Inorganic Chemistry of Life Principles & Properties

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Lecture - 40
Role of Copper in Life - Multicenter copper oxidases & SOD

Welcome you all to the next class on Inorganic Chemistry of Life Principles and Perspectives. In the previous class, we will be looking at the copper proteins and the class prior to that we look at the type 1 and in the previous class, we have again looked at the type 1; how the electron transfer takes place and then we also we looked at variety of type 1 copper proteins having different redox potentials starting from 180 to about 680 millivolts, then we looked at the type 2 there are two examples that we have looked at the type 2 one is galactose oxidase other is amine oxidase.

In both the cases, there are 2 half of the cycles. In the first half, the substrate is converted to the product, in the second half, the converted enzymes regenerated back to the normal and that is what happens in both the cases and so therefore, and to regenerate back use the oxygen; that means, enzyme is in reduced form back to the normal form. So, that requires the oxygen to get reduced. So, though the oxygen is not directly involved in the initial reaction of conversion of the substrate to the product, it is involved in the second step where the converted enzyme, they had reduced enzyme to regenerate back to the normal in order to continue with the cycle. So, this is true both with the galactose oxidase as well as with the amine oxidase.

Then towards the end of the previous class, we looked at another topic on melanin.

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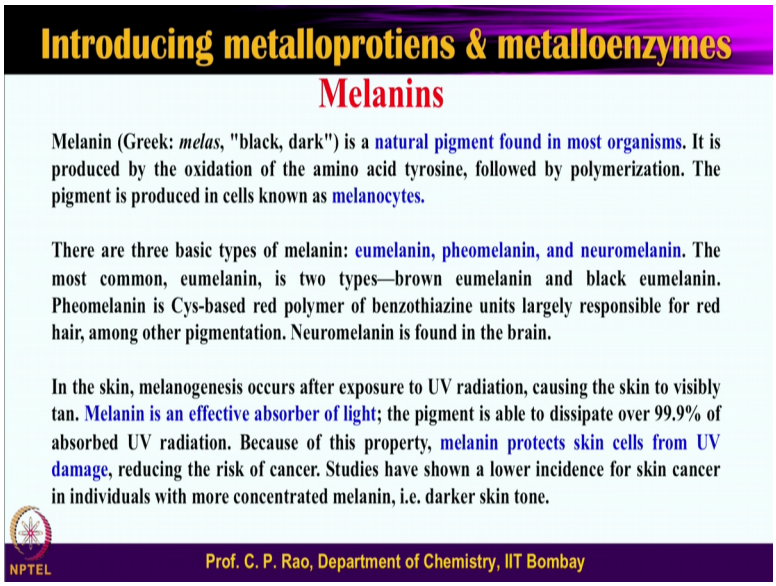

So, melanins as you know that it is from the term comes; it is a black color kind of a color which comes from the oxidation of tyrosine by variety of a enzymes and this leads to pigment black pigment. In fact, this is the black pigment which basically dissipates all that UV radiation that falls on the skin and thereby, it protects a person from attacking by the cancer kind of situation and this is what where the skin where there is no tanning they try to go for tanning so that the tanning will help them from the sun radiation particularly in the UV region of the sun radiation in that.

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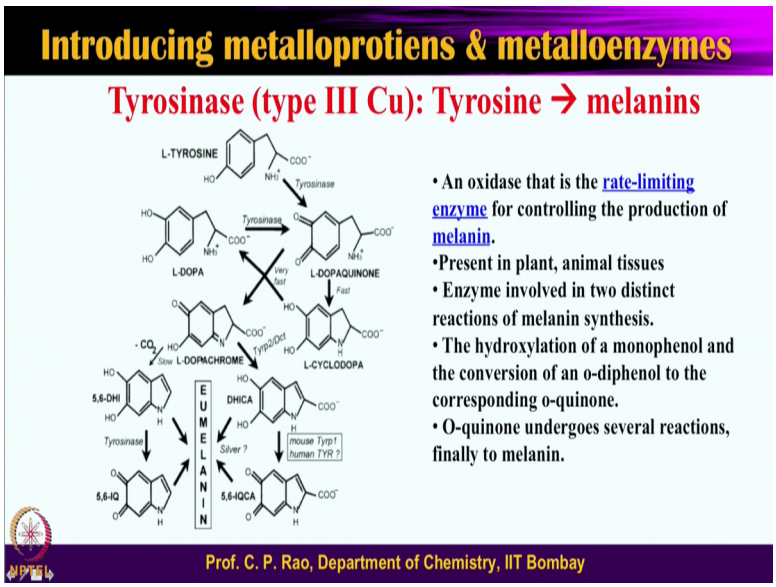

So, we have looked at also this is a enzyme the enzyme is called tyrosinase is a type 3 copper enzyme and we will I will be telling you example little later, here, we will look at how the tyrosine is basically converted to variety of reactions to melanin kind of thing. So, a lot of redox processes goes on ok. So, that there are tyrosinase can convert into dopaquinone and. So, also l dopa can be converted into dopaquinone as well and these can internally convert the cyclodopa and the dopachrome.

So, these dopachrome in turn connects by a variety of enzymatic reactions including the tyrosinase to give the eumelanin which is a polymeric form of this and that is what generates the black color now.

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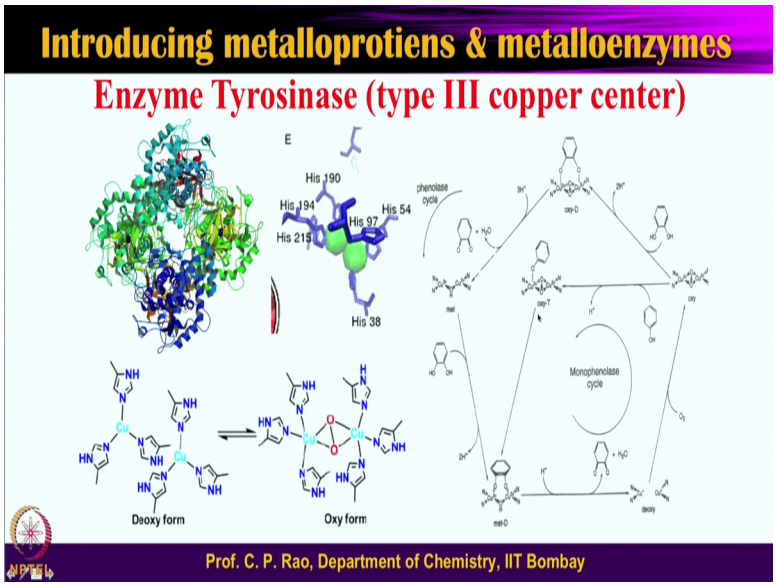

Let us look at an enzyme on tyrosinase; tyrosinase has got a type 3 copper center and this is a two copper centers present together this particular tyrosinase we have a tetrameric structures as you can see and each of this has the reactive center reactive center shown over there where the two these green balls correspond to the two copper centers and no copper copper connected bonding or no copper copper connectivity at all both the copper centers are present in copper 1 state and both the copper centers are connected to the histidines. So, therefore, you have you can see from here a copper with three histidines a copper with three histidines and no copper copper binding in this. So, this is in the copper 1 state; so, in this enzyme; in this enzyme of tyrosinase.

So, let us look at the how the tyrosine is functions. So, before that; let us you have a look at when you have a protein in the resting state is the di copper with the three histidines on each and no connectivity between the copper and each one is the copper 1. Now if you when you add the oxygen molecule to this and each of the copper 1 can donate 1 electron and go to copper 2 therefore, totally 2 electrons can be donated and when the 2 electrons are donated to the O 2, it becomes O 2 2 minus and that is what you see here a bridge peroxo kind of a species and such kind of thing that we have already seen earlier, when we were talking about the oxygen transport proteins hemocyanin and this can be identified from the infrared spectral vibrational frequencies of O O stretching vibrations.

Now, let us come to the mechanistic aspects of the tyrosinase and in the free enzyme which you call it as a deoxy form of it, the copper 1 copper 1 center and since it is a copper 1, it can readily react with the oxygen and then this oxygen will get converted to O 2 two minus as we have shown here it is the same and this O 2 two minus is bridged between the 2 copper centers.

And now copper centers are each of the copper center is copper 2 oxidation state not anymore in one because it donates one of the electrons from each of the copper center now this upon binding to the substrate in this case let us take the substrate as the catechol and the catechol can bind very nicely to the di copper center and this upon the protonation can lead to the oxidized form of the catechol which is called catequinone and the other the oxygen goes is a water kind of thing.

So, you can see a connected species still one more oxygen is there is a connected species. So, therefore, this species is still active, therefore, it can interact with the second molecule. So, so it is this is one of the rare enzymes where the enzyme is regenerated back after doing 2 sets of or 2 cycles of 2 sets of reactions. So, you have first one reaction here and the second reaction. So, another catechol molecule will be bound here and this will be converted to the catequinone and then back to the deoxy ok.

So, understand this the copper 1 state copper 1 state becomes O 2 O 2 2 electrons coming from each O 2 2 minus which is bridge between them and this is sufficiently good enough to re oxidized this and then in the presence of the protonation, you will get the catequinone plus the water; that means, one more oxygen or the O 2 is still left over and that is what you see as a as a bridge species and this form of the enzyme is still active for

can bind to one more mole of the catechol and this will be binding to this and then finally, you get totally two.

On the other hand instead of the dihydroxy compounds, if you have a mono hydroxy which is nothing, but phenol see here the active species and this in presence of the phenol can phenol combined, since phenol has got only one hydroxyl it can only bind more oriented while the catechol combined by the intake because of the two hydroxyl groups. And this will further oxidized this one and that will list the catequinone. So, of which one of the oxygen is coming from this O 2 and the second oxygen goes as a water.

So, the same enzyme if it is a di OH compound diephenolic or catacolic type of a compound, you have one water molecule and it will do a second cycle of catechol and the other hand, if it is a mono phenolic kind of a situation both the oxygens are used one oxygen is used to convert the phenol in to catechol second oxygen is used converted into water and no more second mole. So, therefore, this is what the difference between a mono phenolic or mono hydroxy compounds versus dihydroxy compounds.

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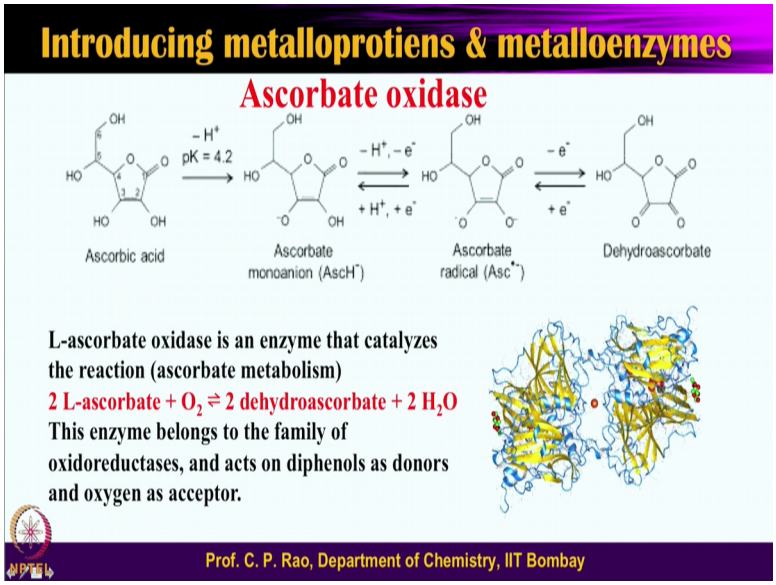

I hope you understand type 3 is a di copper center. There are cases where the enzymes have got type 1, type 2 and also type 3 in the same enzyme. So, we will see a situation of the example here we are looking at ascorbic oxidase look at the ascorbic oxidase. So, this is ascorbic acid and goes to dehydroascorbate. So, these are all steps that you can try to

look at understand that the deprotonation at ph and then one more deprotonation and the oxidation and one more oxidation.

So, you need a di oxidation; obviously, you are getting two a ketone moieties here and from the two phenolic moiety 2 hydroxy moieties over there. So, ascorbic acid dehydro ascorbic acid. So, this is basically or oxidative reaction. So, therefore, the enzyme is a redox kind of an enzyme and it acts on the dye phenols dihydroxy compounds, we have also seen in the previous example and they are as donors and the oxygen has acceptor because oxygen will accept the electrons ok.

So, L ascorbate plus O 2 giving to dehydroascorbate plus 2 H 2 O so; that means, your oxygen what you added is not incorporated into your ascorbic acid both the Os are gone into water we have seen in the initial cases mono oxygenase where mono oxygenase used and the other oxygen goes into water, we have seen dioxygenase both the oxygens are used and no water. And here is an example where both the oxygens are unused, but oxidation takes place and this goes into the water as such a both the oxygens go into water ok.

So, L or ascorbate oxidase is an enzyme that catalyzes the ascorbic acid into dehydroascorbic and this is the 2 dimeric enzyme that you can see.

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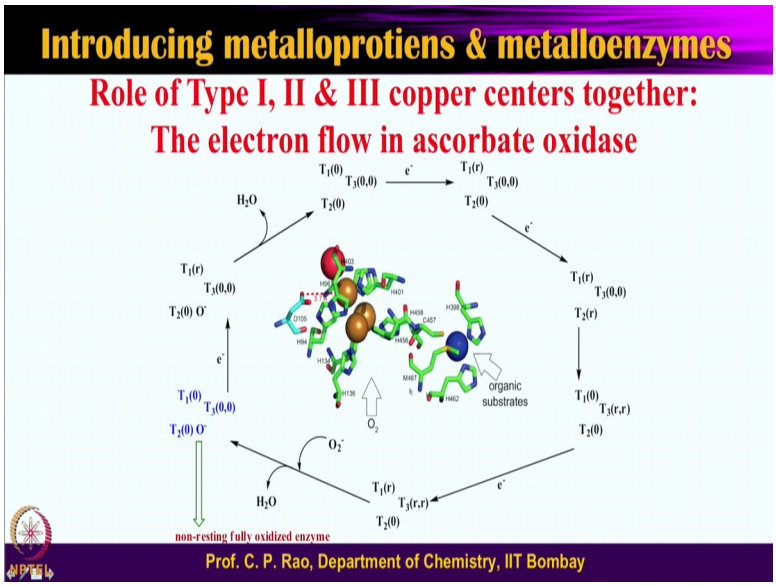

And you know there are 3 different types as I said type 1 which is blue copper protein type 2 single copper, but is a different kind of a one and type 3 which is di copper center which is involved in redox process.

Now, you can see this here this is the type 1 which is a blue copper protein which is a connected by 2 histidines, 1 methane in 1 cysteine, you can see here and you can see here this is the type 2 copper center and you can see here the 2 coppers which are the type 3 copper center. So, there is a distance of about 15 to 20 angstroms from here, but these are within 3 to 4 angstroms distance. So, because these are within three to 4 angstrom distance the type 2 and type 3 joined together and jointly do a catalysis

And whereas, type 1 is involved in what did we study for the type 1? Type 1 is a blue copper protein which is involved in electron transfer. So, therefore, it is basically electron transfer kind of a reaction. So, this will do a part of the electron transfer, this will try to hold activate the oxygen and in between you will have a substrate and the substrate will get oxidized and that is the how the whole reaction will go.

Now, let us look at the sequence or the electron transfers in this, let us call type 1 as T 1 and type 2 as T 2 and type 3 as T 3 and in the bracket, it is shown some value, for example, here 0 that it is to be taken as not 0, it is to be taken as oxidized kind of thing. So, T 2 oxidized T 3 oxidized T 4. So, all the 4 coppers are in oxidized state.

Now, add one electron to this particular enzyme where will the electron go when it go to the T 1 when it go to T 2 when it go to 3 everywhere, you have with the copper two, but it will go only to T 1 because the redox potential of this is very favorable therefore, that will attract the electron. So, that will go to T 1.

The second electron will go to T 2 now you have T 1 is in the reduced state T 2 is reduced state. Now, these two electrons are internally transferred to T 3 and both the coppers of the T 3 will get reduced what is the necessity the necessity of the di copper center is because it can capture the O 2, it can release the 2 electrons; 1 from each and convert the O 2 in to O 2 2 minus, we have seen already that is the mainly the reason why the electrons are transferred from T 1 and T 2 to the T 3 the T 3 has 2 coppers. So, it requires 2.

So, the next step is T 3; that means now the enzyme is ready to activate the oxygen. So, at this stage one more electron reduction will make three electrons and the three electrons will take up the oxygen moiety into this and that will be activated to throw out water and one of the OO is bridged. So, this is out of the 3 oxygens 1 O is bridge 1 O is a z water in presence of the protons, already one is gone now. So now, what it requires it requires only one more oxygen electron because O 2 has got 2 bonds ok. So, therefore, one more electron will be reducing that oxygen again into the water. So, you get two water molecules effectively and the substrate will get accordingly oxidized.

So, substrate will be in this particular region and as you can see the substrate goes from here and the oxygen goes from here ok. So, therefore, you have a substrate getting oxidized and the O 2 getting reduced and the O 2 reduction requires two coppers together. So, I think the whole thing is understandable. Now first electron going into T 1 second electron going into T 2 the both the electrons going into T 3 and in presence of one more electron three electrons will reduce the O 2 to water. And the a bridge oxos species the nature of this is not very well known and this with one more electron will moves out the second water as well and the substrate gets oxidized.

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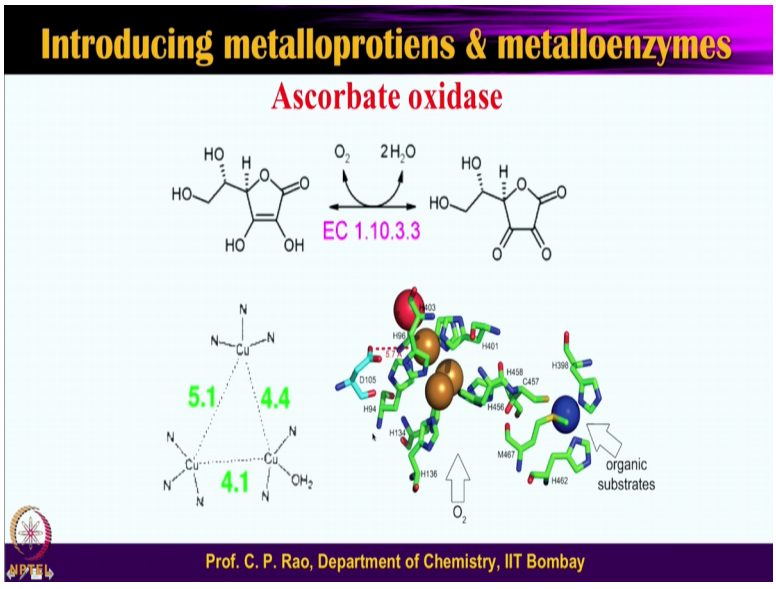

So, as you can see that. So, therefore, you can see this is your ascorbic acid dehydroascorbic; that means water is gone and now you can see that the kind of a copper center here your two of the copper centers here and one is this one. So, therefore, these

are you have all of these are over there and these are your type three kind of things and type 2 and these are roughly within this kind of a triangular shape therefore, O can be activated O 2 can be activated between the two copper centers of a type 3. And then at the end 1 O can be bridged here and that is what you can see; so that requires the arrangement of the three coppers in a very closed fashion and that is what you would see that. So, substrate organic electron transfer kind of thing.

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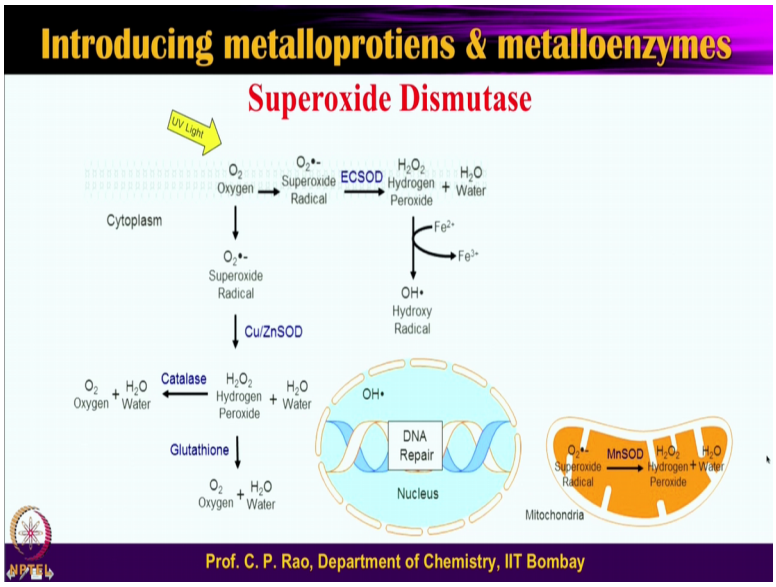

So, this is the kind of a redox reaction that happens.

So, I hope now you understand the type 3, we have seen tyrosinase we also seen a case where both type 1, type 2, type 3, all three are being present and these oxidized ascorbic acid into the dehydroascorbic acid to hydrogen hydrides or lose.

So, therefore, oxidized now, one last enzyme that we would like to look at its very important in the body because body has always has an assault by the oxygen radicals because the cells work on oxygen. So, therefore, oxygen radicals have been continuously formed and these are oxygen radicals are toxic. So, therefore, there should be something that awaits these, remove this, we have already studied a lot on superoxide dismutase in the iron and manganese and other enzymes.

So, now we look at the copper case. So, the general thing this is oxygen going to superoxide and this can be dismuted by the superoxide enzyme dismutase enzyme into

peroxide and water and this can we converted this in presence of the metal ions can convert into radicals, these radicals are very bad for the cell tissue can get damaged, DNA can get damaged on the other side, you can see the oxygen with the superoxide copper zinc superoxide that is what we are going to now study and this will convert this into the H 2 O 2 plus water if you have a catalyst that can further degrade H 2 O 2 2 H 2 O and water.

So, the so, SOD and catalyst together can completely remove the superoxide species into that and as long as there are no iron or metal ion species which in a undergo redox, it can be iron it can be even copper if it can go undergo redox metal ion then that can make the radicals this is what is known as the fenton reaction. So, if such a thing is there it is dangerous.

So in absence of this, this can take care into the oxygen and water and as you can see, it is can get into the mitochondrial system nucleus all this can get damaged.

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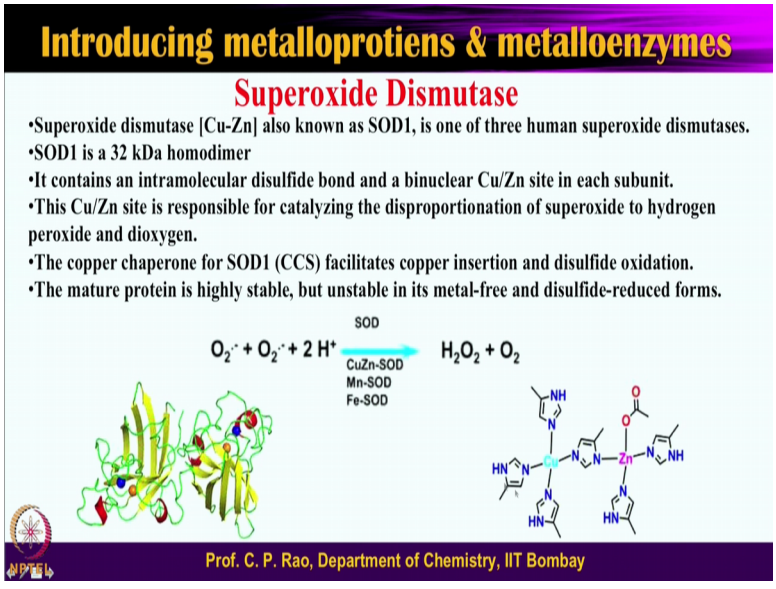

Now, let us look at this last enzyme which is superoxide dismutase superoxide dismutase is a copper and zinc bimetallic of bimetallic one is a copper other is zinc and this is present in the human and this is homo dimer around 32 kilo Dalton ok, it has an intramolecular disulfide bond and by nuclear copper zinc site and this copper zinc site is absolutely necessary for the catalysis for the disproportionate of the hydrogen the superoxide into hydrogen peroxide and dioxygen.

So, if you do not have this no reaction of this the copper is the reactive center catalytic center and the zinc is the structured center, suppose, if I replace zinc by cobalt zinc by nickel, still, I can have the we can have the activity, but if I replace the copper center by something else no activity the activity drops down dramatically.

So, the in fact, in the in the biological system the copper chaperones SOD 1, they facilitate the copper insertion and they also cause disulfide oxidation to form a structurally form this one. So, the mature protein is highly stable, but unstable in its metal free and disulfide if it does not have a disulfide the protein is not stable, if it does not have a metal, it is not a stable. So, in the super oxide, we have seen several times this reaction O 2 minus dot plus O 2 minus dot plus 2 H plus gives H 2 O 2 plus O 2. So, it is a dimer 1, here monomer here and there is a 2 metal ions, 1 zinc, 1 copper, 1 zinc, 1 copper that you have and how is this bridged unlike the previous case to copper centers nothing bridging in case of type three, but there is a bridging here, you see that bridging is nothing, but imidazole one nitrogen towards the copper other nitrogen is towards the zinc so; that means, these 2 are well connected.

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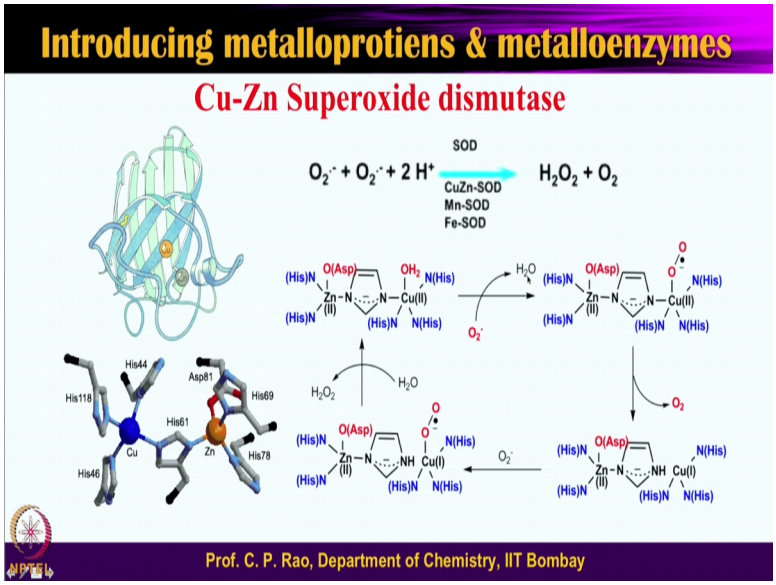

And this is the one this is the histidine, if it is one bridge between the two centers and. So, one is this is the zinc center this is a copper center copper is a catalytic centers zinc is a structural center structural center can be replaced by other kind of ions, but catalytic center cannot be spared. Now, so, you take the native form of the enzyme where you

have a copper 2 and now you add O 2 minus the O 2 minus will bind at the copper 2 center and the redox take place and it will go as the go to O 2 so; that means, it takes the electron out and copper 2 becomes copper 1 and the O 2 minus dot becomes O 2 and the O 2.

Now, this is ready for the second mole of enzyme that will make disconnected kind of thing second mole of O 2 minus dot is bound again at the copper 1 center which will again reoxidized this one and that will replace that will go to the H 2 O 2 and it will go back to the copper 2. So, in one the copper is reduced, in the other copper is oxidized ok. So, similarly the O 2 minus and these oxidize and the O 2 minus is reduced. So, O 2 minus oxidize means O 2 O 2 minus reduce means H 2 O 2 or O 2 2 minus. So, the first step you get the O 2 the second step you get H 2 O 2. So, you could see how the enzyme is undergoing from copper 2 to copper 1 and copper 1 to copper 2 the O 2 minus to O 2 and the O 2 minus to O 2 minus. So, this is the kind of thing that we have in all these that.

So, we have seen quite a good number of examples of all these as you could see that the how a type 3 copper reacts count type 3 copper because it has a two this is also type 3 copper tyrosinase continuously you know bringing redox process on the tyrosine molecule, then end up with the melanin that we have looked at and actually the tyrosine tyrosinase enzyme having 2 copper centers the 2 copper centers can convert the biphenolic compounds or catechol like compounds two moles will be reacting. So, the first reaction if you with the catequinone second reaction with another catequinone and so, in each case the oxygen is conversion to water totally 2 equivalents to the water is converted in all this and.

So, whereas, in the mono phenolic case only one equivalent is reacting and that gives the catequinone and there is that you have seen. So, ascorbic oxidase from ascorbic acid to dehydroascorbic acid as you can see and this is the dehydroascorbate. So, this is again an enzyme.

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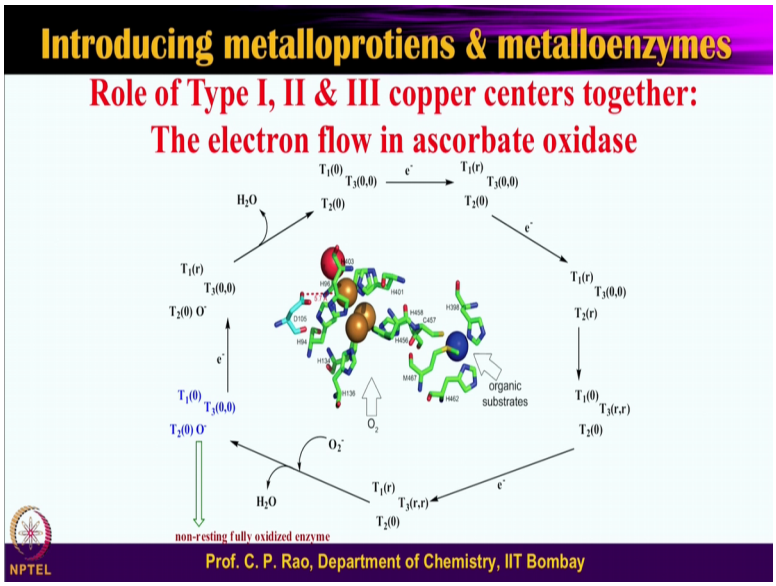

Where the type 1 type 2 type 3 all three are present they work very harmoniously and type 1 to type 2 type 3 you can see that this particular thing distance is around 15 to 20 angstroms and whereas, the type 3 to type 2 is around 4 to 5 angstroms..

So, therefore, type 2 and type 3 together activate the O 2 an and this one do the redox process and overall the substrate is oxidized O 2 is reduced and if the ta substrate is already diphenolic or di O h then both the os will go as water and thats what you see in the dehydroascorbic acid and this is where I explained to you that the all three together sitting over there as a coordination and the last part as we have seen that it is a superoxide dismutase we have already studied this kind of an enzyme several times and this is a bit different from what we have studied in the ion what we are studied in manganese etcetera here do you have a copper and zinc.

So, copper and zinc are connected by the imidazole and that imidazole part is the one which is bridging the two things and here what happens the one O 2 minus is oxidized to O 2; that means, copper is reduced to Cu 1 and the second mole is of O 2 minus is further reduced O 2 2 minus; that means, copper 1 is oxidized to copper 2 and that is what the mechanism is.

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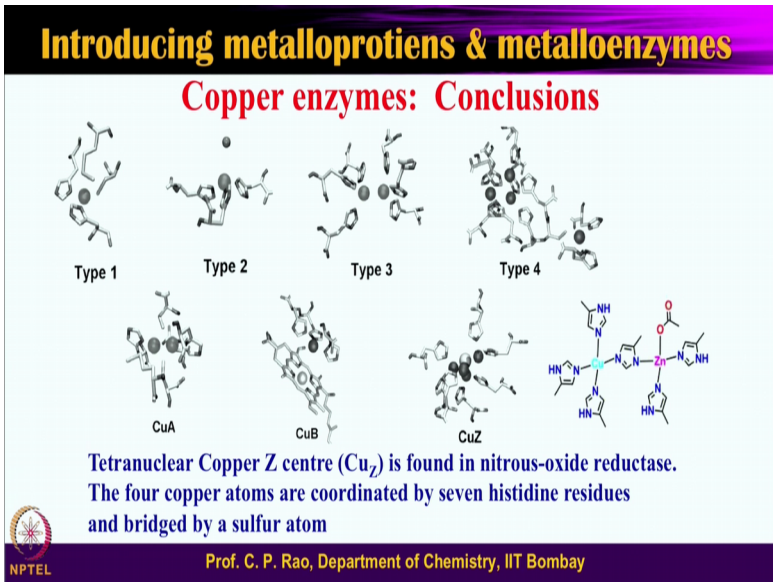

So, in a fact under this particular story of copper bioinorganic chemistry biological inorganic chemistry or inorganic chemistry of copper in life, we have looked at very detailedly type 1 which is a blue copper protein we have looked at the type 2 which is involved in the redox process like galactose oxidase amine oxidase kind of things they dont use the any oxygen of the O 2, but the O 2 is required to bring back the enzyme to the normal state because all these reactions go in 2 half phase.

The first is substrate is converted and enzyme is reduced the second step in presence of the O 2 enzyme is reoxidized type 3 we have seen enzyme tyrosinase tyrosinase which is involved in the melanin information tyrosinase which is involved in the by catechol kind of thing phenol kind of things and the two copper ones can play a role to copper 2 and back type 4 is the one we have already seen type 4 is nothing, but type 1 plus type 2 plus type 3 all the three to the other. So, this is the type 1 this two are the type 3 and this is the type 2 ok.

So, the substrate comes here oxygen goes here. So, you have a mechanism to open and close open and close and copper A, copper B, we have not look at copper Z also, we have not looked at and superoxide dismutase we have of course, looked at this course superoxide dismutase we have looked at earlier and we have seen that one two.

So, in this particular case the zinc is the structure and copper is catalytic, if I replace the zinc by manganese zinc by cobalt 2 plus zinc by nickel 2 plus still reaction goes, but

another hand if I replace a copper by some other ion like manganese or zinc or any other thing, the whole reactivity is gone and that is how you find that the copper is catalytic in nature and the zinc is non catalytic in nature.

So, thus where I think we complete with the copper enzymes in this particular class. And in the next class, we will look at the zinc based enzymes.

Thank you.