Bioinorganic Chemistry
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Module - 1
Lecture - 8
Electron Transport Protein - IV

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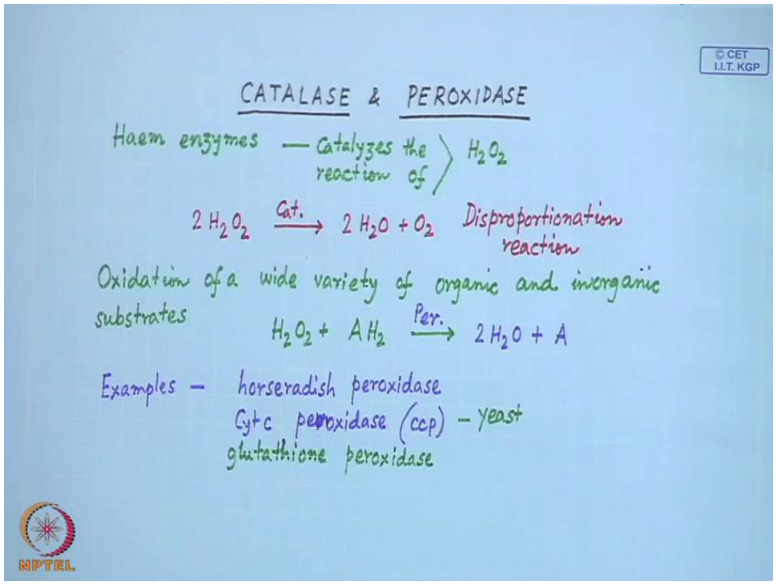

So, welcome back. So, in this class, we will just continue those iron porphyrin system in catalases and peroxidase. So, still we are sitting on the haem enzymes. So, we have in our hand the only example what we are talking so much about the haem enzymes. So, they are also definitely haem enzymes, and now we will bring into the picture, the involvement of H 2 O 2. So, when it catalyzes the reaction of H 2 O 2, these two reactions basically, they are of similar nature. So, side by side we will talk together.

So, you should clearly understand that one is catalase and another is peroxide, and it goes sometime through the same intermediate wherein, the first case, it is the reaction of one molecule of hydrogen peroxide with another molecule involving the catalytic activity of catalase giving rise to water and dioxygen. So, this is basically our well known disproportionation reaction. This you all know. So, disproportionation reaction between one molecule of hydrogen peroxide with the other. So, one molecule of hydrogen peroxide is getting oxidized and another molecule of hydrogen peroxide is getting reduced and at the same time, we will see that some organic substrates or any other

inorganic substrates as well then go for oxidation reaction using hydrogen peroxide. So, that is simple oxidation reaction. So, oxidation of a wide variety of organic and inorganic substrates.

Organic and inorganic substrates gives rise to reaction like H 2 O 2 plus or substrate is now H 2, so is an oxidation reaction. So, this will be converted in presence of the catalyst which is peroxidase part will be giving rise to 2 H 2 O plus A which is your oxidized form H 2 is the reduced form and a is your oxidized form. So, large numbers of these molecules are well known to us and well established form of some of them we should know because these are well studied. So, examples of one such is from the radish which is horseradish peroxidase. So, from the radish root we get this horseradish peroxidase. Then cytochrome C peroxidase is also well known. Cytochrome C peroxidase which is also abbreviated as CCP and we get that from yeast. So, very commonly is available from yeast.

Then, another interesting biological molecule which you all know is very good reducing agent is a glutathione. So, when glutathione is getting oxidized, so when your substitute is glutathione, large numbers of these molecules are well known because they are operating on these substrates. So, when glutathione is getting oxidized by this peroxidase well known as glutathione peroxidase, so these peroxidase as well as catalase molecules what we see that you have the same proto porphyrin 9 and that proto porphyrin backbone we can have.

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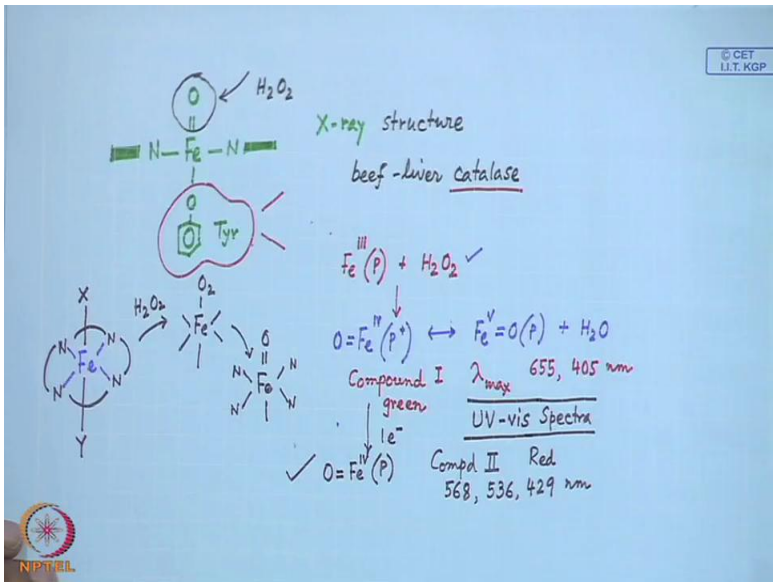

So, we have iron and then this is bound to this nitrogen of your porphyrin ring. So, we all know now that is what are the myoglobin, hemoglobin and cytochromes. Then at one point, it is forming your ferial oxygen. That means, all these cases, it goes through this particular intermediate. So, this intermediate is there and only difference is our binding for this position. That means the position number 5, if it is coming from a terrocyin residue, so if your terrocyin residue is directly using for coordination to iron site, we get a corresponding environment because we know through the protein crystallography and its corresponding x-ray structure gives us a crystal clear picture and x-ray structure of one such species is beef liver catalase. So, beef liver catalase is there. So, when we do not have this particular fifth position bound, it can interact with peroxides.

Now, we know that this we are getting from hydrogen peroxide. So, you have the iron site and iron site is nothing, but your iron porphyrin site and that iron porphyrin site can go for two types of reactions. So, one such environment is for catalase. So, for the other type of activity; that means, the peroxidase activity you can have a different environment. So, by looking at this particular position. That means, this particular site you can control the corresponding catalytic activity of iron in this particular environment, either for catalase or for peroxidase type.

So, in this particular case, when you have separate, isolated sites and for catalase, if we go for any such species which is iron, say in the trivalent state which is bound to or

porphyrin ring, so we get ferric porphyrin system. That means, if you have any ferric chloride in your hand in a test tube and how it will be reacting with hydrogen peroxide because those reactions with simple ferric ion is not so much exciting, but when it is attached to porphyrin and this porphyrin when we put inside a protein structure; that means, your other position; that means, coordination number 5 is occupied by your terrocyin residue. Then it basically controls the reactivity towards hydrogen peroxide. So, this particular binding will control whether it can react with hydrogen peroxide for a catalyst activity or for a peroxidase activity. So, initially if we see and people have identified because already we have in our mind.

Just now what we have seen in case of cytochrome C oxidase that when a iron 3 based porphyrin system is there and which is reacting, now instead of dioxygen molecule because in case of cytochrome C oxidase, we are talking about the binding of dioxygen molecule. That is why we are looking for the reduced form of iron site, but now the ferric form is there. It can very easily go for reacting your hydrogen peroxide system. So, it gives now Fe in plus 4 oxidation state, and now your site is oxidized for porphyrin and you have this oxo.

So, immediately if it gives this particular species and which we already know that in terms of the corresponding redoxity valiant, if we simply ignore the corresponding oxidation of the porphyrin site and if you do not have the corresponding evidence for the oxidized ligand because whenever you have a metal ligand system, you can either oxidize the metal site or you can oxidize the ligand site, but if we are unable to detect the corresponding oxidation of the ligand site, like that of our porphyrin, we can say that no porphyrin has not undergone any oxidation. It is your iron site which has been oxidized, but which is pretty difficult, but if we do not have any such evidence, we can write it as a iron in plus 5 oxidation state. So, that is very simple. So, iron oxo and the porphyrin plus water. So, this is a very simple and straight cut reaction.

So, if you have your iron and it is bound to 4 nitrogen atoms of the porphyrin. So, this is your entire porphyrin ring and whatever thing is happening over there is due to your presence of x as well as y. So, when it is reacting with say, this hydrogen peroxide. So, if this x is there and x is loosely bound, so x will go away. So, there will be removal of x and at intermediate point, it can react with O 2. So, either the vacancy over here or any loosely bound water molecule or any other solvent molecule on this position can be

replaced by your hydrogen peroxide site and it gives you some species which is based on your interaction with peroxide and then it can go for your Fe oxo form. So, this changes now we know.

So, these changes in your hand and for this transformation, what is forming immediately through the addition of hydrogen peroxide over here, we can level it as a compound which is compound one. Why we are leveling it? Because we should characterize it nicely for its different solution properties. That means, what are its corresponding lambda max values and immediately what we should know is its corresponding collar and this collar because there is a change, because all the porphyrins we know the basic ligands collar is purple. When it goes for the compellation with iron, it changes one collar and then if you have hydrogen peroxide, then it changes to a green color and it has some prominent lambda max values at like that of your cytochromes C oxidize at 655 and 405 nanometer. So long you are with this particular species. That means, the oxidized porphyrin and Fe 2 plus. Sorry, Fe 4, we get a corresponding signature for this particular absorption.

So, we get compound one. Then if we allow it for a corresponding one electron reduction because this is the highest possible oxidation state in terms of either five or four with oxidized porphyrin. If you reduce it back, that reduction will give you the same iron in 4. What will happen to the porphyrin? Only the porphyrin is now your reduced one. So, one electron transfer in that particular case, either the porphyrin site or the iron site is that between these two, you can have an electron transfer either it is on the porphyrin or it is from the iron site, but sometimes the covalency is that much that there is no such (()). That means, the corresponding corrector; that means such ligand corrector or the metal corrector is mixed. So, it is very difficult to identify. That means perform the electron is coming out for the oxidation and where the electron is going for the corresponding reduction reaction, but when you allow it to react with hydrogen peroxide, then you can go for one electron reduction. Immediately we get these species.

So, this is our species of choice. That means your iron for porphyrin species which is having a oxo bound and this can be leveled as your compound two and it has a different color. So, the change in color immediately without measuring the corresponding lambda max values without knowing the corresponding spectral detail. You can tell that in this particular case, this is one level of oxidation and this is another level of oxidation. So, in

terms of the electron transfer, you can say that one is your green product, another is your red product and this is green and red product because this red product has corresponding lambda max values of different magnitude which is 568 nanometer. Then another is 536 and last one is at 529 nanometer. So, spectroscopically if you just apply UV visible spectra, so will apply nothing, only UV visible spectra. So, UV visible spectra will tell us that you should be able to generate two different species and these two different species when we have and next, this can be allowed, because we are talking about for this catalyst reaction. This is one hydrogen peroxide. So, we are having one more hydrogen peroxide because one is getting oxidized and another is getting reduced.

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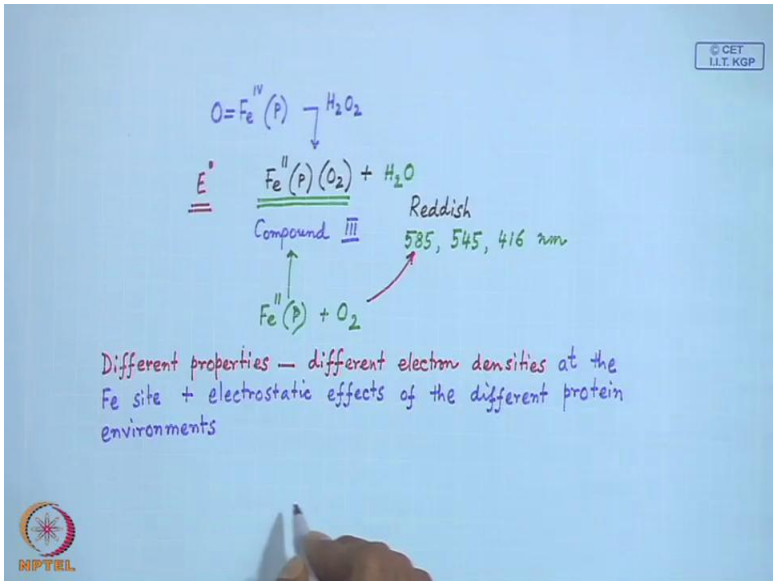

So, you have the ferial iron in your hand and that ferial iron when is allowed to react with another molecule of hydrogen peroxide, it is plus 4 P. So, it is compound, to which we react with H 2 O 2, we get compound 3. So, what is that compound 3? That compound would be that they are utilizing our well known knowledge of hemoglobin and myoglobin isoxi form. So, iron is going down to Fe 2 now and it is bound to dioxygen molecule. So, that is the way that not only forming this ferial species, you can generate the dioxygen molecule. They are with the reaction of second molecule of hydrogen peroxide that gives rise to the generation of compound 3, which is reddish in color. So, it is not completely red.

What is your compound 2? It is reddish in color with the slide shift in the lambda max values from 568 to 585 nanometer, then 536 to 545 nanometer and last one from 429 to 416 nanometer. So, all the three bands in the UV visible spectrum getting changed and color is changing from red to a reddish form and that reddish form is responsible for the generation of compound 3 and this can also be formed because here with the generation of O 2 plus water will be produced from there and this we all know now that this is very easily we can make from Fe to porphyrin plus O 2 because if you just take this in your hand and if you have ferrous porphyrin and that ferrous porphyrin when you allow with the oxygen, we all know that depending upon the corresponding partial pressure and the medium everything, the corresponding oxy form you can generate and this oxy form if you can generate which can easily be characterized by knowing the corresponding lambda max values.

So, simple characterization in terms of this lambda values will tell us whether you have the compound one or compound two or compound three because in one particular case, same iron center is utilized for the oxidation reaction in another case. It is utilized for the reduction reaction, but during the transformation, something is happening. That means, when you start from one particular step; that means ferric porphyrin we are starting and generating the ferial iron center through the use of one hydrogen peroxide and that highly oxidized form of this ferial iron. Now, what ferial iron is your stronger oxidizing agent, now compared to hydrogen peroxide because of this nature. That means, it is a stronger oxidizing agent than hydrogen peroxide. Earlier we are utilizing hydrogen peroxide for oxidizing your iron site. Now, ferial center is more potent oxidized agent which is utilized for the oxidation of hydrogen peroxide. So, you see that iron site we are simply just changing from one site to another. That means, your environment is responsible as well as the binding of the form.

How easily you are able to produce the corresponding ferial species? By manipulating the corresponding E 0 values. So, we get the different properties. So, how this different properties we are getting? Because this is one example for our catalyst compound that we will see in our next example for peroxidase that different properties which we all should keep in our mind because we are starting on discussion from cytochromes C oxidase because one after another, we have to compare all the sites at one point of time. When you know all these things, we should be able to compare that why it is showing

reactivity related to cytochrome C oxidase, why it is peroxidase, why it is catalase and why it is some other molecule which will be cytochrome C 450. That we will see in our next molecule.

So, different properties are originating from different electron densities, which is important. So, different electron densities at the metal site, different electron densities at the iron site, which is very important plus we can have something; that means, we are changing from one protein environment to the other because for catalase, the protein environment is different compared to your peroxidase which is also different for your cytochrome C oxidase. So, the second sphere of environment; that means, you have the corresponding electrostatic effects of the protein. That means, the different of the different protein environments. So, this particular one when we get that, how this protein environment is changing that we will see when we have this particular catalase activity and when this site is loosely bound. That means, if you just see in between related to three-dimensional structure of our simple porphyrin ring.

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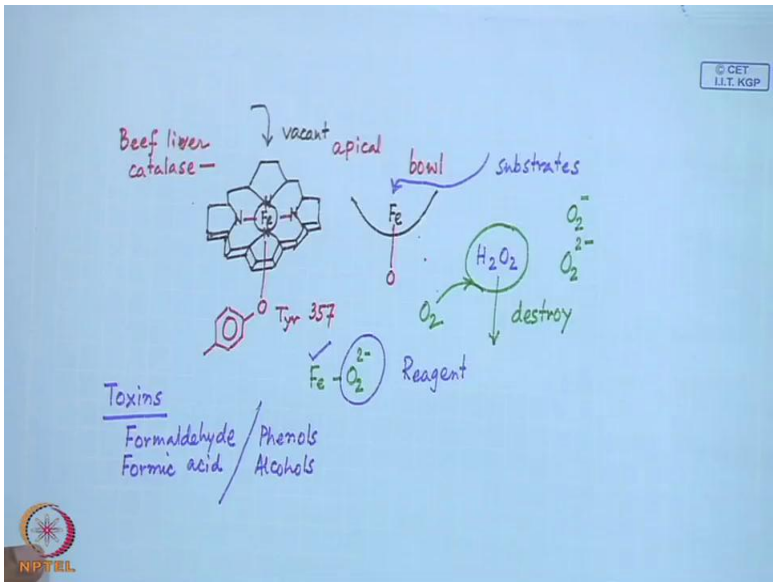

So, it is a very four porphyrin rings. Sorry, four payroll rings should be visible and this iron site should have one nitrogen. This nitrogen and this nitrogen and this nitrogen. So, when this is the vacant site, you get a corresponding bold like structures. So, if you just see, it is visible from this site and you have a typical bowl shaped structure and iron is sitting over there. So, this particular site which is your apical site, because all four bester

sites are occupied, all four bester site around iron are occupied. So, within this particular one and again, you have the same example, the beef liver catalase.

Why it is bowl shaped? Because you have the bonding from the terrocyindeseede. This numbering is 357. So, this particular one, when you get this; that means, you have this particular arrangement and the protein environment is also supporting this arrangement. So, this bowl shaped structure. So, bowl is basically attracting those substrates. So, whatever substrates we are getting, this is getting attracted over here. So, different substrates will come and will just go inside the bowl and the corresponding reactions we see. So, people are trying basically why you talking so much about all this? Because we know that you have the corresponding accumulation of H 2 O 2 on all living organisms including human being, including man. You have tremendous accumulation for this hydrogen peroxide because we are consuming our di oxygen for regular survival.

So, when you put di oxygen into the system for our glucose oxidation or a food oxidation or anything, we all the time know that some amount of super oxide is forming. Then some hydrogen peroxide is also forming. So, there should be some suitable mechanism to destroy this. So, we are able to destroy some of these hydrogen peroxide molecule through the mechanism of this catalase activity that one hydrogen peroxide is reacting with another hydrogen peroxide through this iron site, but at the same time, if we can have some arrangement; that means, you have Fe O 2 2 minus that ferial has not formed. Iron site is there. That means, you have activated hydrogen peroxide because we all know that various reagents, various reactive species sometimes getting activated with the use of our metal site.

So, it is iron site activated hydrogen peroxide. So, that can also be a very good reagent for destruction of some of other toxin molecules, some of other toxin molecules. That means, different toxins if you consider that hydrogen peroxide is also toxic for our body, so accumulation of hydrogen peroxide is not good for our health or any living being that we are able to destroy. That means, the concentration of catalyzing in all living system is important. That means, some person is there which are unable to produce the amount required for the destruction of hydrogen peroxide and some other living species is producing more hydrogen peroxide. So, there will be some imbalance.

So, this particular reactivity can be useful also for destroying some other toxins like formaldehyde also. That is why we are studying this. That means, what information we are getting from this living system that catalase we know and catalase is going through that mechanism.

So, definitely if we are able to use that for destruction of hydrogen peroxide, we should be able to use that system for the destruction of formaldehyde, we can use that for the destruction of formic acid and also, sometimes we can use this particular arrangement for destruction of phenols also and alcohols. So, the different for different species. That means, when you use that catalyst activity, we have the iron porphyrin site and when we go for the corresponding peroxidase, the solution study is also very simple that we should go for knowing the corresponding particular species because the peroxidase are also available everywhere starting from plants.

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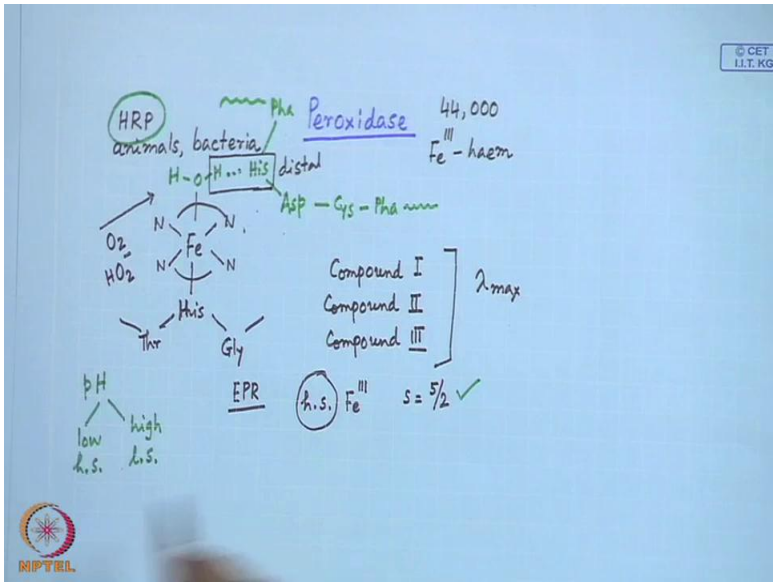

So, the planned origin is basically our HRP, the horseradish peroxidase which is available from the roots of horseradish. Then the peroxidase is available in animals also. It is also available in bacteria and these are very small molecule species and this iron 3 haem unit in peroxidase is. That means, iron 3 porphyrin unit is mono meric having only molecular weight of 44000. So, here also we have iron site which is bound to 4 nitrogen atom of the porphyrin ring and in case of catalase, as we have seen that this particular position can be occupied by some water molecules also and these water molecule has to

be stabilized if it is bound to water. It is not that the only water molecule like our hecsago species, hecsago metal salts. That means, there will be a pocket and a hydrophilic pocket if you are able to form and that bound water molecule which is supported by weak coordination from this iron and this apical side binding is little bit weaker all the time because you have a iron oxygen long bound compared to iron nitrogen bound, and this is hydrogen bounded with some side chain of the protein chain which is histidine.

So, if you have a nearby protein chain, say this protein chain is continued with asparted, then cystine, sulphur cystine group and then phenyl alinine and so on and histidine. Then this side also phenyl alinine and this side. So, hydrogen bonding interaction can be provided by this histidine unit. So, this is all the time very important, this interaction, not only knowing the iron site which is bound to other groups because this histidine binding is important because this site we are utilizing, not only for water binding, but afterwards what we are seeing that this we are utilized for binding of O 2, binding of hydrogen peroxide. That means, sometimes hydro peroxide H O 2 minus. So, they are all related. How oxygen is bound to your iron site, how hydro peroxide is bound to our iron site? So, if this particular species is getting stabilized and if we have some vacancy over here, we will find that this water position can be occupied by your hydro peroxide also.

So, we are actually looking for that binding of hydrogen peroxide or hydro peroxide to the iron site followed by hydrogen bonding interaction with the histidine group. This will also lead to some amount of reactivity pattern, where we will find that instead of this water molecule, there will be O O bound. If it is hydro peroxide with that O bound which is terminally stabilized by histidine residue and that we all know the charge separation will be there delta plus and delta minus, and delta minus end will be stabilized by the histidine unit. Then there will be a possibility for your O O cleavage. So, this hydrogen bound interaction will be useful for our O O bound cleavage. So, this particular case if you have the lower end, you have the histidine unit and then other subunits like glycine, like thryonin, etcetera.

So, in this particular case, we just determine that if we have iron site and this iron site like catalase will find that you have compound one, we have compound two as well as compound three and these particular cases will just can compare by knowing their corresponding different lambda max values and those lambda max values will also

immediately tell us when we talk in terms of the corresponding EPR measurements that what type of iron site you have. This particular case if we have a high spin iron 3 which is very much EPR acting for, it is equal to 5 by 2 value and this particular case, immediately we see since it is high spin. That means, there is some interaction. That means, it is not that all the time when it is bound to your water molecule or it is bound to your histidine.

So, this histidine is your different histidine which is also known as distal histidine and this coordinated histidine also plays some important role to give you an arrangement for iron site whether it is high spin or low spin. So, this particular site depending upon its corresponding p k value. That means, this nitrogen will have a typical p k value. So, if we just monitor the P H and the same example of this your horseradish peroxidase, HRP sample that you have two different P H values. One is low, another is high. So, one is low P H and another is high PH. That means, all these sites EAR by sites are all protonated. Your histidine site is protonated. So, you do not have anything and most of the cases, like your catalase site is also when you have the phenol coordination. So, that phenol can either be unprotonated or protonated depending upon your P H of the medium. So, when it is low, you have the proto netted form of these things. That means, some of these nitrogen groups are proto netted. That means, you have weak field.

So, you will get a corresponding high spin state and in case of high P H where basic in nature you get a low spin state. So, when you talk about these interaction related to your spin state, we should also be careful about the different spin states around the iron and this particular spin state will have different reactivity pattern with that of our water molecule as well as hydrogen peroxide. As we all know, ultimately it is also related to the binding of di oxygen because how you are deoxi of hemoglobin and myoglobin in which form, whether it is in the low spin state or in the high spin state, it is used for binding de oxygen molecule. That we know for our knowledge from your myoglobin and hemoglobin. So, these two spin states are important for binding and your hydrogen bonding interaction from the distal histidine is also important.

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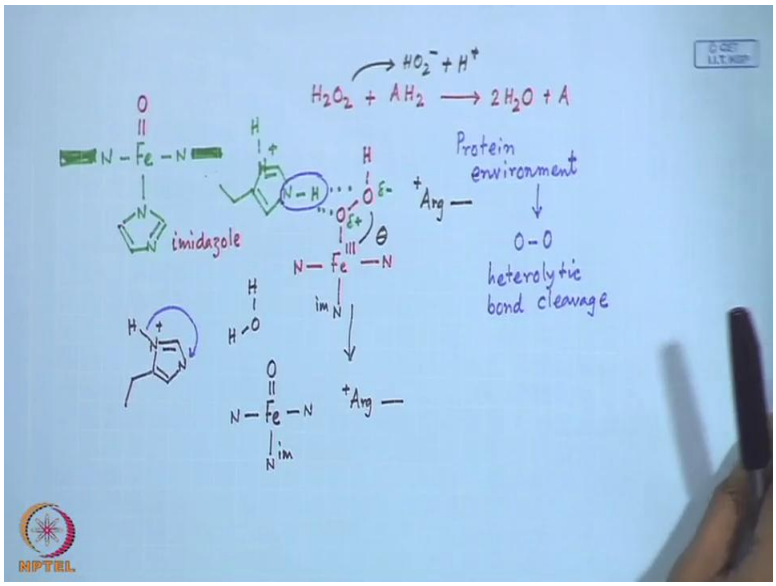

So, now, we can see that this particular form when it is giving the corresponding coordination; that means, you have now nitrogen form, the emote joule unit and these are the porphyrin groups. So, like catalase, it also forms a corresponding ferial unit. So, now, you know that the electronic parameter around iron is different in case of catalase. It was your phenol unit. So, now, is your imidazole site chain. Imidazole group at the coordination number five play some important role for a different type of reactivity for your peroxidase activity because this peroxidase activity is responsible for your oxidation of the sub state. That means, you have H 2 O 2 as well as the substrate in case of catalase. We are talking about two molecules of hydrogen peroxide. Now, you have the substrate as well as the hydrogen peroxide.

So, if you have a typical substrate for this activity of the peroxidase, your substrate should also be nearby. That means, you are talking about something that you transfer this oxy oxygen to this unit or you just simply oxidize it. So, your substrate should also be close to the catalytic site and the generation of this is very important to know that if you have this iron site which is in the trivalent state bound to nitrogen and this is another nitrogen and when it is simply reacting with hydro peroxide. That means, we have used hydrogen peroxide for the reaction, but following de proto nation, it can go for binding with OOH because this is looking for a charge coordination and iron is in the plast three oxidation state. When you talk about plast two oxidation state, we can talk, we can handle about the de oxygen molecule which is a neutral one, but when it is a charged

one, we have to bind this one for this particular type of arrangement. So, here you have a just separation of delta plus and delta minus and this one. Therefore, this oxygen can have hydrogen bonding interaction as well as this will also have hydrogen bonding interaction with the hydrogen of the corresponding nitrogen of the distal histidine. This is the binding.

So, this nitrogen, the second nitrogen, this NH nitrogen is involved in hydrogen bonding, but the second nitrogen is also sometimes depending upon your PH of the medium can be protonated. So, this hydrogen can supply elecproton to the system and can also accept proton from the system. So, this particular unit is very close to your hydrogen, sorry iron site and you see we are having some extra proton from this because we are talking about here the H O 2 minus Plus H plus. So, this proton can go and bind and sit over here, so these particular arrangements for the stabilization of this arrangement as well as the other end. That means the positive end can give raise to something which is coming from positively charged other amino acedent. So, is argenase is a positive discharge, argenase site is available and which is close to this oxygen. So, this arrangement is stabilized within the protein pocket.

So, that is why whenever we determine some x-ray structure of this protein molecule, this bi molecule when we determine this structure, always we talk about the Fe O odis angle and not only the O distance which is also important for its knowing the corresponding bond order that whether it is a single bond or a bond order of 1.5 or a double bond corrector, and how easily it can be clipped how easily we can go for breaking this O O bond. So, that will be known by knowing the corresponding OO bond and at the same time, Fe OO bond angle also you see. So, the hydrogen bonding pulls from this site and another interaction from argenase site will stabilize only this arrangement. That means, at a particular angle, it will be stabilized. This particular arrangement will be stabilized. So, when this weak interaction is taking place and we are getting this particular arrangement, so in the second step what will happen? We should immediately able to write this that you have Fe and this particular one will go for making Fe O. That means, ferial system we are getting this for the nitrogen of emote joule ring.

So, these two nitrogen’s are there and what will happen to this part? This part will go like this nitrogen and you have, this is also remaining. This is going there. So, you have H O H and positively charged argenase. So, the cycle continues. So, the immediate

movement for this; that means, you have this we all know. That means, your N H, this N H is important. So, this proton will now move to this nitrogen to regain this structure. So, you see in this particular case that not only in this particular arrangement; that means, the coordination environment around iron is important, but also the secondary interaction, the second sphere of interaction which we call the protein environment. So, here also, this protein environment is important.

How protein environment plays an important role? That is known from here. So, protein environment, this basically controls the typical arrangement of this O 2 H unit, which can also control the OO heterolytic cleavage because you have the terminal end is attached not only through hydrogen bonding to the emit on joule unit, but also some argenase site chain. So, this arrangement then immediately gives us some idea that we can see some of these reactions because some of these reactions are very useful for that oxidation reaction.

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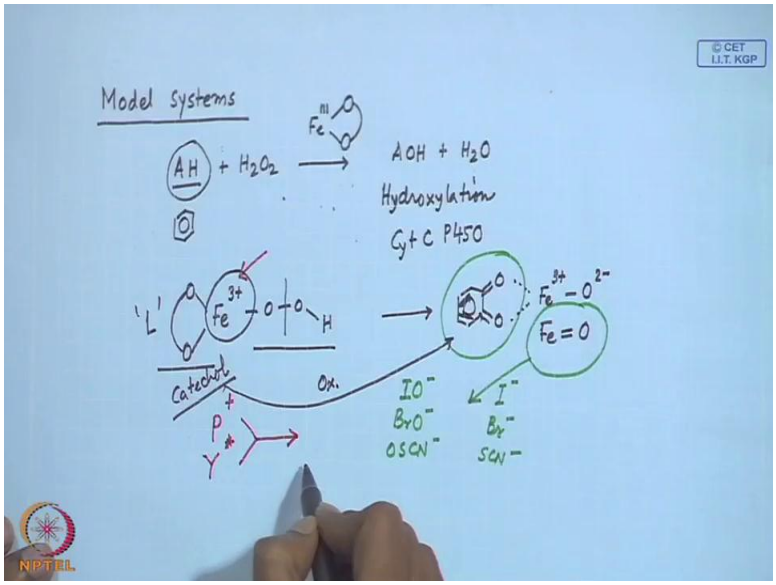

We can utilize this simple information related to our catalase and peroxidase system for oxidation using some model systems. It is very important to design, it is very important to make all these things. That means, if you have some substrate like alcohol or some aromatic compound like phenol, then we can have the AH, which can be oxidized using your hydrogen peroxide, using some iron compound, iron 3 compound and model compound means we have some laboratory peptic compound. So, one such binding

model is that if you have a catechol type of arrangement, so that catechol type of arrangement can be utilized giving you AH. That means this i there; that means, if you have benzene unit like C 6 H 6, you get a corresponding phenol plus H 2 O. So, what we are talking, therefore, basically the next system what we will talk in our next class is the corresponding hydroxylation reaction using some related molecule which is cytochrome C 450, cytochrome C based P 450.

So, here in this particular case, when you have a bidentated relation around iron, we have Fe which is Fe 3 plus this binding is there. So, this definitely reacts with this particular one in this form. That means this interaction whether you have the phantom type of arrangement; that means, iron. That means, it is attached to your ligand system. So, the ligand will play some important role to know all these reactions and then you have the hydrogen peroxide and if this particular unit is there that if it is say catechol which is a very useful ligand, so OO is nothing else, but your catechol ligand. So, that catechol is there and that particular one ultimately can give rise to some of the oxidation reaction to produce quinine. So, quinine system and this quinine at the end, basically can quickly bond to your Fe 2 plus system and O 2 minus.

So, this particular picture tells us so many things that if you have the ligand and if your ligand is functioning as your substrate AH, so if your ligand catechol itself is a substrate, your substrate can be oxidized and at the same time, this particular one whether it is loosely bond to hydrogen peroxide and then you have this OO bond cleavage. That means, some interaction you can have there. You have the Fe O interaction. It can be your Fe double bond O also. That means, you have something in your hand that electron transfer can take place and electron transfer can give rise to the corresponding oxidation of the substrate from catechol to quinine will end up with the quinine system or you should be able to generate a reagent, where you have oxo function is sitting on the iron because not only any organic substrate, but you can utilize iodide, you can utilize B r, you can utilize thiocyanate to get the corresponding hypoiodite species, hypobromite species or hypo thiocyanate species, O S C N minus. That means, it would be a very useful technique to know that how we can activate the hydrogen peroxide and during that transformation, whether your oxidation is equivalent. That means, your oxidation equivalent is residing on the hydrogen peroxide part and that hydrogen peroxide part is moving from this hydrogen peroxide to O 2.

If it moves from O 2, its corresponding reduction equivalent is transferred from this site to other site, but if you can consider this as the reagent and this as the substrate, your mediator is your metal center. That means, the different model system starting from our cytochrome C oxidase, catalase peroxidase, all these things, the first thing what we have identified is that your iron site, which is your metal based catalytic site and you have the other part, the porphyrin unit we have seen or we have the corresponding terocin unit which was y. So, we were at one time, we were able to oxidize it to this or the corresponding stared Watson of terrocyin. So, through this oxidation process, we are able to generate some organic radical. So, this particular case, the generation of these two organic radical are important for different iron based reactions related to hydrogen peroxide and its oxidized form. So, next day, we will see something related to your cytochrome P 450.

Thank you.