one, and when it is directly interacting with your di-oxygen molecule, we immediately go from here to a system, which is copper two and O 2 minus.

So, internal electron transfer will generate a species either a copper one is reacting like your hemocynin, think back that what we know about our hemocynin chemistry. So, from that hemocynin chemistry, but here, the system is little bit different, because we are talking something that where you have a mononuclear copper system and that mononuclear copper system is trying to interact with that of our, this particular oxygen center. So, you can have only one electron transfer because copper is already plus two, if you just next go, try to go for a system where copper is bound to a peraoxide system, your copper center should be plus three, which is not a very common system.

So, something is therefore happening and you have the corresponding radical system; that means, when we go for that binding of this. So, one of this oxygen from this nitrogen oxygen environment is your tyrosine group. So, the amine acid residue tyrosine

is there and it is bound to our copper center. So, like that of our cytochrome C oxidase, what we have seen earlier that in case of cytochrome C oxidase, we are not able to promote the metal center to plus five oxidation state or plus four oxidation state sometimes that these oxidation state, if it is not available from here metal center, through the metal center, you just oxidize the ligand site. So, you have the oxidized ligand site that is providing the electron to the system; that means, this is going. So, in one end, you have this copper and in other end you have this di-oxygen. So, if you are unable to oxidize the copper center, these are able to oxidize the corresponding ligand part. So, you have a oxidized ligand part. So, you get a tyrosine radical. So, this is basically a metal ion which is coordinated to a radical system.

So, you have the tyrosine radical is your ligand, which is bound to the metal site. So, metal bound radical ligand system; that means, the corresponding equivalent of electron transfer, what is coming from there that can be identified very easily. So, when we talk about the corresponding copper two system, we can utilize the electron paramagnetic resonance to identify this site, copper two, this will not show any EPR signal, this will also not show any EPR signal. So, this is your detained system and this is d eight close to your nickel. So, nickel will also not show the EPR spectrum, though you have the paramagnetic system, but it will not show the corresponding EPR spectrum. So, any kind of EPR spectrum, it will not show.

So, when you have this copper two and these O 2 minus, and you have the oxidized ligand, the tyrosine radical is nothing but your oxidize ligand. And to prove that, that means, what is there and which is bound to your metal center, again in this particular case also, we can take the help of EPR; because we know that the reference system that D P P H, diphenyl picrite hydrazide. So, which is also a organic molecule, but having a loan pair of electron like nitroxide radical also. So, nitroxide radical is also a very good reference material for EPR signal and for D P P H we know the value of the corresponding EPR signal which is close to your 2.0037; and in case of all this copper signals, they are either 2.2 or 2.3 etcetera .

So, now, we get something that the same copper two system is there and their EPR pattern is much more complicated; because already, if you have something; that means, this copper which is bound to your radical. So, you get the corresponding EPR for this copper, as well as the EPR for this radical system. So, you get something; that means,

you have the signature for the values of this signal 2.22 2.3 as well as signal for this particular state. So, once you identify these that means, once you get the corresponding EPR spectrum for this one, this will give you the typical signature what we have learnt from your type one system or the type two system which is not further oxidized. So, in this particular case that means, when you have the copper two system, you can indentify by EPR that it is only a copper two bearing system; if you go for the further oxidation for this that means, your radical is forming and this is reduced to peroxide.

So, radical will have some individual signature and these two positions are not overlapping to each other. So, they are clearly visible. So, in E P R spectra, this is a very useful technique because that both you can identify the corresponding copper two site as well as the oxidized tyrosine radical. So, that particular one that means, when we get whether you have the same system for our copper, what we find in case of hemocyanin, that means, once such important molecule is for generation of hormone and all this thing.

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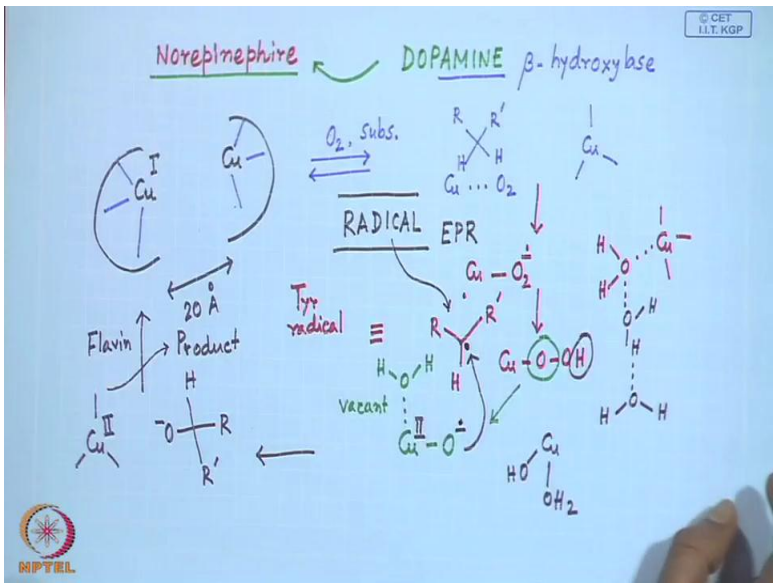

So, for the formation of norepinephrine, epinephrine we require something that means, the production of these hormonal molecules and all these in our body, which also bare the dopamine molecule, you should know the detail structure also; what is the corresponding structure of the dopamine? And this immediately tells us that this is amine. So, if we just react with that of your copper site. So, it will undergo some oxidation reaction.

So, one such important system and or the catalyst required for the production of norepinephrine is dopamine beta hydroxylase, dopamine beta hydroxylase; and there the tyrosine radical type of thing is operating and the system what we have is first you have the copper mononuclear system and another copper one center is also nearby, but these two are individual mononuclear system, these are not your binuclear system, what we call as a binuclear system? If these two coppers are connected by some bridging groups like in the case of hemocynine, and also will see in the case of the other type where you will find that if these two copper are bridge, but you have once particular pocket in this particular area, and another pocket here, it will not go for any kind of this binuclear assembly, but it can provide some long distance electron transfer reactions. So, this particular center, most of the cases, they are tri-coordinated. So, for dopamine beta hydroxylase, you have these two centers and what we have learnt so far is a very simple thing that what you have the copper center, here oxygen is your reagent. So, is a reversible one and you need the substrate which is your dopamine and in other case it is the galactose.

So, the substrate is there and substrate is getting oxidized. So, in the first case where you have this copper site, and this copper site is interacting with your O 2, and the substrate which is based on the carbon hydrogen bond; suppose your substrate is like this, these is H and H and you have the second copper. And in this particular case, we will find that in the next step, where we rate determining step, we can find out the corresponding rate constant for that that you have these copper; and this particular copper is basically now reduced to super oxide that means, it is getting activated. So, electron transfer is taking place as well as, your that other part basically, this one is there that means, this particular substrate is there, and you are generating this particular super oxide there, from internal electron transfer; and then, if we just further go for copper hydrogen peroxide that means, there you will have the corresponding protonation; and this particular case, your second copper, this particular copper is basically sitting there.

But it will not provide anything, but it has some internal interactions with that of your water molecules. So, if there is that means, if something is there, you require some hydroxide group from there for de-protonation that means, not only de-protonation but also you have to have that corresponding oxidation. So, this r and r prime. So, if there is abstraction of that proton that means, proton plus electron H hydrogen atom abstraction.

So, you have r and r prime, and one eight will be there and this will be radical form, which is equivalent to that of your tyrosine radical formation.

So, your substrate is responsible for giving you the corresponding radical, and these oxygen is further bound to, is basically sometime we get a trimeric form, it is very common that mean… So, if you have a very long distance say several Armstrong, say 20 Armstrong or so, but if you find that is a copper activated water chain. So, this copper activated water chain such that is still is approaching the second copper. So, when you have this that means, if you just go for this proton, this is you just supplying this proton to the system that means, you have the charge you can go for the corresponding peroxide then it is getting protonated. So, peroxide protonation can take place from this sort of water molecules connected to the second copper.

So, this particular case. So, if you have that, that means, if we just simply transfer this particular oxygen atom, that means, our goal is that you have to transfer these oxygen atom. So, like that cytochrome C oxidase, in just end of with some oxygen atom over here and some solvent that means, your water molecule is interacting with it that means, it is occupying the vacant site, if some vacant site is available due to this non-occupancy of this particular substrate or any other group. So, most of the cases, the vacant site will be occupied by the water molecule. And the second copper which is already showing some quick interaction with that will be giving you a hydroxo binded, hydroxido group binded copper center. So, this one is there and another copper can go to this molecule to this one. So, this oxygen, this particular oxygen which is charge is also there and this extra electron is also there. So, this basically will attack your this radical form over there. So, this particular one when it is there. So, is a, this particular center will be attacked by these and copper will not transfer this oxygen giving you that oxygen on the substrate backbone. And you have the copper. This coordination is there and this copper is in plus two oxidation state and in the final step, we get something that means, either the flavin or the ascorbic acid that means, some reducing agent is required, such that you reduce this copper two site to copper one site and your product is released from there.

So, this identification, basically how will you identify that it is going through a radical intermediate. So, our EPR is there, you take up this particular hydrogen atom from there and you just generate that unpaired electron density over there and that is finally, collapsing with to give you the corresponding product.

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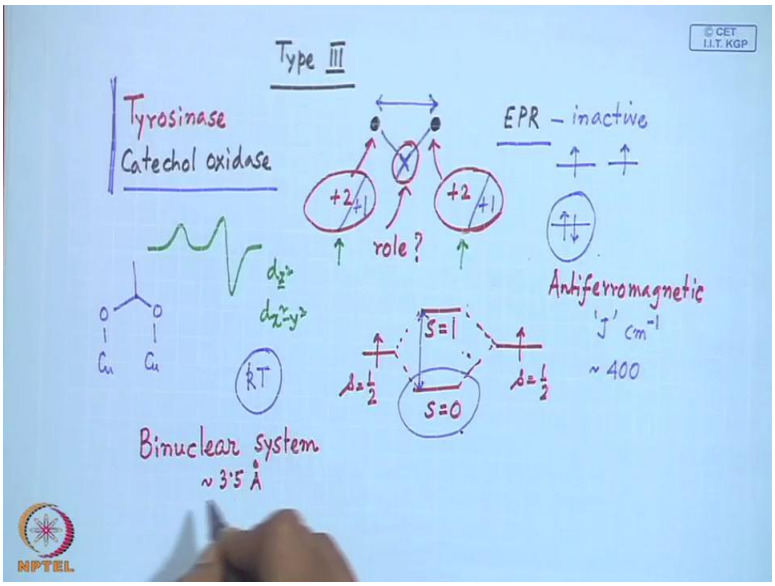

So, this is therefore, a completely different story apart from your type one site, and this type three site which is also little bit different compared to this type one and two. So, if we just simply compare that how this type three is different from type one and type two, for this particular system also, you have the system, we already know the hemocyanin, then another group of molecules is tyrosinase that means, you can work on tyrosine molecule, and you can oxidize the tyrosine also to form a catachol type of thing; and it can also work on catachol oxidase. And in this particular case, when we have that particular type three type system that means, when we go for these that means, if you have one particular site. So, atleast two copper sites are there, and if we just rely on the corresponding characteristics structure in EPR spectroscopy, we will find that in most of the cases, they are EPR inactive compared to your type one site or type two site. So, in most of the cases, when you find that they are EPR inactive, but you have something where this particular copper site, if it is in plus 2 and this is also plus 2, this individually when you talk about in terms of that type one copper, this site is EPR active, this site is also EPR active. So, we get something; that means, this particular site which is EPR active and this particular site which is also.

So, individually both the two sites are EPR active. So, this is a system. So, you have one unpaired electron on this and another unpaired electron of this, and the energy level for those unpaired electrons on the individual copper site will give you the corresponding EPR spectrum; and in most of the cases depending upon its occupancy that means,

whether you can have a unpaired electron in, depending upon the geometry. So, what type of geometry you have? Either it can be on the d z square, or d x square, y square or by twelve. So, when you have this two and these two individually can give the corresponding EPR signal, but in this particular copper two site, we will find that a have the strong oxidase activity, because we are looking for something the biological activity, and we are trying to correlate that with something which is basically dependent on physics. So, this biological activity, whether this particular site, whether your system is paramagnetic or diamagnetic. So, depending upon the of the nature that means, when it is in the plus one oxidation state that means, we are reducing the copper two to copper one, we know that the activity for the copper one sites are completely different compared to your copper two site; but when a system is there and which is increased by something; so that means, you have the bridging group x, when you have the bridging group and then these two electrons are coupled to each other and you get something, the magnetic orbital, we call. So, the magnetic orbital, it can share the same orbital level and these two are paired that means, you have some interaction.

So, this particular case, so when you have this these two combinations that means, either you have two unpaired electrons or a system where you have the paired electron. So, that you all know that this second thing that means, this one is your interaction which is anti-ferromagnetic. So, this anti ferromagnetic nature that means, if you have the levels like these, which that in individually, you have this half electron spin, this is also half electron spin and whether you have that total spin is equal to 0 or is equal to 1.

So, if you are at the ground state that means, this state is 0 or sometime close to 0, that depends on your separation that means, the population of the individual level. So, the Boltzmann distribution law is there. So, Boltzmann constant is also operating your k t value is important and that k t value will dictate that how big your (( )) it is there. So, the coupling constant. So, the anti-ferromagnetic coupling we get, we report that coupling constant. So, this coupling constant J, we report in terms of its centimeter inverse value. So, if the separation is very big. So, if the J is about say four hundred centimeter or so, so your most of the molecules will be in this particular state, your system will show its anti-ferromagnetic property, but if your J value is not so big, you get some population at this level and some population at the above level also.

So, depending upon the distribution, the Boltzmann distribution law will tell you that how much is there the thermal distribution between these two level, will tell you something where you will find that the system is not completely diamagnetic in nature. So, these anti-ferromagnetic coupling which we will show the oxidase activity and you have this particular dimerric system, and these demerric system we can characterize because this super exchange or the direct, because direct exchange, because in this particular case, you do not think that there is a metal-metal bond. So, some mediating group like, we all the time from, we know that the corresponding copper acetate structure, the copper acetate. So, you have the mechanism, we call it as a super exchange.

So, through this acetic bridging your, this unpaired electron density on the first copper with the second copper is coupled and we get some interaction, but there is no such direct copper-copper bond. So, in this particular case, when you have these two and if you are able to know that what type of this bridging group is there and little bit, we can say from there what is it is role? That last case that means, the type two case, what we have seen that you have two individual sites one is one copper site and another is another copper site, but most of the catalytic activities taking place one on the one particular copper that means, this distance is important. So, there you see that the distance is pretty big. So, 10 into 20 Armstrong, but this distance is pretty close. So, this particular reactivity is all the time we level it as a binuclear system that means, you have two copper centers which is close to 3 Armstrong or 3.5 Armstrong; and if we can do something that this one particular copper site that means, the first copper site is responsible for holding your substrate, the second copper site will be utilized for using the reagent.

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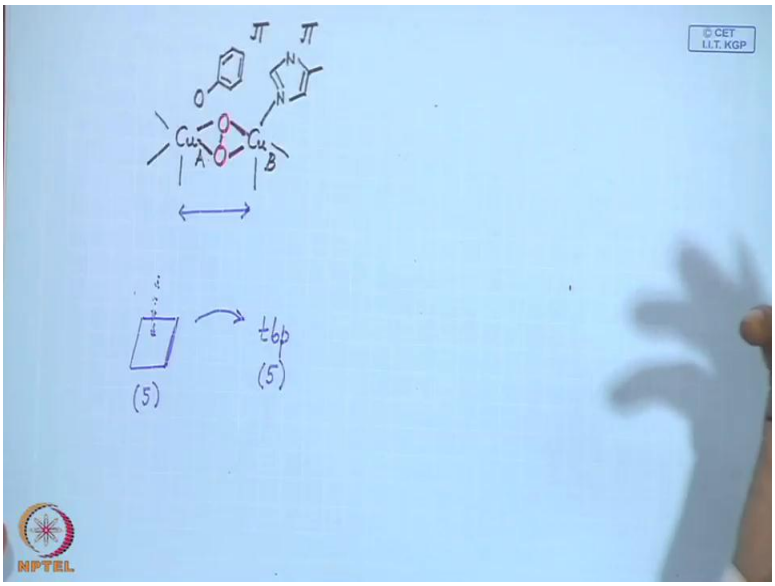

So, that basically, we have seen in case of hemocyanin and also earlier, that you have one copper site, and in the second is also copper, and you have the histidine nitrogens are there, then you have one histidine nitrogen, another histidine nitrogen, and the third one, just we are drawing over here. This is there and the substrate is our tyrosine group, and this tyrosine group, in case of its corresponding tyrosinase activity that means, you have this particular flat ring and this, you have the corresponding flat ring and the positioning of these two. So, this basically the type three copper you have like hemocyanin, you have one as copper a site and another as the corresponding copper b site, and when they are interacting with the di-oxygen. So, in between you have the di-oxygen molecule and that particular identification of these that means, you have the di-oxygen molecule and the typical nature of this bonding, we already identified earlier that if you go and we study the hemocyanin system that the hemocyanin system, we know that this particular type of interaction is different one. So, this perxo linkage is a completely different type of linkage. So, when you have three coordination sites which are coming from the nitrogens of the protein chain and here also the three nitrogens from the protein chain; and this interaction is slowly initially establishing through these two bonds with these di-oxygen. So, copper is moving from a tri coordinated system to a tetra coordinate one, when one bond is forming. Then we all know that whenever you have a copper center and which is a square planar one or a distorted square planer one, it will always attract another group from the fifth coordination site. So, it will try to move from a square planar one to a square pyramidal one, and if there is a severe distortion of the system, it can slowly

approach to a system which can be a tri gonal bi pyramidal geometry so, but these two that means, this is a five coordinated system and this is also a five coordinated system, and that particular one that means, this initially, if this bond is forming first that means, both the copper centers at tetra coordinated.

Then through this interaction. So, this should be a long bond initially; this will try to establish another bond with this oxygen and another bond with this oxygen. So, it is typical bond, and this system was first discovered in the synthetic molecule and later on it was discovered in hemocyanin system because this two will tell you the corresponding copper-copper distance because this is in the perpendicular positions. So, the position is different compared to your linear, this di-oxygen system. And also though that particular group that means, how the secondary interactions are also coming into the picture that in this particular unit which is a flat one and this particular unit is also a flat one. So, it has a pie electron cloud on this tyrosine which is coming into the picture as the substrate molecule, because we are looking for that tyrosinase activity, and this is also a pie system.

So, you have pie electron density, and the biologist also gave us the first of all this sort of informations that what we nowadays consider that hydrogen bonding interactions and the pie-pie interactions in all this super molecular interactions or in the crystal lattice, we find, when we determine some excess structure, we find that some close distances are there, but in this particular case, if we just the pie-pie interactions between this imidazole ring and this imidazole ring that means, the orientation of these two groups are important. This is one flat molecule and this is another flat molecule. Though the stacking of these flat molecules are important that means, this particular group is controlling the corresponding alignment of your substrate molecule.

You see now we just, in our next class we will see that. So, far what we are talking here is that your substrate molecule is not interacting, is not started coordinating with the copper center, it has not established any kind of interaction with the copper center. So, that interaction is not there, only the pie-pie interaction which is the first interaction of that type. So, pie-pie interaction is establishing the corresponding positioning of the substrate molecule at the metallo enzyme site. So, the slight positioning of this tyrosine group is important and the pie-pie interaction is important to attract your substrate

molecule. So, the next day, we will see how this particular one will go for interaction with that center and go for the catalysis.

Thank you.