Biological Inorganic Chemistry
Professor Debashis Ray
Department of Chemistry
Indian Institute of Technology, Kharagpur
Lecture 33
Activators of O2 and Electron Transport Proteins

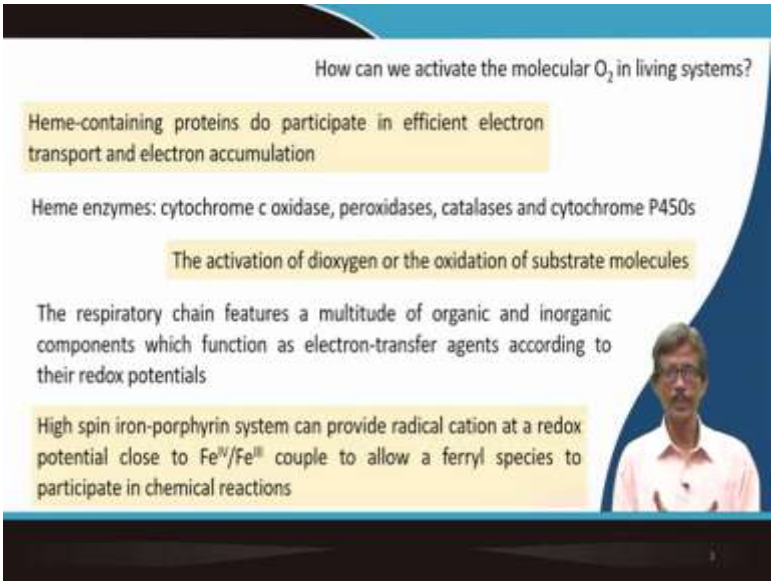
(Refer Slide Time: 00:30)

Hello everybody. So, we are with iron molecules and how iron biomolecules are important we are seeing. So, in this class basically we will now talk about O2 activation. So, in our previous class what we have seen that how we can go for the binding of the O2 molecule, so how we can activate O2 molecule and finally we can see also how these are responsible for your electron transport in the different protein.

(Refer Slide Time: 00:54)

So, the different catalytic processes we see and is iron centered catalytic processes all are biochemical processes then the heme proteins are responsible for this sort of catalysis, O2 molecules should be activated for your metabolism, for your absorption and the electron transferring components how it is transferring from one point to the other such that you can have the electron transfer reactions and the electron transfer chain. And finally we will see about the monooxygenation and the dioxygenation reactions.

(Refer Slide Time: 01:24)



So, how can we activate the molecular oxygen in the living system when it is coming and binding to your Fe center in myoglobin as well as hemoglobin? Let us see. So, you have the heme containing protein so they do participate in efficient electron transport or electron accumulation reactions. What we have seen that your O2 molecule is converted to your superoxide anion that means you have the facile electron transfer reaction which is intra molecular electron transfer reaction iron center is getting oxidized and your O2 center is getting reduced.

But apart from that many other heme enzymes will now bring about the last one we will talk about the cytochrome c oxidase or capital C small c o peroxidases, catalysis and cytochrome p450s a class of molecules or 450s, p450s will also discuss how why we call it as a p450s and all this. So, heme now we are labeling is as the enzyme so these are now catalysts and not only the catalyst for the conversion of a substance a to a b but sometime will find the catalysis for electron transfers only.

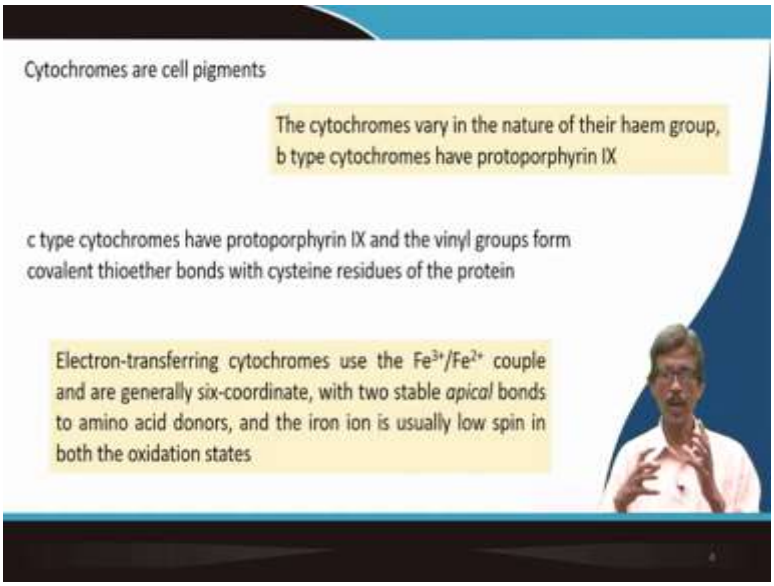
One a is there and is oxidized form or reduced form of a is there and that is also a catalytic process because you are giving the reagent, your reagent is nothing but your electron in the reaction medium. So, you have the activation of the dioxygen as well as the oxidation of the substrate molecule and in your respiratory chain we are talking about lung, we are talking about your mitochondria and your molecule is going to your tissues and cells and all these cases we have the inorganic and organic components and they are functioning as electron transfer agents.

So, only in few cases not in all cases will find that the heme enzymes are responsible that means the iron centers are responsible and those iron centers with the help of the different other molecules like your dioxygen molecule can facilitate those electron transfer reactions. So, initially what we had the high spin iron porphyrin system it can also give the radical cation like your chlorophyll.

We have discussed some point of time that you have magnesium and you have the porphyrin chain and if you go for your corresponding photo activation and you show that the charge separation can take place and you can go for the corresponding one for the charge separation so porphyrin can sustain the oxidized as well as the reduced form.

So, similarly, some values we can have a potential or E0 value which will be close to your ferrous to ferric and then to ferric to the tetravalent iron and that tetravalent iron species bound to your porphyrin chain will consider these as the ferryl species and which are very important and those ferryl species can also be formed at some time without going for your metal centered oxidation but you can go for the ligand center oxidation that means oxidation of the corresponding macrocyclic ring.

(Refer Slide Time: 04:15)



So, cytochromes are your cell pigments cyto, cyto is related to our chemistry which is our cytochemistry that means the chemistry which is happening within the cell and chromes we know that the chromophores are related to that and chromes are the colors or the material which is giving you the color that means the pigments.

So, the cytochromes molecules are giving you some coloration or the cell pigments to us and that is why these are different types of these cell pigments so they are responsible for absorption of h nu the electromagnetic radiation absorption as well as excitation and electron transfer or electron acceptance.

So, the nature of the heme group what sort of heme groups you can have and we already know we are giving examples for your hemoglobin and myoglobin that we can have the photo protoporphyrin 9 which is nothing but your b type cytochromes. Then another variety we can

have, we can have a c type variety which can also have the protoporphyrin 9 and the vinyl groups forming a covalent thioether bonds.

So, in our previous class we have discussed that you have the vinyl functions two of these vinyl functions on that or top part of the peripheral part of the porphyrin ring so these vinyl substitutions if they are available to react with the cysteine residues of the amino acids they can form thioether covalent bonds. So, this particular thing basically covalently linking now to the proteins.

So, the cytochrome unit which is there, which is tightly bound by not only the coordination from the 5^{th} and 6^{th} side but also covalently binding to your protein chain. So, electron transfer molecules or electron transferring these cytochromes can settle between in this particular case only between the ferrous and the ferric state and are generally that is why 6 coordinate. So, we have seen in case of our blood molecules or hemoglobin molecules and myoglobin molecules the preferred coordination number for all these sides are your hexa coordinated sides.

But we are talking little bit about that what you can have you can have the low spin state or you can have the high spin state. So, you can have the 4 possibilities; 2 oxidation states and 2 spin states. So, sometimes it is so difficult to identify which oxidation state your metal ion is and which particular spin state is there that is why we sometimes rely on the spectroscopic measurements which is related to that particular spin state chain which is your Mossbauer spectroscopy.

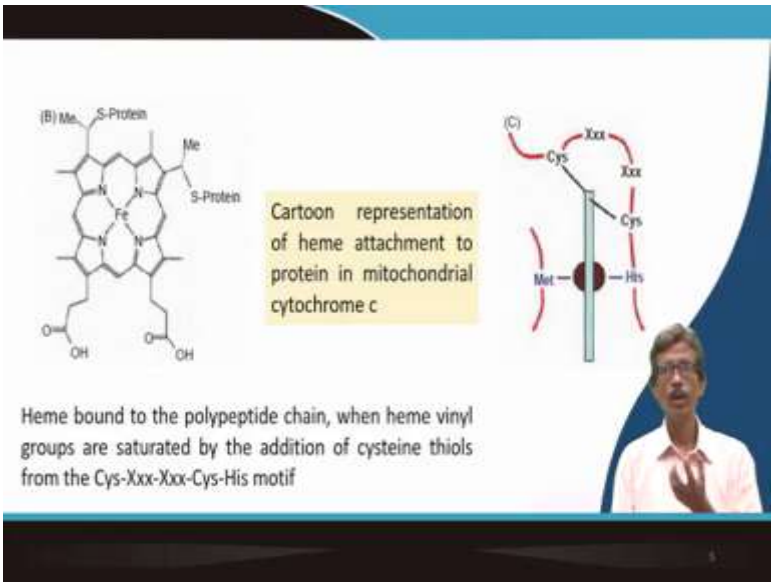
We have discussed some time back that how you can utilize the Mossbauer spectroscopy for identifying the isomer shift for all these species. So, if you have the stable apical bonds because earlier we have seen that you have the globin coordination and you have the O2 coordination in case of your myoglobin.

But now the different amino acids can come and it can coordinate to your iron sides and is basically giving a low spin configuration because it is stabilizing because the corresponding one that means the change in the corresponding magnitudes in the crystal field between the high spin and the low spin state is very less so it is in the spin state equilibrium close to the spin state equilibrium we know the energy is sometimes is decreasing with the change in the crystal field

and in some time it can increase also so you will have in the crossing p range we call it as a spin state crossover region.

So, within that spin state crossover region these molecules are basically citing in that particular point and when you have a subtle change only a very minute change either through coordination of oxygen or through the change in the corresponding tertiary or the quaternary structure of the protein environment that basically moving the iron side from a high spin state to a low spin state even there is no change in the oxidation state.

(Refer Slide Time: 08:10)



So, now you see the same molecule we are showing where we have seen in case of your myoglobin molecule now we are talking about some cytochrome thing and you have now the vinyl thing is there, CH double bond CH2 is there but when the cysteine residue is coming from the S protein chain so you will ending up with the two thioether coordination so covalently coordinating to your protein chain so it will not be able to go away from any pop this particular point so it is highly stable form compared to your myoglobin and hemoglobin.

So, what do you see that if you go for the cartoon representation of that attachment of the heme in the mitochondrial cytochrome C so we are considering that okay we are within the cell we are within the mitochondria, so mitochondrial cytochrome C is there now you see that instead of the flat thing you just now put it as a perpendicular one and you see the methionine sulphur will be coming what you have the corresponding coordination.

So, you see the cysteine residues from the top basically we are showing that the top you have the cysteine thioether coordination and another cysteines are the coordinates such that it is tightly bound to the protein chain and further you are satisfying the coordination sites which is your 5^{th} coordinates site as well as the 6^{th} coordination site, so how much it is different from that of your myoglobin or hemoglobin molecule?

Earlier also we have seen that you have the porphyrin, the corresponding cofactor, the porphyrin heme cofactor is there and your histidine residue is coming but that histidine residue from the globin chain is completely different you do not have any such binding the covalent binding through your cysteine related sulphur thioether coordination, so it is highly bound form so this highly bound form definitely that will affect your coordination from your histidine residues and then you have now the methionine sulphur coordination.

So, this methionine sulphur coordination is basically changing not only the environment of the iron but also the different electronic property if you consider that the electron transfer E0 values and all these things will now be changing, so what we have discussed in our previous class we are talking about the corresponding O2 binding, now we are going somewhere where you are talking about the enzymes which can give rise to the electron transfer reactions and you can talk about the redox reaction which is reversible in nature.

But there a partial charge transfer was taking place your iron is partially oxidized to a trivalent state such that you can stabilize that particular entity in your oxymyoglobin or oxyhemoglobin but once you take out that oxygen from that particular medium it is delivering that oxygen to your myoglobin molecule and at one point also myoglobin will also be delivering to that O2 molecule to your glucose molecules.

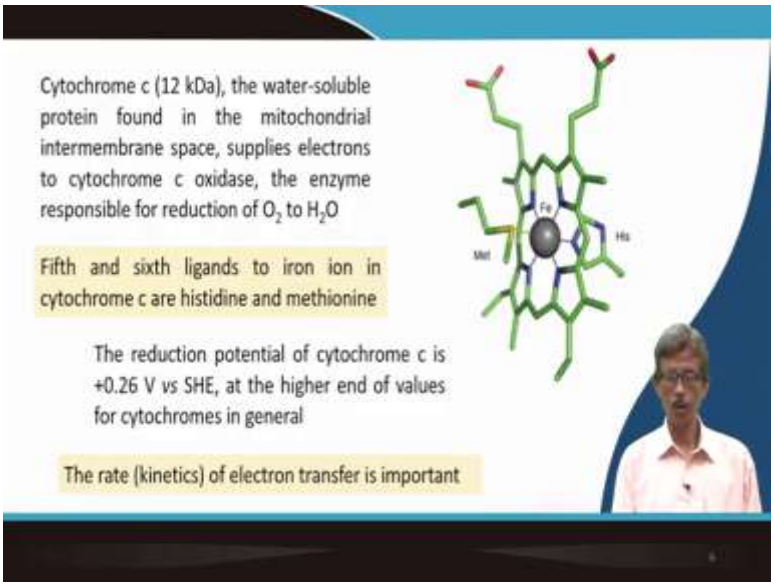
So, during the delivery process when O2 is released your molecule is immediately changing back to your previous condition or previous state so here these two are settling for your electron transfer only is not delivering any O2 molecule is not accepting any CO2 molecule or it is not accepting any H plus or BPG type of molecules that is why it is completely different.

So, if you have the heme bound system a polypeptide chain is there and we are locating only three residues, two cysteine already I told you that is giving you the thioether coordination two cysteine residues and you can have also the immediately the histidine residue so you see these

two are adjacent, so the cysteine and the histidine motive are adjacent, so once you have the covalent linkage to that of your porphyrin chain immediately the histine residue is available for your iron coordination.

So, you see the corresponding coverage of this thing and that basically gives a particular type of stabilization to the system towards electron transfer reactions because if you go for a particular type of electron transfer reaction and if you consider that okay my ferrous state is more stable than that of your ferric state so your electron transfer reaction will give you some weak molecule if you go for the other state of that particular oxidation or reduction.

(Refer Slide Time: 12:24)



So, another cytochrome C which is a 12 kilo Dalton molecule which is can be considered as a water soluble protein found in mitochondrial inter membrane space and which can supply electrons to cytochrome C oxidase I just I told you that is a capital C sometime we have abbreviated form capital C small c o, CC is a cytochrome C and oxidase which is responsible for electron transfer reaction due to the reduction of O2 for making water molecules.

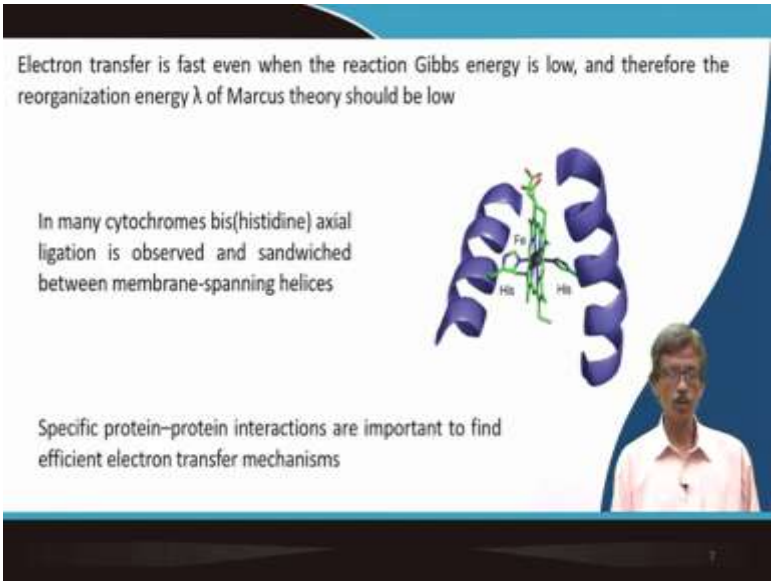
So, that is your terminal cytochrome which we require for our food burning or electron transfer chain or the different types of ATP molecules synthesis and the different types of cytochromes available. So, already we have seen that one is histidine, another is methionine so if you look at the corresponding molecule compare to that of your other coordination side so definitely all these

cytochromes are basically, if you talk about the corresponding covalent binding and then you have the methionine coordination as well as the histidine coordination.

So, this is basically roughly the environment the iron environment because we are looking for electron transfer reaction centered on this particular iron center only. So, the reduction potential basically for this cytochrome C is plus 0.26 volt versus saturated hydrogen electrode and these are towards the higher magnitudes of these cytochromes compared to the other cytochromes if you compare. So, if you have many of these that means if you have number of cytochromes basically and if we can have the measurements for all of them will see that the depending upon the nature of these cytochromes you can have the different E0 values.

So, what we see that not only the electron transfer the E0 values but the rate the small k the rate of electron transfer reactions will also be important that is why Marcus theory is also important not only the Nernst equations are important for taking the advantage of knowing this particular kinetic process for electron transfer that we most of the time we go for the corresponding bioelectrochemistry or bio cyclic voltammetry type of thing.

(Refer Slide Time: 14:38)



So, in this particular case if an electron transfer is very fast even when the reaction Gibbs energy is low that means your potential is not driving that particular reaction but your electron transfer the kinetics basically is governed so is basically we call sometimes about your organic products or many other reaction products we call is the kinetically controlled product or

thermodynamically controlled product so here you can say is a kinetically controlled reaction or thermodynamically controlled reaction.

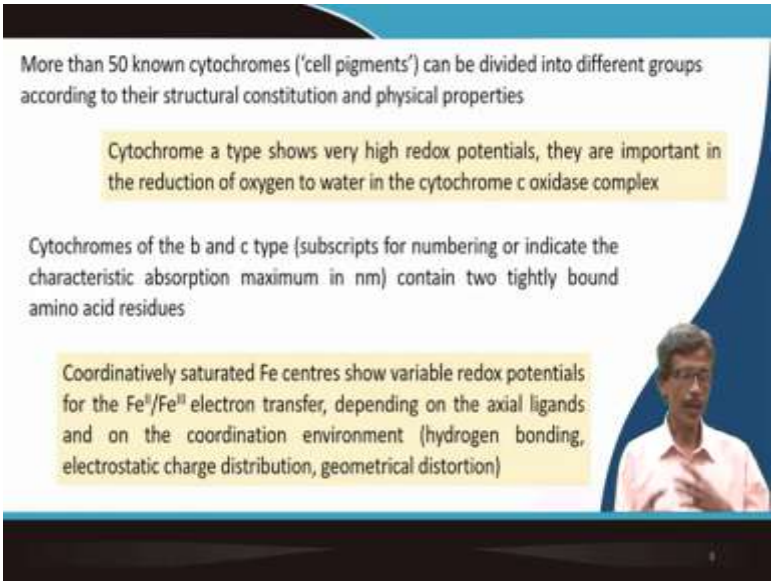
So, kinetically controlled reaction is your electron transfer reaction when you can have a very fast electron transfer reaction because one lambda value is associated with the Marcus theory the Rudolph Marcus another Nobel laureate who did all these works particularly after the experimentally determined work of Henry Taube in 1984, then in 1992 Marcus got this Nobel prize due to finding out all these reorganization energy values and the corresponding electron transfer which can be your outer sphere electron transfer or inner sphere electron transfer so all these things got involved.

But right now what we see that you can have another variety where the thioether can be removed so instead of your thioether coordination you can have two epically or axially coordinated histidine residues and then if you just simply compare the E0 value will find that the sulphur is a bigger donor and easily polarizable donor and it can basically stabilize the high oxidation state of iron as the ferric state.

So, your potential for this will be higher when you go for thioether to another histidine residue so in that way basically we can manipulate the E0 values, so you see when you see that you can get this particular one that means you can have B cysteine residues and the other one which is your thioether histidine residues.

So, these two can be your two side by side or adjacent cytochromes which can be functioning as one as the oxidizing agent having higher E0 value and one will be your reducing agent which can oxidize, reduce that particular cytochrome and move the electron from one site to the other in the long electron transfer chain. So, protein-protein interactions are also important which can also give some good idea about the corresponding efficient electron transfer mechanism or efficient electron transfer chain.

(Refer Slide Time: 17:07)



So, there are more than 50 known cytochromes basically we can have the structurally determined also so they are well known now not only known they are well known about all these big cell pigments and they are divided about their structural constituents and the physical property that means the E0 value and what are the structures basically with this.

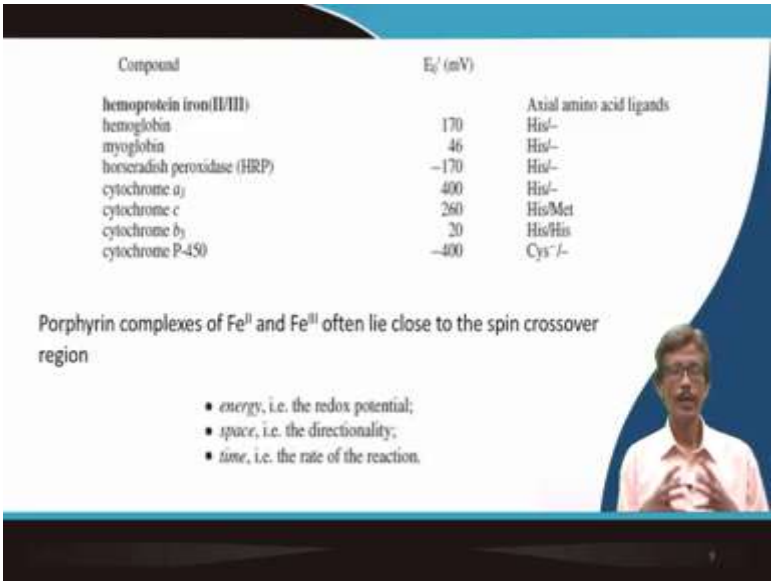
Cytochrome a type shows very high potential values are important in the reduction of oxygen to water in the cytochrome c oxidase, so in cytochrome c oxidase which is a big one but you can have two cytochrome residues and two copper proteins when you talk about copper proteins also will bring together all these and we will talk again and again about this cytochrome c oxidase system which is a very complex system.

But in between you can have the b and c where basically giving you the corresponding characteristic nomenclature for these or the absorption maxima in nanometer sometimes we typically write is a 420 or 410 nanometer for these bound cytochromes due to that of the amino acid residues.

But apart from that they can also have the different redox potential values due to this electron transfer between these two oxidation states those are due to when you change the environment the coordination environment of the RN site how you know the coordination is there you have the corresponding coordination from thioether sulphur or can be simply from the histidine imidazole.

But apart from that you can have the hydrogen bonding electrostatic charge distribution that means the positively charged of the negatively charged donor groups around there and the geometrical distortions also can contribute about your E0 values because we know that the different isomers if you can have two isomers of a particular metal ion centers say nickel or iron if you have typically a facial isomer and a meridional isomer we all know the change in the corresponding donor groups basically you can have a all three nitrogen phase or all three oxygen phase for a tris complex where the ligand is NO type nitrogen of one side and oxygen from the one side. So, these two can have two different E0 values when you determine by doing direct cyclic voltammetry of this sort of isomers.

(Refer Slide Time: 18:48)



So, similarly, for all these molecules what you can have the different types of cytochromes you see that the changing in the axial or the apical sometimes I prefer basically to write is the apical amino acid residues is the basal plane and this is the apical plane to compare with that of your nomenclature what we use in organic chemistry.

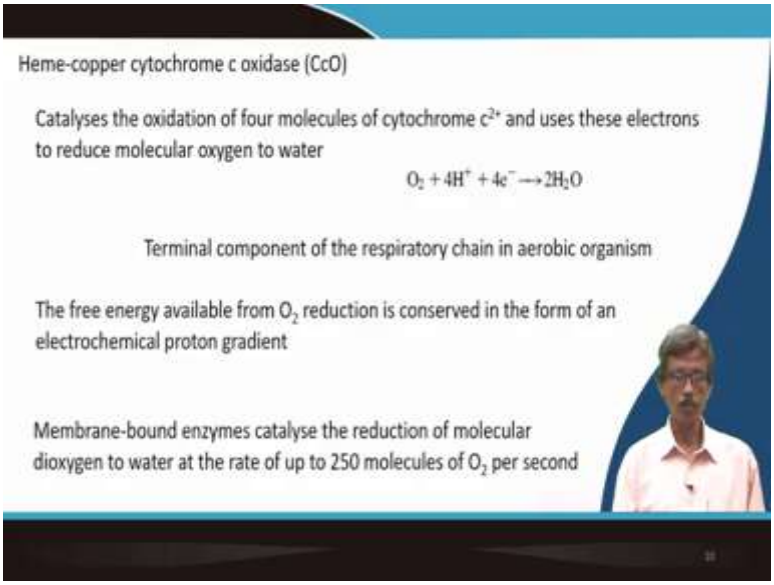
So, that apical amino acids basically histidine to other things and the cysteine residues also sometime you see that the more positive one is for the histidine residues and the histidines are all neutral residues but the nitrogen is a corresponding one is a hard donor and that basically stabilize the trivalent state so you can have the corresponding one as the corresponding high oxidation potential of positively value, positive value of 170 millivolt but when you have the

polarizable charge sulphur from the cysteine residues that basically give you another values of potentials which is minus 400 millivolt.

So, this particular range basically so within the electron transfer change you can move from one point to the other and you can have the corresponding change in the corresponding E0 value because at some point you can have the corresponding change in the E0 value, you can have the free energy change also and due to that free energy change the suitable free energy the magnitude of free energy is such that you can have that particular electron transfer can go for your suitable amount of or suitable numbers of ATP molecules.

So, they are basically as I already told you that they are lying basically close to your spin cross over region that is why you move from one side to the other very quickly because you are not going for a much change in all these cases but we talk about the redox potential then the space that means the spatial orientation of the molecules, then the directionality of the corresponding donor groups and the rate of the reaction that means the kinetics of the reactions and the parameter from the Markus equation we get the rate of the reaction how quickly we are going for those reactions.

(Refer Slide Time: 21:30)



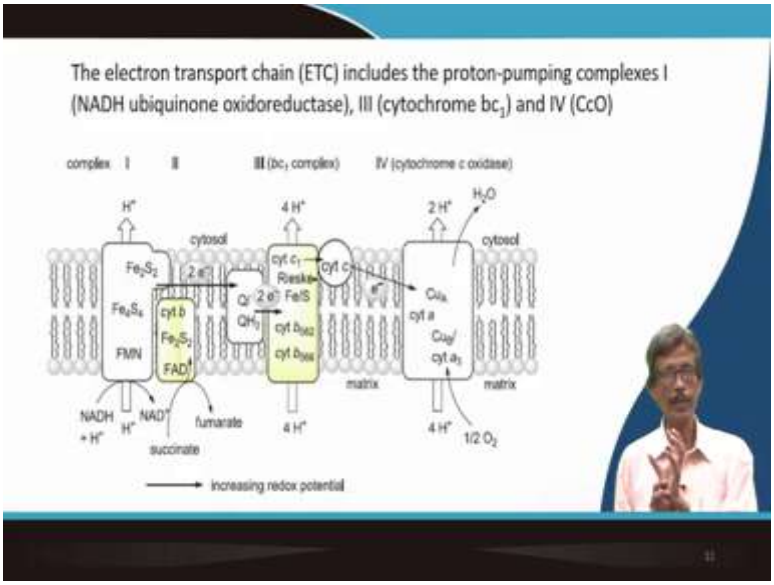
Then now we see that the cytochrome c oxidase form the view point of your heme proteins or the cytochromes only not about the copper sites so when you talk again about the copper proteins in our next week classes we go for the copper proteins or the copper enzymes when will be able to

bring these two together then we will get the real picture but right now what we see about the system where you have the heme system which is attached to the copper system also and we get the corresponding terminal oxidase enzyme which is be responsible for your oxygen reduction to water molecules.

So, these are basically giving some example of oxidase reactions so these oxidase reactions are basically important at some point for all these cases that where you can have the oxidase reactions or oxygenase reactions. So, they are basically catalyzing the four electron reactions and cytochrome or C2 plus is the ferrous ion and basically going giving the oxygen molecule to forming water molecule by this particular reaction.

So, that is the end thing that means the terminal part terminal in the respiratory chain and the free energy available for this O2 reduction is conserved as your electrochemical proton gradient and that electrochemical proton gradient is required for your ATP synthesis. So, all these are membrane bound enzymes and they catalyzes the reduction of molecular dioxygen and basically they going a very high rate that is why the rate is important of 250 molecules of O2 per second they are consuming for the reduction of dioxygen molecule to water.

(Refer Slide Time: 23:16)



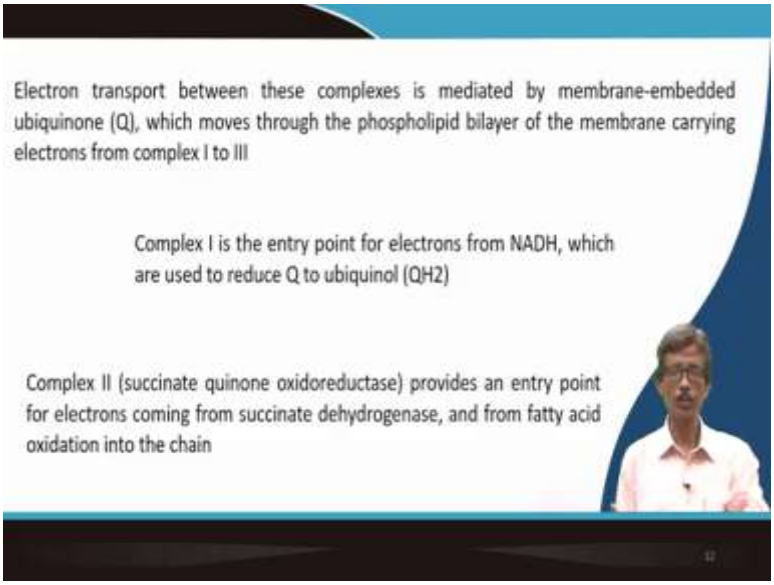
So, what are this big complex structure within this ETC electron transport chain or electron transfer chain and the proton pumping. So, you have NADH, you have the complex 2, you have the complex 4 and all together. So, now you see that all these cytochromes if you put from left to

right on the right you basically get the O2 molecule and on the left you are having the food stuff or the glucose molecules.

So, glucose is the most highly reducing agent and oxygen is the more highly oxidizing agent in between you have the corresponding ETC and you have at different positions you have the corresponding synthesis of the ATP molecule, so you have the succinate fumarate and you have the corresponding lipid bilayer in the cytosol, so the cytosol you have the complex 1, 2, 3, 4 all but you just try to look at where you have the different complexes the cytochrome, b c 1 or in cytochrome c c o and you have the NADH.

So, at the left hand side where we see also the Fe4 S4 molecules iron sulphur proteins, will also talk about the different iron sulphur proteins in our next class and we will talk much about the corresponding oxidases and oxygenases. So, when you get so finally at the end basically your half of the O2 molecule is converting to one of the water molecule basically and giving 2 electron not 4 molecule but you can have also pumping the protons because for that particular purpose your proton gradient should be higher at the other site because their O2 or O is forming O2 minus and that O2 minus is taking up two protons forming your water molecule.

(Refer Slide Time: 24:54)



So, this electron transport between the complexes basically are mediated by membrane embedded ubiquinone, so is a basically nothing but a quinone type of molecule so Q, Q is nothing but a quinone, quinone, what is quinone? Quinone is a very easily reducible species or

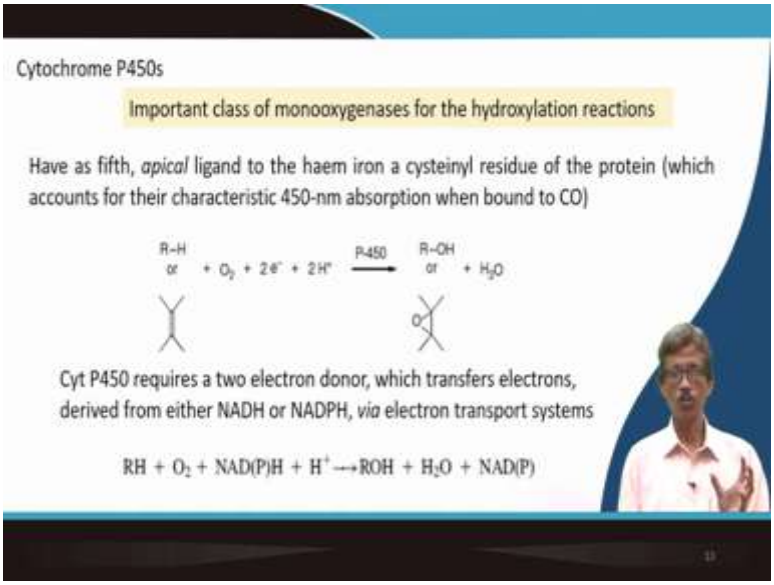
oxidizable species it can go for reduction which moves through the phospholipid bilayer and membrane carrying electrons from the complex 1, 2, 3.

So, you have the electron transfer at the same time you have the proton transfer also but if you have the quinone type of thing when you reduce it you go for the corresponding all form that means the phenolic form and it takes up the corresponding protons also after this electron transfer.

So, complex 1 basically on the left is the entry point for electrons from NADH. So, NADH is the biologically available reducing agent and that is supplying the electrons to the system which is used to reduce the Q to QH2. So, that is why now NADH 2 quinones are now available. So, no metal ion is there but you should learn about or know about all these thing because they are interrelated.

Then the next stage the complex 2 after complex 1 you have the complex 2 and then we have the oxido reductase group of molecule but is on succinate based, so succinate quinone oxido reductance will come that is for complex two and succinate dehydrogenase is there and the corresponding one for your corresponding fatty acid oxidation into the chain. So, not only your glucose molecule or the carbohydrate molecule but also we can go for the corresponding oxidation of the fatty acid.

(Refer Slide Time: 26:38)



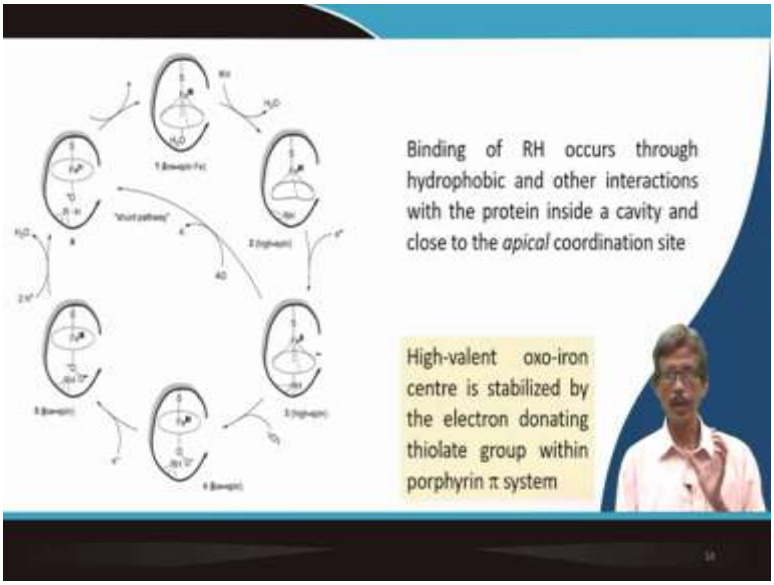
Then now we see about the cytochrome P450 which is a very important molecule to understand because P is the corresponding form or the product of those cytochromes and it can have the corresponding absorption at 450 nanometer because we know all that for corresponding photo system that means the chlorophyll the solid band is coming usually at 420 nanometer. So, it is rate shifted of shifting of magnitude of 30 nanometer from 420 to 450 nanometer.

So now we go for something which is completely different from that of your oxidase type of reaction but it is oxygenase type of reaction that means it is given for your corresponding oxygen atom transfer which is also some kind of oxidation we all know and is responsible for your hydroxylation reaction and that hydroxylation reaction what we can have that cysteine residue you have to bring on the protein.

So, that is why you have the different types of corresponding E0 value and the absorption for the carbon monoxide bound form so a 450 nanometer band that is why it is labeled as the corresponding one, so it can go for the corresponding hydroxylation of your R-H molecule to your corresponding R-OH molecule like methane to methanol or some double bonded species to your corresponding epoxide species but P450 can be useful for that kind of oxidation reaction.

So, you must have the oxygen doner if you have the iron center, so if the iron center is abstracting that oxygen doner so that cytochrome or cytochrome P450 can be a very good reagent for your oxygen transfer reaction or hydroxylation reaction. So, many number of electron transfer reagents are required, NADH is required for this particular type of electron transport to sustain and what is basically required for RH to R-OH conversion is NADPH and NADP formation and your own of the other oxygen atom which is not getting inserted in RH will be converted to your water molecule.

(Refer Slide Time: 28:49)

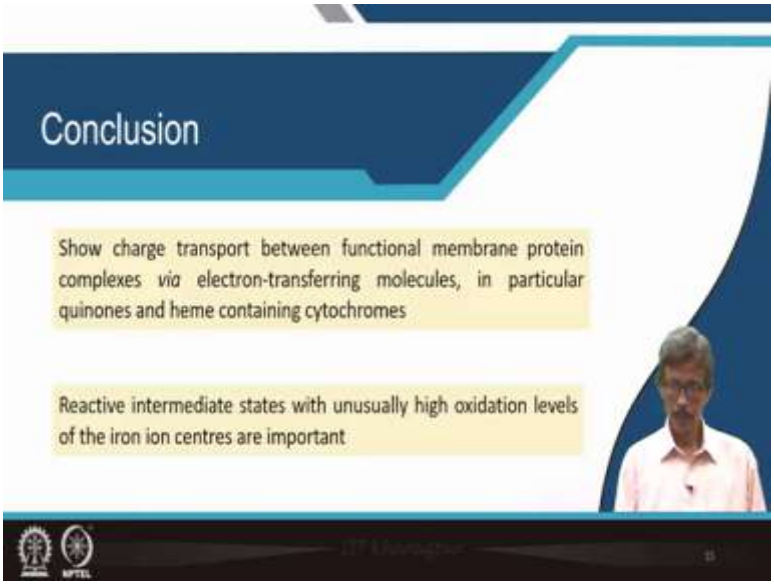


So, the binding of RH can take place and it occurs through the hydrophobic environment and other interactions within the protein inside the cavity and the apical coordination of all this thing. So, do not worry about the big sphere drawing at the end basically of my class but you just see it you just follow it nicely that we are not talking anything else, what we are talking about from our hemoglobin or myoglobin days that you have the ring, the ring is your porphyrin ring, iron is sitting above it and then you have the bottom part you have the coordination, you have the coordination of that oxygen molecule particularly from the lower side if you see, only thing that your apical coordination is different which is your cysteine sulfur.

So, cysteine sulphur is basically changing the electronic property of your iron that is why your activation for dioxygen molecule is completely different unlike your myoglobin hemoglobin it is now activated in the form of something where you go for a corresponding to electron transfer or it can go for a peroxide unit and that peroxide unit can ultimately go for a system where you can have the high oxidation state stabilization or Fe4 at the top left side you have the Fe4 system and the oxidized porphyrin unit which is required basically for your hydroxylation reaction until and unless you have that particular species you will not be able to get the hydroxylated product.

So, you have the high-valent oxo-iron center which will be stabilized and it has the stability due to the thiol coordination as well as the porphyrin Pi system.

(Refer Slide Time: 30:23)



So, what we have seen that we have seen the charge transport between the functional membranes of these proteins and the electron transferring molecules are there and the different quinones are there which are typical biological molecules as well as the heme containing cytochromes are there with you. And all these reactive intermediates are usually having high oxidation states of iron centers and which are very important like that of your last example what we have seen in case of your P450 systems.

(Refer Slide Time: 30:54)

So, references cytochromes you start with the cytochrome page and the book of Crichton. Okay, thank you very much for your kind attention.