Inorganic Chemistry of Life Principles & Properties

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Lecture – 30
Role of Iron in life – Mono - & di- oxygenases

Welcome you to the next class on bioinorganic chemistry, there is Inorganic Chemistry of Life with the Principles and Perspectives. And in the last class I have talked to you about the oxygenase mainly the Mono Oxygenase an example cytochrome P 450. Let us look at one more mono oxygenase. Why are we should look at one more? The one which I have explained in the previous class was that based on the heme.

Let us take another example of mono oxygenase where there is no heme involved, the most popular enzyme for this is methane mono oxygenase, then title itself it is methane mono oxygenase mono oxygen, when you add mono oxygen to methane what will happen methanol.

So, it is a conversion of methane to methanol is a methane mono oxygenase. So, and how easy is this? Suppose you want to make in a in a test show how easy is this, you know what a bond energy for a CH bond because, in the methane you have these 4 CH bonds what are the CH bond you have to activate and insert O 2 becomes COH. So, to do that you have to activate the CH bond, you have to basically break that bond.

So, what is the energy you think is the CH bond, I am sure you are aware covalent bonds are in the range of 100 kilocalories and see this is about 104 kilocalories.

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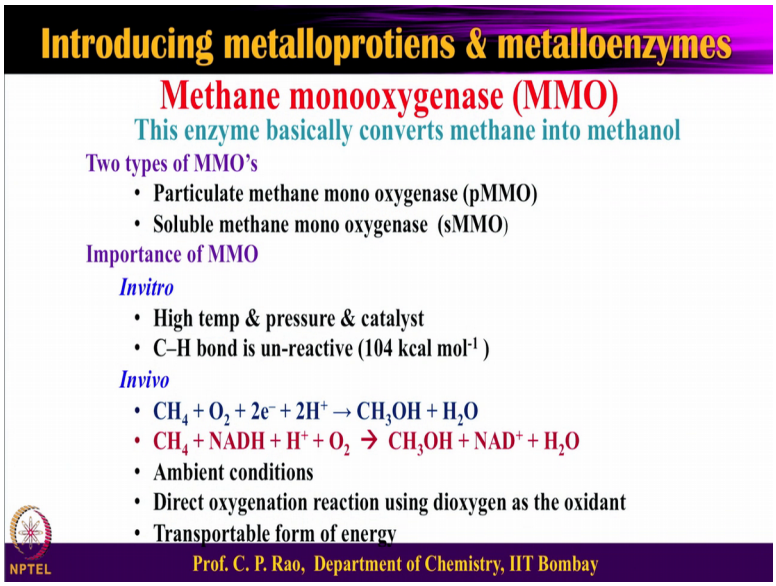

So, can one do this in the invitro? It is yes one can do, but you require very high temperature, pressures, catalysts many things are required. So, without catalyst, pressure, temperature is not there simple, but the enzyme within mono oxygenase, quite nicely does at ambient conditions at the room temperature etcetera and without using any high temperature, high pressure nothing of this thing. And it will also use O 2 as the oxidant.

And once you convert the methane to methanol it is a very safe form and the this can be transported, when you want to transport this energy material from one place to the other, it is dangerous to use in the form of methane, but it is comfortable to be used, or safe to use in the form of methanol ok.

So, what is the kind of a reaction you talk about, a CH 4 plus O 2 plus 2 electron plus 2H plus becomes methanol plus water ok, so, CH 4 plus NADH that is the one, H plus O 2, CH 3 OH plus NADH plus H 2 O. So, this is what is the basically is happening into this particular reaction. And if you look at the methane mono oxygenase there are 2 types; particulate kind of a methane mono oxygenase, which means the insoluble one, the second one is soluble methane mono oxygenase ok.

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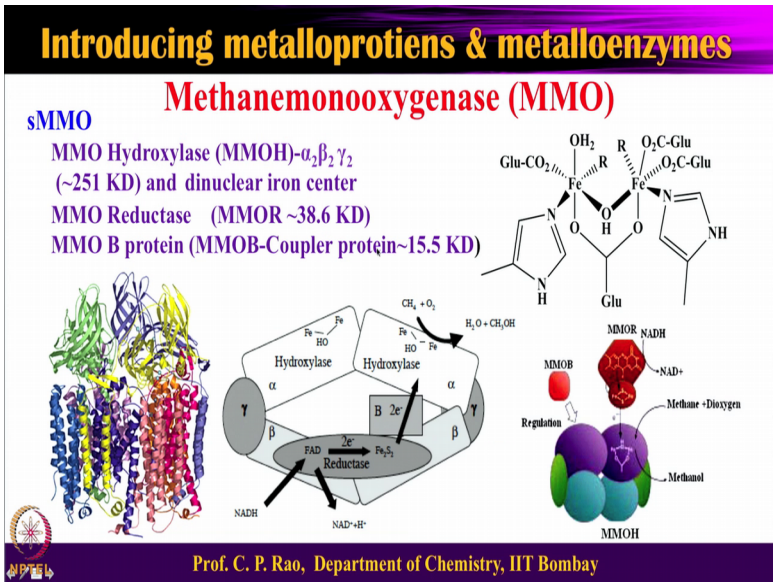

And let us see some characteristics of this enzyme, some characteristics of this enzyme is this enzyme is a very complicated enzyme because, or huge enzyme because it has 6 subunits, 2 alpha type 2 beta type 2 gamma type; alpha, beta, gamma, alpha 2, beta 2, gamma 2. So, it is a hexamer ok, dimer of this alpha beta gamma basically, or what you can say is that. And total molecular weight is that 250 around 250 kilo Dalton so, very huge.

So, what it has? It has one of the thing called hydrolyze portion. The hydrolyze portion is the one where the iron center is there and the iron center in this is not with a heme, but without the heme, but it is a di iron center. So, it is called MMOH. So, this part is called MMOH.

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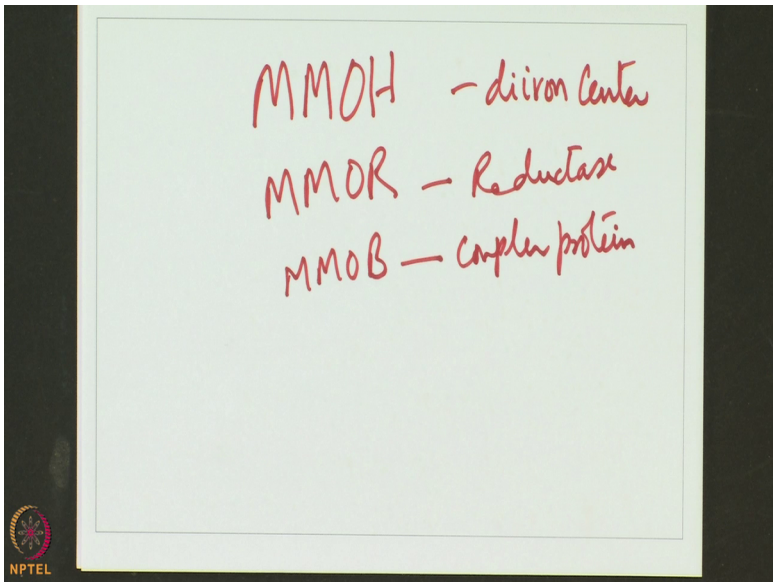

So, the iron that is present in this is called MMOH. So, this is the di iron center and, then you have a reductase part so, MMOR we have seen earlier the electrons have to come from NAD, then NAD to FAD, FAD to iron sulfur cluster all these kinds of things. So, this is the reductase part and there is an another protein which is called MMOB, this is called a coupler protein. So, what is coupler protein? That is whatever the electrons got activated out of this MMOR should be transferred etcetera reaction center to do the reaction.

So; that means, this and that should join together, as that is what is joined by the coupler protein ok. You will understand more now when I explain you using this one, hydrolyze as I told you hydrolyze has a dinuclear iron center. So, you can see this dinuclear iron center this is 2 iron centers to histidine, a metazols, and a bridge glutamic and a bound glutamic one and 2 of the glutamics here ok and these are some internal ligands nothing to do with the substrate or is not a substrate here; some other ligands and those ligands can be replaced when appropriately oxidized. And it is connected by the hydroxyl bridge. So, it is connected by the hydroxyl bridge.

Now, let us come to this portion as I said there are 2 alphas 1 unit of alpha 2 unit alpha, there are 2 betas, there is 1 unit of beta, another unit of beta and there are 2 gammas here 1 here and 1 here and so, therefore, these are the 3.

So, in this basically what is happening was so, this is the reductase part where the reduction electrons generating, this is the reaction part and the gamma is coupling between the alpha and beta. So, it is a coupler so, when something happens here it should attach with this, if something happens and if this part of the protein is far away, then what will happen to the electron that is generated, it will get it will get into the O 2 and then O 2 will be add to the activated O 2 will add to the protein and protein will get destroyed.

So, when the electrons are coming out this protein, this part of the protein should be ready and that is what we basically mean. So, you can see Fe OH Fe is shown and you see the NADH NAD couple and, electron going to the FAD, then going to and Fe 2 S 2 ferredoxin and then going to the iron center finally, to this iron center. And then into the O 2 and then get the OO activator and then make comes the methanol.

You can see in this also it is very clear this is reductase with the NAD will give it to the flavin from flavin to Fe 2 S 2 from Fe to S 2 to the iron center and that is how the electron flow. So, from here to this to this to this electron flow; so, therefore, this kind of an electron flow will ensure. So, when the electron is coming out this two should be coupled that is where the gamma protein is there these two should be coupled ok, and that is what is shown over there. As a result of that actual reaction happens at this center not here, this is all electron releasing junction this is where, the substrate is converted to the protein product.

So, these referred as a hydrolyze part of the enzyme this is referred as a reductase part of the enzyme. So, the total enzyme you can see over there, I hope you understand that so, di iron center is the reaction center and the NAD 2, FAD 2, Fe 2 S 2 is the electron flow and from Fe 2 S 2 the electron will go to the this line.

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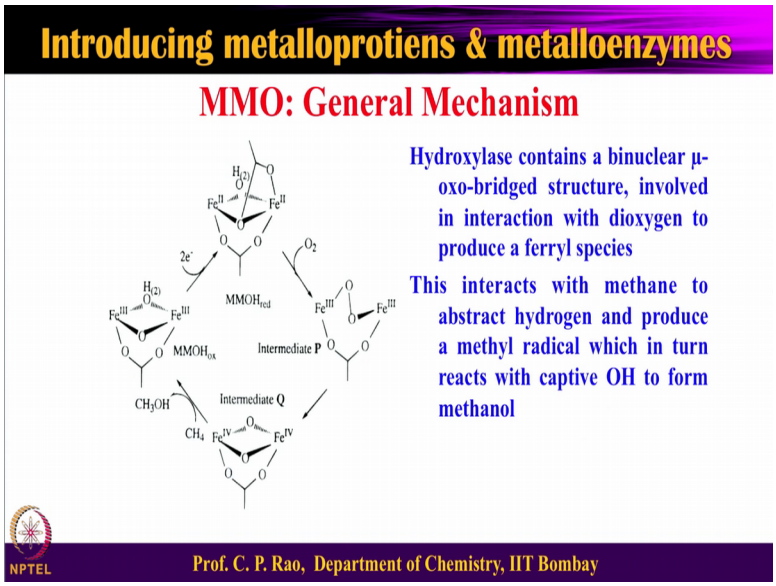

Now, let us look at a general mechanism of these one ok, you have a resting state of this, in the resting state you have the carboxylate bridge as well as a hydroxyl bridge is there. And this one is activated in terms of the electrons there are since there are 2 ions are there, you need 2 electrons 1 each though. So, iron 3 one more iron 3 becomes iron 2 iron 2 now this becomes a very active reduced form.

So, this will grab oxygen because oxygen requires 2 electrons one from this iron, one from this iron can be given when it is given that then it becomes peroxo this is. And this peroxo this is a very reactive it is not that stable for anything, it will break down very fast and form this Fe one of the oxygen will go water which is not shown here, in the next scheme I am showing what it will go water the other 1. And this immediately converts into a ferryl oxo and then that is added to the methane. So, that part is also not shown here.

So, these are called the intermediate P type which is identify intermediate P Q type which is identified in this one, that is why this identified intermediates are being kept here and then so, at the end again (Refer Time: 09:41). So, that is how this whole cycle. So, this is a by nuclear containing oxo bridged species and, interacts with the methane and, abstracts the hydrogen and produces a methyl radical which is not shown here, and the methyl radical in turn reacts with the hydroxyl and which is coming from the iron and, then whole thing will convert into the methanol and, then it will go back.

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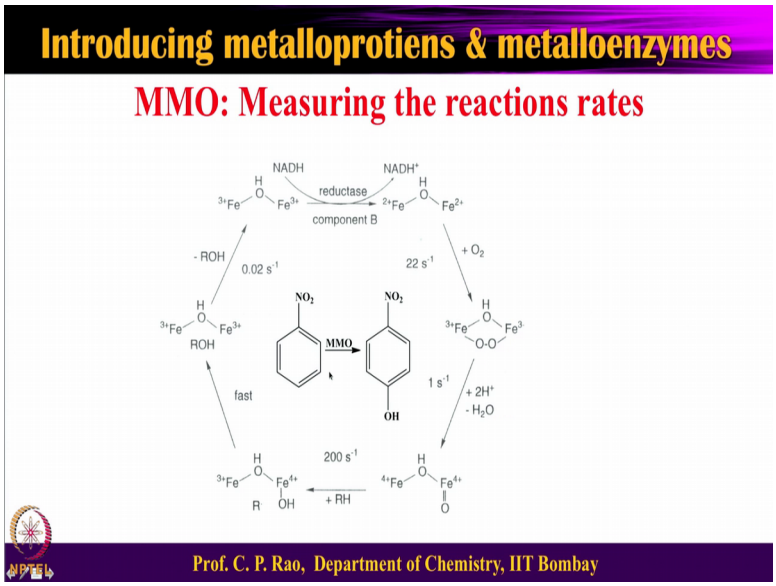

A more understandable mechanism with the reaction rates is shown over here this is your starting enzyme resting part of the enzyme bridged iron 3 iron 3. And this gets active in presence of the NADH with the reductase part that I have shown you and that will give electrons 1 at a time to totally 2 electron very quickly 2 electrons will become iron 2 iron 2 this is a produced enzyme reactive enzyme. This will take up O 2 and convert into initially form some complex and, then break it down. So, these are the peroxo complex and, this is basically broken down.

So, this part will take out basically the OH this is from the bridging this is from this of which one of the O will go as water because 2 hydrogen's are there, H plus are there and the other one is now converted to the substrate. So, the substrate which is bound in the vicinity will form a radical and, then pick up this hydroxyl and therefore, it will go into this.

Now, let us look at the rates. So, initial reductase is of course, the electron transfer is very fast, then the oxygen is a 22 second inverse. And this is and the next one is the breakage of this peroxide peroxo bridge to water and, ferryl and that is only 1 second inverse. And then this activation of the substrate, this is the to form the substrate radical that is also very fast at this stage and, then one more very fast reaction is it will keep transferring and then the product is formed. It is the release of the product which is the low weakest step or slow step.

So, rate determining step in the entire process is the release of the product. So, release of the product, please kindly make a note on this. So, how would one measure all these rates on measure this all this rate, you take this enzyme invitro and put nitrobenzene and look for this one. So, by measuring the concentrations of a how much is converted you can get the rate, as a function of time if you get the do that, you can get the rate ok. So, therefore, this is a very interesting thing.

So, first iron 3 in the resting state then becomes iron 2 iron 2 is very reactive to oxygen O 2 2 minus that will become peroxo; peroxo is not stable to break down to ferryl O and water. And then the ferryl O is now very quickly breaks down the substrate Ca H and then attaches OH to that it will go cycle will go ok.

I hope you understand how a mono oxygenase without the heme is happening. So, with the heme what we have seen ferryl, without the heme when methane mono oxygen again ferryl. So, the ferryl seem to be very important kind of thing and, here what are the steps isolated kind of thing intermediate P intermediate Q these are all understood once.

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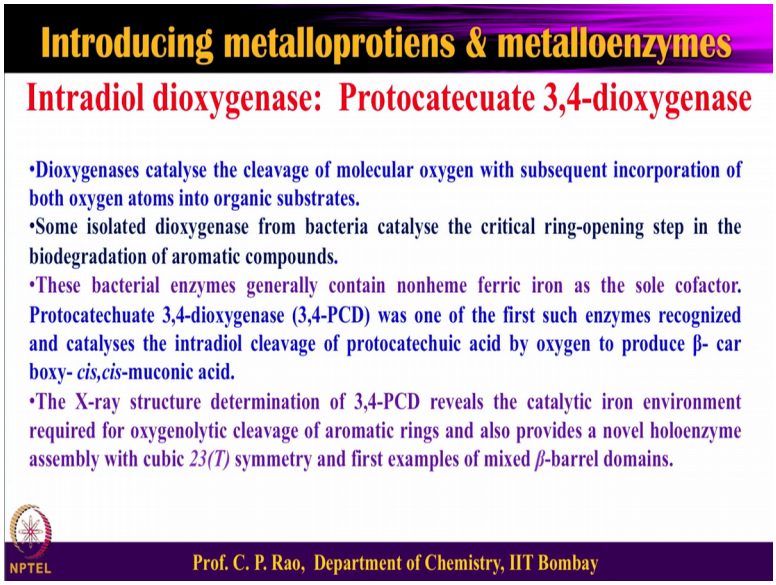

Ok having understood the mono oxygenase based on the cytochrome P 450, mono oxygenase based on methane mono oxygenase. I think now we are graduated to take up dioxygenase. Are not we? Yes. So, in this we will take one example protocatecuate 3 4 dioxygenase. So, dioxygenase as I have already mentioned to you, it will break the molecular oxygen it will 2 O 2 minus and then add both the O's into the substrate ok.

So, some isolated dioxygenase from bacteria catalyze the critical ring openings reactions. And because of this a lot of organic products can be biodegraded. So, there are a lot of pollutions in water. So, what are the carbon pollution is the heavy metal other common thing is it dyes organic dyes, or this kind of thing.

So, therefore one can break these automatic impurities from this particular water to purify water etcetera, by the ring opening mechanism and their dioxygenase is the one which is acting actually. Ok these bacterial enzymes generally contain non heme ferric iron, as the sole cofactor there is no ferry; there is no heme at all. Protocatecuate 3 4 dioxygenase was one of the first such enzymes recognized and catalyzes, they catalyzes the intradiol cleavage of the protocatechuic acid to the oxygen by the oxygen to produce better carboxy cis, cis muconic acid so, protocatechuic acid to the cis, cis muconic acid.

So, there are quite a few structure as a known X ray structure of the 3,4-PCD reveals the catalytic iron required environment required for oxygenolytic. So, oxygenolysis basically you can call it as an oxygenolysis, cleavage of aromatic rings; that means, when you are add when you are adding the oxygen the double bond is broken that is what it basically means oxygenolic cleavage means of aromatic rings. And although provides a novel whole enzyme assembly with some very high symmetry and, cubic 23 symmetric and first example of the mixed beta barrel domains.

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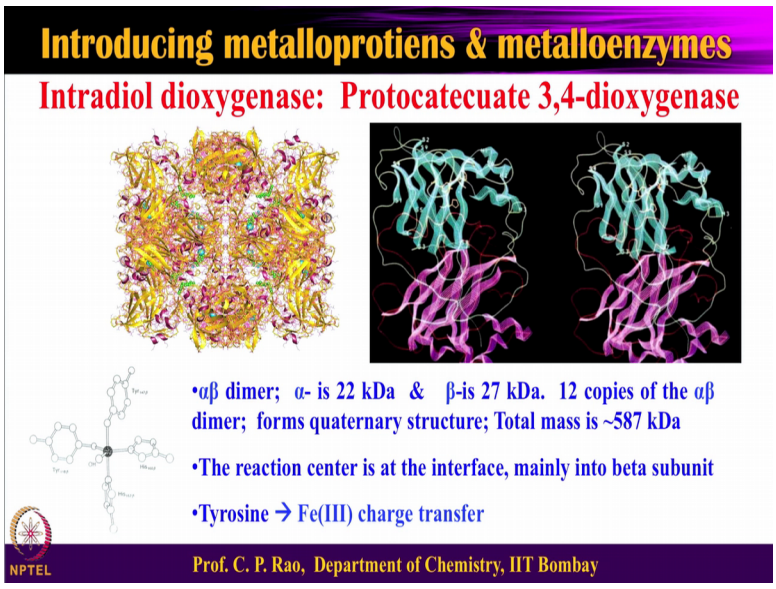

So, this is intradiol dioxygenase with the true, where both the oxygens or added to the substrate. So, this has an alpha beta dimer alpha, has 22 kilo Dalton, beta has got 27 kilo Dalton roughly about 50 total together, there are 12 such copies, 12 such alpha betas; so, 12 into 50; 600. So, 12 into one less so, therefore, 580 590, it forms a quaternary structure in total. So, you can see that, so you can see some in the front some on the backside. So, totally 6 and the 6 total is it 12 so, each one has a pair alpha and beta, alpha and beta, alpha and beta, alpha and beta. So, like that you have a 6 combination 6.

The reaction center is at the interface mainly into the beta subunit. So, alpha subunit means subunit ok, the reaction center is at the bit at the interface mainly into the beta subunit, tyrosine to iron 3 charge transfer. And one another important thing is you can see the center here. So, there are there is 1 tyrosine and the histidine, histidine and another tyrosine.

So, therefore this tyrosine gives a tyrosine to iron 3 charge transfer. So, it is a charge transfer kind of things so, therefore, one can study by absorption, also one can study by resonance Raman as well. Of course, you can study the EPR and NMR as well for binding and other purposes.

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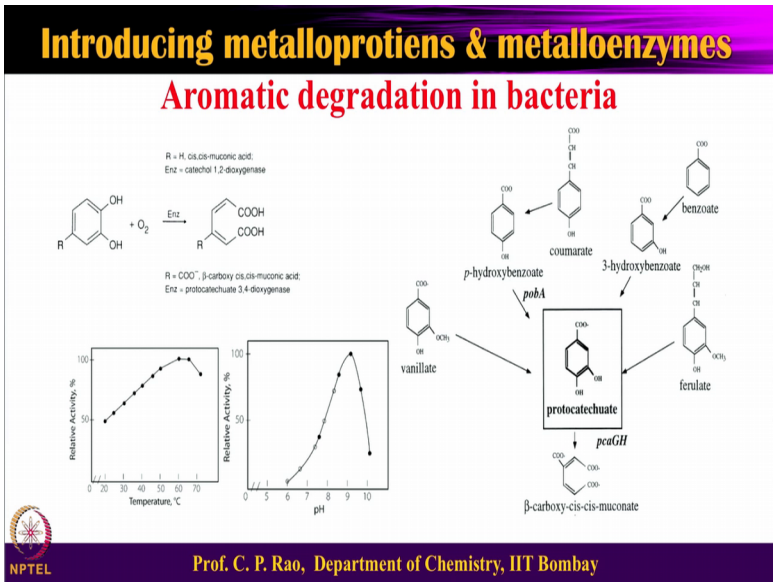

So, as I mentioned to you these dioxygenases intradiol dioxygenases, or also like degrading the organic impurities. So, here we are not taking it as impurity, we are taking it as a reaction of a of a aromatic hydrocarbon. So, you can see that and this cleaves and

as mono oxygen to this mono oxygen to this. So, that is why you get the dicarboxygenase it is called cis, cis muconic acid. So, there are many things in the bacteria in the bacteria in the life, convert into this part of the catechol substituted catechol. It can come from this kind of a background will be at the methoxy, it can come from this where you can add OH here, this can come from here where you can add OH here ok. This can come from this to this to this and mono hydroxyl, they add one more hydroxyl it is meta and then Para, you can come from this.

So, formation of this substituted catechol can be coming from, from the precursors any of these and thereby so, therefore, this is the kind of an impurity you will see, which is called the protocatecuate. And this upon hydrolysis or breakage or additional to the O 2 both the oxygen's, it will break the double bond and gives cis, cis muconic acid.

Now, this enzyme these enzymes are found in lot of bacteria and these enzymes are very robust. When will you say an enzyme is robust? Make a guess. The enzyme is thought to be or is expected to be robust when you show, as a pH it should be sustainable, temperature it should to be sustainable all these kinds of things.

So, if you can show these things, then you can say that that enzyme is robust can work in it. So, you can see here temperature. So, as you increase from 20 to 70, you get somewhere around 60 degree Celsius, it is a very maximum activity is possible. So, around 50 to 60 or 60 degrees Celsius you have a activity is possible high activity still.

And, now if you go to the pH 1 you can see there as you increase the pH from 6 up to about 9 peak point, then there are drops. So, this bacteria will function on all these kind of a molecules, which were forming in terms of this protocatechuate equivalent kind of a species that can be basically cleaved by O 2 of course, it is a weighted O 2 and, then breaks this one and to oxidize to come COO H and COO H kind of thing.

So, now you understand the aromatic degradation. So, aromatic impurities are very common very common water very common in the industries, where the dyes and drugs are synthesized and therefore, the water is contaminated. So, one can use this even for degradation of the organic substrates. So, therefore, enzymes can be taken for their reactivity for their and the reactivity can be converted into this kind of thing, where you have a where you have the substrate and the substrate is basically converted into that ok.

So, you can see that and this, these enzymes in the bacteria work at high temperature like 60 and higher pH like 9. So, quite alkaline and high temperature or ok, with this particular enzyme, with this particular enzyme, these enzymes are a part of several bacteria.

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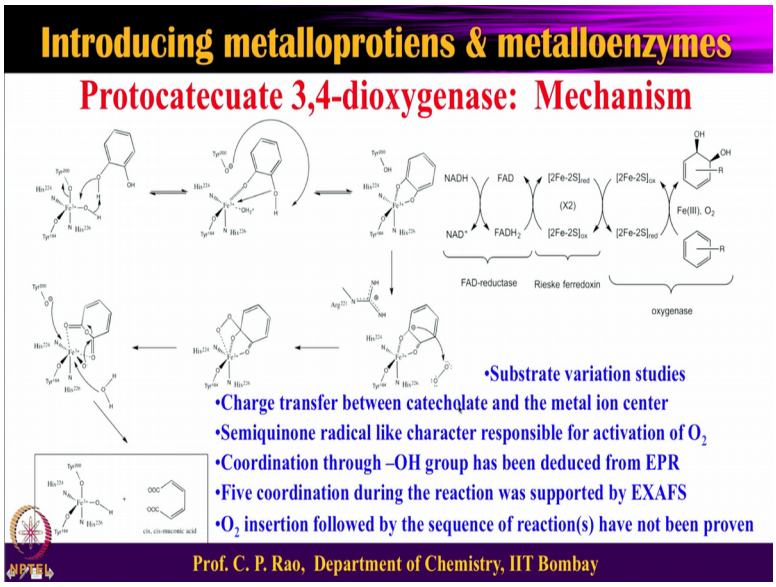

Now, let us look at some mechanistic aspects of this enzyme, we have looked at the mechanistic aspects of the previous enzyme, which is the cytochrome P 450 and now and then we have looked at the one more enzyme methane mono oxygenase, in both the cases it is only one O of the O 2 is being utilized. Let us look at this protocatecuate 3, 4 dioxygenase, a dioxygenase case mechanism.

Now, I mentioned to you it is a 4 5 coordinated center with trigonal bi pyramidal, having to tyrosine groups. And when a catechol kind of a moiety comes catechol will bind. And this will give one proton to this and the tyrosine metal bond can be temptatively disrupted ok. And this can give rise to a kind of a species of this type where both are bonded.

In fact, both the mono binding initially followed by chelation have been identified based on proton NMR spectroscopy, when you have this way you are more of a symmetry, when you have this way you are less symmetry therefore, proton NMR is capable of talking about this, you can also use the EPR because your iron center. Both the iron

center as well as EPR, EPR you have to look at the so, this is a basically the coupling values that can explain you in this ok.

And people have looked at even the exhaust of course they have looked at the resonance Raman of these species. What is a resonance Raman? It is the vibrational frequencies of the attached O, or oxygen species or the substrate which is studied by vibrational spectroscopy, while passing a light which brings an electronic transition.

That means while the electronic transition takes place, that you are trying to study the study the vibrational pattern because, there is a vibronic coupling which will enhance the enhance the bands, in the resonance Raman, I also said that based on the proton NMR whether, it is a monodentate binding and bidentate binding can be determine too.

So, these are all very well worked out. So, what you do is you put some lot of these groups here, which are hindering groups then it will stop at the stage of the binding. So, therefore, that kind of things are studied by proton a proton NMR and EPR. So, the finding and activation by chelation and, now this particular thing; obviously, is involved in the electron transfer etcetera. And there are also some residues which are nearby which is protecting the thing.

At this stage that you have an activated form of the iron 2 activated form of the iron 2 can take up the oxygen and form the this kind of a species and this species is active. So, it will break and one of the water will go and, you have your substrate part of the of course, the substrate into this through. Now what is it happening the substrate, now initially a catechol. Now, then it is becoming a semi quinone in these steps and then becomes the quinine.

So, you have a this quinone kind of a derivative. So, and this derivative can be can very clearly break down here and give the cis muconic acid. So, that is shown over there and give this particular starting material kind of things. So, which is the protein where the iron is a 5 coordinated with two of them are tyrosines in this kind of thing.

So, now do you understand how this takes place? You have active way by a substrate binding it should be always the first in all these oxygen activating enzymes this oxygen should not be the first step because, immediate otherwise immediately the oxygen get

activated, or oxygen bond gets ruptured and then that can lead to the oxidation process to the enzyme so, we do not want that.

So, monodentate can bind bidentated can bind, this has been very well studied by proton NMR and EPR spectroscopy. And this is now ready for electron transfer going into the 2 and then you have the oxygen being activated at this stage. And this is not so, much stable and so, it will form a kind of a ring like structure with the product and, then that will open up and, then it will give the cis cis muconic acid and the original molecule a original center of the iron containing center.

So, now you are understand that very nicely everything is happening here, now earlier we have seen mono oxygenase part here, we have seeing dioxygenase here no water goes out because, both the oxygens are added here, as you can see over there the both the oxygens mono oxygen. And then one more oxygen is added and finally, you get a this kind of thing it breaks cis, cis muconic acid.

So, substrate is being varied and studied you can make the bulky and you can stop the reaction at the stage 2, you can study the charge transfer between the catecholate and the metal center which has been studied. Then you have a semi quinone radical like character, which is also studied and which is responsible for the activation of O 2 coordination through OH group has been reduced from EPR and NMR EPR and NMR, 5 coordination during the reaction was supported by exams go to insertion followed by the sequence of reactions and many of these I have not been proven, but you have the things.

So, this part that means, we have seen 3 different enzymes one is cytochrome P450, one is methane mono oxygenase, both of these are mono oxygenases. Then we looked at one example for the dioxygenase that is the protocatecuate 3, 4 dioxygenase. And these dioxygenases are the kind of one's which break the aromatic organic aromatic substrates because, it can add both the oxygens. Aame thing if you do by mono oxygenase will it happen no because, it will add only one OH. So therefore, there is no breakage or no bond whereas, this particular enzyme, dioxygenase enzyme acts the both the O 2 and both the attraction.

Now, look at for a while this particular scheme of the electron transfer NADH NAD plus is always there, then it will go to FAD H 2, then the 2 iron 2 sulfur one more via 2 iron 2

sulfur, then at the reaction center. So, finally, at the, this is the ion center and that will add the electrons to that.

So, electron flow could you see? Could you see the mechanism of substrate binding monodentate fashion, bidentate fashion, the quinine, internal intramolecular quinone that will become the iron 2 plus this iron 2 plus is ready for activation and, that will add the oxygen and, then that will convert into the peroxide and, then break into the 1 O and that is O is introduced in there is no water goes away, the second O it will basically break this and give this particular starting and this is cis muconic acid.

So, in essential we have studied 3 examples of the oxygenases mono oxygenase by dioxygenases, mono oxygenase, the cytochrome P 450, the another mono oxygenases methane mono oxygenase and the dioxygenase all these and dioxygenase, or also well used from bacteria you dont need to isolate the enzyme. Just grow the bacteria and use the bacteria, incubate the bacteria with the water and containing this kind of a thing or, sample containing this aromatic hydrocarbons they will break. And they will go into the soluble form you know why, because, you are forming at the end the carboxylic other kind of carbonyl carboxylic kind of a groups, therefore they are all very ok.

So, in the next class I will try to start with dioxygen centers in reductase phosphatase like hydrolyzed. So, I have given oxygenase what is left is phosphatase and reductase, these two will be taken in the next I think 3 to 4 classes or so, and then I will try to finish the this part of the iron enzymes; may be at least 3, 4, 5 lectures will be there.

Thank you very much.