Biological Inorganic Chemistry
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Lecture 40
Type 2 Non-Blue Copper Proteins

Hello, everybody, a very good morning to all of you. So, we have reached to the lecture number 38, where we will be talking today on the type 2, but non-blue copper proteins. So, we have seen earlier that how we can talk about or how we can discuss about the type 1, which is definitely a blue electron transfer copper based proteins.

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So, what are the things we will consider or we will discuss in this particular class is basically what we mean, how we can know about the type 2 proteins. Then one very important oxidase, what we know is the first time it is giving some very good information like your copper copper center in case of hemocyanin, when people discovered it people definitely were excited for that. And this is also another good understanding for GO, the galactose oxidase.

Then how we can modify the active sites? So, that the different types of modifications we can have from the biological world, then lastly we can talk about three of them is the amine oxidase, another kind of oxidase, but is working on the amine molecules, then D beta M and PHM.

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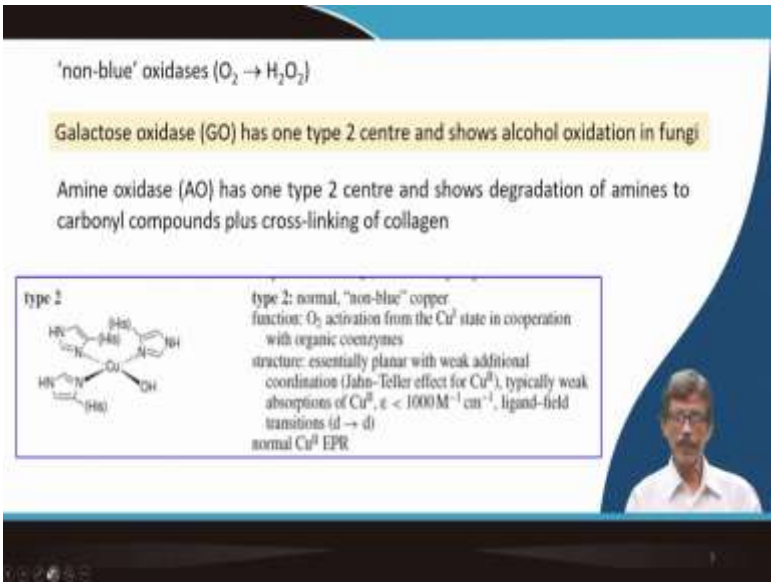

So, what are these and how we can find all these things in this particular category of compounds or the molecules which are active in the biological environment? So, non-blue oxidases So, whenever we see all these things and also try to remember, that these oxidases and at any time you should always keep in mind about the differences with that of your oxygenases. So, you have to have the O2 molecule.

And the very basic idea for all these and what nature is also using for this is that how to activate the O2 molecule. And compared to our knowledge, where we have seen that we can have the O2 molecule binding to your myoglobin center or the hemoglobin center, those are iron centers, but the metal ion is redox active. Similarly, here also, you have the redox active metal ion center try to bind the O2 molecule and a very complex fashion like that of in your oxy hemocyanin.

While doing so, what we are doing basically we are basically activating the O2 molecule. So, this particular type of coordination chemistry or by inorganic chemistry what we study here is that, how these small molecules not only O2, if you have some good idea about the activation of the O2 molecule, you will be knowing about the activation of the nitrogen molecule, the most inert molecule what we know.

So, we will take the example because people have identified structurally characterize the mechanistically well known and everything is determined now, for your copper environment. So,

galactose oxidase, what it has? So, the structural determinations and all these things basically giving us some very good idea that it has only one type 1 copper center. So, is not a by nuclear or a mononuclear type 1, type of center, it is the type 2.

So, which is neither type 1 nor type 3 center, that we should know first. And that we can also characterize from the difference in spectroscopic signatures. And what we can be used is there is very simple reaction, alcohol oxidation in fungi. So, alcohol oxidation in fungi can be studied, but we can get something which is very interesting to understand all these things, and is very similar way what you can see also the different amine oxidases.

Because amines are also very important, and how we can go for the different types of amine oxidations, and oxides formation and all these things are very important. So, what does it mean actually, the copper catalyzed, the copper enzyme catalyzed amine oxidase reaction. Here again, you have the type 1 copper center, and is basically responsible for the conversion of the amine function to the carbonyl function.

Even you can think of if you have a free amino acid, so whether you can go for that particular end of the amine end of the amino acid to be converted to its corresponding aldehyde function. And finally, we will see also whether that is the typical application that the corresponding oxidation, little bit of polarization and the polymerization. And finally to the cross linking one for these protein chains and all these things giving rise to the corresponding formation of the collagen molecules, which are very important.

So, in terms of this typical coordination environment, the ligands, the metal ions, the oxidation states, coordination numbers and all these basically can characterize your type 2 center. So, if you look at the particular geometry, the corresponding figure you will find immediately oh, what is there? So, you have three histidine residue is binding to the copper center and out of that you have one hydroxide (())(05:39).

So, when you see that you try to remember what you have learned in case of your type 1 system or type 1 center. There you have two nitrogen center and two sulfur center. But the environment is not a square planar one, but it is a tetrahedral one. So, definitely this particular center will have

all other properties, the spectroscopic signatures, the spectroscopy properties will completely be different from that of your type 1 center.

So, we call them as the non-blue copper when you label it as the non-blue copper because it is easy to remember also as non-blue copper enzyme. That means, the color of the system is not the typical blue coloration, what we got for your type 1 center which is due to your ligand two metal iron charge transfer transitions.

So, LMCT transition is basically responsible for highly intense transition, in the region of 600 again, but the intensity the color intensity will tell you that you have a blue center and you have a non-blue only typical solution, like that have your copper sulfate or copper acetate. So, it is responsible for your activation of copper oxygen molecule. And when you have this activation definitely the corresponding oxidation state of the copper will be in the Cu plus state.

And in cooperation with other organic coenzymes or cofactors which will be there and structure is essentially the square planer one not tetrahedral one. So, that is the importance of bringing all these three corresponding histidine residues or the midazone donors around the copper center. So, if you have in the facial orientation, we have seen that in case of type 1 center, the facial orientation giving you the corresponding tetrahedral molecule or three plus one situation.

But here typically all three will be in the plane, the meridional plane 1 1 1. So, if you have the meridional plane and a copper is sitting over there, so, it will bind like this. And another hydroxide will come from this side. And it will not show any interaction in the apical sides, two of its available apical side. So, your epsilon max is also very less, sometimes it can go down to 100 million, but centimeter inverts.

And you can have the corresponding only very faint DD transition and you have the typical normal copper to EPR spectrum, what we find for your normal compounds?

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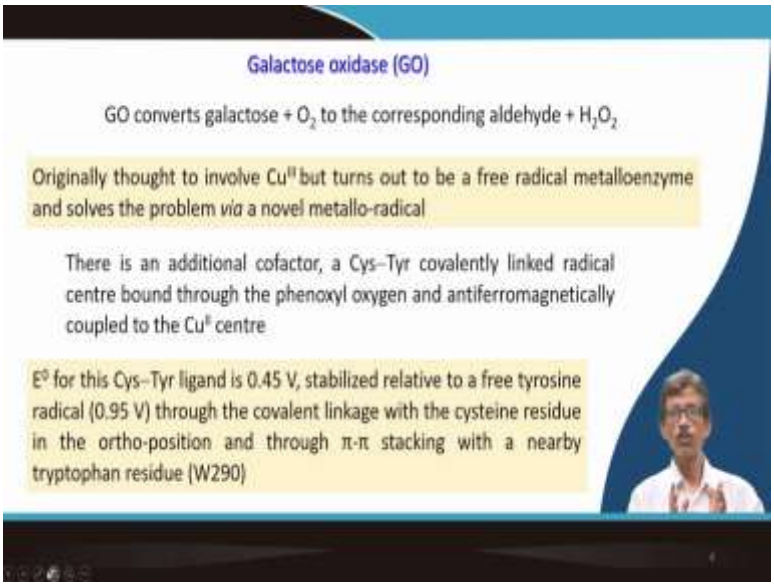

What we have seen in the previous time also. So, what is your galactose oxidase? What is galactose first? So, galactose is a sugar molecule like your glucose, fructose, mannose and all these things whether that galactose is a five membered sugar molecule or the glucose type of molecule or a six membered one that we should know, because it is very much selective, it will only work on galactose.

Because the galactose assimilation in our body, because we are handling only few molecules only in our body while you take your food as your glucose molecule. So, this is being produced from the lactose, not from glucose. So, the lactose hydrolysis is giving you the galactose molecule. And what is other molecules that you should know, I will not tell you here. So, you should know about the hydrolysis of the lactose molecule.

And that gives rise to one molecule plus your galactose and then galactose assimilation is degradation and metabolism is also important. So, the first step of its metabolism is the corresponding reaction of your O2 molecule, which is available as our energy producing molecule for ATP making molecule, all these you can say about the O2 molecule to give you the corresponding aldehyde and the corresponding product, what you get from the O2 molecule is your hydrogen peroxide.

Sometime will find because these are the two components or two species always we are getting either hydrogen peroxide or water molecule. So, what we originally thought about, So, originally we thought about, if you have more than one electron transfer and only one copper center is involved, it is very difficult to conceive that particular idea where from we will be getting two electron. Copper can settle between its two oxidation states the cuprous and cupric.

So, it will be very difficult to thought at that particular time. So, several years gone at that time and then people realized, no, we are getting some other signature for the oxidized enzyme, oxidized oxidase enzyme for the signature of the radical formation. So, what you have it turns out to be a identification, the first identified free radical metalloenzyme.

If the enzyme, it is bound to the metal and center which is your copper center, but at the same time you can have some fragment or some donor group which is free radical itself also. That means, it will have some part, where unpaired electron density is available like your metal ion. So, if it is in the cupric state your copper center is paramagnetic. But if your ligand is also free radical ligand, that will also be a, having unpaired electron.

So, these two centers whether they are coupling antiferromagnetically or ferromagnetically, that will be the question. But first of all how the reaction goes? So, that is the first discovery of novel metallo-radicals. There are many things people can identify, then later on that you just simply run the EPR spectrum, what will you get? We will get the signature of both these two paramagnetic center, you will get the corresponding characteristic axial spectrum.

Which is hyperfine splitting for the copper center as well as you will be getting the free radicals signal like that of your reference molecule, what we use in electron paramagnetic resonance measurements is your DPPH. So, what you have now? If you do not go to that copper trivalent state and your radical center is there. So, there is an additional cofactor, what is that cofactor? which is nothing but is modifying the corresponding protein envelope around the metal ion center.

Which is nothing but, earlier we have seen also that we can have something which we can modify the tyrosine residue. That means, the phenol residue of the amino acid part which is your tyrosine amino acid. So, cysteine can activate that tyrosine and can form the sulfur carbon bond

on the tyrosine residue. And while doing so, we are basically modifying that particular part of the donor point, where the phenol let oxygen is coordinating to your copper center.

But at the same time, it is not redox innocent, it is redox active. So, this phenoxyl radical having unpaired electrons can be coupled now, through anti ferromagnetic interactions. That means, you will be getting less and lesser amount of your magnetic movement. So, e 0 is let us measure the e0 value. The e0 will tell you whether this particular covalent bond formation or the attack of the cysteine in this residue, which is charged one, which is negatively charged.

So, this will be some kind of activation electrophilic and nucleophilic activation of the phenol being all we know. So, you try to understand in from that fashion like your agile funnel coupling and what is the cysteinate coupling. So, two are different, one can be positively charged your diazonium chloride is a positively charged species. But your cysteine ion is a negatively charged species.

But the linkage is for being what you are getting, you are getting a cysteinate sulfur and that of your coupled with your aromatic carbon of the tyrosine residue. So, you measure the e0 value whether we are modifying the electronic structure or the electron transport susceptibility of that particular part, which can help you to understand the corresponding function and the reaction that is fine.

When you have these particular cross linking, the covalent cross linking between these two amino acid part your corresponding potential for oxidation, that means free radical formation is reduced to 0.45 volt which is different from that of your phenol potential or the phenolate potential or tyrosinate potential which is 0.95 volt. So, that can also be stabilized due to that coupling through ortho position and pipe by stacking up nearby tryptophan residue.

Which is W 290, and all of you probably have not forgot that sometimes we write TRP the tryptophan residue, three letter abbreviation for the amine, amino acid. But sometimes, for our convenience, we can sometimes write the single letter abbreviation W for tryptophan.

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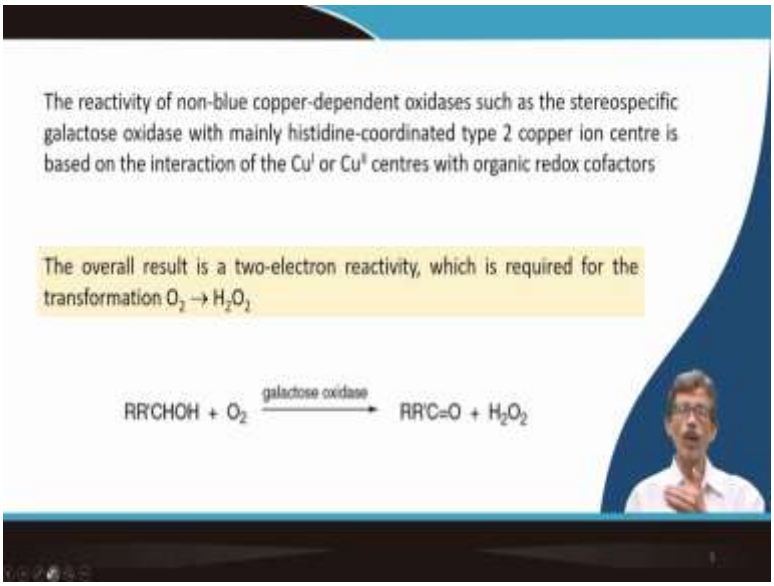

So, what we get? The reactivity of the non-blue copper center, so we are nullifying that trivalent copper set. So, we will have the settling between the copper two and copper one center. And the corresponding free radical form of the tyrosinate residue. So, the overall result is therefore that you can have the two electrons reactivity. So, that two electrons reactivity is therefore a very important thing.

And we can understand these very nicely, that we will be able to now activate the O2 molecule for two electron transfer, to give you the corresponding hydrogen peroxide. So, that particular part is well known now, that if you are able to detect the formation of hydrogen peroxide in these catalytic reactions, you will be able to tell that okay fine, the two electron transfer is taking place on the antibonding molecular orbitals of the O2 molecule making it first superoxide, then peroxide.

So, this is the fundamental reaction you have the secondary alcohol function on the corresponding glucose type molecule, which is your galactose. And that particular ch2 OH can be converted or CH OH type of thing can be converted with your O2 to your CC CO RR CO. That means, if your R or R prime is H, it will be aldehyde otherwise it will be end up with ketone. But what you can detect is that, the presence of hydrogen peroxide is very easy to detect.

You are learning this from your school days again and again I am telling all these things. You have two test tubes, and two colorless liquids you have. One containing water molecule which is your H2O, and another containing H2 O2, you know how to detect that. Similarly, when these catalytic activity or enzymatic activity is operating, your medium is producing more and more hydrogen peroxide molecules.

And you are consuming O2. And apart from that, definitely we will have the water molecule. So, hydrogen peroxide in water and hydrogen peroxide not there in the water molecule or the acquiescent environment you should be able to detect it. So, simple test, simple titration, all will tell you also quantify that how much hydrogen peroxide is forming. And also kinetically, we can monitor forget about the copper part, the colored part its formation and its transformation part.

You can also monitor only the hydrogen peroxide part, that how quickly the hydrogen peroxide is forming? What is the rate of formation of this hydrogen peroxide molecule from the O2? What the system is taking as the gas?

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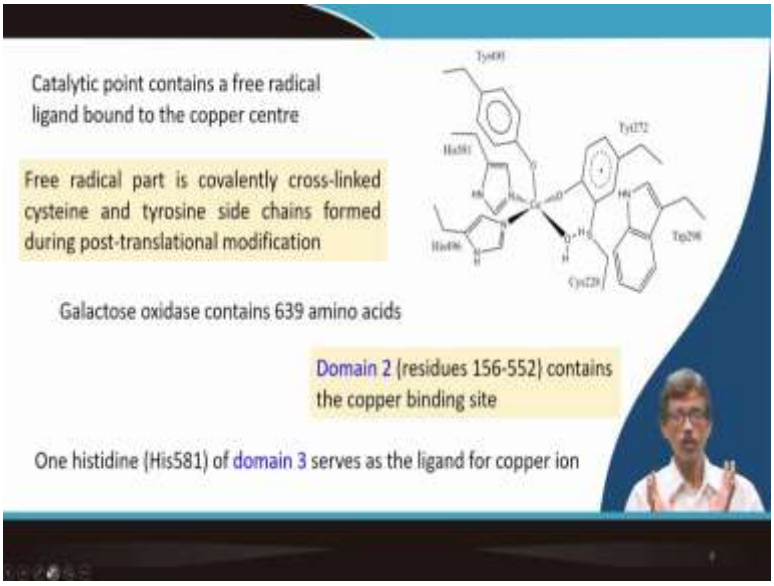

So, the catalytic active site or the catalytic point if you consider, that is a catalytic point what we are seeing here. Therefore, it will definitely have a free radical, which is the ligand, the ligand is binding to the copper center through phenolate oxygen. So, phenolate oxygen, when it is oxidizing, it is converting to your phenoxy radical.

The radical is functioning also as a corresponding ligand system to your metal ion center. Because when it is pH O minus, you have a lone pair of electrons, because H plus has gone. So, that lone pair of electrons having two electron donor, so, that we know that is forming very quickly the coordinate bond. But when you have one unpaired electron and when you have the copper center, so copper center if the unpaired electron from the copper center and if the unpaired electron from the phenoxy radical can come and form the bond.

So, the situation immediately will tell you that you will have the anti ferromagnetic interest and because if you can think about the corresponding interaction of the CU O pH, the phenoxyl radical. So, definitely it will interact antiferromagnetically, such that you can have a good bond, the stability is also an important factor over there. So, this free radical part already I told you should be cross linked. How it can be cross link, that is important.

So, you have the tyrosine residue and the cysteinate residue is coming. The numbering also you should know, we should learn that also because people identified access structure wise all these positions and all these ane acid residue in numbers also. So, the side chain can be formed during post translational modification. So, one particular term we, that corresponding modification, the translational modification we call.

So, post after that translation basically when the protein is getting synthesized, we call it as a translation. The DNA molecules are involved, the translation, transcription translations and all we know. So, after that translation, this modification is taking place that is why it is known as your post translational modification. And you have a huge number of amino acids not 154 like in myoglobin. But it will have more so, 639 amino acids are there.

This number is important because you should know about how big your protein envelop, which is binding to your copper center. That means is a robust protein environment, is not a very small protein environment around the copper center, it is a bigger one. And you can have domain, several domains you have. You have the domain one, you have the domain two and you have the domain three. Three domains are there and roughly if you distribute it, that first domain is running from amino acid number one to 155.

So, it is very much similar to that of your myoglobin molecule. Myoglobin has 154 amino acid residues. So, then you have a long or big domain, which is starting from 156 To 552 amino acid numbering. And that domain is basically required for binding your copper center. That means, the bigger domain or the larger domain, you are utilizing for metal ion coordination. So, let us see that and the domain three is reserved basically for one histidine residue, which is histidine number 581. So, it is going to the another domain, after 552.

So, that is histidine in 581. And serve as a ligand for the copper ion. So, some ligand part is coming from the other domain, it is not that. All the donor groups or the donor points which are available within domain two is utilized for metal and coordination. So, let us see the final structure, what we can see? What we can understand? So, what you should be able to see and know about the corresponding numbering?

So, you have these two basically, so, these two nitrogens like a bidentate thing, 496 number and another is 581. But this 581 is going coming from the other domain. So, it is also a very longer distance. So, what you can have? You can have basically the corresponding histidine 496 and the other one, which is basically responsible for your radical formation, free radical formation which is basically that arrows in 272.

But at the top basically, what you have the corresponding tyrosine one and that tyrosine one again, another tyrosine one which is not redox active. But it is weakly coordinating from the apical side and is showing some very interesting informations, when you see the catalytic cycle. Because whether that can be protonated nicely or the radical part of that particular tyrosine residue can be prorated nicely, that we will see.

So, basically what you can have? You can have a N2 O2 environment around the copper center, but which is not a square planar one. But one water molecule is sitting at the vessel point that is important. Most of the time, whatever or whatever things we have learned so far is that your water molecule is connecting from the apical side.

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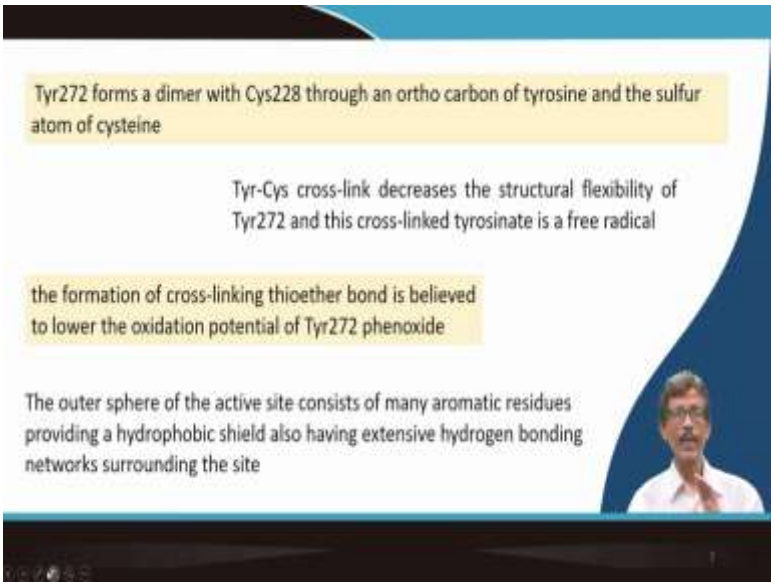

So, that is why these 272 which is forming the dimer and the cysteinate 228 residue is important to give you the corresponding coupling to the ortho carbon of the tyrosine residue. So, that Tyr Cys crosslinking is important and the numbering is also important, where you are getting the corresponding free radicals. So, this is important and is believed to be lowering the potential.

That is why we have the lowering in the potential, what we have identified some minutes back only we have seen how that potential is changing from your higher oxidation potential to a lower oxidation potential. It is due to the weight of your electron density supplied by the sulfur as your thioether donor from the ortho positions. We all know, from the hamet corelation or the hamet plot, that if you have the electron donating group, which is there at the ortho or the para positions your electron bridge.

And at lower potential, you will be able to oxidize that particular system. So, the surrounding is known and you can have certain outer sphere activity also due to the aromatic stacking and the hydrophobic field or hydrophobic environment, which is again helping your hydrogen bonding environment or you can have many hydrogen bonding interactions over there.

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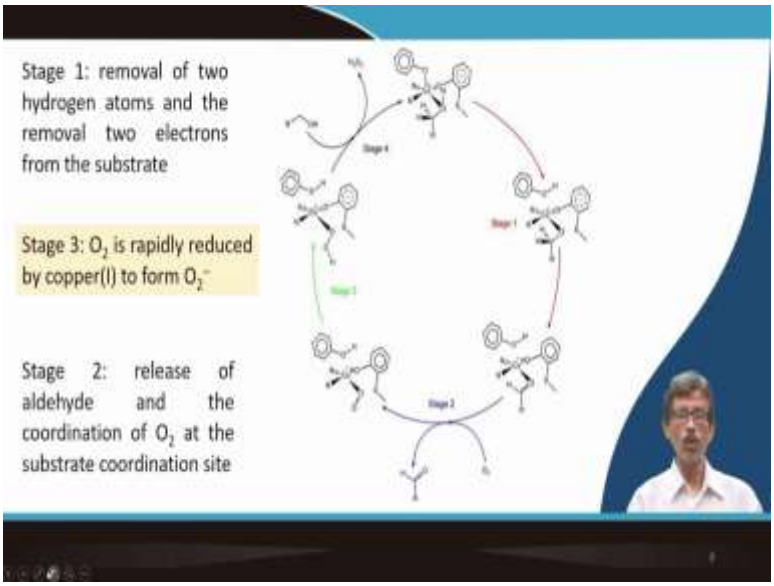

So, do not worry about the corresponding things, that you have 3 plus 2 5 structures, we have written in this catalytic cycle. So, as many as we can have, but thing is that how many we are able to identify? Sometimes we can have two species and we can write down a catalytic cycle, which is settling between these two. And when you talk in terms of the metal ion, what you can write?

We can write as the Cu plus one a copper one center and the copper two center. And you can have the catalytic cycle between these two. We know, that it is settling, but if you are able to find out something different, so another three species if you are able to find, that is why altogether you have five species in this catalytic cycle.

Then you can think of, then you can talk about separately, the electron transfer, the proton transfer as well as the change in the coordination numbers which is very important that how the coordination number is changing during the electron transfer process and the proton transfer process. And some part of the ligand, particularly the phenolate ions, which are available, some is oxidized, some is not for your all these processes.

So, it is a very important and very interesting coordinates in chemistry, nothing is happening. Through that coordination chemistry, what we are trying to learn is that you can have many changes which are occurring in your copper enzymes. The first step, the stage one we have

written over here on the right hand side, what is happening there. In language also I am writing over here, that you are removing two hydrogen atoms.

So look nicely, where you are going for these removal of hydrogen atoms and removal of two electrons. So, if you are talking in terms of the two electron transfer as well as two proton transfer, at the same time you can think of about the activation of the O2 molecule. And O2 is taking up those two electrons, going to the peroxide system and on peroxide and iron which is doubly negatively charged, two minuses charged accepting two protons it will be converting to H2 O2.

So, this is your stage one and stage two in the second step basically, the stage two is not step, stage basically. It is a two three steps can be there. We see that you will have the release of aldehyde. So, aldehyde is forming oxidation is taking place because you have the oxidized free radical ligand as well as the copper in the oxidized state.

Only the, you replace the water molecule and replacing the water molecule you substrate is coming and substrate has been taken over here as R CH2O minus, which can be your typical galactose because for holding galactose, you need a bigger pocket. And holding a smaller molecule like any alcohol, say ethanol, whether ethanol can work in this fashion or not.

That is why the biological people, the Biologist people or biologics or biotechnology is can try to understand all these things in that particular fashion, whether they can use these informations. So, copper one is rapidly reduced by the, O2 is rapidly reduced by the copper one to form the O2 minus the superoxide, what we know which is forming in case of your myoglobin system also.

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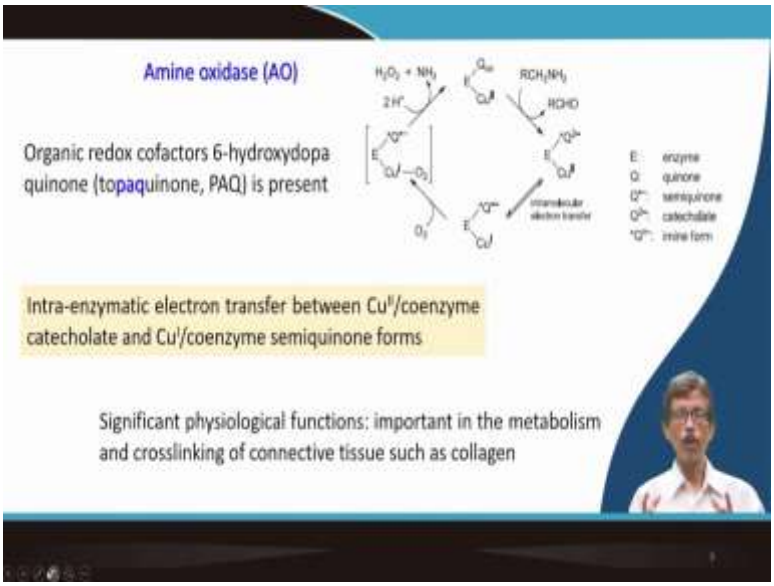

Then we will see amine oxidase, so you have to remove the amine function. But we have to need something which is basically depending upon your topaquinone faction, which is topaquinone. So, topaquinone is a PAQ that inside part is PAQ. So, it is another quinine. So, its dependence on that quinone is basically important and intra enzymatic electron transfer is taking place between copper and coenzyme catecholate.

Because catecholate can be useful for stabilizing your Kubrick oxidation state and your Q plus one is stabilized by the semiquinone form, one oxidized form of catechol we know is the semiquinone form. So, these are the forms basically, you look at it nicelym where you can have the binding of these particular forms and where you can have the corresponding e, that means the corresponding enzymatic function.

And you have the corresponding superoxide formation and all these species are forming and typically that reaction is happening and is a very important reaction for all these cases, where you have the superoxide formation and the copper one formation, copper two formation and all these things. Because finally, you are breaking the carbon nitrogen bond and that carbon nitrogen bond is being broken.

And you are getting something which is in the form of your ammonia molecule. And these are physiologically very important, because they can also useful for many other things where you

can have the electron transfer condensation and the metabolism of these amino acids or some amine, amine type of other molecules also. And finally, they can be useful for the cross linking of the tissues that means the polymerizations finally, for your collagen formation.

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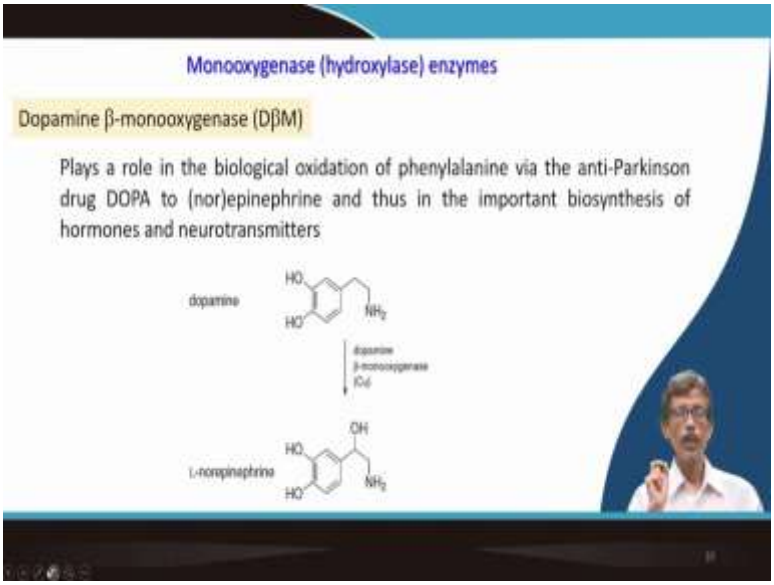

Then some monooxygenase we can see quickly. Now, these are the examples, very extended examples for these particular type where you can have the topamine and the topaquinone we can give. So, quinone parties there, but we will be hydrolyzing something where your dopamine is locked to not epinephrine, which is nothing but your beta position of the amine function is getting hydroxylated.

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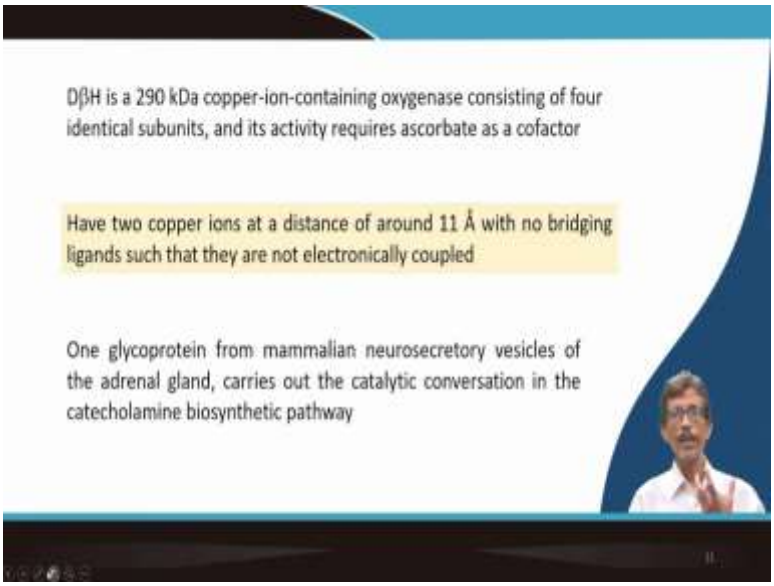

So, that basically getting hydroxylated for a molecule having a 290 kilo dalton molecular weight and can have other molecule copper center because you need to electron transfer again and again all the time and so no bridging ligand. So, it is quite a part which is 11 armstrong distance. But it is basically a glycoprotein, that means the glucose part is attached to the protein part and is a glycoprotein and is a neurosecretory vesicle, it is available in adrenal cortex or adrenal gland. And a catechol amine biosynthetic route is followed through this particular part.

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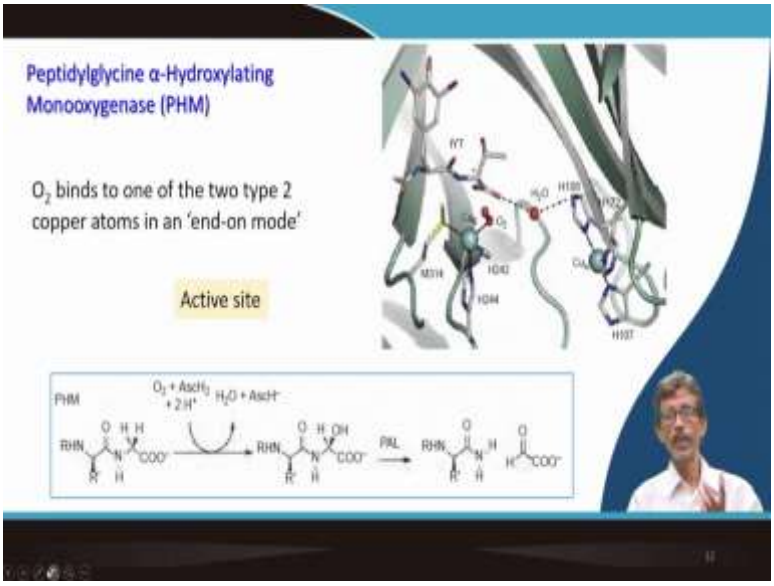

Then the last example is your PHM example, where it is nothing but your peptidyl glycine alpha hydroxylated mono oxygenase. So, think it is a peptide is there, glycine bearing peptide is there, that means the end of that thing, as we know about the carboxypeptidase we will be studying when we talk about the zinc molecules. So, these peptides will glycine alpha hydroxy lytic mono oxygen as is that in part.

Basically it will be trying to cut. And what you have? You have the big protein structure, but you should be able to know about what are the metal ions are there and the ligand environment. That tells us that you can have the O2 molecule and that O2 molecule like your myoglobin molecule can terminally bound in a vain fashion to the copper center in end on mode.

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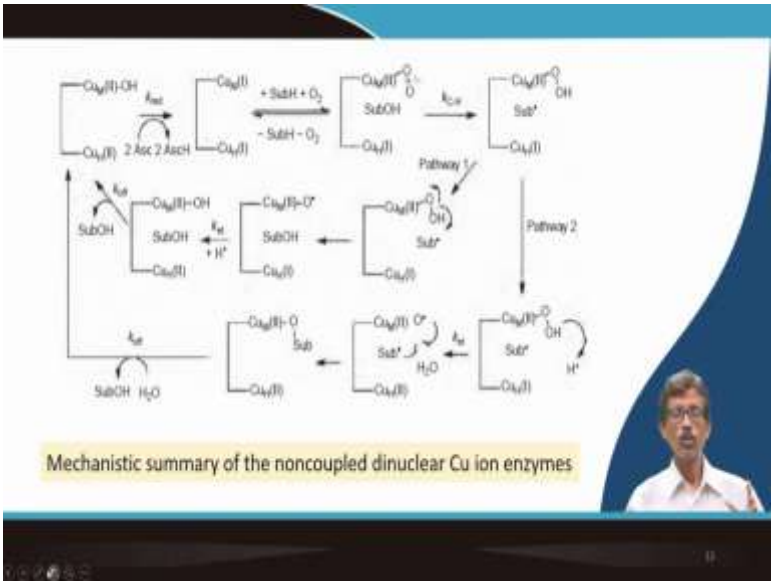

So, finally, we see the entire thing, the mechanistic aspects. So, take your time and go for all these things, everything has been explained what I am talking here. That you can have these copper in two different oxidation states, ascorbate and ions are required sometimes to reduce the copper center from copper 2 to copper 1.

But, the main idea is that you have to oxidize that particular O2 molecule. And substrate can be formed in a radical form and you can have other forms, that O is also can be formed in the radical form. So, this radical reaction can give rise to ultimately the formation of your ammonium molecule.

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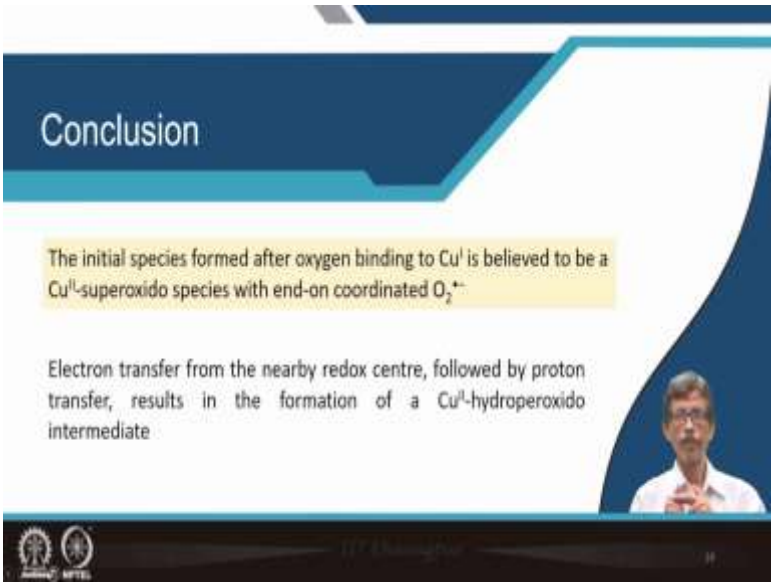

So, in all these cases, what we have seen that the formation of all these oxygen bound form to the copper center, then electron transfer giving you the superoxido species and with an end on coordinating fashion, that means what we have seen for your amine also, the amine oxidase.

And electron transfer from the nearby redox center, sometimes if some other cofactor is required like your corresponding free radical part of the tyrosine residues, which can also be followed by the proton transfer. Because always we know, everything is involved in the electroprotic equilibria. So, electroprotic equilibria will tell you that you can have the corresponding proton transfer. And the formation of then the hydroperoxido species.

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So, another electron transfer, then proton transport. So, you go for the type 2 copper proteins also page, the dopamine page as well as the other page what you can have with you and the book also. So, thank you very much.