Inorganic Chemistry of Life Principles & Properties
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Lecture - 39
Role of Copper in life - Type 1 & Type 2 copper enzymes

Welcome you all to the next class on Inorganic Chemistry of Life Principles and Perspectives. In the previous class, I have talked to you about a few aspects of copper proteins and copper proteins are categorized as.

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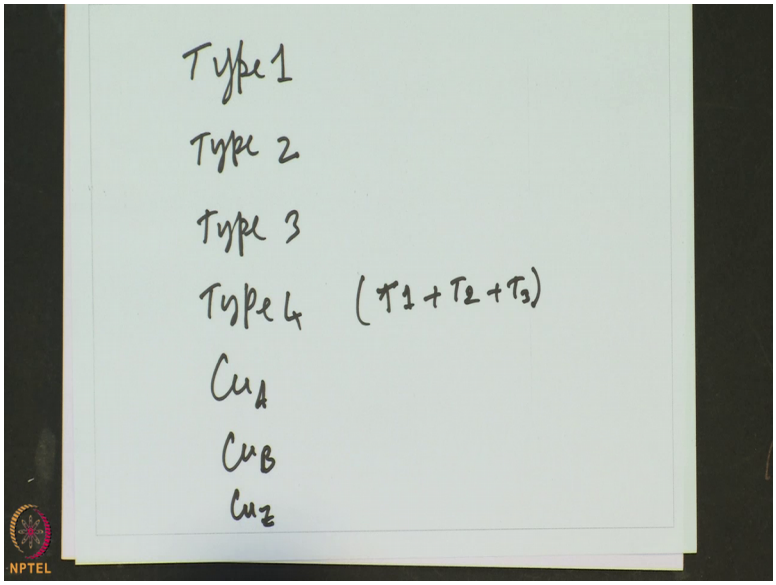

As you can see, the type 1, and the type 2 kind of things and type 3 and type 4 where you have type 1 plus T 1 plus T 2 plus T 3; all of these are present, then you have copper A and then you have a copper B and then you have a copper Z type.

So, we have looked at all of these in the previous class and then we have come to the copper 1 story and as you can see that the copper 1 case.

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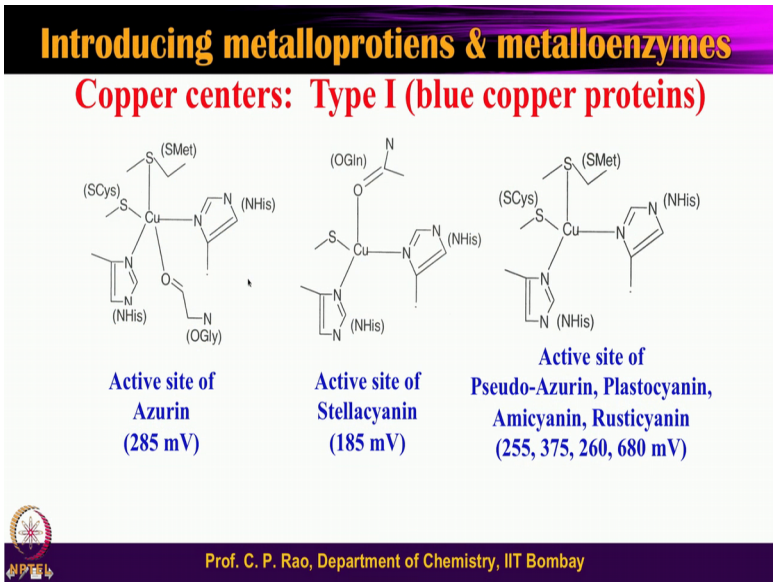

So, a copper 1 centers; there are few proteins structures are shown over here and these are referred as a blue copper protein and the blue copper protein, the copper in the blue copper protein; what you have is the blue color.

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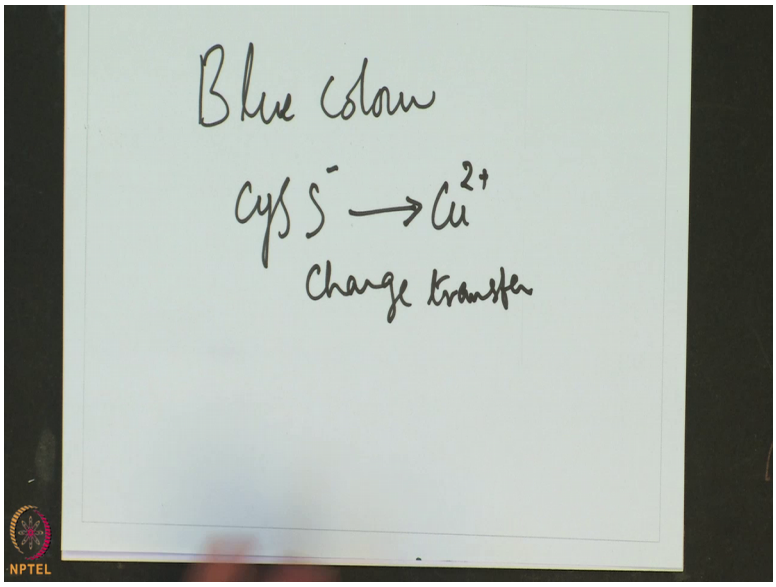

The blue color is coming from the blue color is coming from the cysteinyl sulfur minus to the copper 2 plus charge transfer.

So, this is a charge transfer transition. So, wherever you have a copper 1 site, you will find or type 1 site, you will find this kind of a blue copper color. So, type 1 has nothing

to do with the copper 1 state, copper 2 state, etcetera. So, therefore, this type 1 and copper 1 should not be confused. Now as you can see from this slide that you have a few different copper site structures.

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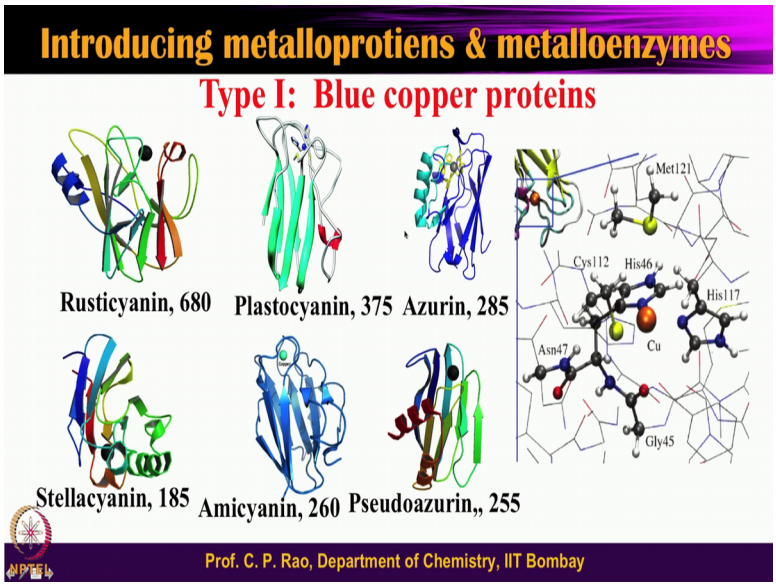

And you see some more proteins over here, these are all type 1 containing ones, you can see in this protein, you have the copper center somewhere in there towards the top and mostly these proteins are more like a cylindrical in shape all of these.

The numbers that you see here 680, 375, 285, etcetera, these are nothing, but the redox potential of the copper that is present in these enzymes; that means, rusticyanin has got 680 millivolts, plastocyanin that has got 375 millivolts, azurin has 285 millivolts, stellacyanin has got 185 millivolts, amicyanin has got 260 millivolts and pseudoazurin has got 255 millivolts and you can see in the protein, this is all the protein moiety close to that and this is the center where you have a copper and the binding moieties of this.

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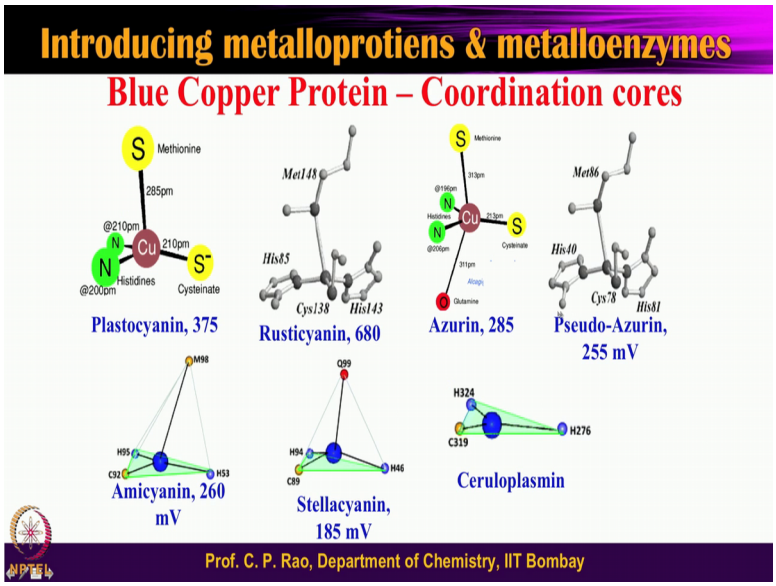

So, on this slide, you would see just the structure centers of the copper center only, this is that again copper coordination sphere copper coordination sphere.

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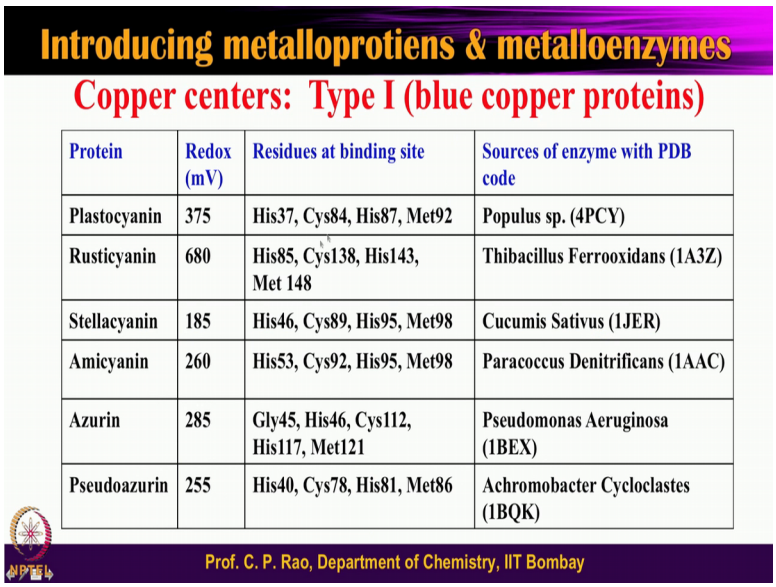

All of these are you are seeing the copper coordination spheres. Now, here what exactly is bound? So, the one does not need to remember these numbers like histidine 37, cysteine 84, all that one needs to know is there are 2 histidines, 1 cysteine, 1 methanin and plastocyanin and if you go to azurin, there is one glycerol Co 2 histidines, 1 cysteine, 1 methynin.

So, this is the one where you have a five coordination these are the ones where you have a 4 coordination. So, I have already told you in the previous class, there are 4 coordinated ones, 5 coordinated ones, tetrahedral and the trigonal bi pyramidal, but none of these structures none of these geometries are or any close to the ideal kind of a structures they are very highly distorted, I will just explain you once again the distortion of how it is helping.

So, before going to the next point, let us look at these different values. So, the lowest being 185, the highest being 680 millivolts; so, a range of almost 400 millivolts difference or 500 millivolts difference, this total difference is coming not only just from the coordination sphere, but kind of a protein structure influencing all this. So, is that ok; have you noticed the point number 1 is the redox potentials very dramatically among the type 1 blue copper proteins going from something like stellacyanin 185 to something like rusticyanin which is 680 millivolts ok.

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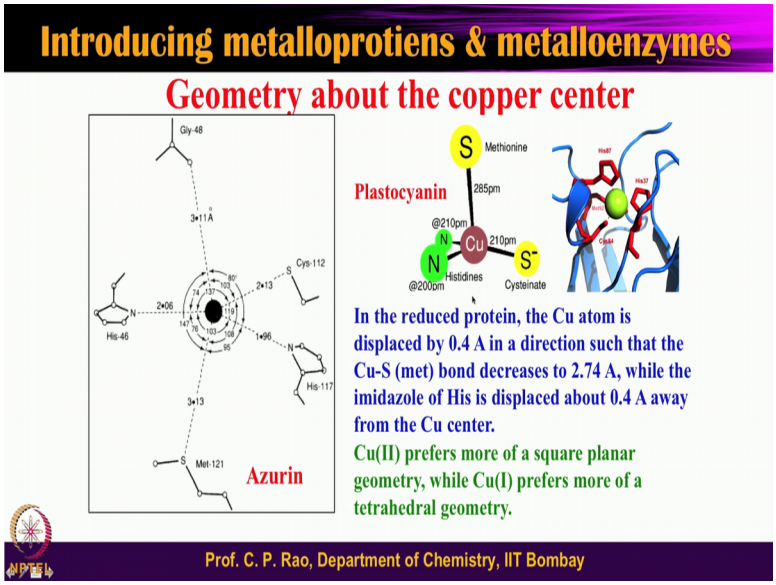

L us look at a little bit more how such a distorted geometry will help you see the kind of a distortion in plastocyanin here. So, plastocyanin here; if you see this methionine bond is 2.85 angstrom; other words 285 pico meter is which is very very far away from the normal one, the normal one methionine is about 2.10. So, during the redox activity, this is from a oxidized protein. Now when you have when the copper 2 plus receives an electron and reduces to copper 1 plus, then you get a reduced protein.

So, therefore, electron transfer occurs between copper 2 plus versus copper 1 plus, then when you have a copper 1 plus, what will happen? This particular methionine sulfur comes closer to this and by at least about 0.4 angstroms and one of these histidines go little further away so; that means, distortion continues to be in a different way in the reduced form as compared to the oxidized form. So, what is that? Why is that kind of thing? So, that is because as you know that the copper 2 plus prefers more towards the square planer and copper 1 plus prefers more towards the tetrahedral and the original structure is neither the tetrahedral nor the square planar, it is aware in between.

So, therefore, during the electron transfer such a transition can take place, what transition? The geometry transition takes place very rapidly. Therefore, you get a very high rate of electron transfer. In other words, during the electron transition the reorganization of the protein is quite quick and the reorganizational energy that is required is very minimal. So, that it acclaims the new protein conformation compared to the old protein that is oxidize oxidized protein has one kind of a protein geometry here around the copper and the reduced protein has a different kind of a geometry.

So, switching over in the geometry from one to the other is phenomenal; a sense that it requires very little reorganizational energy as you know very well about the electron transition; in the during the electron transition, there are no change in the nuclear coordinates and that is the kind of thing we saw; you do so quite quick reorganization. So, similarly, you can see another case which is the azurin, it is also quite well distorted thing. So, all that I wanted to tell you is that the copper geometry and type 1 copper proteins is highly distorted and during the electron transition electron redox properties or electron transition, it goes from copper 2 to copper 1 very quickly and the reorganizational energies it is not so high. So, therefore, it can easily reach the things.

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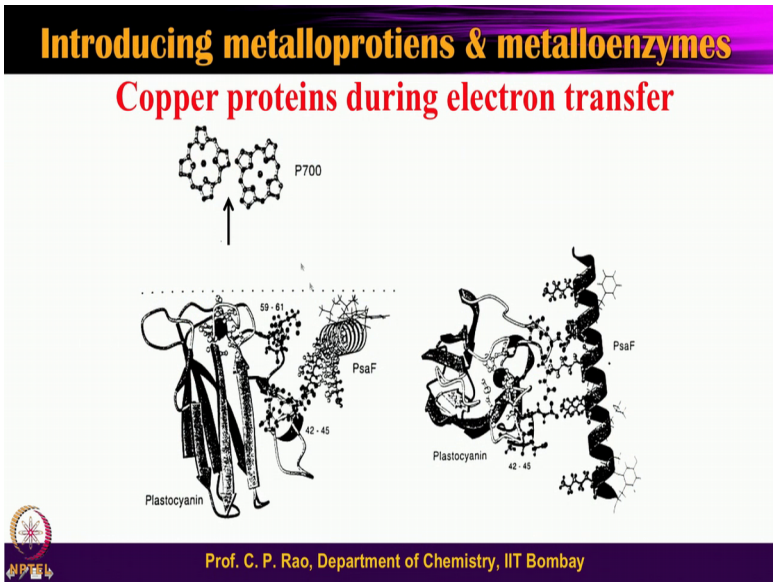

And just to show you an example during the electron transfer, you can see that and this is the kind of a protein that you have this is the centers where you have a redox transfer that you can see the other one two. So, this is just to give a feel that the redox transfer electron transfer during the redox process between the two systems, this is the copper containing system, it is used for plastocyanin, this is another system where the electron transfer is happening.

So, here you have a some kind of a receptors which are based on the a perforin and therefore, the electron transfer occurring. So, now, we have seen that the type 1 has a very unusual geometry which is helping, it is a boon in disguise. So, why is that because during the copper 1, it should have one kind of a geometry favored during copper 2, it should have another kind of a geometry favored and these two geometries should interconvert at very rapid rate and that is what is happening ok.

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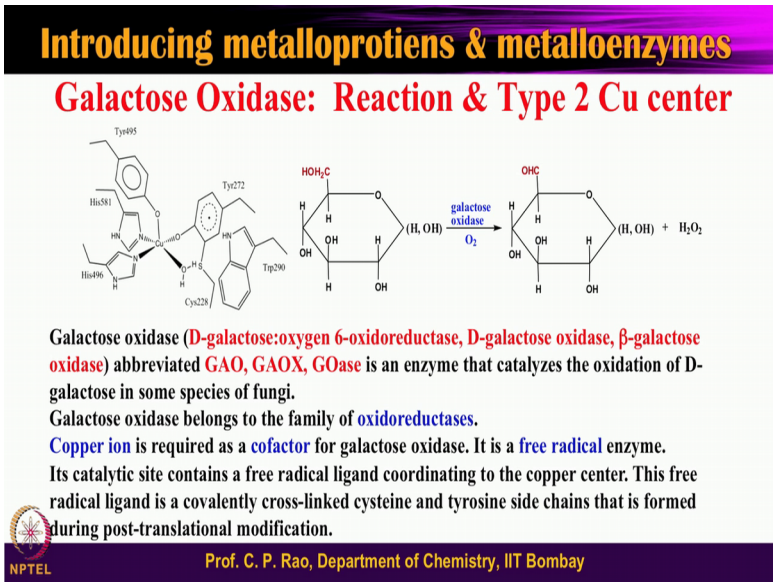

So, having looked at this, now, let us move to the next stage which is the type 2 copper. So, this is type 2 copper. This is nothing to do with the copper 2. Now, type 2; one example I have shown here, it is the galactose oxidase. So, galactose oxidase has a type 2 copper and type 2 copper center is also one single copper center. So, these are the example is galactose oxidase. So, galactose oxidase has what? So, it converts the galactose to the corresponding the aldehyde function. So, then here what you have hydroxyl function which is called the primary alcohol, what do you have here in product aldehyde.

So, the all alcohol to aldehyde; so, if you go back and look at the what I talked to you earlier and this is a oxidative reaction, how many electrons in this? I am sure you would have definitely identify that this is a two electron oxidation, but there is no oxygen number changing at all CH 2 OH becoming CHO and you get rid of two of the hydrogens in this. So, we will look into that and in the process, there is no oxygen taken from outside at all, but in the process the oxygen which is used is converted to H 2 O 2. So, this is quite different from these cytochromes. In the cytochromes, it activates the O 2 utilizes one of the oxygen and throws on the other oxygen as water in dioxygenase, both the oxygens are used, but here in the type 2 galactose oxidase, the oxygen is not used for oxidation, but it is used to bring back the protein to the normal state. So, this is one thing.

So, therefore, these are referred as a oxidoreductase enzymes now. So, it requires; obviously, a copper and it requires one another unusual thing. So, I will bring to you the unusual thing, you just look at the copper site coordination sphere and you have a histidine, another histidine and this is a tyrosine and this is a also a tyrosine, but if you look at on this side, there is something different than what you have. So, this part is coming from cisteine, this part is coming from the tyrosine, these two got coupled and this is the kind of a group which is not a original protein, it is a post translational modification that takes place after the protein binds to the copper center.

So, when the copper binds to this region, this particular activation act happens and as a post translational activity and the whole thing is converted to a new cofactor and this is the cofactor that you have in the galactose and they can show you.

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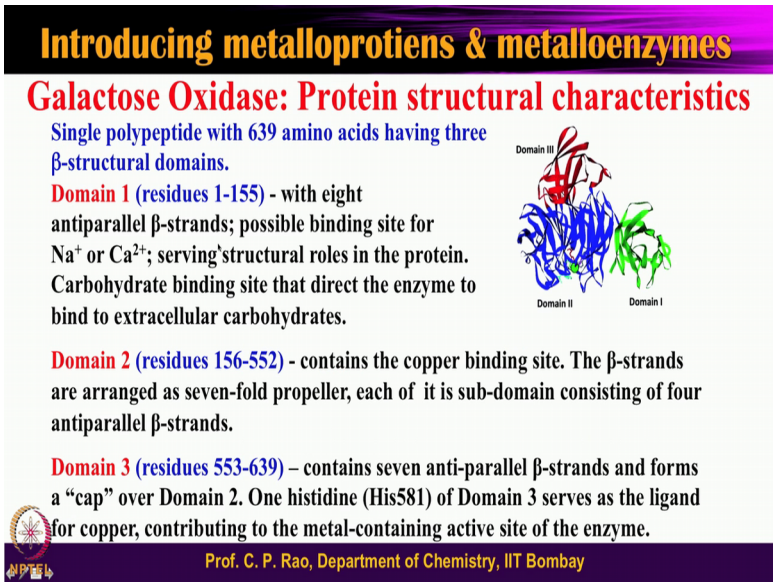

In the next one, let us look at the protein structure features for a while, this is a huge protein having 639 amino acids and it has a three domains you can see the one the green domain the blue domain and the red domain.

So, this is you can call it as a domain 1, domain 2 and domain 3 and total 639 amino acids are broken to up to 155 amino acid residues are present in domain 1 and 156 to 552 is present in domain 2 which is a blue region here and the 553 to 569 is present in a domain 3 which is a red in color and the domain 1 has got some sodium potassium sodium calcium binding sites, but most importantly, it has something like the

carbohydrate binding because here what you are doing is you are taking the galactose and converting into the galactose oxidized form at the C 6 position.

So, therefore, the carbohydrate is important; the carbohydrate binding carbohydrate recognition that is there in the domain 1. Now what is domain 2? Domain 2 has got this type 2 copper centre; that means, catalytic center; that means, reaction center active center. So, all these is present in this particular the blue domain and the red domain is domain 3 is a different thing. So, it forms some kind of a lid on to this one because your active center is somewhere here and therefore, this forms like a lid and it provides a one of the histidine binding histidine 581 and that binds to the capacitor.

So, when the reaction goes on initially, the copper needs to be bound here, it will open up when the copper is bound there, it will it will close it and it can do both these kind of things too. So, so due to the reactions that will close it, once the reaction is finished, you remove the product, it will open. So, the close and a open will be by the domain 3 and domain 1 is where the carbohydrate binding and domain 2 is the reaction center.

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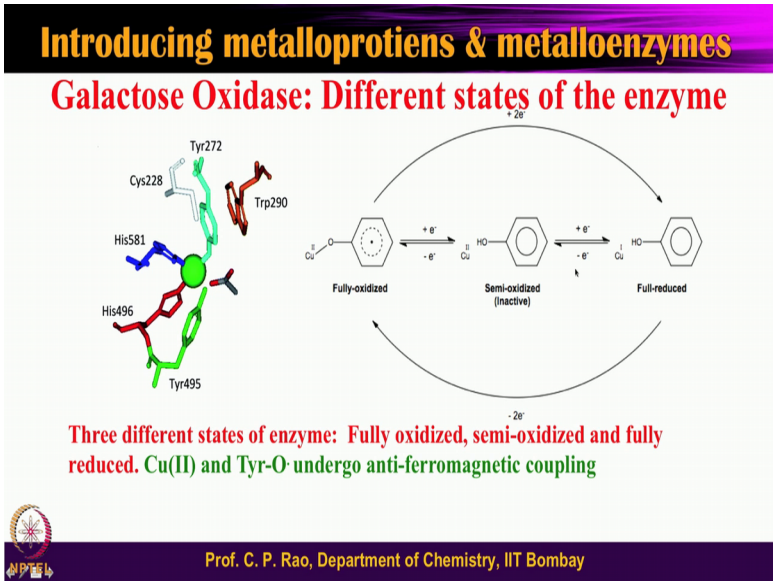

So, as I said that the unusual cofactor that you have and this is the cofactor this one. So, one side cysteine, other side is tyrosine and this cofactor is the one which is stabilizes radical and required on this. So, you can see fully oxidize enzyme have got copper 2 and the dot copper 2 and dot this is oxidized then when you add one electron, then this will be compensated this radical and then neutral and therefore, you have a semi reduced and

then you add a one more electron that is the second electron, then that will become copper 1.

So, copper 2 radical copper 2 neutral copper 1 neutral. So, fully oxidized semi oxidized or semi reduced either way is one and the same and then fully oxidized and this protein going from here to here is two electron, the reduction, then it should get back to the original that is the two electron oxidation ok. So, this kind of a two electron oxidation means it gives any two electrons when it gives any 2 electrons, what will happen to these 2 electron? These 2 electrons; these 2 electrons are taken by the O 2 and that O 2 will become O 2 2 minus and with the presence of the 2 protons that will become H 2 O 2 ok.

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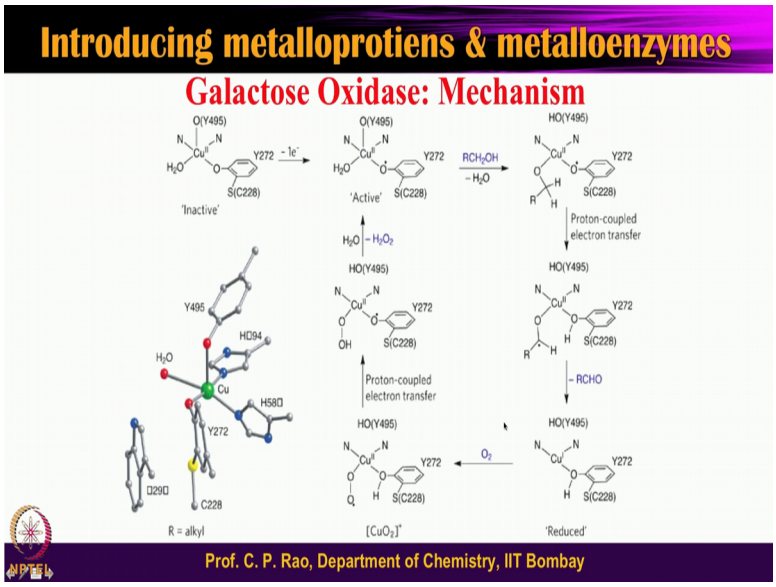

So, the external oxygen is not a part of this cycle, but the second half of the cycle to regenerate the galactose original form of it, ok, if you look at the mechanism, you will understand and appreciate this aspect, see this is the resting state; resting state you have the this binding of these ones and then you have a 1 tyrosine 2 histidines a water and this cofactor and this cofactor in the resting part is not a radical and this when it is when close to the radical; that means, 1 electron oxidation of this 1 electron oxidation of this. So, that will be that will be an electron cation here and this is referred as the active form.

So, once it comes to this, it is an active form, in an active form the substrate will bind; what is substrate here? You are showing for an easiness a or CH 2 OH because in the C 6th position of the galactose, you have a CH 2 OH. So, remaining thing we are showing

like a R. So, therefore, do not worried by this one. So, entire a carbohydrate we are showing by R and sixth position, we are showing by a CH 2 OH. So, this binds where it binds in the position with the copper center by knocking out this water knocking out knocking out means taking out the water that is minus H 2 0 and this is the substrate bound center on this then the proton coupled electron transfer and this proton whatever you have to the alcohol will go to this particular this particular tyrosine and tyrosine will get temporarily detached.

Now, the proton coupled electron transfer will make this into the radical form. So, as you see that the radical is formed at the substrate this is the substrate this is your alcohol; deprotonated alcohol which is bound to the metal center and that creates from here the thing is transfer over here and the hydrogen which is present here is transferred over there. So, you see that this becomes oh; that means, the radical is transferred from here to here ok. So, that is what is called the proton coupled electron transfer.

And now this particular thing which is the one electron a radical and if we transfer one more electron that to the copper, the copper will become the copper 1 and this the corresponding alcohol; it will become aldehyde because already one electron is gone here, one electron has gone here. So, it will become copper 1 with the aldehyde out; aldehyde out is shown as minus RCH over here, as you can see that ok, now the enzyme is fully in the reduced state as you could see in the previous slide here, as you can see.

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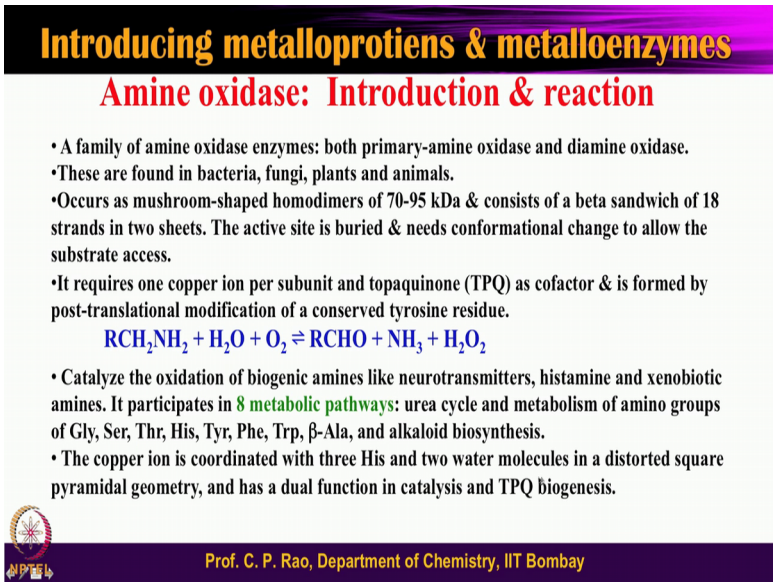

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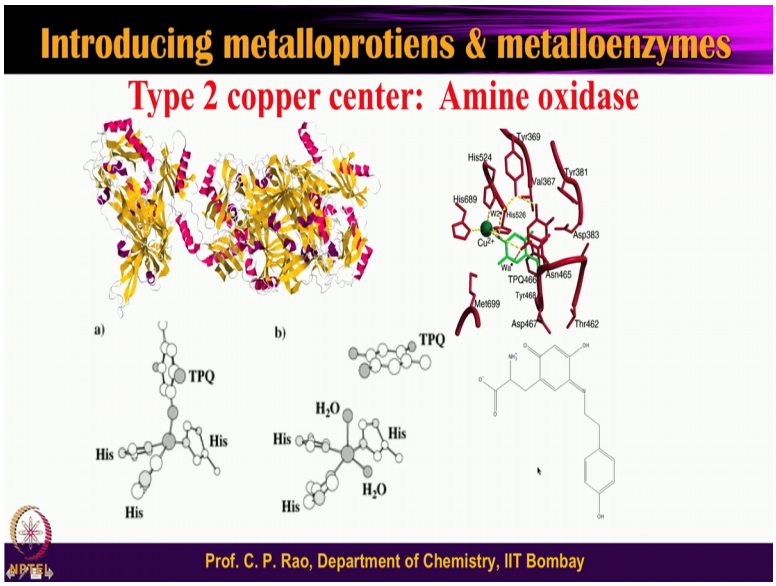

So, it is the fully reduced enzyme, it has to get will oxidized. So, the reoxidation is taking place by this O 2; external O 2 and this O 2 will bind to the copper because copper is in copper 1 state. So, which is very hungry to take the oxygen and takes of the oxygen and transfer this electron, then it becomes copper 2 and then again proton coupled electron transfer and then radical goes back to the cofactor and that will be basically into this and that will give the H 2 O 2. So, OOH is one more proton, H 2 O 2. So, that will give the active form. So, you can see that.

So, actually speaking; your a substrate is bound here and converted to product here and the enzyme is in a different state which is called the fully reduced state and this reduced state is two electron reduced state and this two electron reduced state is brought back to the normal state by O 2 because O 2 is capable of taking two electrons, the first electron will go to this one; the second electron will go to this one into out to make this particular radical and go back to this structure.

So, can you understand that is a very easy kind of a thing if you go through if you follow me it will be very easy. So, active form is radical form and the water is displaced by the alcohol moiety or C 6 position of the carbohydrate or a galactose and this is coupled with the proton transfer proton coupled electron transfer from cofactor that will become copper 1 and the alcohol becomes the aldehyde and goes out and this is re oxidized by the O 2 and the O 2 is reduced by the thing to become the H 2 O 2 ok.

So, this is a one example of the type 2, let us look at another example of type 2. So, another example of type 2 is amine oxidase. So, amine oxidase is an enzyme it is again a family of enzymes where I mean little; oxidize the primary amine, it will also oxidize the di amines. So, this is also called primary amine oxidase diamine oxidase. So, these are found in bacteria fungi plants animals all of these; these are somewhat smaller proteins about seventy to ninety two hundred kilo dalton and these are a homodimers so; that means, each one of the monomer has got the active center in these ones and they have some 18 sandwich beta sheet kind of things and is all you can read from this and find out, but the active site is varied and therefore, needs a conformational change in order to access to the substrate or to remove a the product, etcetera.

So, what is the kind of a reaction? The reaction is RCH 2 NH 2 and with the O 2 going to RCHO plus ammonia plus H 2 O 2. So, for this in the galactose oxidase, you have seen one kind of unusual cofactor and you will see a different kind of an unusual cofactor which is called TPQ topaquinone TPQ is topaquinone. So, you can see that this topaquinone is formed from the tyrosine residue that you will see that and this enzyme oxidizes, the variety of a biological amine containing neurotransmitters like histamine xenobiotic amines, etcetera.

It is also involved in a variety of metabolic pathways of amino amine moieties in glycine serine threonine histidine tyrosine phenylalanine tryptophan beta alanine and also involved in alkaloid biosynthesis. So, the type 2 amine oxidase is a common one across a variety of things that you can see over here. So, the copper is coordinated in these 2-3 histidines; 2 water molecules with a distorted trigonal bi pyramidal and has a dual function in making the topaquinone. So, now, you can see huge size; the protein that you have as a dimer in each di monomer has got one catalytic center and this is the catalytic center you can see nearby a amino acids and the catalytic center this is your TPQ.

The same thing is showing over here nothing to worry about it you can see the TPQ is labeled and this is the TPQ is very crucial for converting the amine finally, in the reaction. So, how does it convert?

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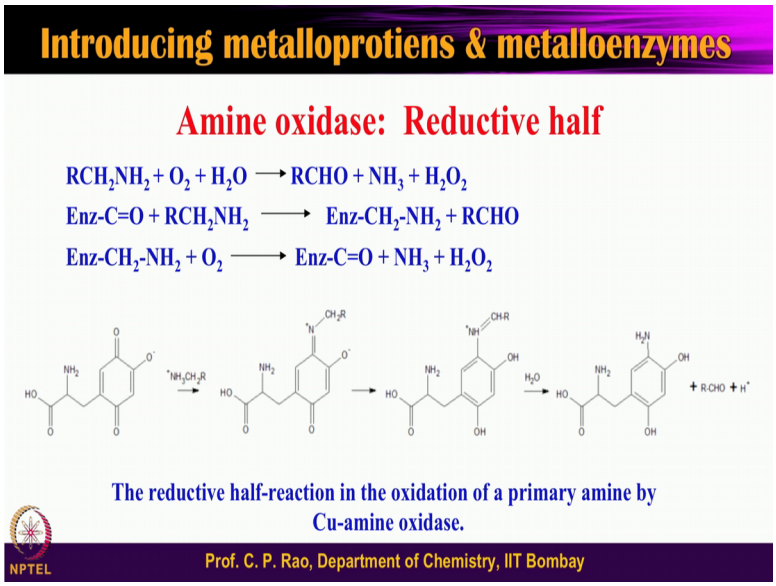

Let us look at the next slide. In this slide, you have the two things here the two half type of reactions in the galactose oxidase, you have seen two off side half reactions, what are the half? First half is the galactose is oxidized that is the first half and the second half the reduced enzyme is reoxidized in the second half.

So, now in this case also, you have 2 halves, but little differently in this in this case, the the amine substrate is converted to aldehyde, this is the total reaction aldehyde ammonia and H 2 O 2, but this goes by 2 halves. First half the enzyme based carbonyl reacting with the substrate amine to form you know the ship based via this amine which is very popular reaction and oxidizing the oxidizing the amine to convert into aldehyde. So, this is; obviously, an oxidative reaction there is only half of the reaction because at this stage the enzyme is reduced..

So, the reduced enzyme is reoxidized by O 2 and to get back to your normal state of this and that will release the ammonia and the H 2 O 2 also. So, at this stage the aldehyde is formed, but the ammonia is not released for the release you need to reoxidize the enzyme. So, just like earlier case. So, you have a reduced the oxidized form of the enzyme which converts a all galactose alcohol into aldehyde and then converts the reduced enzyme back to the normal form of the enzyme.

In this case similarly; so, you have a amine and aldehyde and, but then this is the ammonia is released out of this only in the second part of the enzyme. So, let us look at

the first half of the enzyme which you can call it as a reductive half, you can see that and this is the cofactor TPQ part of it and this interacts with your substrate which is a amine as an example is shown NH 2 CH 2 R and that will form in ship base and this ship base is inter converted to this by using the water and the amine moiety and this releases the aldehyde.

So, you have the aldehyde come, but still you have no ammonia seen yet. So, in the first half of the part, then you have this amine formation, but still bound to the TPQ, but aldehyde is only released still ammonia is not released in the second half in the second half.

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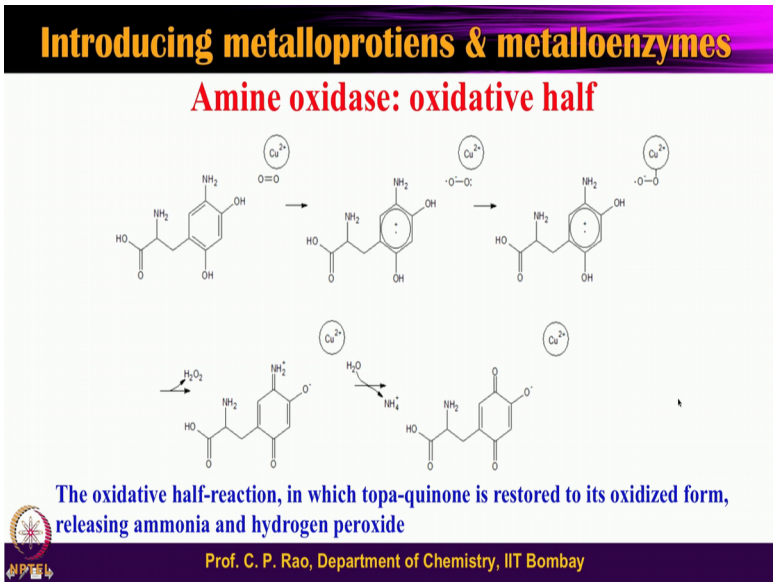

So, this particular amine converted one is now undergoes for the redox with the O 2 and the O 2 reacting with this enzyme and then converting the redox process between the TPQ and the O 2 and converting into binding to the copper center and then all these transformations from here, it goes into this.

So, that will release the H 2 O 2 because you already made the O 2 2 O 2 2 O 2 2 minus and that will with the protonation will go to as a H 2 O 2 and still the enzyme is has in one more stage and this particular stage you have to get the ammonia out. So, to get the ammonia out you need the protonation for this and thats by the water and that will give the ammonium out see.

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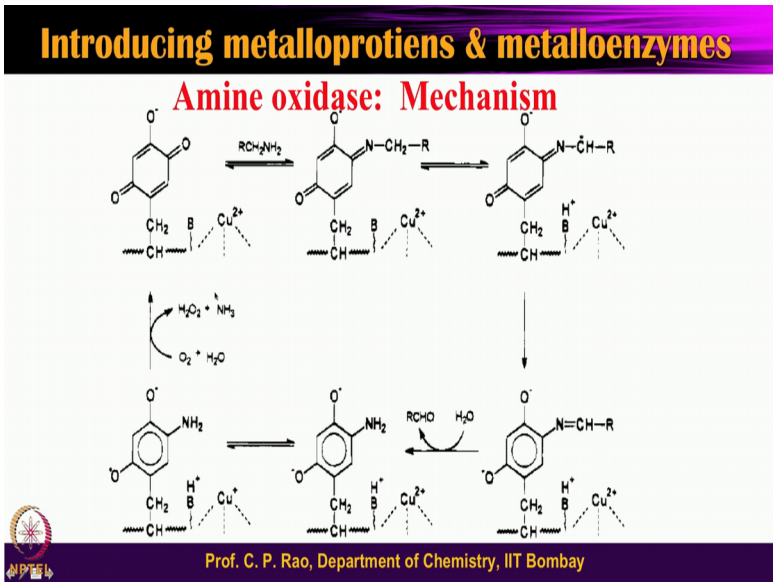

So, you have the total thing this; let us look at this one. So, first half and second half and first half will give you the aldehyde and amine bound one second half will give the back the enzyme ammonia and H 2 O 2.

So, in that second half, again you can see 2 parts here in the in the first part the H 2 O 2 is coming the second part ammonia is coming. So, this further, but anyway this is the whole oxidative and there is the reductive half. So, putting together amine oxidase mechanism, you can see the enzyme you substrate in the oxidized form of the quinone enzyme forming this ship based reporting into these ones and losing the aldehyde and then binding to O 2 and then O 2.

So, the clarity of this is not shown here, but in the previous case that we have shown you how the O 2 reaction goes here. So, in this is not shown very well. So, take the previous one for this. So, you can look at the overall you get the H 2 O 2 ammonia and of course, your aldehyde. So, what you get you get in one stage aldehyde and the second stage you get the ammonia hydrogen peroxide plus the enzyme in the oxidized form.

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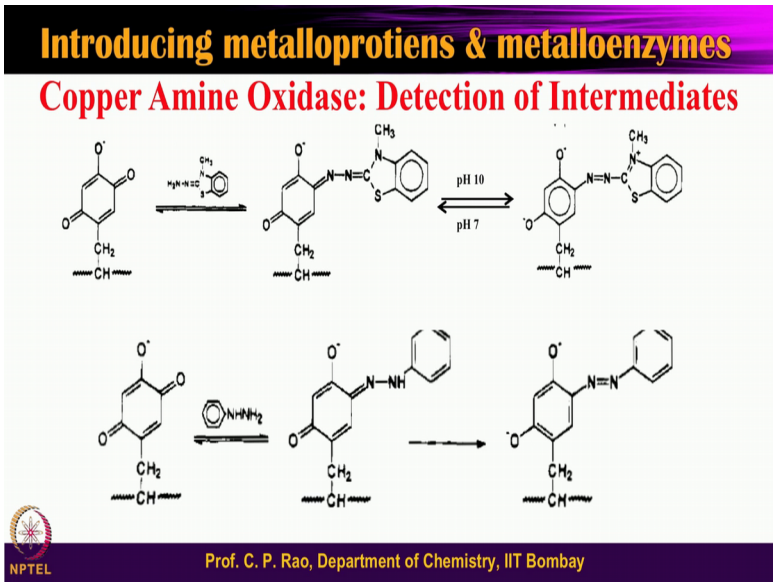

So, this is a one which is in the process of type 2, let us just begin with the type 3 before we conclude type 3 is again as an enzyme where you have the oxidizing the centers of like tyrosines.

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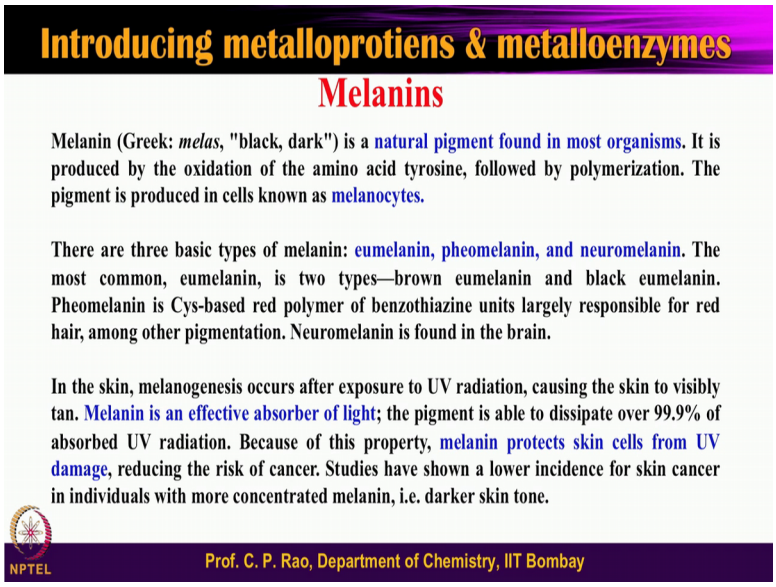

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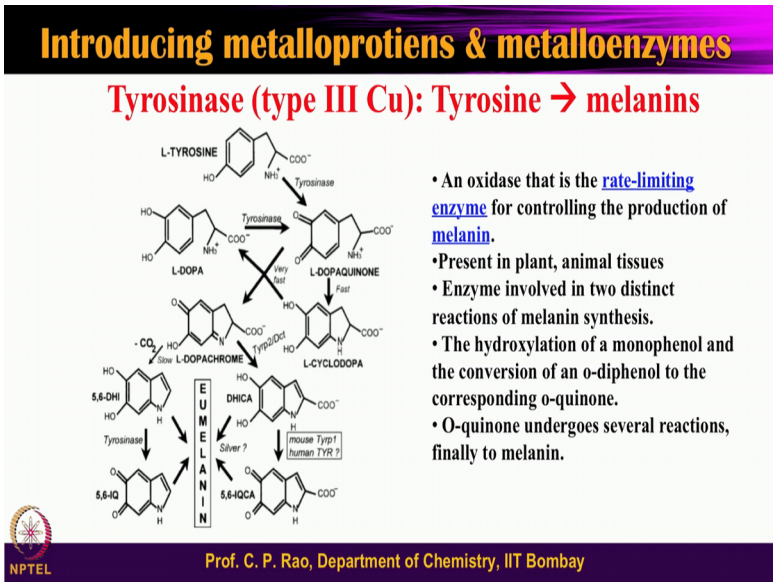

So, oxidate oxidized tyrosines will join together polymer form a polymers and these polymers will result in some molecules called melanin you know melanin is referred for color black color kind of skin, there are variety of melanins are there this you can read, but I am not going to go into the thing the one which is with the skin that you can see and this particular thing is very important; why the black color of this is very why is it very important because when the some radiation impinges incidents on our skin, there will be some UV radiation and this UV radiation should not damage the skin.

So; that means this pigment should do something. So, it should not absorb it should reflect or it should dissipate and that is what it does and that is why the people who do not have this pigment they try to try to tan their skin and that is what you see in western countries the people tan their skin to get black pigment and the back black pigment will save you from the UV radiation; otherwise, what will happen and this radiation can cause cancer the body and therefore, the black color is buring this guys.

So, therefore, our skins in the Asia and in particularly in India, our skin is very well suited for less damage from the sunlight as compared to the western kind of thing. So, how does this melanin happen is a basically tyrosine with type 3 copper center; type 3 copper center is a oxidative you see that. So, dopamine and converted to this particular thing with this enzyme called tyrosinase. So, the enzyme is referred as the tyrosinase and it has a type 3 copper center. So, this will oxidize this and further, slowly it will convert

into these two and so, this one the dopa dopachrome, this is referred as a dopachrome and this is a referred as a dopaquinone; why? There are 2 W 1 goes here that is called dopaquinone and this is called dopachrome because one W 1 O 1 OH and this one can form again in presence of the enzyme it can lose the carbon dioxide and then from this di all kind of thing and with one more enzyme again you can go back.

And or even going through this one it can form. So, therefore, you can see variety of oxidative processes and this will make the eumelanin form of it ok. So, what we have seen in this particular class I have brought to you a recapsulation of the type all different types of enzymes; which are talked to in the previous class and then I talked to you a bit more on the type copper 1 type 1 center, though I have talked to you in the previous class, I have again given you that and then I talked to you about type 2 two examples; one is galactose oxidase, other is amine oxidase, both of these are very important enzymes that I have explained to you they have two cycles one cycle.

So, half for other cycle is the second half in the galactose oxidase, the first part will oxidize the substrate and reduce the enzyme 2 and then the second part O 2 will reoxidize the enzyme. Similarly, in the amine oxidize, the first form you have a aldehyde is formed and the amine part that is attached to the cofactor TPQ will be further converted to H 2 O 2 in presence of O 2 and ammonia and now just started with the type 3 where how the melanin is formed.

So, I will explain the mechanism of type 3 in the next class.

Thank you very much.