Basics in Inorganic Chemistry
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Lecture - 18
Metalloprotein (Hb, Mb, and Transferrin)
And Metalloenzyme (Plastocyanin)

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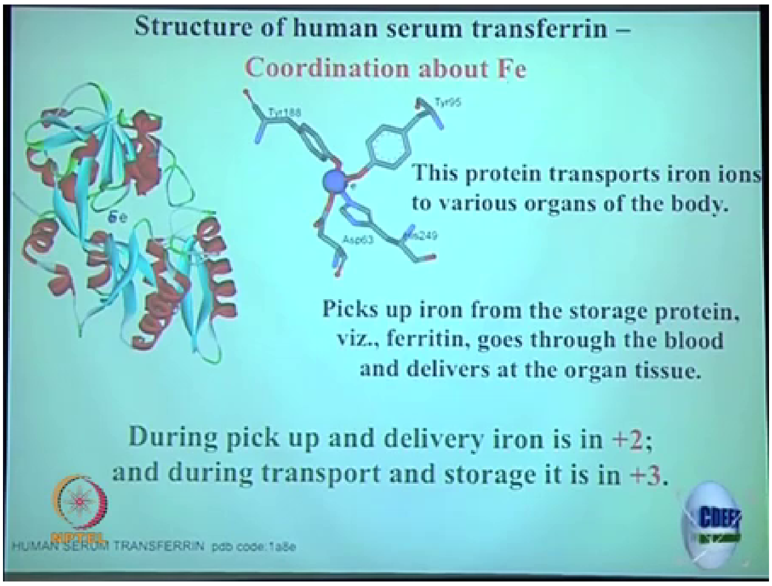

Now, this is the one example I am trying to tell you. Again, you do not have to worry about few things, let me get it very clear. You should not be worrying about these drawing ok. These are the protein backbone; protein backbone means amino acid put together it is forming. It is a huge polymer; polymer means let us say it is a huge length, it could be a lot of things together lot of amino acid together. And that is how these sort of different orientations are given, because that is how they look like different you know way of they are structural aspects that is going on over here structure are different.

So, you do not have to worry about all these protein backbone or the peptide backbone. If you zoom on over here, you see that amino acid backbone these are from different amino acid; all those. Amino acid if you look at you can see their structure they are nothing but organic compound ok. For example over here tyrosine, tyrosine is nothing but phenol containing; amino acid which has a phenol let us say phenol. Tyrosine oxygen can bind with the iron centre, another tyrosine oxygen phenyl ring phenol oxygen can bind with the iron centre. This is histidine and this is aspartate. Aspartate is having carboxylic acid, CO 2 H; carboxylic acid that can bind with the iron centre this is having histidine unit just nothing but imidazole. Imidazole you have heard of imidazole, phenol, carboxylic acid; these are the side chain of amino acid.

All those amino acid we know which it is having some side chain which can bind with the iron centre or any other metal centre. So, this is the one, let us say its transport iron that is what I was telling this is a pizza delivery guy; it is a delivery guy iron delivery guy right. Now, it picks up iron from different side and it see it is not like they have to move they can just pass on also. You can I can take I can give it is a it can be a relay process as well right.

Overall, this is where it picks up or that is how one of the example we are showing how the metal centre is going to be picked up. It binds with the amino acid backbone and it gets picked up and then delivered at a certain point, where it can be stored. Pickup iron from storage, iron protein you can read. But usually what we see is iron centre’s during this movement, when it is moving, this is pickup and delivery time it is going to be plus 2 during transport and storage it is stabilize in plus 3 stage.

Because plus 3 at the one which is oxidized state little bit stable state, during this storage it has to be stable. So, their redox chemistry is used for their transport as well right. Storing is in plus 3 form and during transport also it is in plus 3 form. During picking up and during delivery it is in plus 2 form ok, all right.

So, what essentially we are trying to tell you is a metal ion gets delivered by different approach. One of the main approach is simply different protein backbone, that is nothing but amino acid backbone; protein backbone is nothing but amino acid backbone. Amino acid has this phenol unit, this imidazole unit, carboxylic acid unit; these are ligand these usually acts as a monodentate ligand and thereby can bind with either let us say iron 2 plus or iron 3 plus or zinc 2 plus or copper 1 plus copper 2 plus ok.

Of course, you have to also understand that hard soft I told you hard soft acid base, when you have a harder one for example. So, harder anion will come right, when you have let us say you have copper 1 plus versus copper 2 plus; copper 2 plus is harder one.

So, if copper 2 plus has to be delivered it and let us say the ligands are very hard copper 2 plus is hard, so the ligands associated with it during the delivery time its let us say very hard right; hard anions are bound. Now the place where it is going to get delivered it is not having the harder anion. What copper has to do or copper has to undergo is reduction; copper 2 plus will undergo reduction to come to copper 1 plus, since copper 1 plus in soft that is high previously which was having hard ligand it will not bind it very tightly.

Now, that softer copper one can be exchanged from one place to another. Let us say storage place if you say or the delivery place wherever it is delivery, so that is how things gets also delivered. You have very hard situation, very strong situation hard anion all the oxides let us say are bound. Let us say carboxylate O minus or phenolate pH O minus differ whatever you know as hard; hard ligands are bound with a hard cation.

Now, then you want to deliver it to a softer place. So, the metal centre has to undergo a reduction and thereby softer centre will pick it up, and the reverse is true also the other case; soft to going to hard. So, all this simple principal actually matters; what ligands is there, what ligand environment is there, what oxidation state is there and thereby you can deliver in a particular manner.

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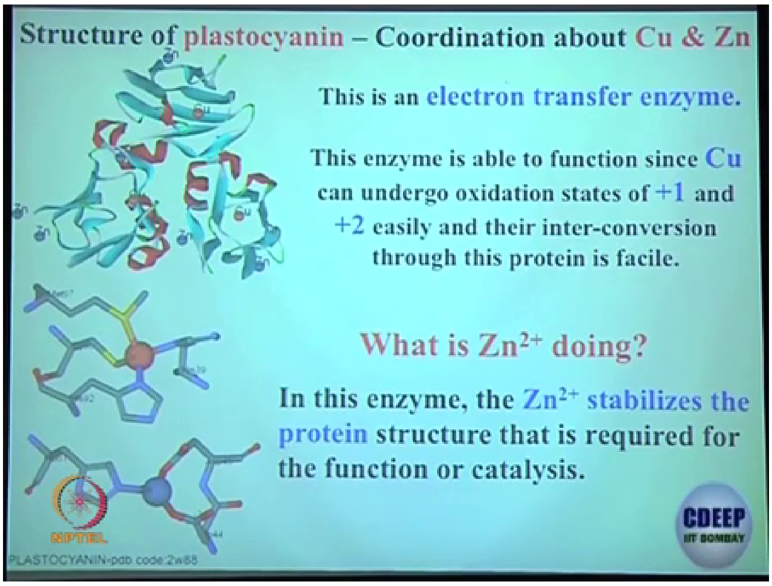

So, this is another enzyme. I am just giving again you do not try to memorize too much because you may not be able to get into too much detail, we do not want you also at this point. These are protein backbone, it looks quite funky and big. But if you made it down, if you zoom it down what you see is this. So, different protein backbone is coordinated with metal centre and another different protein backbone is coordinated with another centre.

Essentially once again hard soft acid base principle will be followed. Of course, also another thing you have to see what is available. Sometime whatever is available they can still go with it, if there is no competition between hard and soft centre. Even if it is in a soft centre hard if metal centre is soft, but hard anions at the one which is available. It is it will go with it, it is not like they cannot bind binding efficiency may not the great that is it. Overall I think the what we are trying to say, this enzyme is able to function since copper can undergo oxidation state blah you read it I think I explain it. Copper 1 plus and copper 2 plus, iron 2 plus and iron

3 plus these oxidation state change help you to do a lot of chemistry; one or two chemistry will discuss today that is number one.

Now, zinc plus does not undergo usually this oxidation state change, they can give the structural aspect they whole things together. And thereby provide some chemistry to be done at the centre. Let us move on.

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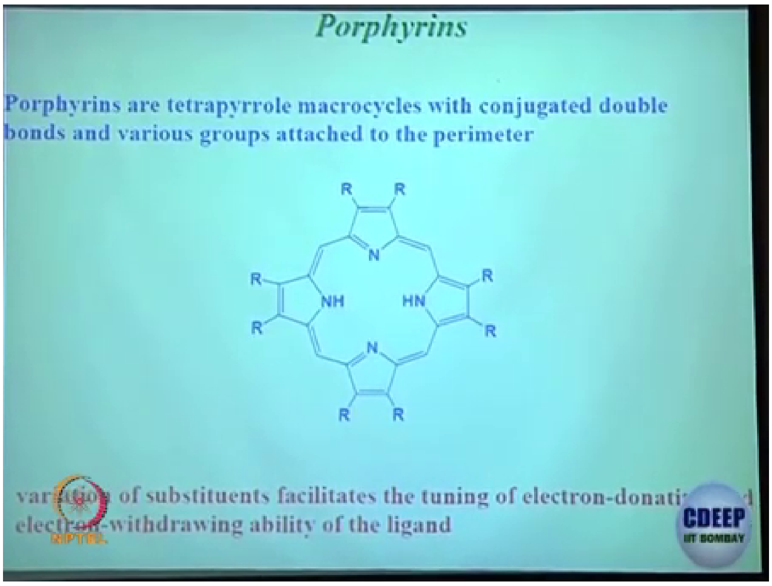

Now, I think the major part of the syllabus is on hemoglobin and myoglobin or so called the porphyrin centres, right. I do not know whether you have studied little bit before, fine.

So, porphyrin centre is something, this is one thing I i would ask you to remember and draw it again and again. Usually, what people do it they cannot provide the double bond in a rational manner; you try to draw it usually in the exam it is given, ok. Draw porphyrin or you will be ask in a question in a way that you have to draw porphyrin ok. Draw the porphyrin; what is not important is these R, what substituents are there.

Of course, these substituent these R group will determine whether a complex is going to be soluble or how soluble, they have a role ok. But for this class you do not have to understand or know what the role they are actually playing. By changing the R group you can change or modulate the properties to some extent ok.

Now, this is the porphyrin you should be very familiar with, this is nothing but a ligand, this is a chelator; it chelate the metal centre actually it is a tetradentate ligand. Usually what we have seen so far in the amino acid backbone is monodentate ligand usually, right. Means only one coordination sides are there: one let us say phenol, phenol oxygen can bind right; imidazole, imidazole nitrogen can bind.

But porphyrin these 1, 2, 3, 4; 4 nitrogen can bind and if you put a metal centre over there you can see 1, 2, 3, 4, 5, 6; 6 membered metal that ring will be formed. You have seen the bidentate ligand how they are forming then I mean how they are biting it bidentate they are biting it this is like tetradentate. And this is a very good chelate, it can you know it is almost like those EDTA we were talking (Refer Time: 11:12) a can be extract out porphyrin is a strong ligand. It can extract out iron let us say are any metal centre very easily ok. Not I mean of course, not neutral form let us say iron 2 plus or iron 3 plus. These are these can be extracted or these can be put very easily over here.

Of course there is a side right, there is a cavity. So, anything that does not fit into that cavity will not have strong binding with the porphyrin. So, the size of the iron let us say iron 2 plus versus iron 3 plus will matter whether it can fit in here or not properly, ok. High spin and low spin will matter and thereby of course, high spin low spin size will be different, right. And thereby you can see whether metal can be sitting over there or not, ok.

Now this is what I was talking about iron binding.

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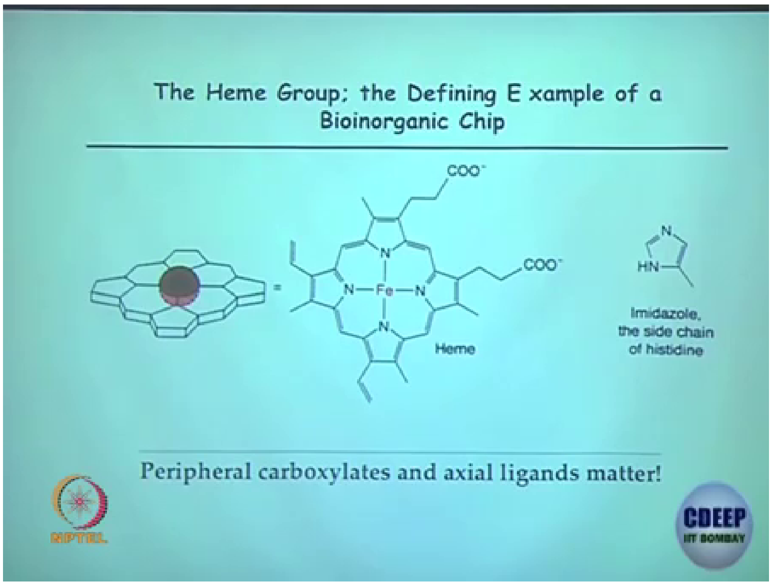

You see 6 member ring, 6 member ring, 6 member ring, and 6 member ring its holding it very tightly. You do not have to memorize this side chain, you have to just remember the core structure. Core structure includes these pearl ring right. So, these side chain whether this is a double bond or a methyl double bond or methyl-methyl or carboxylic acid these are the one you do not have to remember anything, ok.

But the porphyrin ring itself, porphyrin ring means without those substituent, that is the simple porphyrin ok. You can mean substitute, so you should be a very familiar with this. This is the imidazole which also binds at the. So, if porphyrin actually flat like this flat, so iron is right let us say in the middle of my thumb and then imidazole is right over here in the middle. So, below this plane this is a plane, below this plane imidazole is bind like is bound like that ok.

So, it is a tetradentate ligand porphyrin is a tetradentate ligand, this is the fifth coordination imidazole ok. The sixth coordination is over here which is open or almost let us say something is there very far interacting not too close, this is the centre where oxygen binds, when we are inhaling right oxygen gets in this is the side on top of it. On top of the flat porphyrin ring that binds the oxygen and that determines the life.

You can now imagine; I will come to that. If this oxygen binding side this is this is imidazole, this is oxygen binding side if these oxygen binding side is occupied by carbon monoxide or cyanide ok; I will see you again after life ok. So, that is what happens. Those bindings are going to be stronger right compared to oxygen binding those bindings are very strong, so oxygen cannot displace them. The ligand exchange between oxygen and C O will not happen other or cyanide will not happen. Other way around will may happen, oxygen can go of cyanide can come in. That is why it become so poisonous**.** So, deadly carbon monoxide cyanide ion and so on it is also one of your tutorial question, ok.

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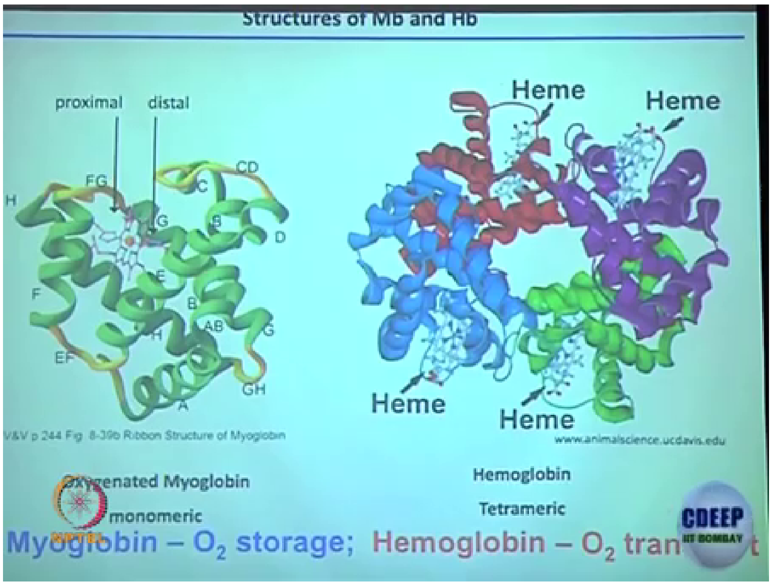

Now, I will go into again once again do not get; I mean some time we do get very there is feedback also right. Please give your feedback not the end of the class at the usually I think at the end of the semester or something you are allowed to evaluate us as a teacher. The way we do also get an opportunity to evaluate your performance by taking exam; I think that is kind of our exam it determines a lot of things for us. So, take it seriously for all of all of you or for all of the teachers, because a it determines of course you know not too serious, it is not life threatening, but it determines something, ok.

So, the major one of the major objective or topic of this chapter is myoglobin and hemoglobin. how molecular oxygen or oxygen which is in the air how it binds in the blood. In the blood we have porphyrin centre, the porphyrin centre we were discussing over here right.

So, this is there in the blood. And how oxygen from air binds with this centre and what happens during this binding. What happens when oxygen gets released ok.

So, as you know usually what we have learn or what happens oxygen goes in for our body we are showing just human; it goes in the lungs it gets in and then it gets delivered from all our body parts right. Now, let us say in a muscles it gets stored right, it gets stored in muscle so how oxygen is going to get transported and then how it is get stored in the muscle. That is the one kind of the major things will be discussing today.

So, the storage protein let us say in muscle what we have is myoglobin, where that is we are trying to say iron storage is there, some other metal storage is there, then oxygen storage is there, oxygen transport is there right. So, hemoglobin is the one which transports oxygen.

Myoglobin is a single unit, means one porphyrin centre is there; only one. Hemoglobin is the one where 4 units are there; 4 iron centres are there, 4 porphyrins are there ok. Where are they roughly; again you do not have to even attempt to memorize any of these structure, please do not ok. This is a heme centre heme centre, this is another heme centre, this is another heme centre; this is another heme centre. Heme means porphyrin ring plus iron that is called Heme. The porphyrin rings flat plus iron that is the heme.

So, we have a heme centre in here, we have 4 heme centres are in there. How they are connected? They are connected by these protein backbone ok, it could be let us say I mean heme centre is here another heme center is here, in between heme centre is there another heme centre is there, in between there is those protein backbone amino acid side chain lots of amino acid side chain putting together.

Of course, is peptide backbone those protein backbone peptide put together is forming protein; those protein backbones are together. It is not like all of them has to be covalently link; covalent bond we know some of them are covalent bond some of them are just link by hydrogen bonding. Let us say this is a protein, this is a another protein they are linked by hydrogen bonding, right. So, heme centre heme centre they are linked by let us say simply said amino, acid amino acid residue which is having let us say your covalent bonding ok.

So, all of them are connected if not directly none of them are directly connected, like iron centre bound with nitrogen that is a direct coordination ok, but there is indirect coordination such as hydrogen bonding and other loose forces which becomes important. So, they are connected, they can communicate with each other they can talk, it just like we can communicate they can communicate with each other that is essential ok.

Communicate means what happens to this centre, when something happens to this centre this centre comes to know, if something happens to this centre this centre comes to know and it relays. That is that relay of information will be learning in form of cooperativity; they corporate with each other ok, that is the cooperativity. That thing is missing from myoglobin because they have nothing to no one to communicate, they are isolated kind of system. One porphyrin centre it is not like their unhappy just they do not want to communicate ok.

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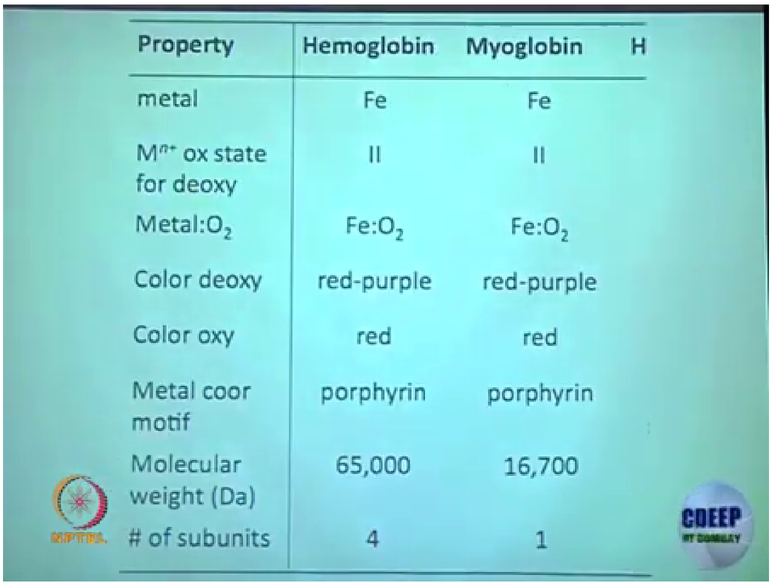

Property	Hemoglobin	Myoglobin	H
metal	Fe	Fe	
M^{n+} ox state for deoxy	II	II	
Metal:O_2	Fe:O_2	Fe:O_2	
Color deoxy	red-purple	red-purple	
Color oxy	red	red	
Metal coor motif	porphyrin	porphyrin	
Molecular weight (Da)	65,000	16,700	
# of subunits	4	1	

Now of course, if you want to compare hemoglobin and myoglobin first thing I said is; this is the myoglobin 1 hemoglobin 1 having 4 iron centre, so 4 unit versus 1 unit ok. Of course, they are molecular weight is going to be you know related by 4 times. So, myoglobin sorry hemoglobin is approximately 4 times compared to myoglobin ok. Of course, both of them are having porphyrin, both of them are red in color in the oxy form. What is oxy form? Oxy form simply means that oxygen is bound; deoxy form means that oxygen is not bound period ok. Oxyhemoglobin, deoxyhemoglobin, oxymyoglobin deoxymyoglobin; these terms will be used, deoxy means no oxygen is there, yeah.

What is there to remember, I do not see anything to remember. It is this, what I am saying you remember this nothing to remember. This is a porphyrin centre iron is there, porphyrin you have to remember; yes porphyrin you have to remember. That is the only thing I am asking you to remember, rest of the things you can just read or you know understand as a story. I think that is good enough, story we do remember I guess we can tell the tale, right now ok.

So, the color as you know the blood color is red, simple right it is going to be red. Now deoxy form when if you do not have any oxygen in it, it is going to be red purple ok. If you do not want to remember do not remember ok. Now, ok I did mean in that way ok. Of course, iron is in reduced form you are going to add oxygen ok, so iron will be in reduce form ok.

What will not be discussing today is the redox chemistry. What happens to the iron, is it going to iron 3 plus; yes, it goes but that information will not be discussing for the class. What will discuss is initially what happens. Initially redox chemistry does not happen, redox chemistry happen write after it you do not need to know this, we will discuss that.

Oxygen comes and binds with iron centre that is it, that is the syllabus. Right after that binding oxygen actually gets reduced, iron gets oxidized. That is not part of the syllabus do not even write in the exam, ok.

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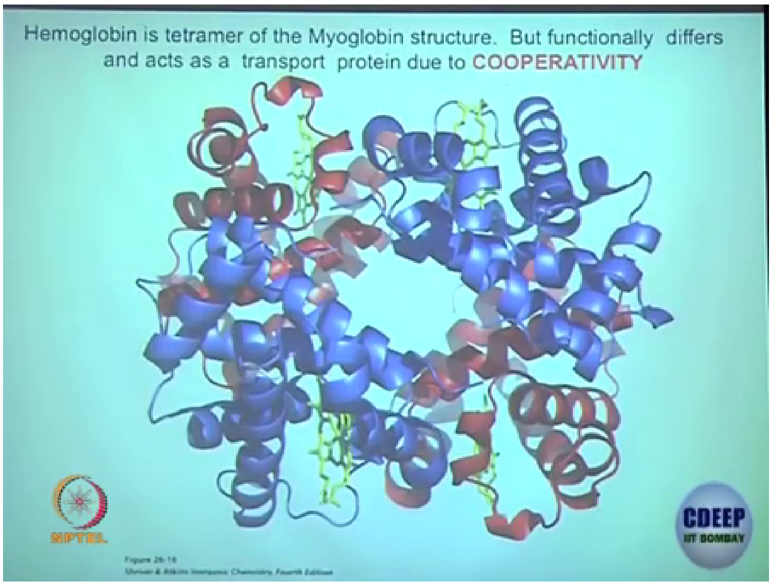

I will be discussing whatever part in the syllabus ok. This is the same thing again 1, 2, 3, 4 iron centre nothing else ok. 1, 2, 3, 4 iron centre; we are discussing myoglobin no hemoglobin see you understand by now, ok.

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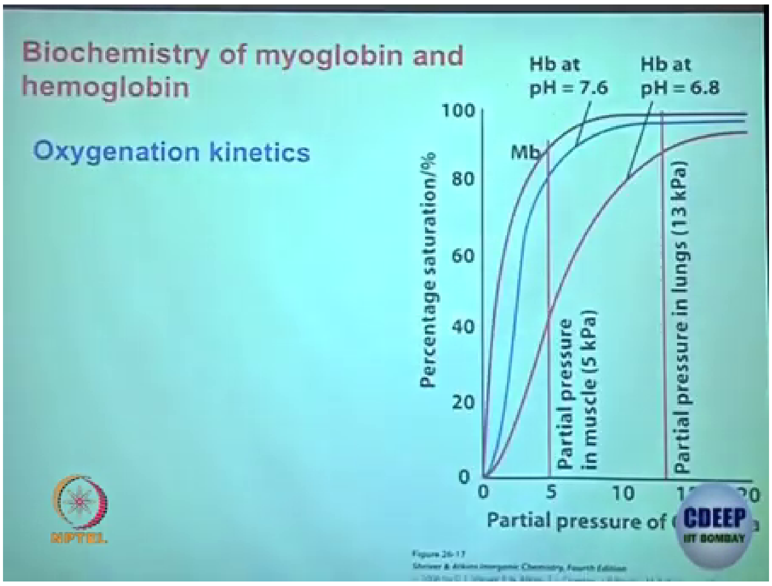

Now, see something like if you put a lot of oxygen in, a lot of if or other way around. If lot of oxygen is not there you will be suffocating right you feel breathless, that means oxygen is not there. Breathless situation is less oxygen right.

That situation and then if you compare to breathless situation if you want pressurize oxygen too much oxygen is there, that oxygen pressure you can vary. And then you can major how oxygen is uptake; uptake means how it is taking how porphyrin centre in hemoglobin and myoglobin is taking up oxygen with respect to oxygen pressure. You pressurize oxygen, say gas cylinder let us say you can pressurize gas cylinder, you can have not so pressurized gas cylinder.

With respect to partial pressure of oxygen as you increasing the pressure of oxygen and you see how oxygen pickup is happening at the myoglobin and hemoglobin. What do you do?

This is the curve for hemoglobin ok, this is the curve for myoglobin, and this is the curve for hemoglobin at pH 7.6, hemoglobin pH 6.8 is red curve lot of things are red this one sigmoidal curve is at pH 6.8 hemoglobin, pH 7.6 is the blue curve for hemoglobin and then myoglobin that is the curve.

Now, so what we are seen as you increase the partial pressure of oxygen, oxygen uptake goes up; percentage saturation means you can keep on bind the iron centre. You have more oxygen pressure it will go up the curve sigmoidal curve you are saying it will go up and after certain point it will saturate, it will not take up any more oxygen. That means, that is the maximum that is the almost like the equilibrium is reached, after that no more oxygen pick up will happened.

So, you pressure, you know you take pure oxygen we have 16 percent oxygen, pure oxygen you can take or pressurize oxygen you can take. So therefore, what we have major here is oxygen pressure is going up how saturation level is going, how are hemoglobin and myoglobin is up taking.

Now, in lungs what happens: in lungs we have this is the partial pressure. Now its almost the its very high oxygen amount we can take it up ok. At the at the at the low pressure if you see ok, at the low pressure you see the myoglobin is having higher affinity, that is in the muscles where low pressure of oxygen is there oxygen is going to transform transfer from hemoglobin to myoglobin in muscles.

Firstly, because at a low pressure of or you know myoglobin has a higher affinity or higher affinity for oxygen compared to hemoglobin ok. And you know myoglobin also has pH something like 6.8 at a lower pH. So, at a lower pH this is the pH curve with respect to pH hemoglobin oxygen uptake is shown in here; at higher pH oxygen uptake in shown in the there.

Since, in muscles pH is low then what happens from hemoglobin so hemoglobin is over here that much oxygen it can pick up, myoglobin write over there is having high affinity for oxygen. Therefore, hemoglobin to myoglobin transfer will happen ok. That is how the transfer mainly happens. Both at a lower pressure myoglobin has higher binding and the pH for pH 6.8; this is pH 6.8 curve you see hemoglobin binding is over here myoglobin binding is over there. And therefore, hemoglobin to myoglobin oxygen transfer occurs ok.

It is very beautifully written in this here, we you can read it say Shriver Atkins book which is your text book, you can read in there in detail.

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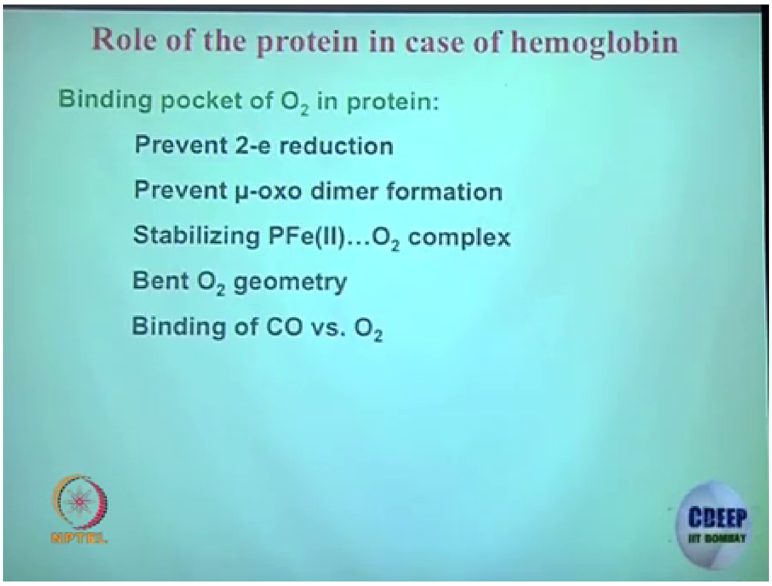

Now, let us go on. So, what is what is essential for oxygen binding, irrespective of myoglobin and hemoglobin what is essential? The thing that is most essential is we have to prevent reduction of oxygen. Oxygen can get reduced by 2 electron that is oxygen to peroxo formation can happened. Iron centre can give 1 electron, another electron the second electron can also come from either that iron centre or from another iron centre.

Since, you are seeing that 2 iron centres are really far apart from each other ok, although they communicate but the electron transfer will not be that very fast ok. So therefore, what happens is oxygen to peroxo formation does not happen, that would have been a irreversible thing. So, what all happens in our body is a reversible oxygen binding; oxygen does not get reduced completely to peroxide; hydrogen peroxide let us say, that that reduce reduction does not happen fully.

Oxygen to peroxide level reduction is prevented by default by design, because that 2 electrons are required oxygen to paroxo; the 2 electron reduction cannot be possible because this that reducing equivalent mean the you need something to give electron right, that is how you will reduce. Reducing equivalent is not available readily there is nothing to reduce very readily right over there. That is one aspect.

Another aspect is you of course you have to prevent the oxygen from going out. It has to bind those binding even though it s not that very strong. What happens over here is, let us say it binds over here for example, oxygen is over here this oxygen will have after binding oxygen will have hydrogen bonding. Some sort of, so let us say this is my porphyrin, this is my iron centre, oxygen binds these non-pair right. And this is the axial position top.

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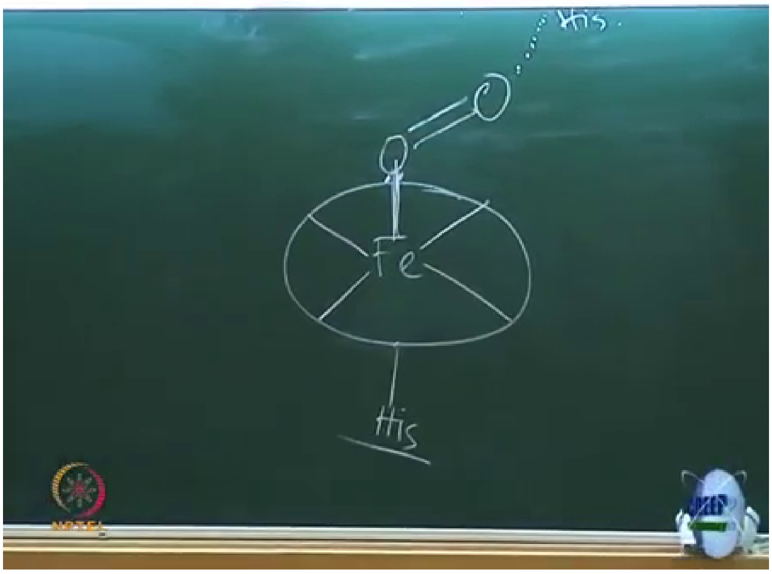

The below is again your histidine or histidine unit right, this is your porphyrin. Now these oxygen you will see there is a hydrogen bonding from another histidine. So, this hydrogen bonding also gives the stability to the structure ok.

Overall it is a bent oxygen geometry and it prevents it prevents the reduction oxygen to peroxide.

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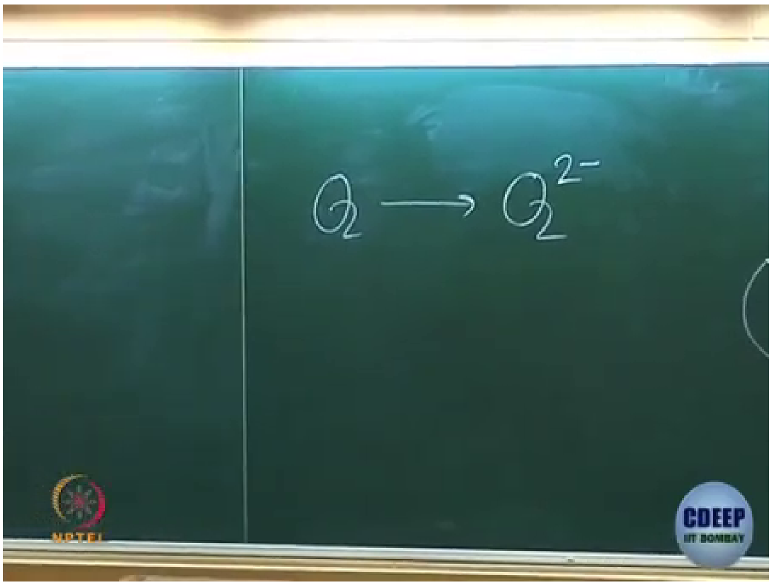

This is peroxide reduction. That is what happens in our body. When you try to synthesize porphyrin which I have done let us say, actually I have done; if you take porphyrin and iron synthetically prepared, so that is why synthetic blood is very difficult to prepare.

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Why? Because the movement you have porphyrin, it reacts with oxygen. If you have porphyrin synthetically made it reacts with oxygen overall what you get is here iron III.

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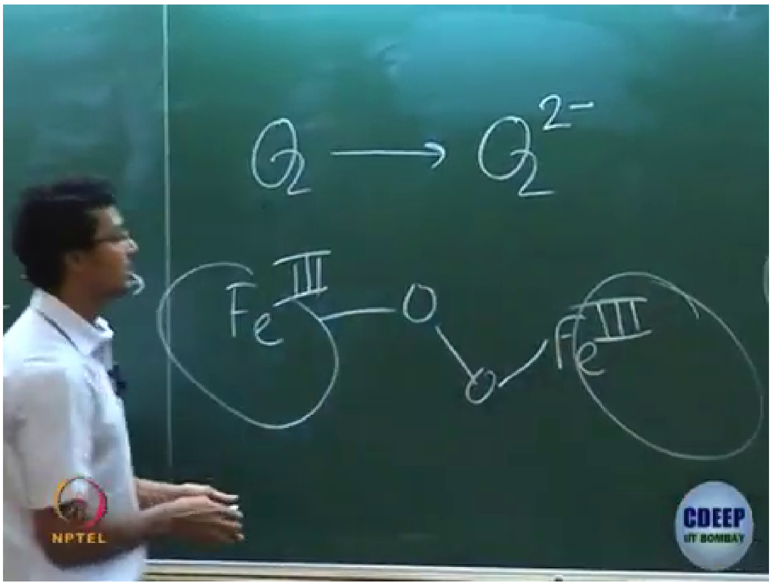

See, what happens here is porphyrin porphyrin is too far, oxygen can come over here, its it cannot bridge between 2 porphyrin. But in synthetic system 2 porphyrin can come very close because there is no restriction, there is your globular protein that is again another tutorial question which is preventing the dimerization and also providing the stability for the monomeric structure. In synthetic system what is happening is or what can happen invariably 99.99 percent cases it happens that, porphyrin P means porphyrin oxygen reacts and overall; you do not have to remember all these again this, this one.

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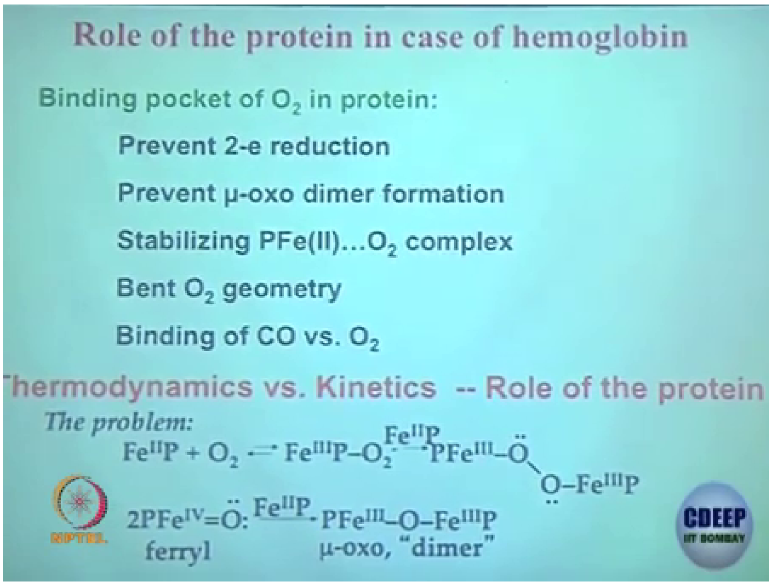

Iron III: iron III each iron is giving 1 electron therefore iron II plus going to iron III plus oxygen is getting reduced by 2 unit; oxygen to peroxo is for me. This reaction is killing the porphyrin or the oxygen binding. This is not too much of a reversible reaction.

So, in our body what happens is it is a reversible oxygen binding, oxygen can bind and popup it can go it can be delivered to myoglobin; hemoglobin takes up transport and deliver. In synthetic system it is very rare to deliver. How you can deliver? That can be done in synthetic system; it requires if you put some steric bulk steric bulk means let us say you have you put an umbrella.

You put an umbrella on top up porphyrin, of course that will take a lot of synthetic effort to put an umbrella on porphyrin. If you put an umbrella another umbrella will not be able to come very close and effect. So, another umbrella even if it comes it will come over here, but iron side is here iron side is here, so they two cannot communicate.

So, these are called steric effect, you put bulk and thereby you prevent, but that is very expensive. I mean it is a lot of synthetic effort. But by default nature has it, in our body we do have that by default. We do have what? We do have these protein backbone. Protein backbone prevent these 2 porphyrin to come close and thereby we do not have any problem to deal with, right.