Bioinorganic Chemistry
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Lecture - 6
Electron Transport Proteins-II

Hello. Just now, we were talking about the redox gradient for the different E 0 values of cytochromes.

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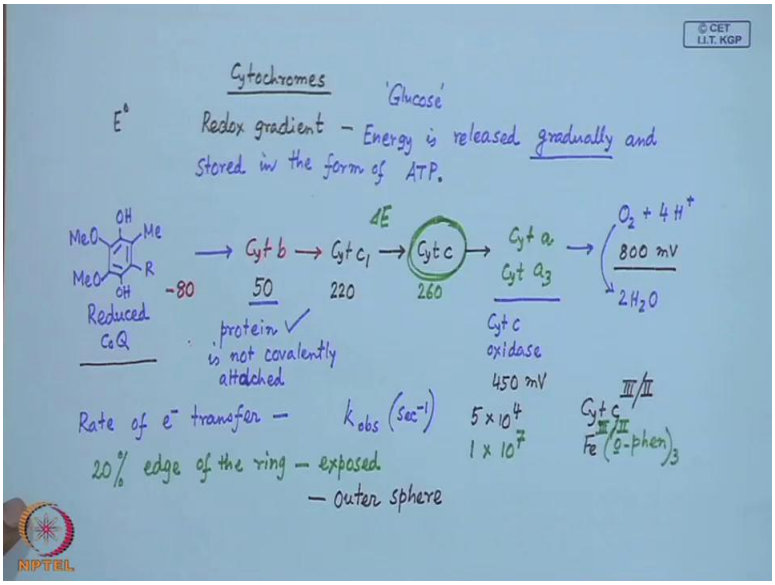

We have the different cytochromes. And those cytochromes can be considered from their different E 0 values such that we can have a typical generation for redox gradient from one particular species to the other. In that case, if we just want to burn glucose, the processes is basically, the glucose burning. And, that glucose burning process takes place in such a way that, the corresponding energy for that is released gradually in slowest possible manner and stored in the form of ATP. So, this would be able to synthesize large amount of ATP molecules during this burning process. Like any deplas, we can have on the other hand, several other biological reducing agent; one such is quinone-based one, which is reduced coenzyme – quinone coenzyme reduced – CoQ – coenzyme quinone, which is OH-OH-Me-R – some typical substitution; then, on the left, you can have two MeO functions as oxide functions.

This particular biological reducing agent – we can determine its corresponding E 0 value; that means the E half value, which is minus 80 milivolt. So, this particular system is stacked for that. So, at a particular pH value, you can give this particular system for its corresponding oxidation as well as reduction, because experimentally, when we measure by any cyclic voltammetric technique, we always get some value, which is the E half value. So, at that potential, if you push it from left to right or right to left, you get the corresponding either reduction potential or oxidation potential. That is why, we consider this as the E half potential.

Then, this is useful to reduce cytochrome b. And, this cytochrome b has a corresponding potential of 50 milivolt, which is negative one. So, this will be reduced by this reduced coenzyme Q. Then, we have cytochrome c 1, which has a potential of 220 milivolt. So, gradually, we are moving from left to right. So, E 0 value is increasing. So, as we are moving from left to right, we are getting strongest possible oxidizing agent on the right-hand side. Then, cytochrome c 1; then, cytochrome c.

So, we see that, we are talking so much about the corresponding metal environment; how it is related to the protein part; but, the basic interest lying on the corresponding redox potential, which is iron centered. So, iron is giving you electron and iron is taking up the electron. So, the corresponding electron transfer proteins will be responsible for its redox catalysis. So, this electron transfer shuttle will be useful for the redox catalytic reactions. So, for cytochrome c, it is 260.

Then, comes one important group of system, which we will slowly study that; one of (( )) is a very complex molecule, which is a combined form of cytochrome a and cytochrome a 3. So, all these levels we will see that… We are talking about the different cytochrome, but different levels or their different porphyrin units. And, these two together; that means you have now a complex system; that means you have one cytochrome a system as well as a cytochrome a 3 system.

So, basically, if we do not tell anything more beyond that, you should be able to tell that, it has one iron center, which is in cytochrome a environment; and, another cytochrome center, which is in cytochrome a 3 environment. But, it is much more complex. It has two other metal ions also present. So, collectively, this system is known as, in a simplest way, is cytochrome c oxidase, because this is on the right-hand side of the cytochrome c

and it will oxidize the cytochrome c. So, it is cytochrome c oxidase, because this is a little bit complex molecule.

Then, this is attached to the most important reaction, what we are thinking about; which is the reduction of dioxygen molecule to water in presence of four protons. So, it is a four electron reduction process. And, that four electron reduction process is taking place around 800 milivolt. So, these – all these potentials and this one is 450. So, all these species, when we talk about the corresponding reduction of the O 2 system; experimentally, we can all determine these values, because in the intermediate form, we will find that O 2 by getting one single electron can be reduced to its superoxide. Then, when it gets another electron, it goes to peroxide unit. And, those E 0 values are all different.

Then, it can go for the different radical pathways. So, it can go for OH dot. And, that OH dot is also reacting with again for O 2 molecule or water molecule. So, there are large numbers of oxygen-based species available. And, those oxygen species will have all different E 0 values. So, right now, what you see that, this 800 milivolt is pretty high; which can be utilized for the oxidation of this species; which in turn can be utilized for the oxidation for cytochrome c, cytochrome c 1, cytochrome b, and ultimately, the biologically available reducing agent.

This particular case when this cytochrome b we get; the cytochrome b – you see that, this potential is pretty less compared to the value, what we get for cytochrome c as 260 milivolt; that means it has a different environment around iron. And, the protein part in this case is not covalently attached; protein is not covalently attached. So, not only that, porphyrin environment, but also, the protein environment can control the different E 0 values. So, this E 0 value can control the corresponding thermodynamic aspect of this electron transfer behavior. But, we should little bit aware of the thing that, what is the corresponding rate of electron transfer.

So, experimently, these rate of electron transfer as well as theoretically, we can calculate it out from some theoretical values. So, in this case, we will find that, if we just simply determine the k absorbed values in second inverse, we will find there is a drastic change in values for all these typical biological molecules compared to that of the iron center, which has typically ligated to some simple ligand system.

These k abs values for a system, where it is cytochrome c and which is shuttling between the iron oxidation states of plus 2 and plus 3 giving a value of 5 into 10 to the power 4 second inverse. Compared to that, if we use an iron-tris complex; that means, if Fe – orthophenanthroline like bipyridinium; Fe orthophenanthroline. Again, it is shuttling between 2 and 3, but the determined rate constant in this case is in the order of 10 to the power 7. So, we see that, the simple molecule like iron-tris-phenanthroline, which has iron center surrounded by 6 nitrogen donor atoms of orthophenanthroline. But, it is showing some faster electron transfer rate. So, sometime the slowing down of the electron transfer rate is also important not only the corresponding E 0 values, because in some of these cases, we will basically find out some delta E values; say threshold delta E value is required for one step to another.

And, that threshold E 0 value or the delta E value will be responsible for a corresponding free energy change. And, that free energy change will be utilized for the ATP synthesis. Not that all these delta E values are useful for the ATP synthesis. So, this rate for that particular electron transfer is also important. So, what is happening there; that means in case of cytochrome c, you have the protein envelope. And, only a part, which is nothing but 20 percent of the porphyrin ring. So, 20 percent of the edge of the ring; that means the porphyrin ring is exposed to the system; rest is covered by the protein chain. So, that basically slows down the electron transfer rate.

So, when it is covered with the protein chain, only a part is available there for electron transfer. And, basically, in all these biological system, the electron transfer will take place through a mechanism, which is outer sphere in nature, because we know that, there are two types of electron transfer: one is outer sphere electron transfer; another is the inner sphere electron transfer. But, in all these, important biological molecule based on iron, because the availability of a very less amount of exposed area for electron transfer, only the outer sphere mechanism is operating; and, which is responsible for slowing down the corresponding electron transfer rate.

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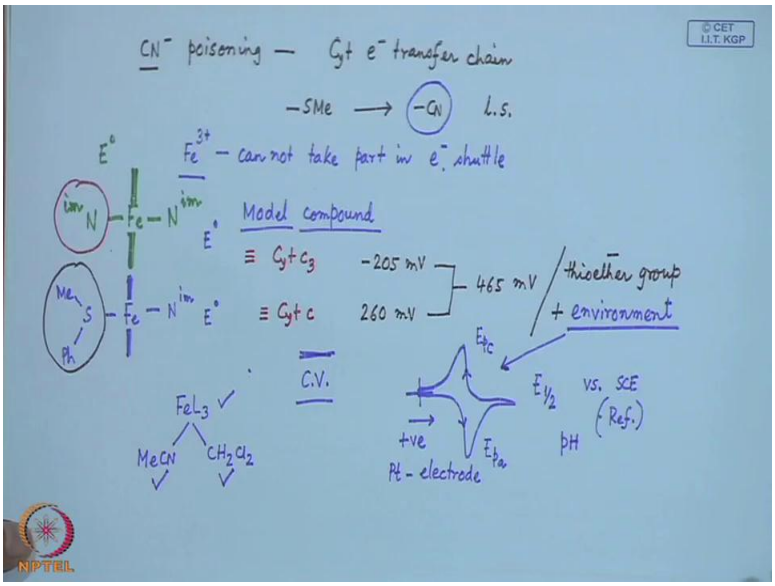

This particular thing also tells us that, some of these molecules, what we know that, from our childhood days about the cyanide poisoning. This also basically disrupts the cytochrome electron transfer chain. Cytochrome electron transfer chain is hampered. It is not that the cyanide poisoning is due to the binding of cyanide to the hemoglobin or myoglobin molecule, but now, you see the same type of molecules is available for binding the cyanide group.

So, the thioether binding will go when cytochrome c is directly involved. And, that sixth position is now occupied by the cyanide group. And, already, we have seen in these cases of molecules that, corresponding electron shuttle is also important. And, in cytochrome molecules, we have only the low spin states. So, these low spin states are there and… So long, it is bound to a methyl function; it can shuttle between the ferrous state and the ferric state. But, when it is bound to the cyanide function, it cannot shuttle between these two oxidation states. So, when there is a binding of cyanide function, the Fe 3 plus, which is getting stabilized there and cannot take part in electron transfer or electron shuttle from one side to the other.

These also tells us that, how some groups are available in the biological system. If these groups are available there; which can stabilize the high oxidization state of the metal center; that means in this particular case, we are stabilizing the ferric state; but, if it is stabilized more; that means we are changing the corresponding E 0 value. So, E 0 value

is changing. So, it is stabilized at that particular point and you cannot reduce back to the corresponding ferrous state. So, this particular information prompted us to know that, this particular porphyrin coordination is we are keeping constant; we are talking about the coordination from the fifth site as well as from the sixth sites. So, in one case, it is thioethersulphur and the imidazole unit.

But, you can have some nitrogen from this side as well as nitrogen from this side and both of them are imidazole nitrogen. So, this gives us an opportunity to know the corresponding change in the E 0 values for synthetic molecules as well. So, if you have a laboratory-prepared porphyrin ligand, you get the corresponding iron compound and then its corresponding bis-imidazole adduct; that means the coordination number 5 and 6 is occupied by imidazole groups only. So, it is basically a simple model compound, which can be made in the laboratory.

And, this particular function can be compared with some other system, where you have this as well as one by S-Me – any group say; it can be Ph-S-Me; and, other by N im. And, if you determine experimentally by cyclic voltammetric measurements, the corresponding E 0 values for these two compounds will find that, related to the same cytochrome c, where you have… So, this is equivalent to the cytochrome c. And, this particular one is equivalent to some other molecule, which is cytochrome c 3 basically; where, the thioether coordination is not there; instead of that, you have the imidazole group of these. So, determination of these two basically tells us that… Already, we have seen that, for cytochrome c, the potential was 260 milivolt. And, this can be determined, which is minus 205 milivolt.

So, what basically you get? You get the corresponding difference of these two; which is 465 milivolt. And, that difference is due to these two coordinations. So, from the entirely the same type of environment and entirely same type of molecule, if we are able to substitute one coordinating group from one position; that means the position number 6 say; by the other group, we will find that, there is a drastic change in the corresponding potential value. And, this change in potential value can be correlated to the binding from the thioether function – thioether group and some amount of the environment. So, there will be some effect from the environment also, if we are unable to restrict the same environment.

Protein environment or some solvent environment, because when we go for cyclic voltametric measurements… During this cyclic voltammetric measurements, what we will find? We will find that, we all know that is a typical response for a voltammogram we get. So, if it is in the positive direction, it is a cyclic one. And, this gives us a corresponding anodic peak potential and this gives us a corresponding cathodic peak. And, from the average of these two, we get the E half values.

So, these E half values we report against some electrodes; that means against normal hydrogen electrode or saturated calamine electrode or silver chloride electrode; that means you have some reference electrodes into the system. And, when we measure in aqua's medium, we should also report the corresponding pH values. But in this particular case, when we talk about the environment, we immediately see that, if some of the compound, say, some iron compound is a model compound say; if it is a Fe L 3 compound and if we can measure in a different non aqua solvents; non aqua solvents like… Simple example for that is acetonitrile one and another is dichloromethane. So, that gives us some very good idea that, how the environment is also playing some role to find out the corresponding E 0 values.

So, when we report this potential, not only the reference electrode, not only the pH, not only the solvent; but, working electrode also we report; that means what type of working electrode, because in most of this measurement, we use platinum electrode for the measurement. But, in this particular case, we will find there is a difference in potential when we measured the corresponding E half value by cyclic voltammetry inacetonitrile solvent or dichloromethane solvent. So, dielectric constant for the medium is different. And, that dielectric constant is basically changing the corresponding E 0 values for simple corresponding E half values for any iron 3 compound. It can be the iron bipyridine compound or it can be the iron orthophenanthroline compound. So, environment has always some role to play.

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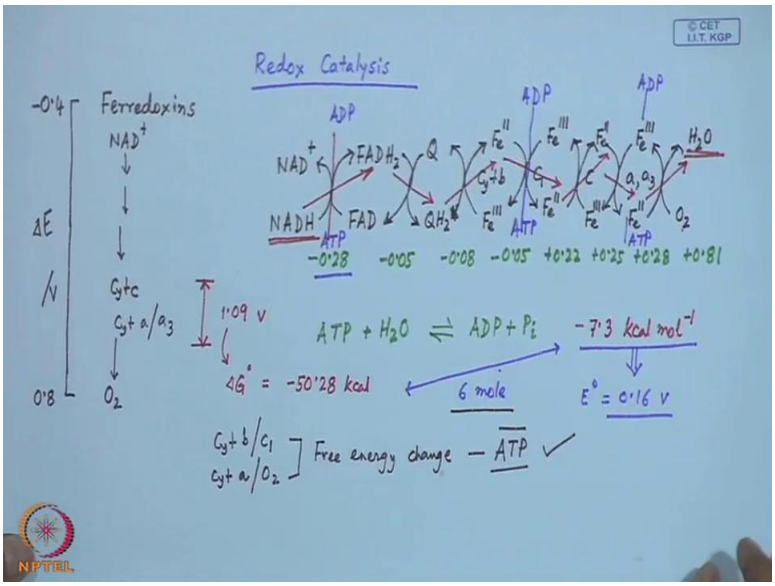

But, in this particular case, the protein environment, which is the bigger one; and, that protein environment is basically controlling the corresponding E 0 values; and, basically, it is controlling the corresponding catalysis, which is dependent on the redox property of the system. So, if we see this typical redox catalysis for this system; and, we will be talking for a potential skill now, because we had seen the corresponding electron transfer chain. Now, the potential for this is minus 0.4 volt to plus 0.8 volt.

We know now. What is that? 0.82 is the O 2. And, in this particular area, that means, minus 0.4 is some ferridoxin molecule now, because we all know this ferridoxins are iron-sulfur proteins. And, those are very good electron transfer agents. So, ferridoxin; then, the entire chain, what we have written just now – NAD plus; then, flavin protein; then, all cytochromes –cytochrome c, cytochrome a and a 3. Then, it is going for O 2. So, now, we know the corresponding E 0 value. So, this is in volt. So, this particular case, when you have the different steps; and already, we know the different steps.

And now, the cycle is like this; that if you have NAD plus, which is shuttling between NADH. So, this is the oxidized form. And, another is the reduced form. And, which is attached to the corresponding reducing agent, which is flavin-based; nicotinamide-based and then the flavin-based. So, FAD; and, the corresponding reduced form is FAD H 2. Then, we are bringing the quinone – the coenzyme quinone. So, you have quinone and hydro quinone. It is oxidized by cytochrome b now, which is based on Fe 3; and, the

reduced one is based on Fe 2. So, quinine, hydro quinone – this arrow will be like this. Both the arrows on the downward and both will be upward. So, Fe 2, Fe 3. Then, cytochrome b; then, c 1. Again, it is a Fe 2 and Fe 3 only. So, this part is quite simple. It is only Fe 2 and Fe 3. Then, after cytochromes c 1, we have c. This is also based on Fe 3; this is a Fe 2; then, a and a 3.; O 2 to H 2 O.

This will move from one step to another. Within this scale, this NADH and NAD plus is close to minus 0.28 volt. Then, this one – minus 0.05 volt; next one is 0.08 volt; then, 0.05. But, significantly, these are changing from this place to plus 0.22, then plus 0.25, then plus 0.28, and plus 0.81 for oxygen. This particular change when we calculate for the reaction of ATP plus water giving rise to ADP plus inorganic phosphate. So, that particular reaction, we know the corresponding energy involvement; that for this particular synthesis or the hydrolysis – the phosphate hydrolysis from ATP to ADP requires minus 7.3 kilo calorie per mole. If we can think of the total change from this end to that end; the total change from this particular end to that end is basically covering a window. So, it is the potential window we call it. So, this potential window of 1.09 volt. So, that 1.09 volt can be calculated for its corresponding free energy change delta G 0; which will be equal to minus 50.28 kilo calorie. The total window if we consider; that this much is the window. And, within this window, you have the corresponding free energy change.

And now, this is known for the hydrolysis of the ATP. So, you can think of… that means if we just correlate these two, you expect that, 6 mole of ATP will be synthesized, but it is not that, because we will have a corresponding threshold; that means if you go back calculating this one; from here, you can calculate the corresponding E 0 values required for the hydrolysis or synthesis of ATP, because these are connected – the E 0 value and the free energy change. So, this value basically gives us some value, which is nothing but 0.16 volt. So, this is the threshold magnitude for ATP synthesis. Then, until and unless within this redox change, you have a corresponding change of this magnitude; you cannot get a corresponding ATP synthesis.

So, here we will find that, in this case, you have the electron flow from here to there; then, electron flow from this to that. So, in this way, it will go; that means from the reduced form. This is the reduced form; this is the reduced form; this is the reduced form; this is the reduced form. So, electron is moving like this. So, red arrows are

pointing towards only the reductions – reduced forms. So, we are starting from reduction from NADH; and, we are ending over here. So, the electron is dumped on the O 2 molecule and producing the water molecule. These junctions basically – when the junction is 0.28; at this point, you will get a corresponding synthesis; that means ADP on this side; and, at this side, you will have ATP, because this 0.28 is higher than 0.16. So, at this step only, you will have one ATP synthesis; then at this, that means, 0.22 and this minus 0.05, you will have that… that means between these points ADP, ATP. And lastly, within this a a 3 unit.

Basic things, what we are talking about here that, instead of this 6 mole of ATP, we will be getting 1, 2 and 3 ATP molecules. So, within this short chain, because we have a huge and long chain and that can be broken up. So, between the cases, where you have a cytochrome b and c 1 – this particular case; cytochrome b and c 1 – this would be nicely tell that, where you have the large free energy change and between a and O 2. So, this a and O 2 – in this region; a and O 2 – there will be large free energy chain apart from this NAD and FAD part. So, in these two parts, that means, in the cytochrome chain, if we consider from here – the chain involving cytochrome b, cytochrome c 1, cytochrome c and cytochrome a and a 3. So, this particular part is a very small cytochrome electron transfer chain. So, this – if we can consider cytochrome electron transfer chain, we get only 2 mole of ATP synthesis.

So, you have the sufficient amount of free energy change responsible for the synthesis of ATP molecules, which is the energy store. We can store the energy, because always when we bond the food material, when we bond the corresponding that glucose molecule, we always try to produce large number of ATP molecules. So, this energy stored we can have; and, in a stepwise manner.

When we have the reaction of NADH with O 2, the entire chain can be broken off into several steps. And, all these discrete steps are very important. And, some of these steps can produce only the right number of ATP molecules; it is not that the entire change in corresponding delta E value will tell us that, the total number of ATP molecules, what will be synthesized in this particular process. So, this thing and the electron transfer from this cytochrome c to cytochrome a and a 3 is also very important, because in this particular part, we will get the synthesis of ATP molecules.

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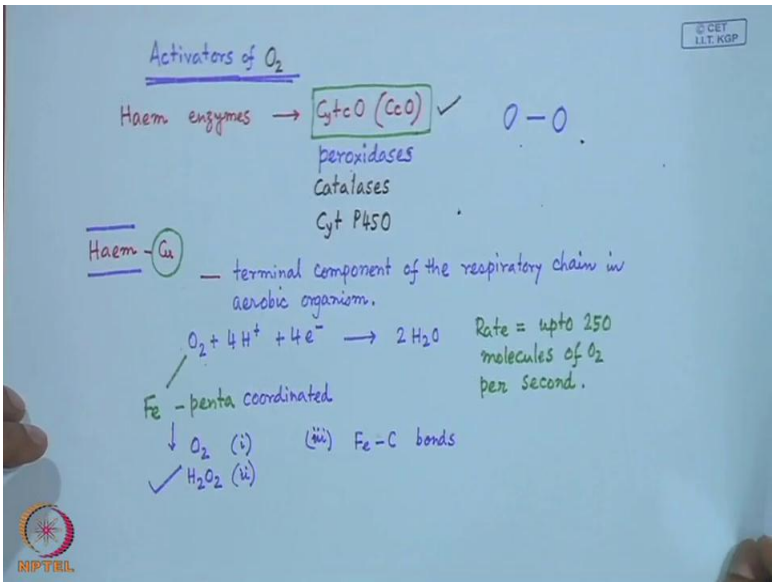

With the involvement of O 2, we have the O 2 molecule in our hand; and, how we can activate this O 2 molecule; that we should know. There are some molecules, which we will consider as activators of O 2. The way we have seen that, there are some molecules like myoglobin and hemoglobin, which are very good for binding the O 2. But, in this particular case, there are some molecules, which are responsible for activating the O 2. So, these are the haem enzymes.

And, these haem enzymes – one, we have seen that is cytochrome c; now, the next one, that means, the last one before the binding of this O 2 molecule is cytochrome c oxidase or cytochrome is abbreviated as C c O. So, this is the different type of cytochrome molecule. And, that cytochrome molecule will show us that, this is basically a system, is a complex system, which has haem protein plus some copper. Some copper is brought to the system. And, copper has some important role to play to function as a cytochrome c oxidase, which is nothing but the terminal component – the end component – terminal component of the respiratory chain in all aerobic organisms, because we are talking about thing, which is utilizing oxygen.

The simple reaction, which is… We all know that, involving 4 proton and 4 electron giving rise to 2 of the water molecule. And, the rate of the reaction is now is pretty well-known to us also. We have determined for that also that, it is a very first reaction. So, up to 250 molecules of oxygen is consumed per second. This – very first reaction for the

corresponding reduction of dioxygen involving one such species, which is the cytochrome c oxidase. And, this particular one – when we talk about that, there is a point that, this O 2 will be there. So, you have this iron. If iron is involved; not that copper for dioxygen binding; but, copper is also known for dioxygen binding in hemocyanin we know. This iron – if it is only penta coordinated, then we can think of binding of O 2 from the sixth site. So, one such example for this cytochrome c oxidase – we will find that, iron center is made penta-coordinated; and then, the O 2 is allowed to bind to that iron center and it goes for its corresponding reactions.

Then, in another case, we will find that, this particular site can bind H 2 O 2 as well. So, this is another group of molecules known as the activators of dioxygen. And, thirdly, we will see that, we can have some interactions, where iron carbon bonds can be formed with the substrate. So, here the choice is very open now; that either the haem group, which can be penta-coordinated can bind O 2; or, the copper centers are involved for finding the dioxygen molecules. So, together with this system; that means after studying this cytochrome c oxidase behavior, if the iron porphyrin system is involved in a typical fashion, we will see how they are behaving in some other molecules like peroxidases, because we are looking at something, where the iron centre is involved in binding the H 2 O 2.

So, if we can recall the corresponding binding of the O 2 to the iron center in the myoglobin molecule; that is, if there is some internal electron transfer and if we can feed one extra electron to that O 2, it can be converted to O 2 minus; that means the superoxide anion. If we can put 2 electron to the system, it can be a peroxide system. But, we are bit… We will be talking about something, where you have the activators of O; that means in this particular case, whether we should be able to break the O-O bond. That is important. So, one such reactivity pattern for this will be for peroxidases. Then, we will be talking about catalases and another different group of cytochrome molecule, but involving again iron and porphyrin, is cytochrome P450. So, cytochrome c oxidase, peroxidases, catalases and cytochrome P450. So, this we will see in detail for this corresponding binding as well as the electron transfer to the adjacent cytochrome.

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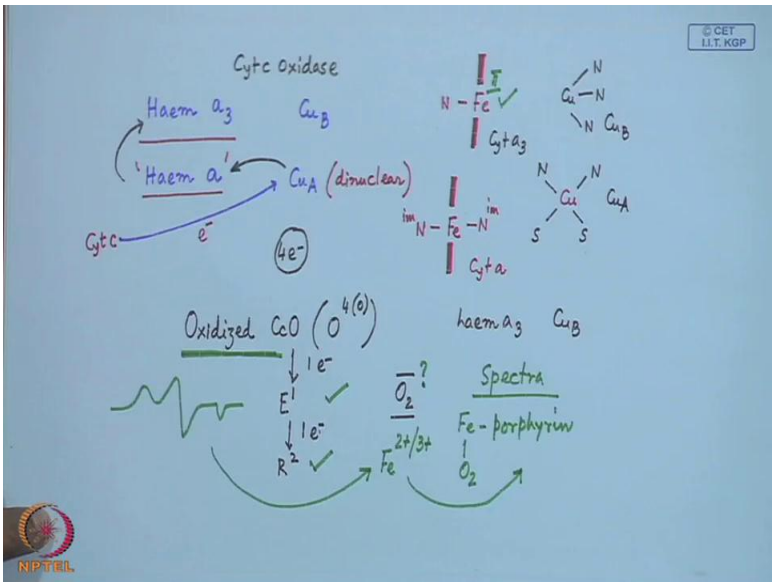

When we have cytochrome c oxidase bearing haem group; but, now, this haem group is a different one; we will level it as a 3. Already, I told you that, one is a 3 and another is haem a. So, two iron centers: one in a 3 and another in a. Then, we have a copper center, which is designated as B; and, another copper center, which is known as A. So, these particular cases, copper A is basically receiving the electron from the cytochrome c, what we are studying so far. So, cytochrome c is giving electron to copper A site. In this copper A site is a dinuclear one. And, once this copper site is given, and this copper site is nothing but we have one center as copper bound to 2 nitrogen and 2 sulphur, which is C u A. And, left to that; that means the haem a site is imidazole nitrogen; both of them are imidazole nitrogens. So, this imidazole nitrogen, which is the copper A; and, this is the cytochrome a there containing haem a function. So, the porphyrin has that suitable substitution, which we consider as haem a.

And, when that haem a is attached to that protein part and the corresponding environment with respect to that bis-imidazole unit, we get a cytochrome a 1. And, other one; that means haem a 3, which is the desired penta-coordinated species. So, haem a 3 is the desired penta-coordinated species, which is the cytochrome a 3. And, this copper, which is copper B, which has 3 nitrogen donors. So, one side is vacant. So, you have the possibility of binding the O 2 molecule to this iron as well as this copper. So, this is consecutively transferring one electron at a time.

When electron is posted from cytochrome c to cytochrome a site; and, this particular case, it can move from here to the haem site; and then, haem a site to haem a 3 site. So, oxidized cytochrome c; what we will see in the next part that, this oxidized one – oxidized cytochrome c oxidase; which we will consider as that, is a particular state, which is correspondingly, the number of electron transfer to the catalytic site; that means we are talking about only transfer of food electrons.

So, this particular state; that means the oxidized form; the number of electron transfer to the catalytic site of this haema 3 and copper B. Then, we see that, this can go for sequential electron transfer to another state, which is E 1 and another state, which is R 2. So, these are basically we will see; we will just discuss all these. These are the different states; that means in all these cases, what will see; that means the fully oxidized form or the fully reduced form and the intermediate form; that means you can get something; that means one electron reduced form or the one electron oxidized form.

Then, after reduction, what we will find that, after reduction, if the system is allowed to bind to dioxygen molecule; that is interesting to know, because we have seen that, you do not have anything in your hand, which we will study in detail – the corresponding binding and the elimination of this species. But, we can monitor the corresponding spectra.

So, the spectra of iron porphyrin system in your hand; then, this particular one – when it is binding to O 2 – that is also in our hand. So, this is different that, we know that, the oxihemoglobin or oximyoglobin spectra is completely different from the corresponding deoxy form. In this particular case, if you have some form of the cytochrome c oxidase in your hand, then like we go for the synthetic molecule – the corresponding oxidation or reduction; which we can go by electrochemical oxidation as well as electrochemical reduction. So, in this particular case, we will find that, we can go for this reduction as well as oxidation.

Then, in one particular state, you get; you monitor spectroscopically that particular state; then, you reduce the other state; go to the other state. So, these are typical levels basically; that E and R form – these are the typical level. But, spectroscopically, you can monitor this that, one is the reduced form by one electron; another is the reduced form by

the one electron. Then, what you can do; from there, basically, you can run in the same time, the corresponding EPR spectrum.

So, these two techniques – basically, the electronic spectra or UV-visible spectra or electronic absorption spectra together with the EPR spectra, will confirm the corresponding nature of this O 2 species, because O 2 thing is a notorious one in that sense, because it is a paramagnetic one; you have two unpaired electron on the O 2. Then, you are monitoring these two unpaired electrons by moving it to superoxide species or the peroxide species, which is the diamagnetic one. And, at the same time, the corresponding iron state, which is 2 plus or 3 plus, because this ferrous and ferric system has a characteristic EPS spectrum as well as the UV-visible spectrum.

If we can consider then that, during this reduction process, we already know that, we are starting from cytochromes, which is in the plus 3 oxidation state; if we are able to the reduce that particular center to a plus 2 one – a plus 2 site; and, we know that, the plus 2 site has little affinity for the 6 coordination site. So, it can be a penta-coordinated species. And, by reduction, if you are able to generate a penta-coordinated ferrous one, if this is a ferrous one; then, that particular center will be utilized for dioxygen binding. So, not only the electron transfer; now, the system is much more complicated, because now, with the help of copper and the iron center, you have to bind the dioxygen and then you have to put the electron on those bound dioxygen molecules. So, that will see; how this dioxygen is bound to the system and how you can transfer the electron to the dioxygen molecule.

Thank you.