Biological Inorganic Chemistry
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Lecture 32
Heme Proteins for O2 Transport and Storage

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A very good morning to everybody so we are now in the week where we are talking about the iron ions so the different life processes how they are dependent on iron ions that will see so start with we have now a interesting group of molecules which we consider as the heme proteins so these heme proteins will take as for this particular class that how they are utilized for O2 transport and storage.

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Because the family tree of the metal of biomolecules what we have seen that at one point when you are talking in terms of the corresponding oxygen abstraction, oxygen passage and oxygen storage we use hemoglobin as well as myoglobin.

So, if you have a typical heme iron ion which is very important to get you or give you the corresponding myoglobin and hemoglobin molecules then we will talk about the fold structures of the globin protein unit then how the different O2 binding curves we see Hill's equation we derived that way from a very simple proposition, then board effect and the related allosteric effectors.

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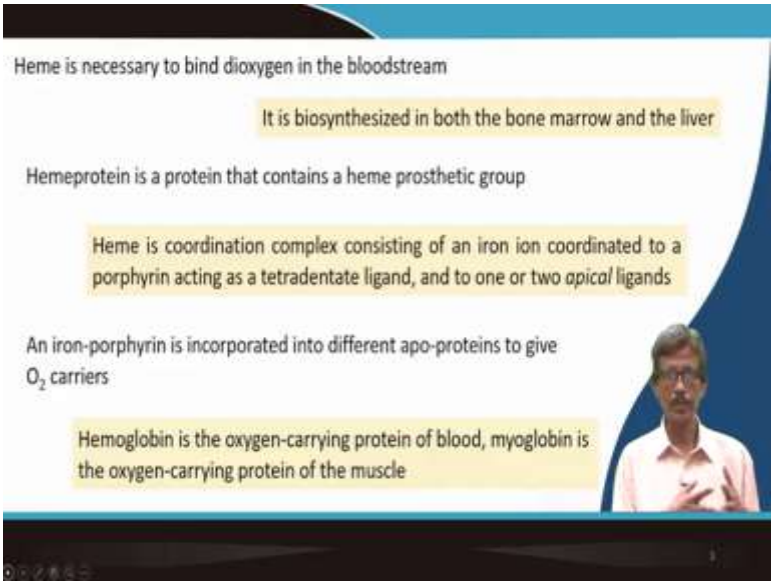

So, what we know now that heme is necessary, if you have the O2 molecule in your bloodstream, so this heme will come and try to grab that by a very simple bio coordination chemistry where the sixth coordination side of the iron site you should always try to remember which iron side it can be your ferrous side or it can be your ferric side.

So, how these are basically absorbed the typical O2 which is available in our blood stream. So, where we synthesize these molecules where is you have the corresponding machine or the machinery where we can synthesize this very important class of molecules because you have a very huge porphyrin ring around the iron center that we call as the heme system.

So, it is basically synthesized in your liver as well as in bone marrow and these class of proteins basically when you have the heme unit so iron you put in porphyrin unit you get the heme unit and then heme unit is attached with the protein chain either through some coordination or through some covalent connections that will see for your cytochromes.

So, will be considering this particular unit that means the iron bound to your porphyrin unit as your prosthetic group. So, typically, therefore if you have the macro-cyclic ring and the metal ion what will you get then? You get the coordination complex so this coordination complex will have the corresponding tetradentate ligand and two apical coordinations because you have to conserve the octahedral coordination site which is very much familiar or which basically both the

iron side that the ferrous site as well as ferric site always try to have or always try to gather that particular octahedral coordination environment.

So, when you have the apo-protein that means without metal ion so an iron porphyrin is incorporated in different these sorts of apo-proteins where the iron porphyrin or the immunity is not there so what you have to do you have to synthesize in your body your bone marrow and liver is utilizing the synthesis of that particular heme protein before that you have the corresponding synthesis of the porphyrin unit so biosynthesis of the porphyrin molecule the macrocyclic ligand molecule.

Then it is coordinating to your iron site and then this particular unit will be incorporated within the corresponding fold of the protein such that your globin chain will come and coordinate to the iron center at the fifth coordination site. So, everywhere we will have this particular oxygen carrying protein in our blood and oxygen carrying protein in the muscle. So, one particular type or variety will be reserved for oxygen transport and another will be reserved for your oxygen storage in muscles.

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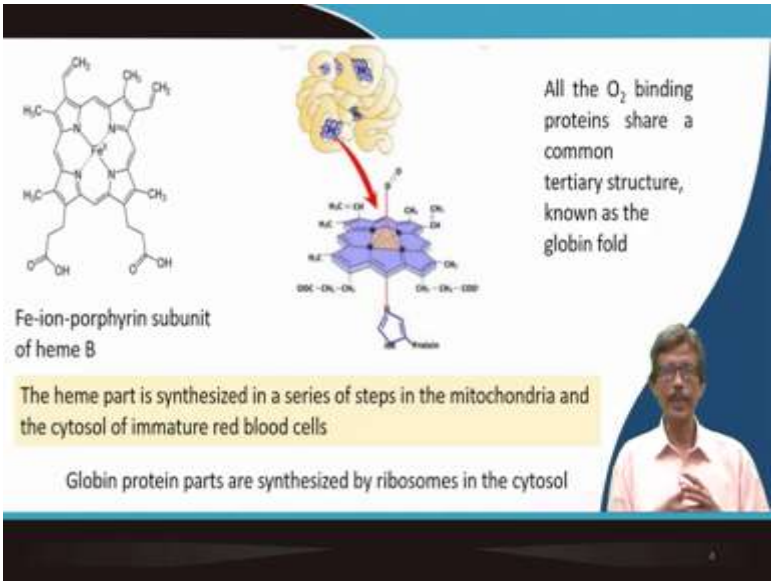

So, how does it look like that Fe ion is there then the porphyrin unit is there and we will have the different nomenclature heme B, heme C and all these things depending upon the substitutions at the peripheral part of the big porphyrin unit.

So basically, this is the most simplest one what we can have in our blood so is a tetrapyral unit and a tetrapyral macrocyclic unit with the different substitutions okay, so basically, it basically gets synthesized or biosynthesized from some amino substituted carboxylic acid part so this amine function the NH2 function is finally converted to your pyrrole unit.

And then four such pyrrole units are getting cyclized and while doing so you have the different substitutions but try to remember these two substitutions the vinyl substitutions the CH double bond CH2 two positions at the top at the right hand side at the upper part of this molecule so you try to look at these two particular substitutions when we will talk about another class of molecules which are cytochromes that these are very much useful because these two functions will be utilized for covalent binding to that of your cysteine residues.

So, when you find this particular part firing sub unit and the iron porphyrin unit is there you immediately get okay I have the restriction for four coordination sites one two three four nitrogen sites and iron is sitting at the center, so you have the square planar type of arrangements, in all these sorts of O2 binding proteins you have this particular structure and this particular structure is a tetra pyrrole structure.

But when your protein folding is coming which we consider as a globin fold I will have a tertiary structure we all know the you have the polypeptide chain and the coiling of the polypeptide chain that alpha helix and the beta seed gives rise to the corresponding secondary structure then you can have the hydrogen bonded units to give you the tertiary structure and finally like hemoglobin you have the quadratic structure.

So, initially you must remember about this particular tertiary structure and this tertiary structure will give you a protein fold such that you can trap this particular group so you see when you show this particular one from this particular planar unit that your not these four units the four nitrogens what you are getting but your nitrogen cannot go inside this particular four nitrogen pocket or N4 pocket it will sitting above this particular N4 unit but one such unit with that particular pocket basically what you gives this particular structure that means the folding that the tertiary structure we call it is the globin fold.

So, when you have the globin fold the protein chain is basically giving something which is your fifth coordination site or from the bottom of the porphyrin ring it is coming, which is your

histidine amino acid residue that means it will give you the imidazole unit and immediately nitrogen which is not your NH nitrogen but the tertiary nitrogen which is only nitrogen the basic nitrogen like your tri ethylamine or pyridine will come and coordinate to your iron site from its fifth coordination site.

So, what you get the heme part is synthesized in a series of steps in the mitochondria and the cytosol of immature red blood cell. So, if you have immature red blood cell then the synthesis or the biosynthesis part will take place for this particular conversions and when you have this particular heme part you definitely get also the corresponding metallation that means the metal ion will be coordinating or metal ion will be sitting within the tetradentate tetrapyrrole pocket then the globin part is getting synthesized in ribosomes in the cytosol, so these are the two different places but the globin chain will be directed towards that particular coordination to this particular iron site.

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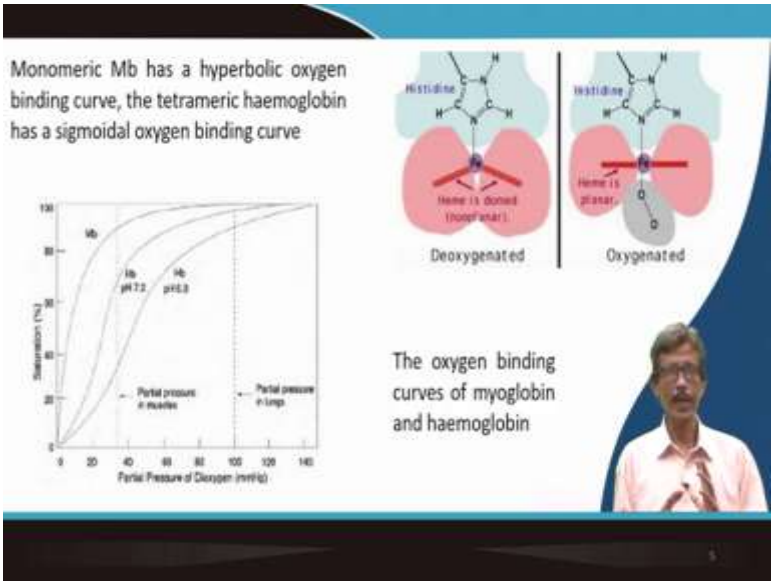

Then when it is coordinating as I just now I told you that you have this four unit and iron is basically the ferrous iron so ferrous iron Fe2 plus and that Fe2 plus if it is high speed is bigger in size so that bigger size is not be allowed to go inside the cavity because the cavity has a particular diameter to accommodate a spherical unit or a sphere like that of your iron.

So, you have this particular dome shape structure which is non-planar and if you look at in this particular way that means the direction where the globin chain is coming in the previous drawing

we are showing that it is like this and the globin is coming downwards but you try to remember it in that way which direction you are showing and keep it in mind also nicely that which direction your globin is approaching your molecule and your O2 will be approaching at the end.

So, if you have the approach of the globin chain is there so that means you have initially that particular tetrapyrrole unit 4 nitrogen donors are there and your iron center is sitting above that then it will be attracting globin from this particular area that means the top of the particular plane of the porphyrin ring. So, globin will come and try to fulfill the 5^{th} coordination site around iron.

And at the same time, it will try to push little bit if you think that okay it is also available for pushing that particular iron or fixing that iron in a square pyramidal geometry. Earlier you have the square plane then you have the 5^{th} coordination side you get the square pyramidal geometry. So, in the next step when you go for the oxygenated myoglobin or hemoglobin you have the corresponding oxygenated species such that your dioxygen molecule will come from the bottom.

So, that will see either you can talk in terms of the O2 is coming from the top or O2 is coming from the bottom is you cannot distinguish it depending upon your corresponding structure of the protein unit. Then when the oxygenation is taking place what is happening therefore that how what sort of curves you can look at if you just simply monitor the oxygenation process for your hemoglobin as well as for your myoglobin.

So, you have the percent saturation will be able to monitor the oxy the corresponding blood which is available and percent saturation you just increase the partial pressure of the dioxygen. So, depending upon the partial pressure of oxygen your hemoglobin show this first plot but in case of myoglobin the plots are different you see this is S shaped or we call this S is very easy to remember is sigmoidal shape.

For myoglobin it is the hyperbolic curve, so this is the plot is hyperbolic typically and then you have the sigmoidal end and also the average blood pH we all know it is close to 6.8 but if it is towards that of your pH of 7.2 that means little bit basic unit so you are oxygenated quickly. So, when the partial pressure of muscle at means around 40 millimeter of mercury you have the corresponding percentage of hemoglobin oxygenated say around 70 percent.

But when you go for another pH of 6.8 which is your blood pH it will be different. So, you have the different pH that means you are looking for something which is your pH dependence. So, it has something or some important implications basically so that is why for microbial you get one type of plot and for the hemoglobin you get another type of plot. One is hyperbolic and another is sigmoidal for your oxygen binding curve.

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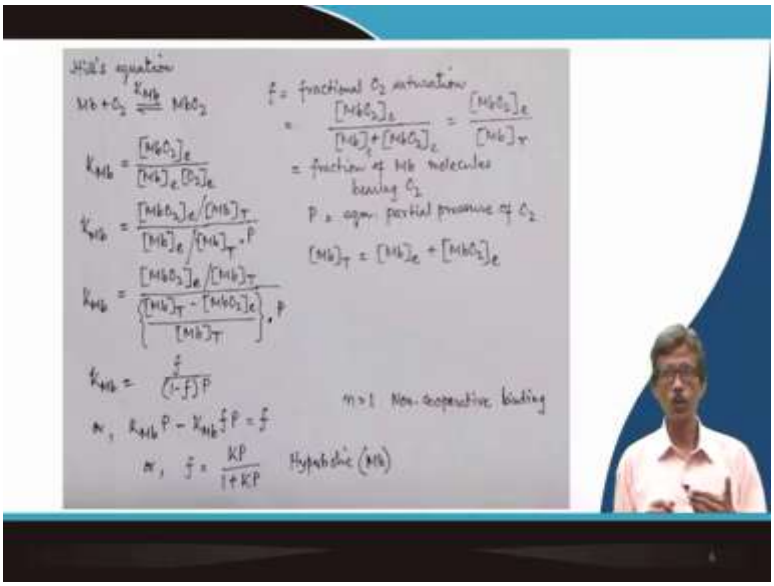

Now, take your own time and just try to understand this particular equation which we call as the Hills equation; what is that? At the bottom what we have derived which is f k p by 1 plus k p and whose nature is hyperbolic in particular shape. So, when myoglobin 1 unit of myoglobin is coordinating your MbO2 you are getting k m b and that k m b is nothing but your equilibrium concentration of the oxygenated myoglobin divided by the equilibrium concentration of the free myoglobin and the equilibrium concentration of your O2 so which is your partial pressure.

Now, if you define f the fractional O2 saturation which is nothing but the ratio of MbO2 divided by total Mb which is nothing but the fraction of molecules bearing O2 molecule and which can also be substituted by the position where you can have the corresponding concentration at the equilibrium concentration of O2.

So, just simply manipulating these the k m b is equal to then you divided by Mb total from your numerator and the denominator then you bring the p which is your equilibrium partial pressure

then some adjustment will give you the bringing of f now how you just put f within this particular equation of these concentrations.

So, now if you realize that your MbO2 e by m b t is nothing but your f so the numerator will be f and denominator will be 1 minus f into p. Then if you rearrange also will get something where k m b p minus k m b f p is equal to f where you can define something that you do not have any power thing for the p, p to the power 1 dependence for this that means f because f you are plotting what you have seen is basically the saturation or the fractional saturation against that of your p is your partial pressure of dioxygen.

So, when you plot it f against p you will be getting a hyperbolic plot which is also similar to many other cases in physical chemistry you know the corresponding land mode surface area coverage and all these cases these are all hyperbolic in nature but when you have n is equal to 1 that means p powered 1 you get a non-cooperative binding so mathematically also you can have some idea that okay we will have the corresponding binding of myoglobin with that of your O2 molecule is non-cooperative in nature and that is why your plot is hyperbolic in nature. So, next, how you get the corresponding one for the hemoglobin molecule.

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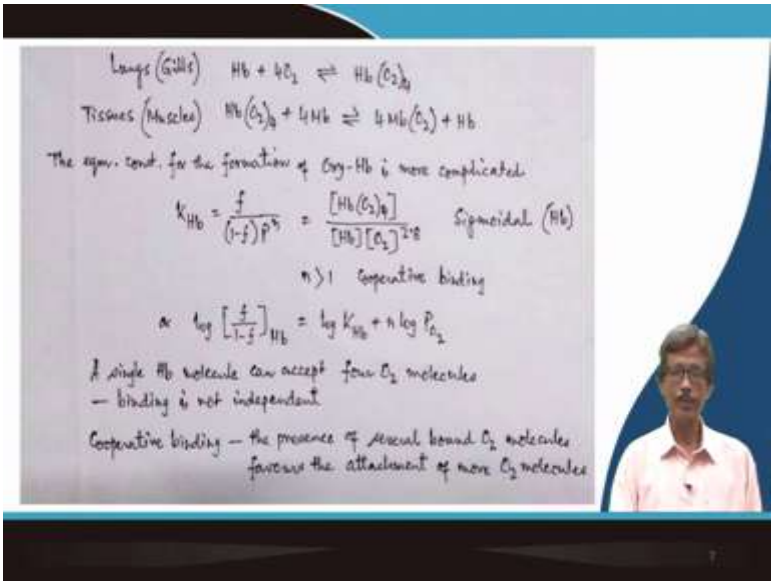

So, for the hemoglobin molecule we see is the detail one but what are the equilibrium basically that we try to see that in case of myoglobin what we see that Mb plus O2 is giving you the corresponding reactions for giving you MbO2 but in lungs or gills in our lungs or the gills of the

feces what you get the hemoglobin is basically a tetrameric unit four such units of the monomeric myoglobins are there.

So, it will try to attract four O2 molecules, so you will have four equilibrium constants k 1, k 2, k 3 and k 4 and they are not independent to each other the k 1 is just modulating the k 2 and k 3 and k 4, but if you consider that okay after following all these four that means the capital K basically which are product of k 1, k 2, k 3 and k 4 you get HbO2 whole 4 but when this particular blood is basically carried to the muscles or the tissues it will be delivered to your myoglobin molecule which is your oxygen storage molecules.

So, we will get 4 MbO2 plus hemoglobin which is deoxygenated and which will again carried back to your lungs and the cycle is going on, but if you consider if you look at the corresponding plot the oxygenation plot what we find here is now that your kHb will be nothing but the same formulation f by 1 minus f p to the power n now.

Earlier we have seen that n is equal to 1 but now for hemoglobin case you will have some power. So, you can also try to match the experimentally observed plot with the different values of these n then at one point you will get the corresponding reproducible nature what we get experimentally. So, at a magnitude of 2.8 of n value we get a plot which is exactly matching with the corresponding oxygenation plot of hemoglobin with a value of says particular pH so is basically a sigmoidal plot then the logarithmic values for this also will get a straight line plot of all these things so either this sigmoidal plot you go for or you can go for the logarithmic plot also.

So, what we see now we just begin the co-operativity that way why it is cooperative in nature that is why the binding of one iron center for O2 is dependent on the second binding or the third binding. So, when it is accepting the four oxygen molecules the binding is not independent to each other that means k 1 is not independent of k 2 or k 3 or k 4.

So, we will see that several bound O2 molecules when you have the binding when binding is going on it is basically favoring the binding of more molecules that means when k 1 is there it will favor to go for the k three also but when one is deoxygenated it will again go back very quickly so that is the process of co-operativity.

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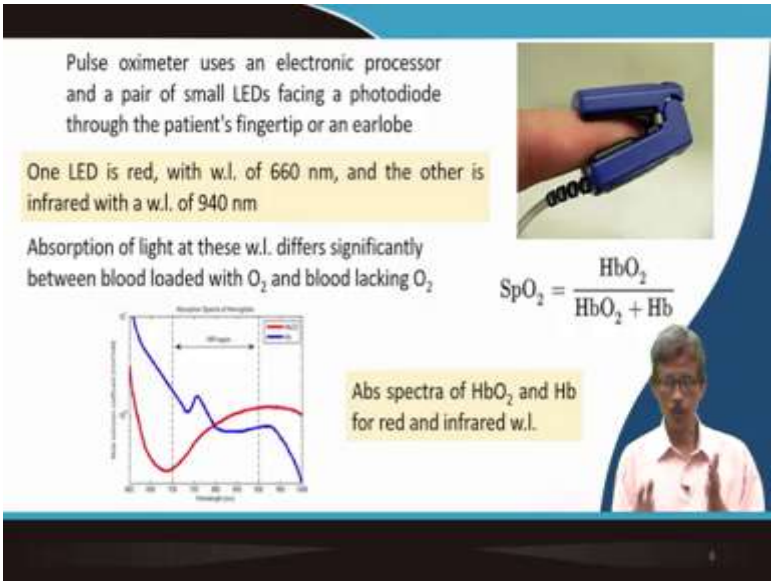

How we monitor basically in this particular time in the Covid time we now that everybody we are having now the oximeter and oximeter is basically giving us the corresponding thing that the how much your blood is saturated by your oxygen molecule so the same thing what we are learning here and what we are monitoring is very important because your blood should get the oxygen and that oxygen should be supplied to your muscles or in the tissues such that we can utilize it for your food burning process and you should get the corresponding energy for that particular purpose.

So, how a typical oximeter can be formulated or can be designed or can be manufactured so is a you will have a electronic processor and a pair of small LEDs only nothing else and a photodiode we all know the photodiode cells are nothing but your detectors. So, you have the cell that means the blood, you have the corresponding LED, LED light is falling not the corresponding visible light or UV light so LED up to particular frequencies or wavelength is falling on this blood sample and you have the photodiode detector.

So, all these complicated things is basically a spectrophotometer type of thing within the pulse oximeter and we all know how the fingertip or your ear lobe can be utilized to detect that thing is basically a stapler type of thing so basically your finger is getting stapled with that particular oximeter but here what we required, we required to know like that of your equilibrium what we are showing now that how much you go for the oxygenation process now the saturation pressure

or saturation O2 or SpO2 will be determined by how much O2 molecules in your hemoglobin is getting saturated so we must have more and more HbO2 molecules in your hand.

So, we use only two LEDs for this particular purpose, one is the red LED and another is infrared LED because your system we all know the red blood and the blue blood but in this particular case you will be able to determine nicely at these two particular wavelengths one is 660 nanometer and there is 940 nanometer.

So, in these two places we will have the wavelength differences significantly between the blood loaded with O2 and lacking of O2 and what the absorption spectra will be getting for these two species the your hemoglobin and hemoglobin we are not talking in terms of your myoglobin.

Here we are interested to know about the hemoglobin saturation because hemoglobin is carrying from your lung because your lung is infected due to Covid and your when your lung is infected your HbO2 formation is hampered so that is why we will talk in terms of your Hb and HbO2. So, any simple kind of your absorption but is not a very good looking one what we get in your laboratory spectrum is a bad one but you have to find out these two values for these two nanometers that means one is around 660 nanometer and another is basically at 900 nanometer or 940 nanometer.

So, these two range basically is what we get because 700 above 700 you have the corresponding one as NIR region so NIR region or NIR spectroscopy basically is utilized to get the corresponding responses in your oximeter.

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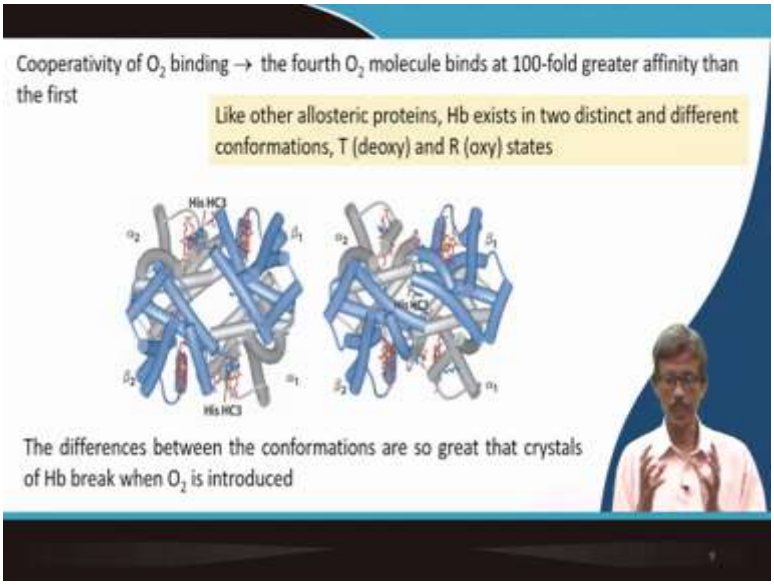

So, what we have seen now that you have the cooperative O2 binding in your hemoglobin molecule, so now we see that your 4th O2 molecule binds therefore basically 100 folds greater having affinity than the first one, so if you go for the first one then the second one and the fourth one.

So, something is happening for your co-operativity and we consider that these all are allosteric protein that means the globin part and also the hemoglobin, hemoglobin itself is now your protein and two distinct forms you can have one is the deoxy form another is the oxy form one is the T for the tensed form or the tau form and R is the relaxed form where your system is getting relaxed after coordination.

So, if you look at those two figures one is your the corresponding one for the deoxy form and on the right hand side you have the oxy form and you see now that due to that particular change if you have the huge structure the three dimensional structure of the protein molecule what you see now that there are differences because only you look at the cavity at the central part, so the differences between the conformations are so great that when you just expose the hemoglobin crystal because people took huge trouble to find out the excess structures for these particular systems.

So, when you have the hemoglobin exposed to your O2 it will be cracking that means you have to take the oxyhemoglobin then you try to crystallize it so there are some methods or there are

some techniques which can give you the crystals separately one is your deoxyhemoglobin another is your oxymoglobin but the central part, the central cavity is important so the cavity size can change and cavity structure can change or the conformation can change due to oxygenation that means the whole structure of this huge molecule is getting changed.

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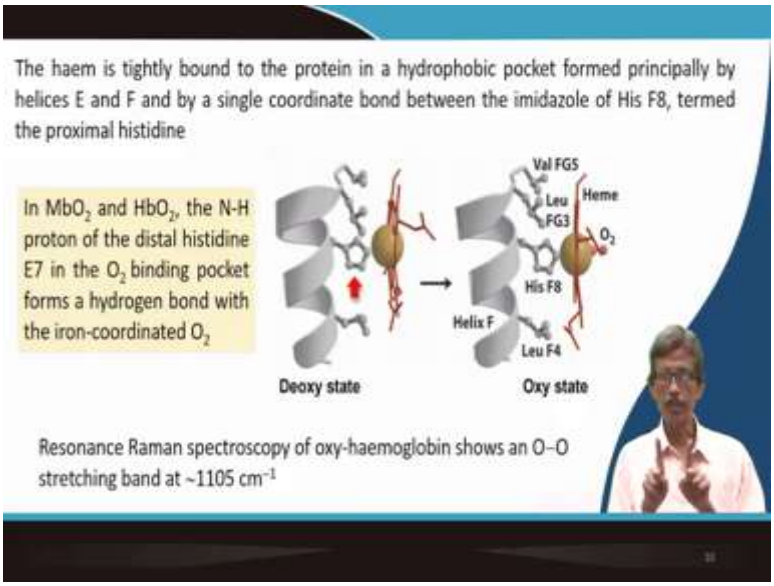

So, when it is tightly bound to the protein in the hydrophobic pocket which is formed principally by the helices E and F. So, we will now bring the different helices because we have talked about only the corresponding coordination of the globin chain which is coordinating to the 5th side. Now if you bring the histidine F8 that means corresponding F helix you use and the position there as the F8 and is basically your proximal histidine.

So, in both these two cases your N-H proton of another histidine we are bringing now on the other side where your system is getting oxygenated where you have the oxygen site you have another histidine molecule is available from E7, one is F8 another is E7 so in that particular case is basically that N-H is now into the operation because your O2 when it is bound to your iron center through one electron transfer from the iron side, iron was your high spin ferrous it will go to low spin ferric and while doing so one electron charge density will be transferred to the anti-bonding orbital of your O2 molecule and O2 will be converted to your super oxide that means it is charged it will have O2 minus one charge like your chloride ion.

So, once it is charged you can have the attraction for the N-H proton of the distal histidine residues that is why you have the corresponding hydrogen bonding interactions. So, the deoxy state you see your iron is above the porphyrin chain or the porphyrin ring and your the red arrow thing is your the corresponding proximal histidine residue His F8.

When O2 is binding so from the right hand side basically on the other side you get this particular one that means your O2 is when it is bound then the electron transfer is taking place it has become super oxide and then it will be stabilized by hydrogen bonding interactions with the N-H proton of the distal histidine.

So, while doing so you can have a corresponding change in the bond order of the O-O we know the O-O the pure oxygen molecule what we are taking during our respiration having O-O double bond and that double bond will be destroyed due to the corresponding occupancy of one single electron in the anti-bonding orbital the Pi star orbital of the O2 molecule will go for the corresponding lengthening of the O-O bond in super oxide.

So, that is why if you go for the resonance Raman spectroscopy the stretching frequency the O-O stretching frequency O-O is a longer one unlike your O2 molecule so this longer stretching frequency will give you a corresponding stretching frequency at a value of 1105 centimeter inverse.

So, 1105 centimeter inverse is the characteristic one that not only tells us that O2 binding is taking place your stabilization is also taking place but also the character of this O2 molecule as your superoxide and your stabilization of the iron center in low spin ferric state and you have the low spin ferric state is a D5 system having one unpaired electron and superoxide also will have one unpaired electron.

In O2 molecule you have two unpaired electron, one electron is being donated to the Pi star level and that will be paired so you will have remaining with one unpaired electron, so these two centers now you see the metal ion center as well as the ligand, ligand is a paramagnetic, still it is paramagnetic, single electron paramagnetism that O2 minus molecule has and you know iron center is also parametric and they can show some magnetic interactions. And if they are anti ferromagnetic coupled the whole product of the system or whole oxyhemoglobin system will be diamagnetic in nature so that is your typical explanation for that particular type of thing.

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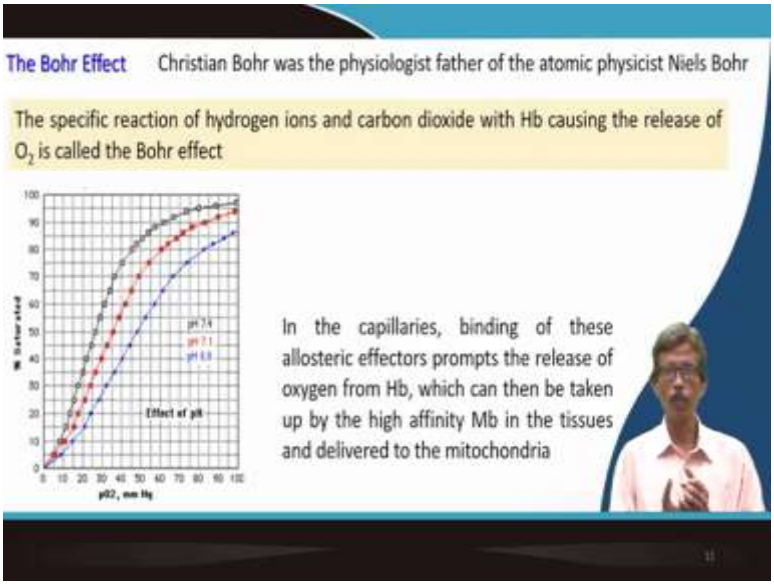

So, now quickly we will see about what is the Bohr effect we have already seen that you can have two different plots for the different pH values one is at 7.2 another is 6.8 why that change is there so that is due to your Bohr effect which is due to the Christian Bohr, means the father of Niels Bohr the atomic physicists Niels Bohr's father who gave it so it is not Niels Bohr, it is the Christian Bohr.

So, he developed all these things studied all this thing basically the oxygenation thing and all and this particular case what we see that the specific reaction of hydrogen ions that means the protons and the carbon dioxide when we produce carbon dioxide through burning of our food material we have to carry that carbon dioxide along with your emptied hemoglobin the deoxyhemoglobin is delivered your oxygen to your myoglobin storage house then the deoxyform is basically going back from your tissue and the cells to your lung.

While doing so and this protonation as well as binding of that of your carbon dioxide and its protonated form that means the bicarbonate anion so if your thing is getting protonated it will positively charge center and your bicarbonate is negatively charged center so at the peripheral part you have the corresponding ion pair and that carbon dioxide will be dragged by this particular hemoglobin in the deoxy form and carried back to your lung for your exhalation.

So, this we already have seen the saturation versus pO2 or the capital P that means the partial pressure of oxygen and we have also seen the two different plots and now you can have also in

between the red plot. Earlier we have seen that you can have the 7.2 or 7.4 and 6.8, now you have, so you have the gradual change basically if you change the pH so that is due to the corresponding functioning in the capillaries and binding of these allosteric effectors we call now because due to some reason basically your thing is changing.

So, one is your pH so definitely you have the corresponding change in the corresponding protonation level or the proton level as well as the bicarbonate anion because it is the buffered medium, the carbonic acid or the bicarbonate anion is the buffer, the blood is in buffered medium so the pH of that buffered medium is important and due to this particular case hemoglobin is again responsible for your release of your oxygen to your myoglobins in the tissues and delivered to ultimately to the mitochondria.

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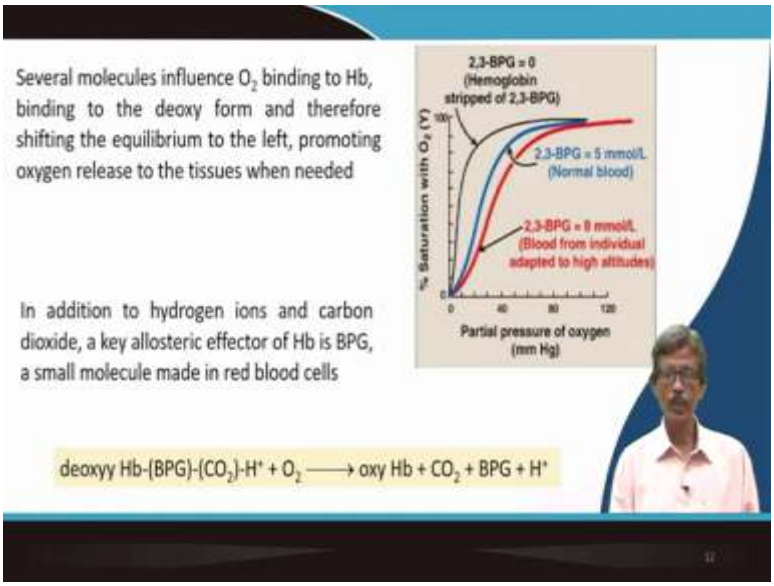

So, several such molecules which can influence the O2 binding in hemoglobin and other related molecules will see and we can have one is the 2,3BPG this phosphoryl, phosphorylate glycerol so that particular thing is very important molecule and we synthesize in our body and that can also be function as your allosteric that phosphoglycerate basically can function as your allosteric effector.

And more or more of all these things are there so concentration of these like your pH can also change the same plot the saturation plot or S we have seen from oximeter SHbO2 with that of your partial pressure how much oxygen you get when you are not getting oxygen from the

atmosphere you have to take the cylinder help of the cylinder or some oxygen producing machine. So, this is the equilibrium where you have the deoxyhemoglobin attached with BPG Co2 and H plus and is basically going to the release of all three together.

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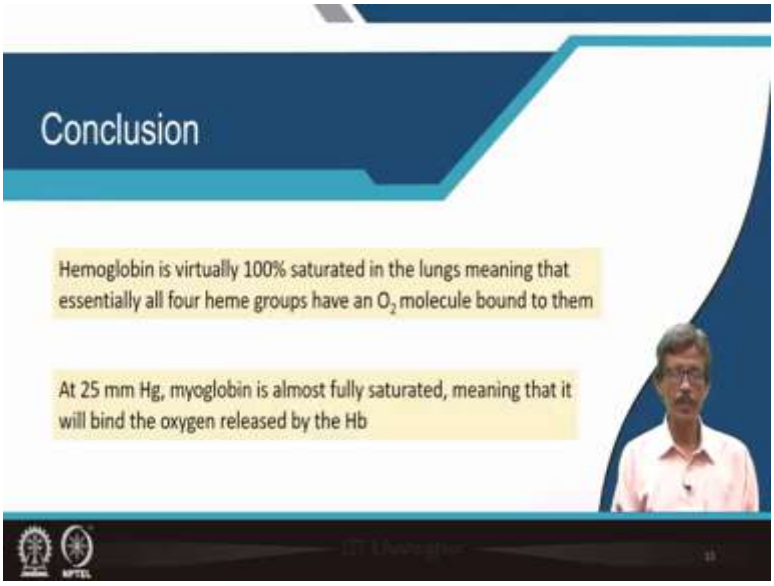

So, what we have seen that basically virtually 100 percent saturation in your lungs essentially all four hemoglobin molecules are oxygenated and bound to them and at a lower partial pressure of oxygen myoglobin is fully saturated because you have the corresponding hyperbolic plot meaning that your hemoglobin loaded with oxygen will deliver all the oxygens to your myoglobin molecules and your hemoglobin will be deoxygenated form and will be released from the system.

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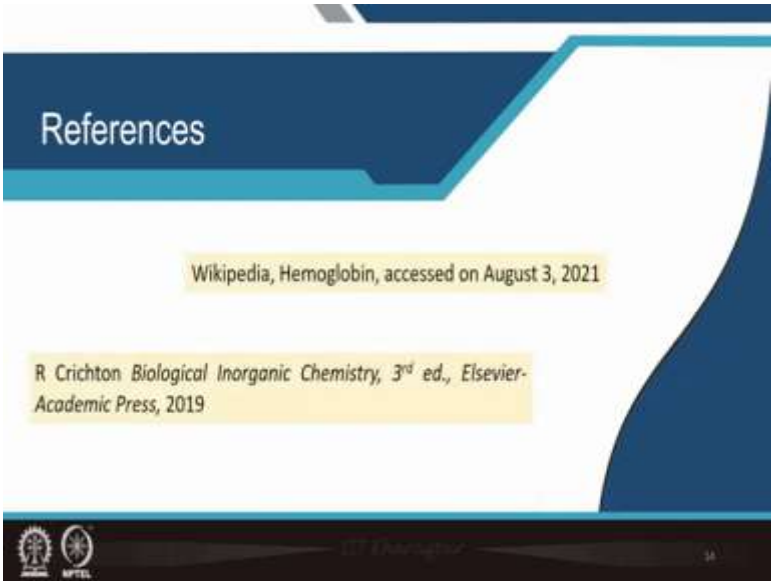

So, you go for the Wikipedia page on hemoglobin and then the book of Crichton. Okay, so thank you very much.