Bioinorganic Chemistry
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Lecture - 9
Electron Transfer Proteins - V

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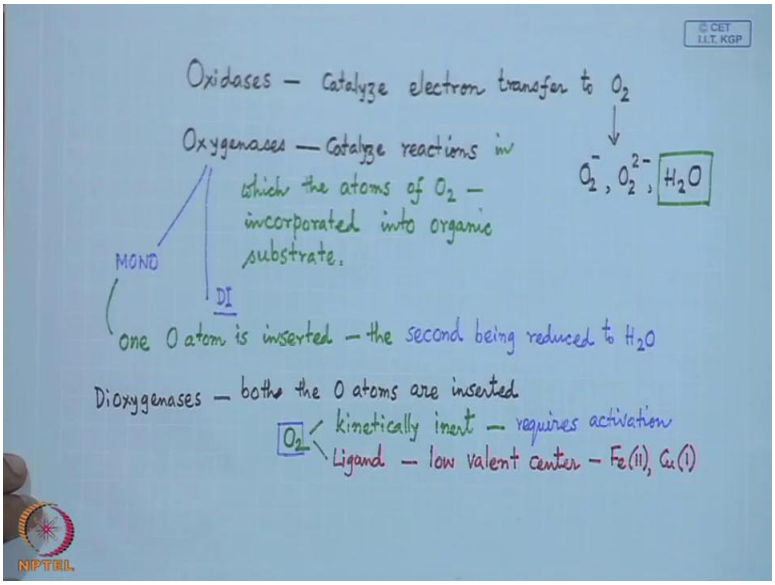

Welcome. So, still we are within that electron transfer proteins. And today we will be discussing on oxidases and oxygenases which we have some clear idea about what we are taking by saying that some of them are iron based oxidases, and some others are oxygenases, and since we are all utilizing iron center and this particular oxidases, they catalyze electron transfer to our di-oxygen molecule.

So, that is why they are all belongs to that category of electron transfer proteins. So, when we transfer electron to this O 2, we all know now that they can be reduced to O 2 minus O 2 2 minus or H 2 O. So, this particular electron transfer is first of all very important. How we can transfer this di-oxygen molecule containing the some extra electrons which can activate this di-oxygen molecule.

So, when we utilize the iron centers, we see that then when iron is interacting, not with this single bond which is forming with O 2, but when it is interacting with this O 2 by both outer sphere and inner sphere mechanism, this iron can donate the electron to this O 2 by reducing it to the iron two center, but these oxygenases are different; they basically

catalyze some reactions in which we use directly, in which the atoms of O 2 are incorporated into the substrate molecule. So, if our substrate molecule is organic molecule, it would be then a organic substrate. So, we will have the opportunity to basically transfer both the oxygen atoms from O 2 molecule to a organic substrate.

So, these two basically can now be sub divided into two, one will be the mono-oxygenases where we can insert only single oxygen atom. So, in case of this mono-oxygenases, one oxygen atom is inserted within the substrate molecule. So, what happens to the second?The second one will go for reduction to our water molecule. So, the secondthat means, the second oxygen atom being reduced to H 2 O. So, this particular oxygen is when we consider it for its behavior for di-oxygenases. So, the other category will be di-oxygenases. So, it is now obvious in that particular case; that means, in case of di-oxygenases we can insert both the oxygen atoms into the substrate.

So, both the oxygen atoms areinserted within the substrate molecule. So, it is very important that we can have at one hand; that means, we can just simply go for mono oxygenases reaction; that means, we utilize one of the oxygen atom for activation and corresponding conversion, in other case, both the two oxygen atoms. So, that is why we have two different types of catalytic systems, one is your mono oxygenase and another is di-oxygenase. So, this O 2 molecule, all we know is a very interesting molecule for us because this we can level it as a kinetically inert species because we know it remains in a triplet state. So, what happens? It requires all the time, some amount of activation. So, its requires activation. So, this particular part from the biological system, what we find that how the different avenues are available, how we can activate the di-oxygen molecule for some useful purposes.

So, one such purpose is your simple binding that means, we know that this is a very good ligand. So, if we can have a transition metal base system say iron based system or any other metal center, particularly in the low valent state. So, if we have a low valent metal center. So, that low valent metal center can bind initially to di-oxygen molecule can and then can activate it. So, the most useful metal centers will be the ferrous center and the cuprous center.In all this cases, we know that whenever such system is available, internally we can transfer the electron from the iron side to the oxygen or from the copper side to the oxygen, and at that particular point your metals centers showing variable oxidization state that means, it can settle between the two oxidization states plus

2 and plus 3 in case of iron, and plus 1 and plus 2 in case of copper. So, this gives us something, some idea that metal ions are playing some important role.

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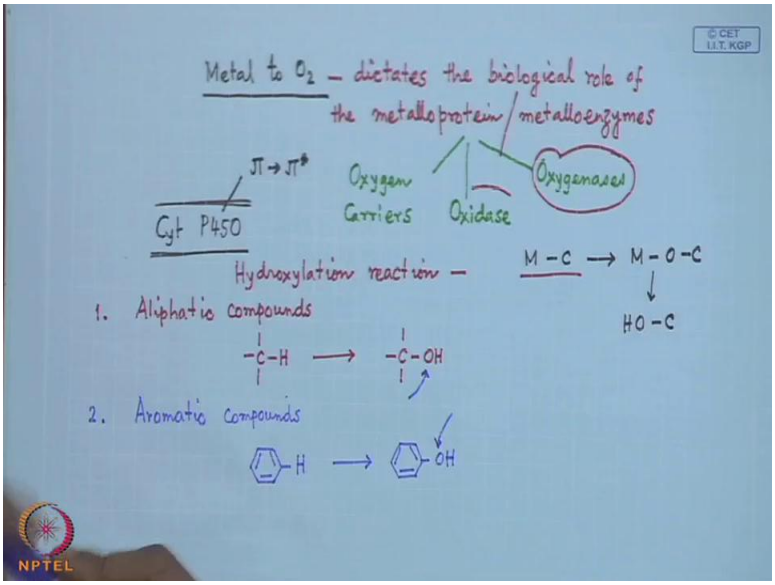

So, the binding that means, metal to O 2 bond, this metal to O 2 bond is therefore very important; and if we are able to identify the corresponding nature of this metal to O 2 bond, starting from the interactions what we know in case of our myoglobinand hemoglobinmolecules. So, this metal to O 2 bond basically and this interaction dictates the biological role. That is the reasons why we are gettingsomany different types of reactions where the same iron center and the oxygen is involving. So, this basically dictates the biological role of the metallo protein, metallo protein or sometime the corresponding metallo enzymes. So, the binding initially and then this corresponding activation is important. So, that is why we have different types of species.

In some case when O2 is simply attaching and transferring from one side to the other, we get simple oxygen carriers.It can be your hemoglobin molecule, it can be your hemocyanin molecule; then in another case, we are getting oxidase molecules and lastly we get the oxygenases. So, the critical role of the metal center as well as its corresponding biological environment or the protein environment play some important role whether that particular assembly will go for your function has oxygen carrier or it can show its corresponding oxidase behavior or the oxygenase behavior.

So, one such example we all know very well, that in a particular case of cytochromewhich is cytochrome p 450 this. So, we get therefore, the iron porphyrin system that means, the iron heme protein and that iron heme protein has some important propertythat means, it has the corresponding chargetransfer band for pie-pie-star at 450 nano meter. So, it is in the visible range where because otherwise we can get mostly in the ultraviolet region, U V region only the pie-pie star transition, we usually get. So, the delocalization and the extended delocalization of it moves it to the visible region at 450 nano meter.

So, this particular cytochrome p 450 can function as oxygenase and that particular oxygenase reaction, we simply say like this corresponding hydroxylation reaction.This sort of hydroxylation reaction, we all know very well that if you have some organometallic compound or anything. If you have a corresponding metal carbon bond,if we able to do some reaction over there, and if we can convert it toM O C species to finally if we can eliminate it to this species.

So, we can insert basically, this is a basic insertion reaction between metal and carbon bond a oxygen atom. So, direct oxygen atominsertion reaction giving you something where your system is getting hydroxylated, and this sort of hydroxylation has tremendous application in different area. So, there are large number of this sort of reactions. So, one after another we should know little bit about the nature of this reaction. So, when hydroxylation is taking place on aliphatic compound,on aliphatic compound.

So, you have in that particular case we will be looking for the C H bond. So, C H bond will have to be activated and there are some avenues for our synthetic molecules, non biological molecules, what we make in the laboratory. Sometime if you find that this particular C H can be activated for a corresponding CM type of bond middle carbon bond, if we are able to make it, and afterwards by giving some reagent, one such reagent would be your cytochrome P 450, you have the iron heme system. And that iron heme system is activating the di-oxygen molecule, in some fashion and that oxygen is transferred ultimately from the iron site to the substrate site. So, this will be hydroxcilated, therefore to C H to C O H. So, you can convert methane to methanol.

Then the next category immediately, once you know that the aliphatic hydroxylation can take place using cytochrome P 450 and some other related molecules. So, hydroxylationof the aromatic compounds are also favorable. So, for aromatic compounds, we have aromatic carbon and then we have the C H there. So, if we are able to activate it. So, any benzene like substrate can be activated to a phenol like of species. So, this basically gives us some important ideas that on all these cases, we are going for a corresponding oxygenase reaction. So, these are all mono oxygenase reactions. We are just basically inserting one oxygen atom between the carbon and the hydrogen atom, thenthe next category.

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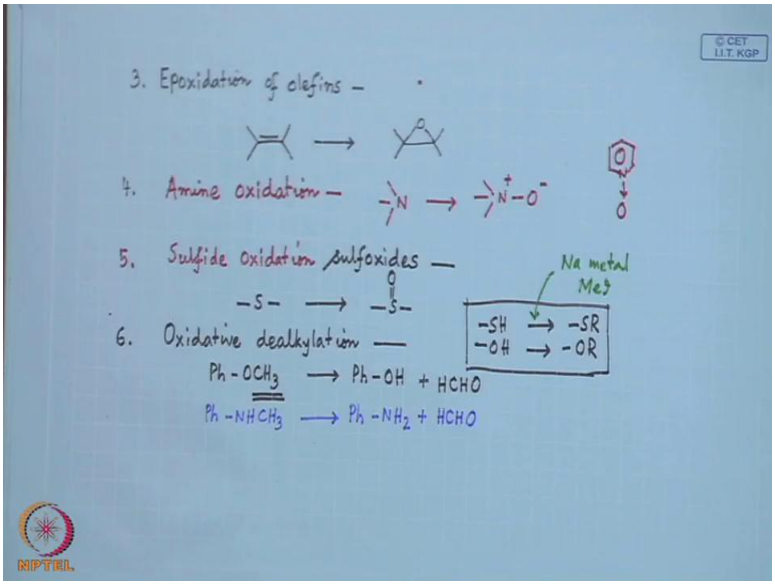

What are the difference types of reactions, we can think of and is the epoxidation reaction of olefins. This is also very useful reaction that how we easily, we covert the oneC-C double bond to a epoxide, then amine oxidation, amine oxidation reaction giving rise to this amine through an oxide, like we all know that we can make very easily in some cases, the pyridine anoxide or bi pyridineanoxide, this sort molecules are very easily made. But sometimes, if the amine is complicated one we cannot makesoeasily using some standard peroxide reagents.

Then sulphides can also be similarly oxidized to there are two different possibilities because we all know that thioether sulphur can be oxidized to sulfoxides and sulfon. So, in most of these cases, they end up with the most difficult one that means, the sulfoxides

and some time we cannot control the sulfoxides, we go for the sulfon, but here we are looking for something where only one oxygen atom is utilized from the di-oxygen molecule.

So, we have sulfon then another intersecting reaction which is known as oxidative reaction, oxidative dealkylation, because the standard practice for the different types of alkylation reactions, we all know that how we can convert S H to S RO H to O R, these are the standard alkylation reactions we do, but using this cyto chrome P 450. We can go for some reaction where P H O C H 3 giving rise do your phenolplus H C H O formaldehyde which is coming from this C H 3 group, because in this particular case for the corresponding alkylation reaction. We all know that the standard reagent in all this cases,you get the sodium metal in dryethanol and methyliodidefor alkylation reaction. Similarly for the corresponding methyl group on nitrogen like n methylaniline, Nmethylanilinewill end of to aniline plus H C H O. So, these gives us some idea that how the same cytochrome P 450 center which is theactivated by the oxygen from the air or the any other reagent molecule can go for different types of reaction.

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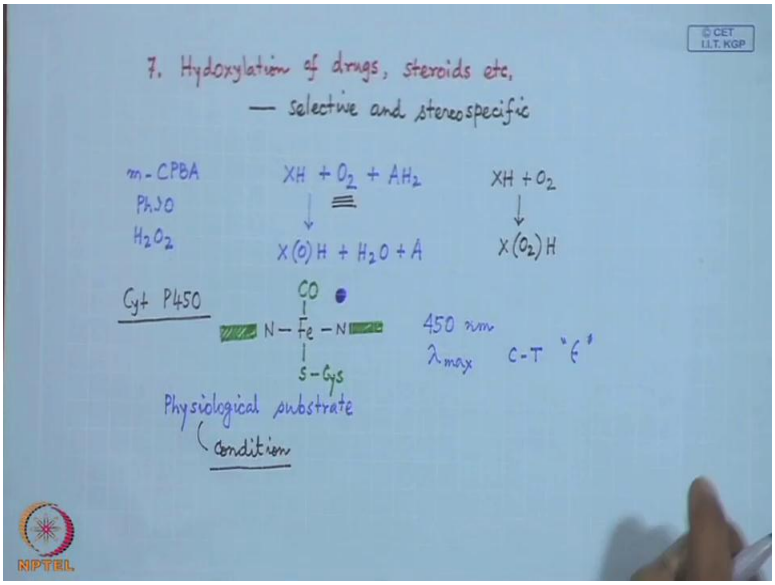

And the last one of these category is the corresponding important hydroxylation reaction for different drugs and steroids, we know that when we consume the different drug molecule and a steroid molecules in our body. We have the corresponding fate of that hydrox, that corresponding drug and steroid molecule should be hydoxilated, otherwise,

it will not go out from our body. So, that is also a very important reaction that is hydroxylation of drug molecule,the drugs then steroids,etcetera; and in that particular case, the reaction issoselective that some reagent or some molecules are only applicable for some type of drug molecule or some type of steroid molecule. So, these reactions are sometime very much selective as well as stereo specific.

So, from all this sort of reactions if we want to have some step of these reaction, if we just some go for some conversation reaction in the laboratory also for some synthetic molecule to make, all this seven categories of reactions, we just simply go for some insertion reaction like that oxygen atom or the single oxygen atom. We can follow this reaction where the single oxygen atom is being transferred from some of the useful reagent like M C P B a meta chloroperbenzoic acid or P H I O iodosylbenzene or hydrogen peroxide.

So, all these reactions, when they go for the corresponding mono oxygenase reactions, we see that in case of mono oxygenase reaction if your substrate of all or any of these substrate, what we have written now that means, if it is reacting with O2. So, x H is your substrate, it is bearingC H group, if you have a carbon hydrogen. So, you can go for the corresponding reaction with that of your O 2 molecule. So, it will be converting to xO H,if it is carbon hydrogen it will C O H plus H 2 O and at the same time, we use some reductant like a H 2 which is supplying this hydrogen's as well has reduction equivalent and will be converted it to a. So, this is for theinsertion of single oxygen atom into the substrate, but in case of the dioxigenase reaction, the same substrate if you have x H plus O 2, what will happen? It can go for x O 2 H. So, it is not that some peroxide linkage is forming, but there are several points of attachment that means, if you have two carbon centers. So, both the carbon centers can be attached with the oxygen atom or sometime it can be converted directly to carboxylic acids. Sothat we will see how these two differ from one reaction to another.

So, this cythochrome P 450 therefore, the basic unit what you have here, we allknow now that is simply the basal nitrogen that means, the full nitrogen we can draw quickly there that means, this is the base and this is the corresponding plane of this prophyrine. So, it is the basal plane and this particular one, now has a different binding from the fifth coordination site which is cycteinesulphur that means, anionicsulphur.

So, you bring cysteinesulpur attach it to iron site, because we not looking much about the prophyrinentity, but this prophyrinentity has some important role to play, the electronic structure of this prophyrinring can sometime play some important role. So, it can modulate little bit on the reactivity pattern on the iron, but most of reactivity pattern is solely dependent on coordination site which is in epical site.

So, this one when it binds,so, this is the, our active site like our hemoglobin and myoglobin where this particular site was available for di-oxygen binding. So, this can also bind to carbon monoxide and in that particular case,that is basically determine whether it is functioning as a cythochrome P 450 or not, because these gives that characteristic band of 450 nano-meter for its pie-pie star transition.

So, this is a corresponding characteristic lambda max value is 450 and since it is a charge transfer type, pie-piestar charge transfer type, it has several thousands of your epislon values, the molar absorptivity of several thousand's only to know that particular reaction. So, there are therefore, from these drug and steroid molecules. So, not only the drug molecule, there are large number of other molecules are there that we can go for the typical hydroxzilation reaction of the physiological substrate, which is very important, because in our body and in the bodies of different living system, this particular a very interesting reaction is taking place in physiological substrate.

So, we have some physiological substrate, what we want to convert, for hydroxylation reactions some useful conversionand obviously, that is also happening in physiological condition.We are not using any drastic condition, we are not going to have some high temperature condition or some inert atmosphere condition. So, that is why these reactions are very useful reaction that it can go for a particular transformation involving a physiological substrate as well has a physiological condition such thatyou can basically go for the corresponding activation of the di-oxygen. So, if this O 2 from this reaction can be activated by this iron center.

So, basic idea behind that is the for that how we can activate this O 2 molecule and basically, we have nearby the substrate molecule which is nearby. So, you will have somewhere the substrate molecule. So, you have the catalytic site, you are generating something. So, that gives us some idea that how catalyst are functioning. So, all sorts of this catalytic or bio catalytic reactions, the substrate will be nearby and you have this

particular iron and if we are able to activate the di-oxygen molecule. So, what is that particular species which is forming, what we had have seen in case cythochormev C oxidase molecule which is ultimately transferring that oxygen atom to our substrate.

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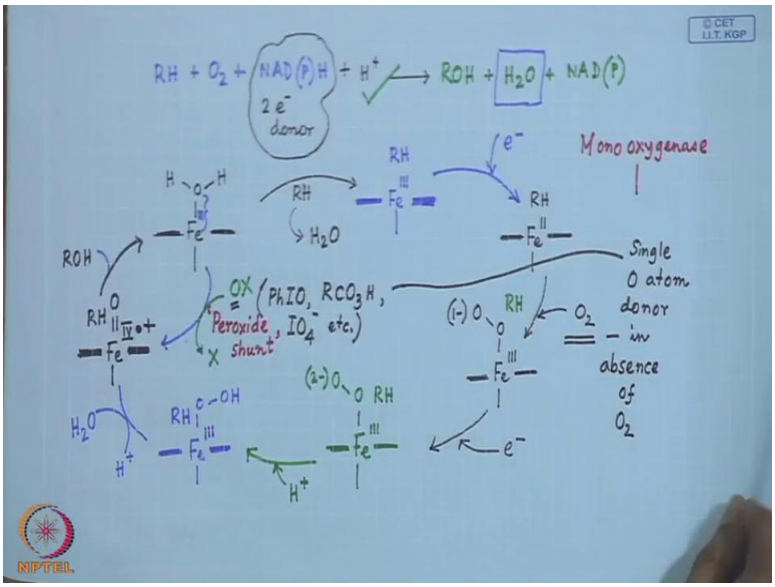

So, this number of electron donors which we are getting for this sort of electron transfer protein, if we consider that your substrate is RH, it can be your P H H also, it can be aliphatic as well asaromatic, your O 2 is there, what is your A H 2? A H is 2is nothing, but N A D P H or N A D H. So, N A D P H or N A D H which is required for your reduction. So, in presence of a reductant and some amount of H plus. So, this is well known has a two electron donor system.

So, basically we are taking something where we all the time just discussing about the transfer of oxygen atom, whether we are talking for a single oxygen atom or two oxygen atoms and at the sametime, we are also linking the number of electron transfer. Because all these will be controlled by a corresponding thermodynamic burden that means, thermodynamic control that means, the potential at what potential, you are supplying that electron, and if it is a two electron step that means, one after another, your supplying the first electron. That means, your having a reduced product then your supplying the second product and it is sometimessodifficult that for a metal center, we all know that if you have a metal center, if you put the first electron to it is reduced. So, it will not accept the second electron for further reduction.

So, you have to have some species or some metal center where you can go for the second electron for the reduction reaction, but in this particular case, this N A D H or N A D P H is functioning that electron transferssoyou have a biological reductant. So, this is very important therefore, not that you are externally using some ascorbic acid or any other type of reagent what we do in the laboratory.

So, this particular biological reductant is useful for converting RH toR O H plus H 2 O plus either N A D or N A D P, nicotinamide adenine dinucleotide simple or the corresponding phosphate. Soyou, now you see that you have two oxygen atom, one is taken by the substrate and another is coming out has your water molecule. So, this we can start from now that how our catalytic cycle goes from here. So now, we know the structure that means, you have the prophyrin in the bassel plane then the binding from the cysteinesulphur.

And this side which was utilizedfor binding carbon monooxide for your 450 band and like your deoxy hemoglobin or deoxymyogolbin system, it is always have a tendency for binding two a water molecule. So, your iron echo complex. So, iron monoecho complex, only one water molecule is there and that basically, initially react with RH and we are bringing in the first step, the substrate molecule itself. So, when we bring the substrate, what happens?You can monitor basically, there are several spectroscopictechniquesor any other type of technique, whether your particular site that cythochrome P 450 site is bound to water or is divide of water.

That we can, if we can monitor in the solution state by our streching frequency also, that also gives us some idea that how it can go for binding any other anionic groups also or neutral groups like carbon mono oxide or it can bind to some agite or any other anionic species. So, when carbon mono oxide is bound to this particular site,you can monitor the corresponding shift in the corresponding C O stretching frequency in the higher. So, that stretching frequency change immediately dictates us that your carbon mono oxide is bound to your iron site.Similarly, this water molecule if you can able to monitor it that whether water is directly attached to this iron site or it is somewhere else. So, when it is reacting with your substrate,it is basically removing your bound water molecule, you are losing the bound water molecule.

So, that gives us some clue thatyour this particular site that means, the 6 coordination site, if this particular coordination site it has some available area and that available area is only required for coordination of this H 2 O, if the pocket is not very much useful that means, it is a angular molecule and it goes and approaches to the iron site and for the standard molecules we know that this corresponding Fe O distance, we all know. So, if we can determine the corresponding structure and if we find that it is in the regular range of this corresponding iron oxygen bound distance, then you can consider it has a corresponding bound which is forming with that iron site, but otherwise, if and have some weak interaction also. So, if that is a weak interaction.

So, there your iron oxygen separation is longer and only some weak interaction is taking place and that weak interaction is required for blocking that coordination site. So, whether we are blocking that coordination site by water or something else. So, when you bring the substrate. So, close approach of that is substrate to iron site is important that means, water molecule is removing and this site which is definitely the ferric site,this is our ferric site. So, this is the prophyrin plane and is bound to this cysteinesulphur.Now,you safely write RH nearby, and when you write this particular assembly that RH nearby,you say that your water is not there that means, this particular position. So, RH will have.

So, it is three dimensional system is octahedral molecule, only the 6coordination site you have the corresponding RH group; that means, your catalytic site is this site, your RH is not coming over here, your RH is not here, your RH is not in the other site, because all other position, o, basically all five positions hold by the prophyrin, and fit by the cysteine sulphur is blocking that iron site and those bindings are required for the corresponding reactivity or that activation of this iron site. So, once you make this particular site available for some amount of binding and the simple strategynow you can say immediately that… Now, your N A D P will be active, it will supply the electron because for some kind of reactivity for this iron site, it has to be in the reduced from. So, that is why, we all the time, we use the reducing agent and iron center will be reduced to Fe 2.

Here the cysteine sulphur and RH is still sitting nearby, then one set is reduced to the ferrous state, it will be equivalent to your myoglobin or hemoglobin molecule. So, nowyou can go to your di-oxygen within it. So, this di-oxygen now can giveyou O O minus. So, if it is again going back to the ferric state, it will be a super oxide then is O 2

minus then.These O 2 minus, when in the next step, we have seen that role of this N A D P H or N A D P is for the donation of two electron. So, this is first step of electron transfer from N A D P H it is getting reduced. Soin between your O 2 is attaching that means, your ligand substitution reaction is taking place over here, basically we are replacing the water molecule by your di-oxygen molecule. So, in the second step,you see interestingly because this biological reducing agent playing some important role that is why, is not that both the two electrons are supplied, in a single step in first step it is getting reduced then O 2 is attaching into it, then we are putting the second electron into the system.

So, that second electron after putting it, you just simply will convert this iron, still in plus 3 state, but these oxygen is now your peroxide. So, electron is now moving here and inall these cases, RH was nearby in this particular case, here RH is still there, here you have the RH also close by. Then we just take the help of this proton. So, H plus is now coming into the picture and that basically is utilized for the protonation of the peroxide species. So, you have a Fe 3O O H and RH is there. So, this also gives us some idea that why some of these reactions will be studying, we know that for the synthetic reactions also, you are reacting it with O 2 and some reducing agent that means,youcan provide it by hydrogen peroxide also. So, we are basically providing hydrogen peroxide to the system, and reaction is going like this, but this particular step that means, the intermediate what is forming over there, is the hydro peroxide species.

So, if you have a corresponding hydro peroxideas the reagent and the iron prophyrin system, you can directly from this hydro peroxide species. So, there are some groups, very useful groups that the tertiarybutal hydro peroxide are the reagents, what you can utilize directly to establish that it is basically forming the corresponding and it is going via the hydro peroxide state, because getting this particular step is very important, because we have already studiedsomany of this type of reactions including your reactions for the catalysis, the reactions for the peroxides that in this particular case, when you have these as well has your RH over there.

Now, if you look for this that means, now the catalytically active species,you can generate that means, again the same mechanism of formation that means, the ferrylspecies. So, cythochromeP 450 is going through this ferryl species. So, it is Fe 4 and a cation radical. So, it is not the exotic iron five species. So, it is ion four cation

radical is forming and we have cycteinesulphur bound to it.And this particular RH will now be transferred to R O H,because you have the same RH is there. So, what is living behind? Because this particular species is very easy to identify the ferryl species, there are large number spectroscopic technique is available whether you have a iron double bound oxygen, whether you have a prophyrin in a cationic, as a cationic radical that once you established that means, from these two, one of this oxygen is remaining or attaching to this iron side that means, it is going for a double bound formation and your iron-oxygen bound is getting stronger and stronger, and this O H is living behind.

So, in this particular case, here you have added this proton. So, here if you add one more proton over here,you can takeoff the other oxygen as water. So, that is basically forming in our reaction as H 2 O. So, this RH is nearby. So, when it is living our R O H from here,you can go back to this particular species with the use of some water molecules. So, water again complete the cycle and that water next bound to that particular iron site, and we can regenerate this particular cycle. So, this particular case, in some of these molecules, the whole catalytic cycle is operating and operation of this particular case, basically what we can see that if we have this one that means, the species which is bound to water molecule and if we are able to short-circuit the cycle that means, how we can move directly from there to this particular species by giving some reagentthat means, not through this stepwise introduction of the electron, in this particular state as well as the next particular state as well as a use of di-oxygen, but some other oxo transfer reagent, if we can gives some oxo transfer reagent which we consider has O X,do not confuse it to X O.

If x is the substrate the hydroxilated product is X O. So, this is O X that means, it will simply donate that oxygen to the system and it is forming something where we do not get this peroxide intermediate, the peroxide, the super oxidestate, the peroxide state and the hydro peroxide state. So, this, all these three steps are getting avoided. So, this particular state can be avoided by using something that is nothing but just now what I told you is your iodosylbenzeneisoxotransfer reagent, then meta chloroperobenzoic acid or any other peracid, RCO three H or sometimes iodate group is also helpful,IO four minus, etcetera. So, these reagentsand biological system, we are not getting this reagent, but proving that corresponding catalytic cycle, how it is going from one step to another, particularly the corresponding ferryl species in some highly oxidized form that is iron in

plus four as well as prophyrin is in the oxidized form, that is a very common phenomenon. Because the people earlier used tothink that the iron is oxidized to the plus 5, but the your they are not getting the corresponding signature from the EPR spectra or others spectroscopic technique, it has a iron 5.

So, your ligand is getting oxidized. So, this particular step, when we are avoiding the formation of super oxide peroxide or hydro peroxide, we call it has a peroxide shunt, this particular state thereforeis known as peroxide shunt, we get that. So, this therefore, giving us some idea that this thereforedefinitely a reaction where we are getting some example for reaction for mono oxygenase. So, the reaction is definitely a mono oxygenase reaction because a single oxygen atom is utilizefor the conversation of our substrate. So, in this particular case,this when we are going for this peroxide shunt step, these reagents, these donors, one oxygen we have written over here.

So, this is basically a single oxygen donor. So, these all are, these species all are single oxygen atom donor. So, remember that very nicely that when we are taking about the mono oxygenase as well as di-oxygenase, we will find that in one particular case, we will be able to get one oxygen atom into the system such that your system is getting hydroxilated, if it is a methane molecule, it will be giving you the methanol molecule, if it is benzeneyou will be ending up with phenol molecule and the other oxygen atom is utilized for the formation of H 2 O.

So, this definitely not going through the corresponding utilization. So, you cannot utilize both the oxygen atoms for its corresponding di-oxygenase behavior. So, the di-oxygenase behavior is littlebit different that we will see nowin our next class, that how we can utilize these two oxygens. So, the challenge for us is alwaysthere that whether we should be utilize because if it is going through the same type of intermediate, whether we should be able to utilize both the oxygen atoms for the corresponding substrate oxidization, where this particular center is being utilized for its di-oxygenase behavior.

So, this basically proofs us that the single oxygen atom oxidant and this particular state is available and we can go for, in absence of di-oxygen. So, single at oxygen atom donor, we can use, even you can do the reaction in inert atmosphere, in nitrogen atmosphere or in a urban atmosphere by simply giving the reagent. And the single oxygen atom donor

can go for the corresponding hydroxylation reaction, because we will able to do this reaction in absence of air or di-oxygen, which is very important.

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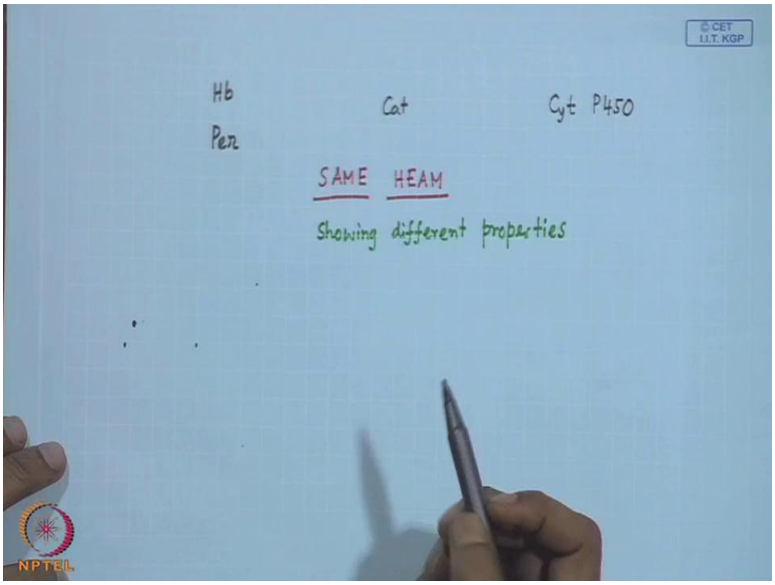

So, so far what we have seen that slowly, we are moving from one particular molecule to the other that means, we have seen so far that we have the hemoglobin, then we have the peroxidase molecule, they are all similar type of molecules. Then you can have the catalase, and now we see your cytochrome P 450, and we are extracting out different types of reactions. The same iron is there, same porphyrin surroundings is there in the bassel plane. Therefore, all of them that means, hemoglobin, peroxidase, catalase, cytochrome p 450, all basically contains the same heme. So, that is very important and you should be able to remember it that the same heme, showing different types of properties, showing different properties. So, that gives us a very important idea that one particular coordination site which is different.

So, answer lies there, what is your fifth coordination site? So, if we can monitor, not that your changing the prophyrin ring, if we just simply vary the fifth coordination site. So, the apical binding from the fifth coordination site is basically important, if you move from the hysterin nitrogen. So, on this hysterin nitrogen, tyrosineto now in case cytochrome P 450, it is cysteine sulphur. So, as you move from one after another and which is also true for the synthetic molecules that if can monitor, because it also true for the cobalt system or any other system, the coordination from the epical side that means,

the fifth coordination side is basically important to change your catalytic activity from the sixth site, so that we will see for the molecules in our next class.

Thank you.