Biological Inorganic Chemistry
Professor Debashis Ray
Department of Chemistry
Indian Institute of Technology, Kharagpur
Lecture 39
Type 3 Dinuclear Copper Proteins

Hello, everybody, so welcome back where we finished last time. So, in this class, we will talk now, after one, two, we will go for the type three.

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So, type three copper proteins, where we can see these. So, the nature of the center already we have learnt it, we have seen in some other examples, but again we can just concisely again we can see some new examples, some more things, all these things. So, there are coupled dinuclear, not uncoupled long distance copper center. So, they are coupled, they are nearby.

And they are responsible for O2 activation again but you require two copper centers, unlike your problem that we have seen earlier, in case of your galactose oxidase, you have to oxidize the protein part or the ligand part to get the electron. Then these two important molecules, the tyrosinase activity and the catechol oxidase activity, we will see.

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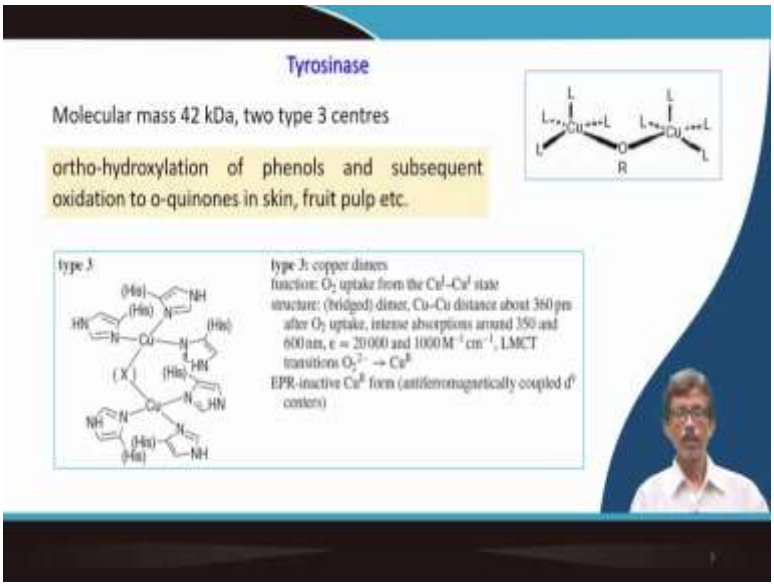

So, what is tyrosinase? So, tyrosinase you should know in that way, that we know the tyrosine amine acid. And as we all know, so something we are going with that of your tyrosine residues. So, we will be going for the corresponding hydroxylation or oxidation of this particular enzyme. We see on the substrate site tyrosine. So, it is not very huge molecule, is only 42 kilo dalton having again only two type 3 centers.

So, if you have two copper centers, like copper one and copper two, and you have the corresponding donor groups L1 L2 L3 L4 and again one is also one. So, how we can just breeze these these two? Because we have seen earlier that hemocyanin is also a good example of your type 3 copper center. But you have only three donor groups around copper one and another three around copper two.

So, what is happening now here? We will be looking for some reactions which are giving you the corresponding hydroxylation as your ortho hydroxylation. And that ortho hydroxylation of phenols and subsequent oxidation to ortho quinone is very important reaction. That is why, we know that in potato skin or any other fruit part, there is catechol oxidises. Tyrosinase is also responsible for our skin melon information.

So, what do we get? We get some phenol part, you oxidize it to make it a diphenol or bisphenol or catechol. So, phenol can be converted to catechol and catechol can further be converted to

your quinone. Because quinone is important, for all these cases you have the huge conjugation. That is why it is the color absorb, is colored as well as it will absorb the radiation, it will absorb nicely the h nu, the sunlight also.

So, like one, like two, your three we have these here now three. So, how schematically you will be able to identify you should be able to know it, if this site is given to you. Identify the nature of the type, you should be able to tell it is not type one, it is not type two, it is type three. So, only the positioning, the detailed positioning of the histidine residuals or histidine amino acid residual so with that of your imidazole side chain coordinating to your copper center giving rise to these and you have the one group where we have seen in this particular box, that is OR.

And here it is writing as x, that means you can have many groups over there. You can have the water molecule, you can have the hydroxide group, you can have the alkoxide functions or many other things. So, oxygen uptake can take place two copper centers, where it is happy with supplying two electrons from sapling of the copper oxidation states between ox and x.

So, the distance of these two are important because you are trying to activate that O2, whether it is again in the same fashion like that of our emotion. And most of the time people developed all these literature, people study it, people researched it on these is that, they compared it with the well-known hemocyanin molecule. So, with hemocyanin you have, which is the oxygen transport molecule, you see these tyrosine is a copper enzyme now.

But your active site is very much similar. So, electronic transition, the corresponding resonance Raman spectroscopy signature as well as the EPR can tell you or identify the nature of this site, what is there in type threes center.

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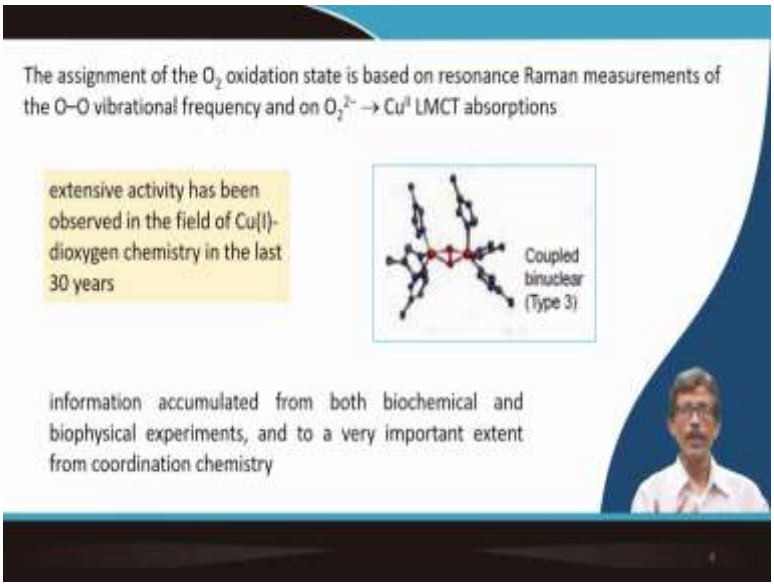

So, the assignment of that particular O2 oxidation state, whether you are with the oxidation state of 1 minus or 2 minus in superoxide or peroxide, so use the corresponding vibrational stretching frequency measuring technique, which is your FITR. And here since your O2 is symmetric, we will be using resonance Raman technique or RR technique. And also the electronic transitions for to identify the LMCT transition.

So, charge transfer transition you can identify and they extensively you can studied it for the last 20, 30 years, we have studied it. That is why the area is matured now, is reaching all these informations. And you can have the more number of informations and which are justified nicely for your transfer also. So, you see that the active site is nothing but your CU2 O2 diamonds saying, you have one copper here another copper here and you have the two, one of these two fingers in the oxygen.

So, the diamond code Cu2 O2 or Cu O Cu O diamond code is important is a coupled binuclear copper system with a type three center. So, these are very important and has been studied by chemically, by physical measurements, as well as all these informations are supplemented further throughout the understanding about the coordination chemistry, the redox chemistry or non-redox coordination chemistry also.

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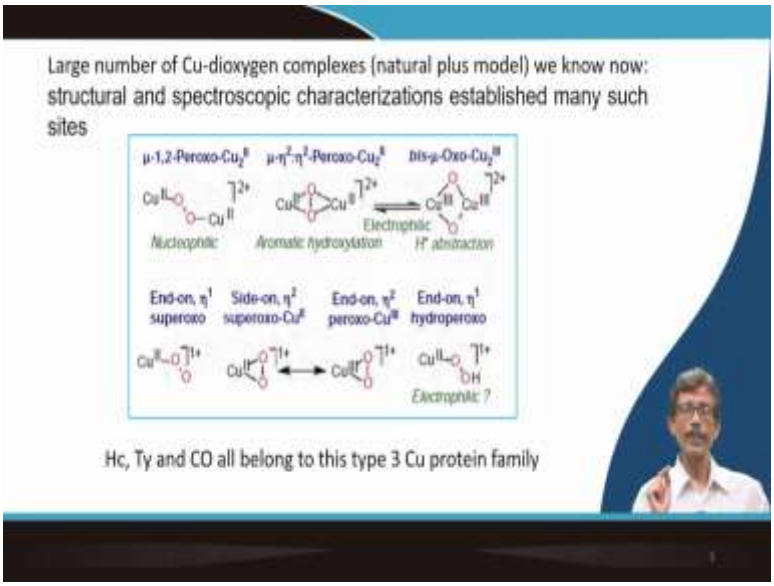

What we see that there are a large number of such systems, we all know. And the structural and spectroscopy characterizations, basically could be able to establish many such sites, not that with the hemocyanin, but we can identify those sites in the tyrosinase molecule or in the catechol oxidasis also. So, what basically they are doing? So, not only the naturally occurring system, but also with that of our synthetically prepared model compounds, what we see now is the model compounds are also very important to tell us many informations.

If you take these three examples, Hc is hemocyanin, Ty is the tyrosinase molecule and CO is not carbon monoxide, but it is catechol oxidase. Why we abbreviate in a particular point, in immediately discussing all this? Because you should be able to know, that without telling hemocyanin and all big thing you should know it is Hc. You, as you know as Hb is hemoglobin, Mb is myoglobin.

So, these three species they are very big species, but we are abbreviating it is a small thought, even less than that of your amino acid residue, where we are using three letters, but sometimes the single letter abbreviation is important. So, these three molecules and these particular categories of activation, you can have the 1 2 peroxide or bridging between these two copper center is all we know.

So, this sort of thing or you can have these two oxygens coming from these two copper in this particular fashion, this is binding. Two copper sorry, two oxygen and by two finger heads and come and bind to two copper centers. Then you can have the corresponding nu eta2 eta2 peroxide or bridging which is well known for your hemocyanin molecule. And the bis Oxo, how the Oxo is forming?

Which we are writing as a highly electrophilic in nature compared to that of your new 12 peroxide 1 which is nucleophilic in nature because the substitutions, the catalytic functions and all these things are dependent on your nature of this reactivity site, whether it is nucleophilic or electrophilic. So, when you have these nu eta2 eta2 binding between these two copper centers, what you see?

That at one point, not in your biological world, which is a soft world, but if you go for any (())(08:48) and hard world like your synthetic molecules or laboratory prepared model compound, you can break the O bond. Many model compounds you can have, where you can break the O bond, where you have the initially form this nu eta2 eta2 bonding hemocyanin type of molecule.

If you break it, the individual oxygen centers can take up more electron from the copper centers, making those two copper centers to the tribal instead which is very unusual. But sometimes you can get in the synthetic molecules. And you have two oxygen centers which are your oxide centers. Similarly, for the examples what we have seen for your dopamine beta hydroxylase or pH m, we have the mononuclear center and you have the terminal binding like that of your binding in your myoglobin molecule.

So, you have the end on eta1 binding which is superoxide O, site on eta2 binding for superoxide O molecule is also possible. And on peroxide O (())(09:46) fashion and end on eta1 binding is also possible which is available for our corresponding superoxide O molecule at the left bottom and then one electron transfer making it peroxide O, but immediate proton transfer will make that center as the hydro peroxide O species. And that can also be functioning as electrophilic center, for your all the transformations and the reactions.

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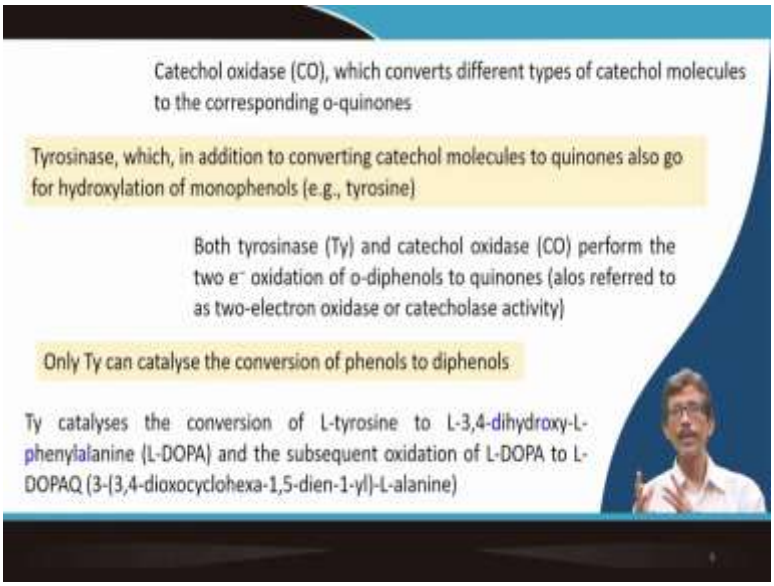

So, we will talk all three together basically, because they are very much similar. So, site by site it will be very easy to understand the reactivity pattern of all three together. Then what you can do? You just compare, which is there in hemocyanin and why hemocyanin is functioning only in this fashion? And why tyrosinase is functioning in a different fashion and your catechol oxidase also.

So, the name will tell you, CO will tell you that it converts the different types of catechol to its quinone. That is why it is known as catechol oxidase, the electron transfer reaction as well as the removal of your protons, the catechols all having OH function. So, when it is binding to your copper center, your O OR O minus O minus. Then when oxidation is taking place, it is accommodated in such a fashion, through semi quinone, it will go to your quinone form.

So, this catechol molecule when it is going to the quinone, but in case of tyrosinase we see that, it is going for the hydroxylation of the mono phenol. On the tyrosine, the tyrosine amino acid is nothing but a phenol part on the amino acid part. So, that basically going for the hydroxylation, the second hydroxylation already you have one phenol OH, phenolic OH. The second phenolic OH will come through this particular reaction.

So, both these Ty and Co therefore can go for two electron transfer, and oxidation of these finally, to the quinone. And since you have these two electron oxidation, we can also consider it as you are seeing some catecholase activity. So, you should be able to remember it in that

fashion, that which one is your catecholase activity. And where catechol is your substrate, and when the catechol is substrate, two electron transfer and two proton transfer in the different direction basically, giving you the quinone.

But in case of tyrosine, it is working on the phenol molecule. So, it will catalyze the conversion of phenols to diphenol. Then phenol is there and the ortho position of that phenol will be hydroxylated. So, the tyrosinase basically when it is converting L tyrosine molecule, what happens then? So, L tyrosine molecule you should know about, that is why all these things in terms of your exam, in terms of your questions and all these things, you try how will you remember all these things nicely?

Do not go for memorizing blindly, you know, you should know also the L tyrosine because all the time, I should not give you the chemical structure or the chemical drawing of L tyrosine. But it should be in your brain. Whenever you reading L tyrosine, like your alphabet, identification of the alphabet A B C D, we know that how to identify what is A, what is B. Similarly, when you are uttering something that L tyrosine things should be in your mind, from your memory you should not tell it is delta, you should be able to know and understand about the structure.

Then if I say that something is happening, but the name it tells you that something is related to phenylalanine. So, how it is related, what is alanine then? You should know about alanine and the phenol substitution will give you the phenylalanine, that is why you are bring in the phenyl ring or alanine substrate or alanine backbone. That phenyl ring, when it is first time hydroxylated giving you the para cresol type of molecule, we all know para cresol is.

You have the methyl function opposite to that of your OH of the phenyl ring. For methyl, phenol or paramethyl phenol is your para cresol. Similarly, you have the substitution, the amino acid is your bigger substitution. So, that bigger amino acid substitution away from that your OH function, that means in the opposite direction, is para position is giving you the L tyrosine molecules. And when it is hydroxylated, you are getting adjacent OH groups.

So, it is 3 4 dihydroxy L phenyl alanine, so abbreviated in that way also. D has been taken from the entire name, O is also taken and PA is your phenyl alanine. So, you will be forming L DOPA

is a very useful name, doctors also known as a drug molecule and all these things are basically important and in our brain basically we are producing these important molecules.

So, oxidation further of these, so it is one part of oxidation. That means, you are forming tyrosine send to L DOPA then L DOPA will be forming something that to L DOPAQ. Once we know that catechol type of molecule if it is in your hand, you can immediately oxidize to its quinone form, it is a very simple thing when you take in the laboratory, when you take it in the laboratory when the bottle catechol is there.

If you open the bottle and take a, keep it for some time in air also, the air oxidation can take place, but that amount of DOPAQ formation is very less. So, this particular formation is basically catalytic one and is a huge amount of this will be forming.

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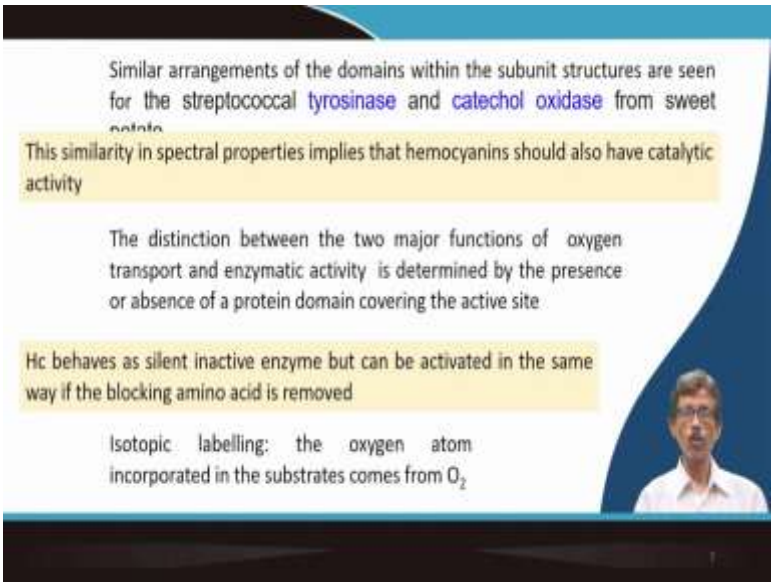

So, the arrangements what you can have now in your hand, the domains where the subunit structure you should know about that means for the tyrosinase molecule, origin is streptococcal, the streptococcal functions, a streptococcus is important, where you can study all these, you can isolate the tyrosinase molecule from there and the catechol oxidase from sweet potato. So, these are the two sources of these molecules basically.

And this similarity in spectral properties basically implies that, this hemocyanin should also have a catalytic activity of this type, but it is not showing us. So, the distinction between these two

major functions of oxygen transport and oxygen activation, in this enzymatic activity is determined by the presence and absence of the protein domain covering the active site. So, like your corresponding covalent bond formation in galactose oxidase, you can have something here also happening.

Such that, you can see that it is enzyme, it is not your oxygen binding and transport protein like your hemocyanin. So, hemocyanin behaves as a silent inactive enzyme. So, what we can now consider that if you consider the what is the hemocyanin there? Hemocyanin is used for only oxygen binding and oxygen transport, but it is not your enzyme. But it can be activated if you go for the blocking of the amino acid just discarding, that means removal.

So, the blocking part that means, the active site access basically is important. If it is not there, it is only functioning as oxygen trapping and oxygen releasing molecule. So, isotopic leveling with oxygen 16 and 17 and even 18 can give rise to all these informations nicely, that the incorporation of the substrate which is taking place over there the oxygen atom which is getting incorporated within the substrate is coming from these leveled oxygen, which we are using in your O2 molecule as your oxygen gas.

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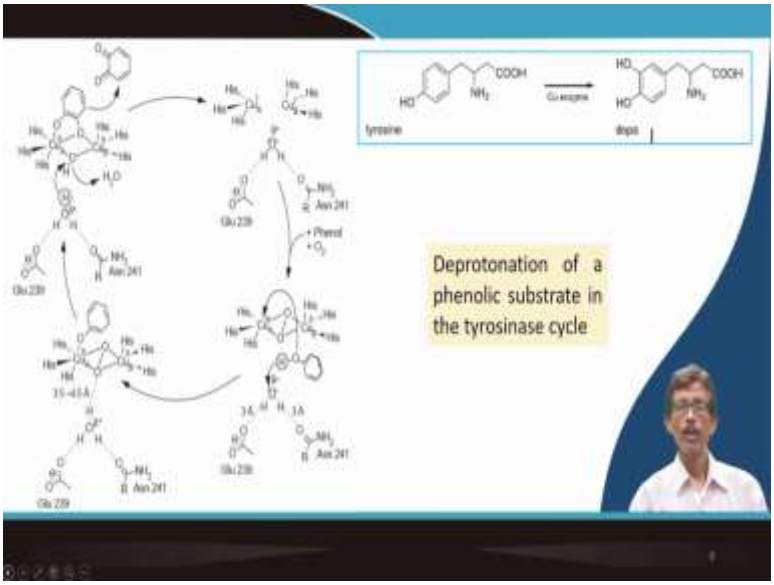

So, you have the tyrosine molecule. So, schematically what you see quickly we will see that if you go for the corresponding thing, that means the corresponding hydroxylation reactions. And

that hydroxylation reaction is basically giving rise to the corresponding OH orthoposition to that of the already existing OH molecule. So, what you see now is a bigger description, you can have the simplified description in other way.

So, on the right hand top you look at, what you know nicely from your hemocyanin days. So, from the right hand top, what do you see? You have the two copper centers instead of leveling as copper 1, copper 2 we level it has copper a and copper b, it is fine. But the overall it is the type three center. Then something is happening over there, that hydrogen bonding, water molecule all these are nearby, but is not directly coordinating to these two copper centers.

But you bring the phenol center so, that means that particular deoxy form or the lower oxidation form that means the Q plus transform is part of the enzyme. So, when the O2 is getting activated, your phenol can be a substrate like your tyrosine. Tyrosine is also a phenol, substituted phenol, para substituted phenol. To us for this catalytic activity, phenol is your there and your para phenol. the para substituted phenol is there.

But when it is substituted like tyrosine, it is also substituted phenol. So, it is basically binding and is trying to interact with the copper a center and you all know that you can have the corresponding binding, so epical coordination can take place. And that particular copper you see, the copper a is converting to a octahedral geometry. So, not only electron transfer your changing coordination number is important.

So, that is why phenol is coordinating like your galactose oxidase, from the top. So, now you have more complex copper copper center but your phenol is coming from the top and is coordinating there, through this particular site and only the copper a is involved. So, it is engaged over there. Now, you see that, what can happen over there that this particular dioxygen molecule is there.

And that dioxygen molecule is responsible for your formation of the hydroxide obliging. So, proton is coming from the hydrogen bonded water molecule from here, the lower bottom part. So, this hydro, proton basically is coming from this protonation of this particular oxygen of this dioxygen molecule. And then that basically gives us for the model studies we have also identified that you will be able to break the O bond.

So, while you break the O bond, what is happening? In the model compounds we have seen, we have the dihydroxide oblique dicopper system. But, once you break it, one is coming out as the hydroxide and will stay like that bridging part. But the other hydroxide O function, but is not hydroxide is activated oxygen. So, that activated oxygen, if it is peroxide, if we break it what you are having with?

You are having with O and O with a single charge. But again you have the further electron transfer and you go for the corresponding one as the corresponding oxide. If one is oxide and another is radical, and that can also attack the corresponding phenyl ring. So, that activated oxygen, do not bother about other thing. That activated oxygen from the top site is basically now attacking your phenyl ring at the ortho position of the phenol.

So, it is basically a very good hydroxylating agent. Chemical hydroxylating agents are well known to us, we know meta chloroperbenzoic acid, iocl benzene and many other are there. Like your cytochrome p 450, which is phenyl species are an oxygen. Why that for phenyl species is important because that oxygen on iron is a very good reagent for oxo transfer reactions.

So, here in situ we are activating all these things, we are not getting any copper double bonded oxygen or copper bound this oxide species. Because it is not very well known, people sometimes have that idea also that we can have like feral species we can have, the cuprite species. So, once it is got, now, you are already transformed molecules. That means your catechol part, catechol part is used as a bridging ligand, unique bridging between these two copper centers.

One of the phenol at function, one of the phenol at O minus is bridging these two copper center. But another one, the original one, original one is coordinating like that of your epical coordination to that particular copper side. So, you understand in that way and the elaborate thing is also that the bringing of this water molecule. And water molecule is basically pushes by two other amino acid residues from the bottom. And they are hydrogen bonded. So, these water molecule is not a free water molecule.

In the biological system, most of the time you see that your water molecule is not free like your test tube water. It is always bound or hydrogen bonded to that particular site. So, when you determine the access structure, why the protein crystallography is so difficult and so tedious, that

you have to identify not only the copper environment, the entire protein part, entire carbon, hydrogen, nitrogen, sulfur and all. As well as the other part, which are nearby to that particular active site.

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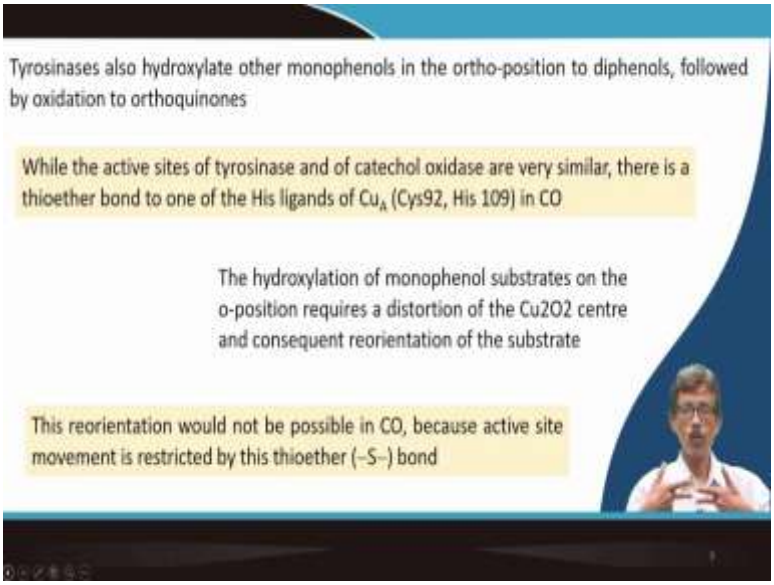

So, the deprotonation of a phenolic substrate in the tyrosinase cycle that we are looking here, the cycle that is why is basically devoted for that particular purpose. So, what we see that is the tyrosinases also hydroxylate other mono phenols in the ortho position that is why to diphenols, followed by oxidation to ortho quinone. So, hydroxylation followed by oxidation if we allow and these two molecules are therefore very similar type.

And in one case, you see that there is a thioether bond to one of the histidine ligands of Cu A, which is by cysteine at 92 and histidine 109 like that of your galactose oxidase. So, if you nicely or coolly read the galactose oxidase thing, you will now have that particular idea that cysteine anions are so interesting, that it can give you new bond to your phenol residue or the phenolic residue.

It can also give you the histidine residue, many other examples are also there, in future also we will take that particular example in different form. So, the hydroxylation of monophenol substrate at the ortho position require a distortion of the Cu2O2, these 2 are super subscript,

Cu2O2, that is your diamond thing, Cu2, two Cu and two O. And its consequent reorientation of the substrate, what does it mean?

That you write like this, and you can have the corresponding movement of all this, so all these movements. So, basically in this particular plane, your copper is fixed. So, this copper basically can allow, so only the movement of the copper is moved there and this particular so, you can have also this sort of bending also, this O. So, these are the things basically. So, these sort of distortion, and at one point it is fixed over there.

So, this reorientation, sometimes it is allowed and sometimes not. When it is allowed, is not be possible in catechol oxidase case. Because the active site movement is restricted because of this corresponding covalent bond formation, that means with thioether bond formation. The cystinate sulfur, what is coming as S minus, is bonding to your histident residue, giving a new sulfur carbon bond to that of your residue, that imidazole residue, which is coordinating to your copper center.

So, that means your ligand part, the immediate ligand part around copper center is getting modified. And due to that modification, basically you have the restriction, restriction in the movement. So, this is one site of this ligand coordination and you are talking about the movement of this particular. See, if you are able to restrict that your functional property will be changing.

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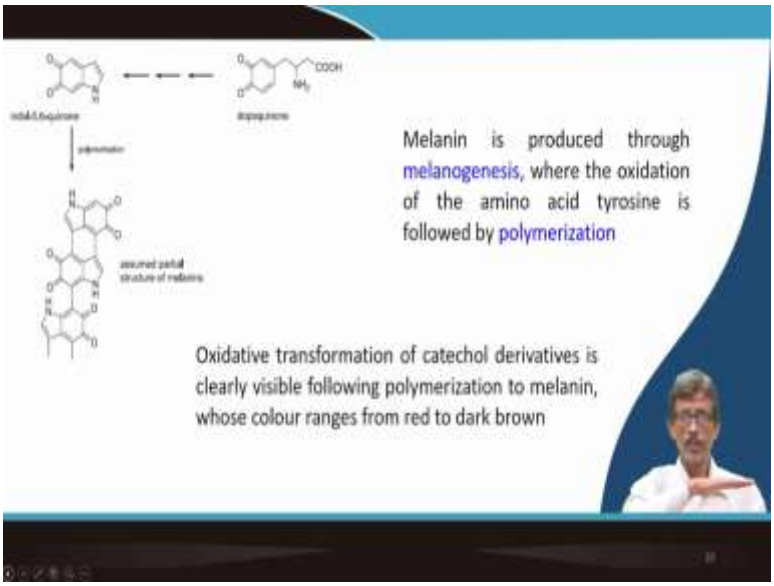

That we see, the then at the, in this particular example that where you have the melanin. Where we have the skin color, our skin pigment is produced through melanogenesis. What is that melanogenesis? Melanogenesis is nothing but the oxidation of the amino acid tyrosine followed by polymerization. So, if all these statements are given to you, what you can understand nicely close your eyes and think it.

That we are talking about oxidation. And we are talking about the oxidation of tyrosine molecule, that very useful amino acid molecule which is a part of the protein chain also or it can be a separate thing also or it can be a separate thing also. So, when you have the oxidation of the amino acid tyrosine followed by polymerization, what can happen? So, this particular oxidation, what sort of oxidation?

So, oxidative transformation of the catechol derivatives, we can see if we allow the polymerization reaction so, you cannot stop that polymerization reaction. So, it is the bio polymerization reaction and melanin is nothing but your biopolymer on your skin or epidermis, the lower part of the skin, not at the upper part, epidermis of your skin. And which is very important, this color is very important for absorbing UVB radiation.

That is why, the brown the color brown is also very important. So, that is why the red to dark brown color is also important, people will not suffer the degradation of this particular melanin

part and all these things and the corresponding skin also, they do not suffer from the corresponding skin cancer. So, these things so, what we have understood so far that you have the corresponding catechol molecule convert it to quinone molecule.

And this quinone molecule, you have the para position substitution you see. And that substitution can further be transferred, you see that three arrows, three left hand arrows. So, these three left hand arrows basically what tells us, that you can have different steps of other reaction. Such that, the amino acid functions, the amine functions can be cyclized, to give you something where you can have the indole function.

Indole with substitution and indole with the catechol function. So, it is indole 5 6 quinone. And that is very much susceptible for your polymerization reaction. So, you see, one part is, only a fragment at part, a partial structure of the melanin is a huge structure. But you should be able to identify these indole, quinone form one, then in the opposite one. So, one is this, one is this and another one. So, this is basically crosslinking.

Like that of your seat, from the graphite we get the graphine seat. So, it is typically a seat like structure, this is nothing but your melanin pigment, which is forming over our skin basically. So, this is a very important reaction, but this is basically catalyzed by the copper center.

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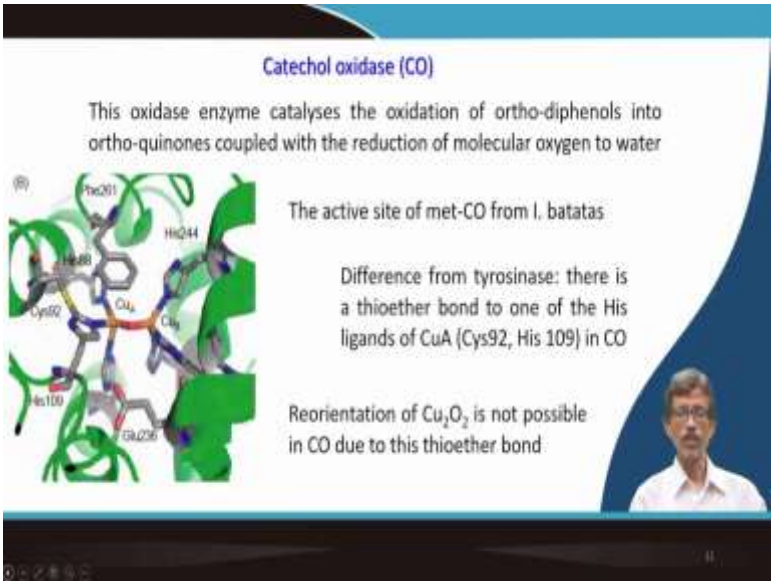

So, quickly, we see now at the end, the catechol oxidase. So, these enzymes basically also can catalyze this thing, what we have seen. So, the restriction, in another case you do not have the restriction. If you determine the structure, we will find that the active site of the met form of cadigal oxidase, a from the sweet potato, I batatas. So, that basically gives us that the copper a and the copper b site to identify then another histidine nearby, so you can have the corresponding bond.

So, the difference from tyrosinase is that there is a thioether bond. So, that we are discussing so far, that if you had the thioether bond your catlytic function will be changing. So, you have a thioeter bond on one of the histidine ligand of Cu A, which is coupled between these two numbers of this histidine residue and the histidine residues like the tyrosine residue. And the reorientation is now prevented. So, reorientation due to the formation of this thioeter bond is top and we are not getting this thing.

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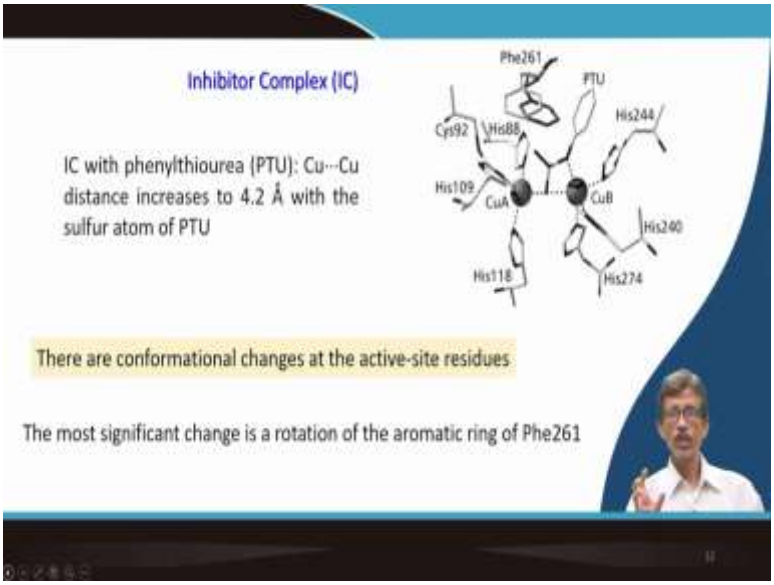

And sometimes the function of this catechol oxidase can be inhibited. If your thing is overactive, sometimes we required the drug molecule or the medicine to innovate some function. The catechol oxidase, if it is giving some problem and maybe it is genetically also transformed also. So, you go for the inhibition.

So, phenylthiourea if you take, and you take it that particular the native catechol oxidase, crystallize it in presence of that phenylthiourea and you get a very interesting molecule, which was similar to that of your tyrosinase function of the bisphenol or the catechol binding two copper center. So, phenylthiourea can bind in that fashion or sulfur is bridging these two copper centers.

And one of the point, basically the nitrogen point is coordinating to one of the copper centers in that fashion. So, similar type of coordination. So, there will be a conformational changes at the active site residues and is the rotation of the aromatic ring of your corresponding amine acid Phe261, which is your phenylalanine. So, phenylalanine 261 is responsible and restricting. So, that means the secondary interactions can also be important for inhabiting all these things.

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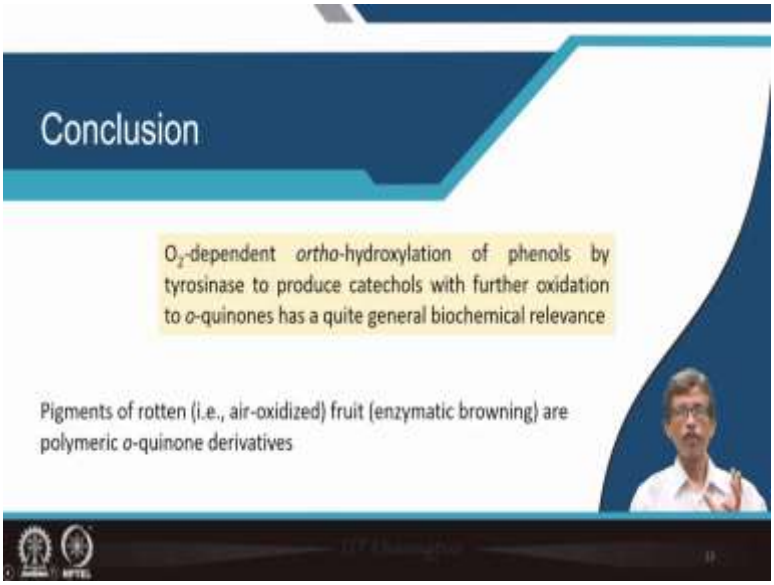

So, what we have seen therefore, whatever we are talking nothing but the typical enzymatic ortho hydroxylation reactions, in organometallic chemistry in many other parts we also studied ortho hydroxylation reactions. They are not only hydroxylation reactions, but also we can go for the other thing that means the (())(32:01) formation, your melanin formation, which are very important in all these studies in the biochemical world.

Then not only the, your melanin but the rotten pigments, the pigments for the rotten fruits due to the enzymatic browning, we know the yellow is then browning and then finally the rotten thing.

But what we are producing basically due to that, that oxidation of all these things, we are producing more and more number of your orthogonal molecules within it.

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So, you read catechol oxidase nicely in the Wikipedia page and your related other items, which is coming from that particular page and the book. Thank you very much for your attention.