Inorganic Chemistry of Life Principles & Properties
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Lecture – 29
Role of Iron in life – Monooxygenases: Cytochrome P450

Welcome you all to the next class on Inorganic Chemistry of Life Principles and Perspectives. In the past 2 classes, I have been trying to explain the electron transfer in general as well as in electron transfer in with reference to the enzymes ok. Now having learned the transport storage of oxygen transport storage of iron transport of electrons etcetera of course, you do not store electrons.

So, with all these now we are very well equipped to go to the next topic which is the enzyme reactions of ion, which are oxidative reactions, reductive reactions and hydrolytic kind of reactions; all these 3 we will see some example or the other. We will start with oxidative type for the first as a first part of it and in the oxidative type; these are referred as the oxygenases.

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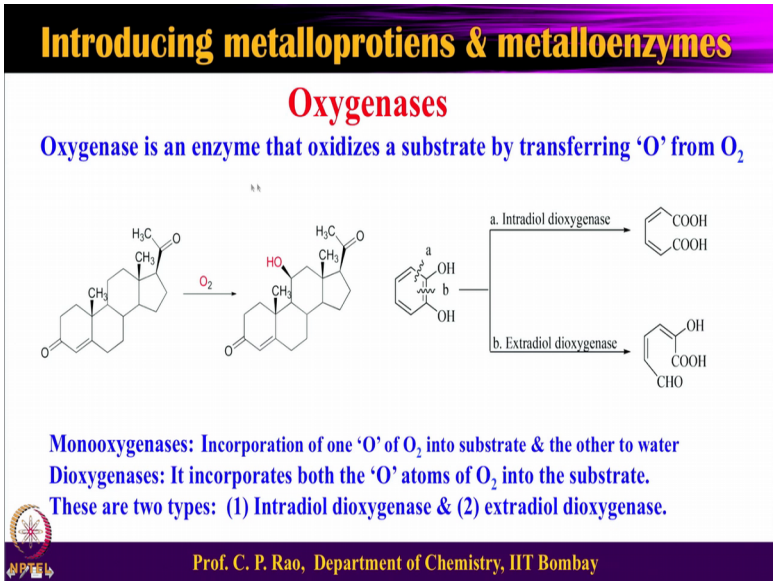

So, oxygenases are the enzymes which oxidize substrate by using one or both of the oxygens of the O 2. It will use either one O of the O 2 or both the O was an O 2; if it uses one O it is called monooxygenases, if it uses both the O's it is called the dioxygenase.

So; that means, monooxidation and dioxidation and that is what basically means. So, when it does the monooxygenase property; that means, only one oxygen from the O 2 is used for substrate oxidation, the second O will go as water. In case of dioxygenase, both the Os of the O 2 or utilized for the substrate oxidation; just look at an example some kind of you know heterocyclic kind of an organic substrate and this with oxygen and giving the hydroxylation of this.

And the second oxygen will go as water. In fact, one can one can use this labeled one and find out the labeled water as well. So, this is called the monooxygenase because it is using only one O of the O 2 to oxidize the substrate ok. Second part of this is the dioxygenase; the dioxygenase means both the oxygens are added to the substrate. Now in this there are 2 ways for with respect to a double bond, this is the double bond you can just add across the double bond in a equal way.

So, O you can use outside this double bond; so, if you use within this it is called the intradiol dioxygenase and if it is outside it is called extradiol dioxygenase ok. So, when the intradiol dioxygenase; it will give this kind of a product and if it is a extradiol dioxygenase it gives the other product which very well. So, we will look at later on some of these enzyme, their you know mechanistic aspects etcetera.

So, I hope from this slide you understand what is oxygenase, what is monooxygenase, what is dioxygenase. In the dioxygenase you have intradiol dioxygenase, extradiol dioxygenase; now, how does that add how does that add the O?

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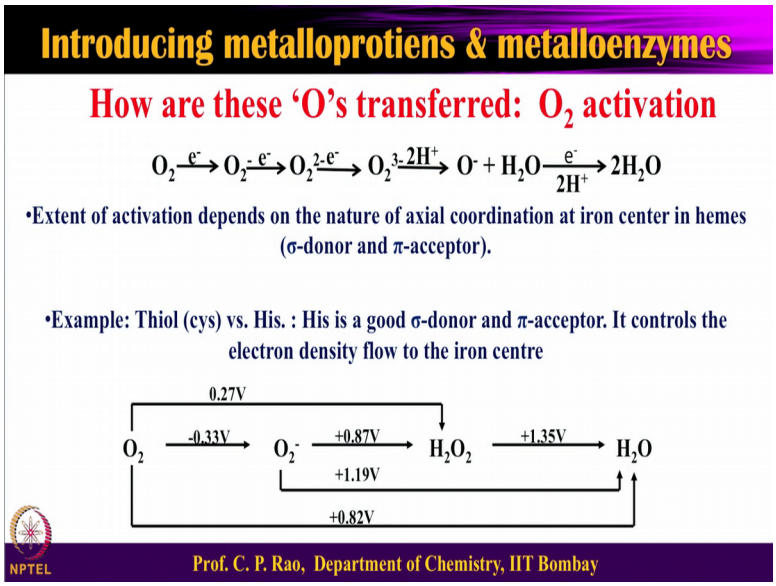

So, how is O transferred? Or in other words what is a O 2 activation? Say why O is required; why how can you get O out of the O 2?

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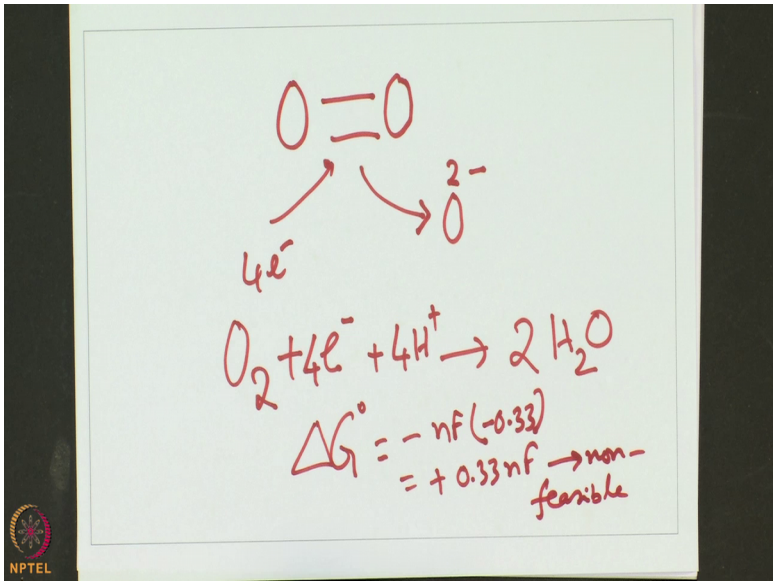

So, O 2 is a diatomic molecule with double bond. So, if we want to get from this O 2 minus; obviously, you have to pump 4 electrons inside. So, if you do not have this you will not get this. So, first 2 electrons will break one of the bond, next 2 electrons will break the other bond totally 4. So, that is because the number of bonding electrons minus

n number of antibonding electrons and that should become 0. So, in order to take the bonding out; so when this happens that will be the thing.

So, in other words the O 2 add 4 electrons and 4 in presence of the 4 protons, it give 2 H 2 O. So, if there is no substrate both the oxygen will go to the water; so, this is what we called as a O 2 activation. So, as you can see 4 electrons have to be added; so, you cannot add all the 4 electrons at one time. You have to add one electron, add one more electron, add one more electron, add 2 protons add this; so, that will become 2 H 2 O.

So, O 2 one electron will go to O 2 minus turn, one more electron becomes O 2 2 minus turn, one more electron becomes O 2 3 minus because that will not be very stable and that will break down to O minus and O 2 minus. And O 2 minus will combine with 2 hydrogen H plus and become H 2 O and one more electron that will become O minus will become O 2 minus and take up the 2 protons and the water.

So, as you can see in case of this the reduction or the O 2 or activation of the O 2; you also have concomitantly protons are associated with it. So, all this depends on what? Depends on the redox potentials, so the activation; obviously, depends on the redox potentials and the redox potential in turn depends upon the in case of a hints, it depends on the axial coordination at the iron centre.

So, whether the coordination is more; sigma donor more; acceptor pi acceptor type or a combination of these that will determine how easy that you can get the electron, how easy you can reduce. So, that is most important because the iron redox potentials will depend upon what is the surrounding, what is the bonded immediately, what is the hydrophobic; hydrophilic environment that is being created? All these will influence the potential of that; so, therefore.

So, for example, if you take the coordination we have seen earlier; either you can have a thiol or you can have a histidine etcetera. So, thiol or histidine when you compare the histidine versus the thiol or cystine; histidine is a good sigma donor and pi acceptor. So, it controls the electron density flow to the iron center. So, therefore, the enzyme the nature is small and nature tries to arrange the requisite group so, that the potential shifts as per the requirement.

Now, whatever we have seen this electron transfer process let us look in terms of the potentials, then we will be able to discuss and debate. Now you take O 2 and one electron, it will become O 2 minus dot and what is the potential here? Minus 0.33; that means, its negative potential ok. So, if it is negative potential what will happen? Delta G is and this is minus n F and minus 0.33. So, that will become plus 0.33 n F. So, this is what is this feasible, this is non feasible; clearly it is a non feasible reaction.

So, this means the first electron which we are talking about here cannot go easily spontaneously. But once let us assume for some reason, we are getting this in; how we will see that is why the enzyme is required, otherwise you would have just thrown the electron into that. The next part is O 2 minus one more electron O 2 2 minus inference of 2 protons it will become H 2 O 2 and this is a plus 0.87. So, it is very plus large therefore, delta G is minus therefore, it is favorable. The next one going from here with 2 more electrons and 2 more protons, we will get 2 waters not one water 2 waters is again positive.

So, in entire step of this the first step is the negative potential; suppose it has to go to O 2 directly to H 2 O 2 is a positive O 2 to O 2 minus to H 2 O that is also positive; O 2 to overall H 2 O is also positive. So; that means, the conversion of O 2 to water is a favorable reaction though the first reaction is formational superoxide is not a favorable one. So, this is thermodynamically unfavorable therefore, the protein has to bring down the activation energy and make it make it accessible and that is where the proteins role is coming into the picture.

Now, you understand how the O 2; so, O 2 breaking down to O to minus requires 4 electrons then to become water for more protons. So, O 2 plus 4 electron plus 4 H plus is to H 2 O and that is with. Now having studied the O 2 activation; how the OO breaks that now we understand that is how the enzyme breaks it that is how the enzyme breaks; enzyme adds first electron, then second electrons, then third, fourth electron. Once it goes the third electron reaction is very fast it will go to the fourth electron without any realization of that ok.

And then we put on pump parallely and protons will be added therefore, at the enzyme center the O 2 can be. So, at this stage if you have a substrate; it can add the one of the activated O or both the activated Os can be added to the substrate and thereby it will turn

out to be a monooxygenase or a dioxygenase. Let us look at very popular enzyme of example; so, a popular enzyme for this is cytochromes P-450 why is it popular?

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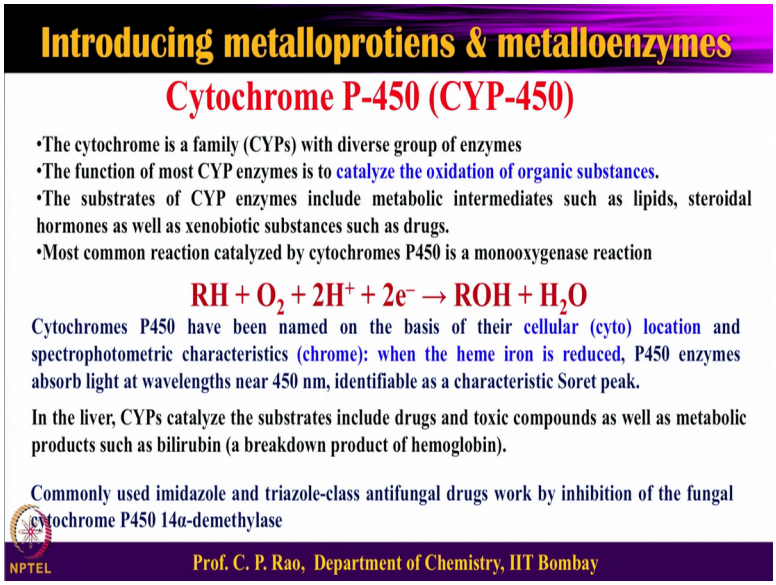

So, why is that so, popular?

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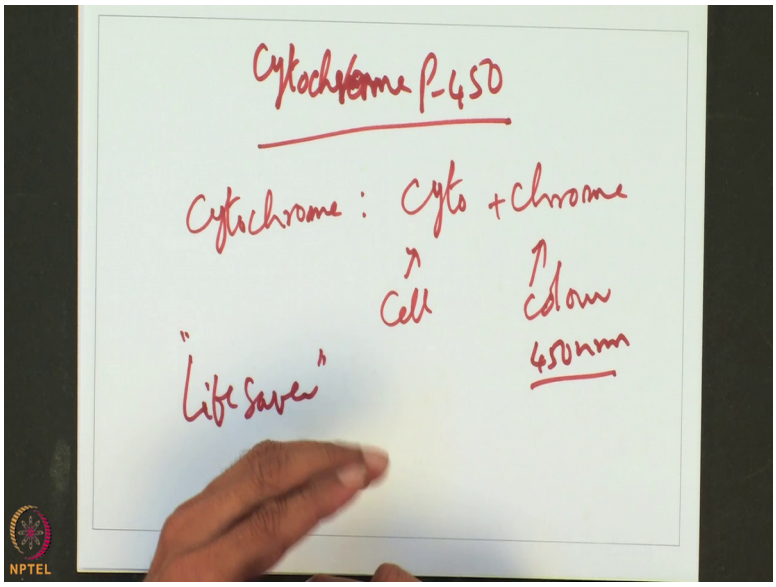

See so, cytochrome sorry cytochrome P-450; so, before we come to the question of why it is popular? Let us come to the word cytochrome has got 2 words cyto and chrome. So, cyto is cell and chrome is color; so, that is why this is these are the cellular proteins. So, cyto and color and the color will be absorption that will be in the nanometer and P-450

cytochrome P-450 is 450 is 450 is the nanometer absorption. So, that is our nomenclature in cytochromes will come and you do not know.

Now, let us look at why is this cytochrome P-450 is so, popular? So, cytochrome P-450 once upon a time was considered as a lifesaver, it is considered as a lifesaver, but later on it was found it is not true. The reason why the reason; reason is cytochrome P-450 as I told in the beginning of this course is involved in oxidizing the a polar substrates, nonpolar substrates, organic substrates to ads the hydroxyl group or oxide oxidizes and makes it more polar or soluble kind of thing; that is were the fatty acids fats that we eat can be digested that is where I talk to you.

But now we can also learn there they are involved not only in converting those oil stuffs, a variety of metabolic intermediates in the cell; they could be lipids, they could be steroids, they could be hormones; all of these can be hydroxylated by this enzyme. So, the main thing is it catalyzes the oxidation and cytochrome is a is a family of enzymes and, but though we are talking about this particular enzyme here.

So, the main reaction RH is categorized as some kind of a hydrocarbon; oxidize, oxidation 2 H plus 2 electron. When oxygen activation what did we study? 4 H plus for 4 electrons, the reason is you have to convert that into both of them into water, here you do not need to convert into water. So, you need only one O 2 convert into water that is why you require 2 H plus and 2 electrons and therefore, this RH.

So, it is basically inserting one of the O into the RH bond that will become ROH; inserting one O of the O 2 into RH bond ok. So, these are the cellular it catalyzes a lot of reactions particularly of toxic compounds also; drugs metabolites as I said. So, therefore, this is not a lifesaver later on people found and if you see the recent books, they will not write this as a lifesaver, but if you see the very old version of biochemistry books; obviously, you will see there is a lifesaver ok.

So, this can be in that and you know lot of imidazoles and triazole kind of a molecules; they will basically inhibit the activity of these there they act as a inhibitors of this ok. So, we need to look at some details of this enzyme, how it functions etcetera. This is a very highly prevalent enzyme in the entire biological systems and it is based on the cellular based systems and if you can is involved in the oxidative process and you can have the in

this case the monooxygenase. Having studied some basic aspects of cytochrome class and cytochrome P-450, let us look at the electron transfer; electron transport.

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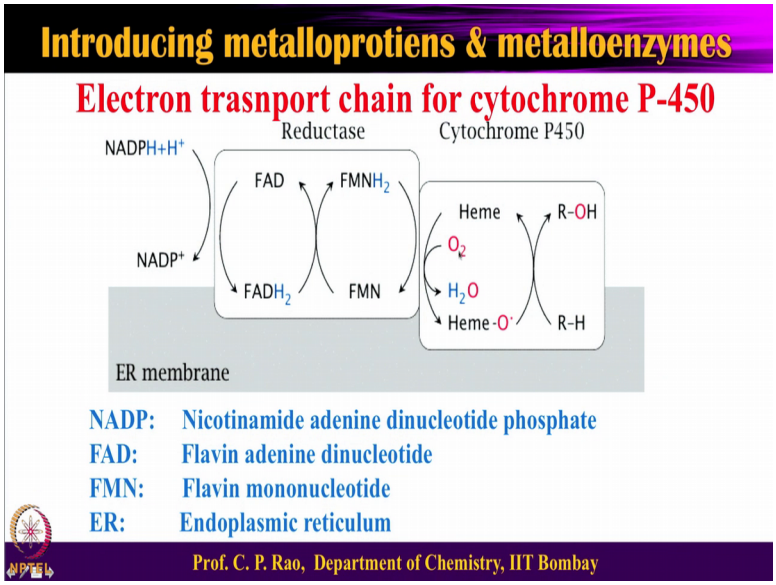

So, this is in this particular scheme you have some part here, some part here, some part here you have; 3 portions we are talking about ok. This is the last part where the cytochrome P-450 is involved and this is the last part where RH is converted to ROH and this is coupled with the heme a cytochrome P-450; this part is connected with the cytochrome P-450. But cytochrome P-450 on its own cannot do, it has to get electrons and taken from something, that is taken from something, that is taken from something.

So, you see that; so, at the end the beginner in this is the NADPH NADP plus. So, when NADPH oxidizes to NAD plus what will do? It will release a one electron. This electron will convert FAD which is oxidized form into FADH 2 which is a reduced form. When this reduced form of flavin adenine dinucleotide turns back to oxidized that will give an electron. And that electron will convert the flavin mononucleotide into flavin mononucleotide which is reduced form.

So, when this reduced form of the flavin mononucleotide return backs to the oxidized form; that will transfer the electron that will transfer the electron to cytochrome P-450; which is in the oxidized form and takes of the electron goes to the reduced form. And this in turn will break the O 2 to one part of the O 2 H 2 O, other O is added to ROH; how it is done? That is called mechanism; I will be explaining in the later slides; maybe

after one or 2 slides later I will be explaining how this O 2 would give one O 2 this, one O 2 this; so, that is all.

So, is that understandable? NADH, NAD plus is a standard source of electron and this is give this gives the electron to the FAD and makes FAD reduced. And then FAD reduced when return backs to the oxidized form will give electron to the FMN and then that is flavin mononucleotide will get reduced, when the reduced flavin mononucleotide returns back to the oxidized form will give the electron to the oxidized form of the cytochrome P-450 and cytochrome P-450 will go to the reduced form and that will give the electron and then electron that will break the oxygen and then you have one or going with this; other one going is the water this is the kind of act.

But generally we do not look at all these all the time and we look at these one. So, the mean this enzyme must have a set of FAD cofactor; it should also FMN cofactor and then by the heme cofactor. Of course, NAD plus is always available in the cell and that will give on this one, but this cofactor and this cofactor has to be a part of that kind of things.

So, why so many things are why cannot this directly give to this? So, if you look at the redox potentials of this with respect to the heme, they will not match at all they are highly negative. And so, therefore, it needs to go through one more cycle, one more cycle and then finally. As I told you in the previous class, the difference between the donor acceptor potentials within 0.2, 0.3 is good enough; greater than that it will not be it will not be a reversible electron transfer process ok; so, is that clear?

So, that is you cannot have directly an electron going from NADPH to the heme because of the redox potential differences that we have. Now let us get into the get into this enzyme structure, other things etcetera.

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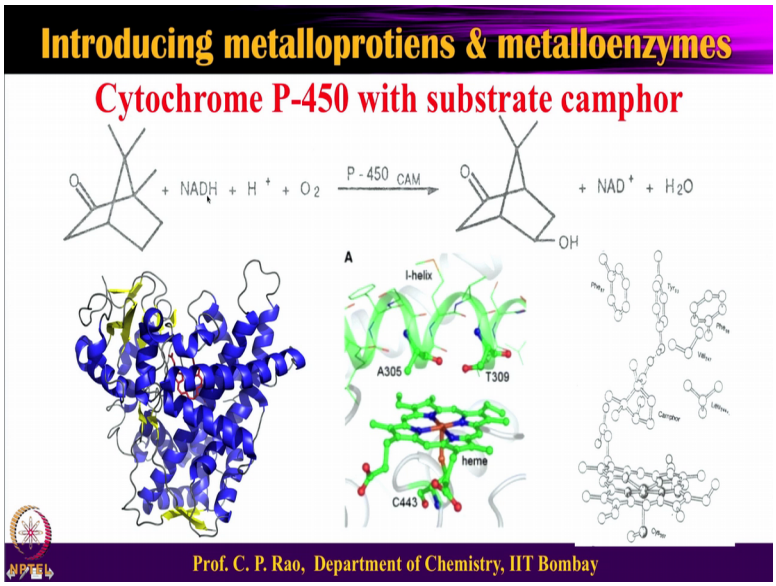

Now, this there is several cytochrome P-450 have been studied for the structure and the reactivity, but I will be taking only one example that is cytochrome P-450; which oxidizes the camphor, which oxidizes the camphor.

So, therefore, this is the; so this is the camphor and this requires as I mention to NADH of course, the other equivalents are already there H plus O 2 and what is required? This enzyme cytochrome P-450 camphor; CAM refers to camphor, P-450 refers to cytochrome P-450 camphor that will give a product where hydroxylation is here; not here, not there not there not there use hydroxylation is only here.

So; that means, it is the position selector and also in this particular kind of a geometry. So, it is stereo selective and regio selective; so the product of this camphor upon intra upon catalysis by the cytochrome P-450; the product has a selectivity and specificity and this is both regio and stereo selective. So, this is the total protein and has some way here the heme and then you have FAD FMN, which we are not showing over there.

Now, if you look at this expand this region and look at this where you can see that. So, what is this has? This has the heme iron is a other coordination here and this side is empty, but very close proximity; you have a lot of residues are there. So lot of residues are there; now this is where what we need.

How come this is getting such a very high selectivity in terms of regio and stereo; you see this particular thing. This is from a structure not from a apoprotein before the substrate is bound; it is regular protein, but before substrate is bound this is a structure after the substrate is bound.

So, actually if you put substrate like molecule you can always get the structure because that will be inhibitor. So, you can see there are residues here and there and there; phenylalanine tyrosine all these kinds of flavin all these residues and this is your substrate. And this substrate is not just left alone there are some interactions between these ones, these ones and these ones. So, there are some hydrophobic as well as this interactions.

So, all these interactions and the size of the core gives selectivity to recognize the camphor; if some other molecule comes here, it will not be recognized. So, they feel that will not be considered as a substrate and will throw it out. So, the enzyme is active; it will understand what is its right substrate; how do it understands? From the weak interactions coming from all these, they will touch in some way; they will touch in the form of hydrogen bond, they will touch in the form of hydrophobic interaction, they will touch in the form of Van der Waal interactions etcetera all these interactions will call will make it whether the right substrate has come or not and this you can see that all of these are there.

Because this is a held like this only this portion only this portion is accessible therefore, only that portion is exactly on the top of the iron is exactly on the top of the iron and therefore, oxidation or hydroxylation takes place at here not there, not there, not there not any other place. So, how come is the camphor is getting the stereo and regio selectivity Stereo and regio selectivity of the camphor is coming because camphor is held; in other words the substrate is held on this enzyme only in one fixed manner, where that particular carbon is straight exposed on top of the iron center.

Why should it be on the top of the iron center? That will be answered when we get into the mechanism because the iron center is the one which are which activates the O 2, creates the active O species therefore, it should be close to that proximity. So, therefore, this substrate and this position is exactly aligned on top of that; for aligning this whole

protein is playing a role. So, therefore, all these secondary interactions are reacts absolutely important to do this one ok.

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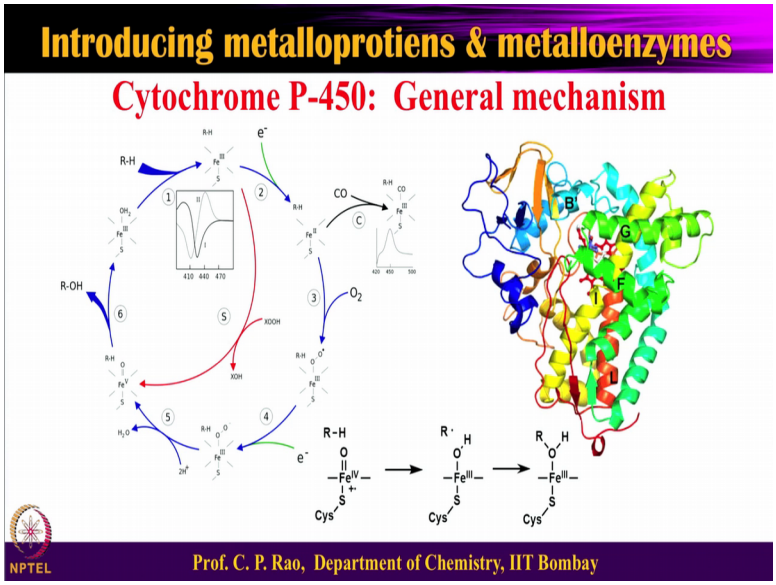

So, you understand that? The selectivity how it is coming; now let us look at a little bit more interior into this that is the kind of a mechanism, how is this able to do that.

So, as you can see that this particular is the starting step and this is in the iron 3. And you have a cysteine on this side and you have a empty space on this side, but in the resting protein the sixth position which is empty is never empty is always with water; so, that is called that will indicate is a resting part of the enzyme. Now this resting part of the enzyme when the substrate RH is a substrate comes in proximity to that particular protein in that, the protein gets activated.

So, protein starts thinking that I must do a reaction and that is how it is gets activated. So, it comes in the vicinity; you already seen in the previous thing where it binds, how it binds etcetera. So, now, the protein is active therefore, the step start working. So, the it will we ready to take up the electron; from the electron transport series which I showed you on the 2 slides behind. Once the electron comes that will be that will be iron 3 becomes iron 2.

And this first step has been identified; how it is identified? This is trapped. How it is trapped? You just pass CO at this stage and then that will convert into one of the complex

and this complex has a absorption IR; all kinds of things can be monitored and studied and then that study. So, this is not a part of the; this part is not a part of the mechanism, this part is a part of identifying this intermediate.

Now, once that becomes this iron 2 and now iron 2 is active towards the oxygen because iron 2 can always shuttle to iron 3. So, it means it can add an electron; so now this is ready to O 2. So, therefore, now you see tell me suppose if this O 2 is ready at this stage itself at this stage itself or what will happen before the before the substrate comes? Suppose at this stage itself O 2 comes, suppose if the O 2 is a first step what would you expect? If the O 2 is first step then the O 2 gets reduced and that can add to the protein and protein can get destroyed.

So, that is why it has to get activated only after the substrate comes. So, this is a substrate then the activation electron transfer iron 2; now iron 2 you now is ready it will take up the oxygen and it will add one electron from itself to into the O 2. So, when you add first electron that becomes O 2 minus dot is superoxide; you know this is favorable now. Because it is activated by the enzyme this is activated by the enzyme therefore, it is ok; it is not a free O 2 it is activated by the enzyme.

So, therefore, first step which is otherwise not favorable has made been made favorable therefore, the role of the enzyme is to hold the oxygen, to activate the oxygen. Then at this stage one more electron will be added and that will become O 2 minus. So, that is O 2 minus; now the O 2 2 minus already one bond is broken. So, it is very active in stage can in presence of the 2 protons can take out this OO, break this OO into water and bring an internal redox; these electrons are not coming from the other internal redox.

So, internal redox you require 2 electrons; so therefore, iron. So, the iron gets oxidized either 4 plus and with the powerful and radical or 5 plus. So, therefore, therefore, this thing is now activated this becomes a ferryl oxo species. This oxo species is a very active species, it will very immediately add to the substrate and substrate becomes RH becomes ROH and then returns the back.

So, in all these from here the steps are very fast. So, this this part and this part are very fast; so, the first part; the first electron and the O 2 these are somewhat lowest lower in step very likely this may be the may be the kind of a thing where you will have a determining kind of a step. So, you can see that all of these are the kind of a

intermediates and the spectrum overlap over here for one and the 2 and that is how one can make out the difference that is what is happening.

So, what is happening? The now this forms a ferryl oxo; ferryl oxo will oxidize the RH to R dot and OH, then this will become ROH and this is kind of a rebound mechanism ok. So, understand now iron is an iron through; first step should be the addition of the substrate, substrate should add if the O 2 gets added then it is a devastation. Because it will get activated and the protein will get hydroxylated; that means, protein commits suicide protein commits suicide.

So, therefore, to avoid this then first you have the substrate; then you have the electron transfer then followed by the 2 electrons and that breaks and then gives a ferryl oxo and then that may continues to form this kind of a thing ok. So, therefore, overall what you have seen? That one is a ferryl oxo formation, other is the CH activation. So, upto ferryl oxo nothing will happen to the substrate; substrate is just being sitting there, but substrate has to be there in the beginning.

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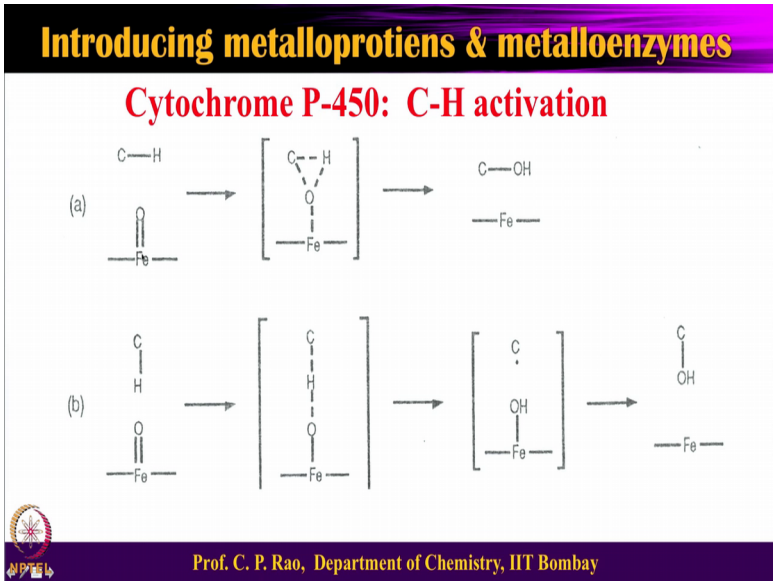

So, now ferryl oxo is formed like this C H can be that way; C H can be this way. So, in either of these you can see in this kind of a thing you can have a transition state of this try you know cyclic and then this can break down into co instead iron. Or it can need not form a cycle kind of thing it in form a linear bond or this and this can break down to a Fe OH and C dot and then this will recombine to form the C OH recombine to form.

So, these things are possible there is lot of mechanistic approaches have already been ordered in the literature; it is not that the further details are not known, but for this particular course beyond this will be become it too much more. So, in this lecture what we have learned? We have learned there is a class of enzymes called cytochrome P, in that we have taken an example of cytochrome P-450 cyto refers to the cell, chrome refers to the color and the here in this case the color absorption is at 450 nanometer. And these are all the they are involved in oxidative process they oxidize substrates, lot of substrates even metabolites, intermediates, drugs all these kinds of things and they give other kinds of formation of the product and those are all dangerous products.

In fact, I tell you at this stage I should bring one aspect to your notice; the cigarette smoke has the benz of iron. You know we know that the cigarette smoke is carcinogenic is carcinogenic people think it is becomes the benz of iron, it is not true; it is the metabolite of the benz of iron where the double bond is converted to epoxide that epoxide is the one which is carcinogenic; not the benz of iron itself. So, therefore, the P-450 cannot be lifesaver because it is a converting lot of drugs intermediates everything into OH and therefore, there is a dangerous kind of a part.

And then we have looked at the electron transfer first substrate binds, then the electron transfer then 2 electrons transfer peroxo. The peroxo will quickly break down to ferryl and water and the ferryl will quickly add oxygen to the substrate. So, the slow steps in this or the substrate binding and the first electron transfer or other processor very fast and they go at the rapid rate.

So, that gives the and then I have talked to you the 2 possible ways of C H activation and the and the ferryl oxo species. The specificity in selectivity of the camphor is coming because the camphor is oriented very nicely on top of the iron, where they have ferryl oxo species is formed. So, in the next class we will look at the dioxygenase and some example or even monooxygenase; which is not aromatic substrate or any kind of these ones, then look at some other methane activation also we can see.

Thank you very much.