Bioinorganic Chemistry
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Lecture - 15
Life with oxygen: O_2 - Carrying Proteins: Hemocyanin and Hemerythrin

Hi everybody and welcome back to the short course of "Bioinorganic Chemistry". We have been discussing our life with dioxygen. Most organisms require molecular oxygen in order to survive in this earth. We are going to discuss about oxygen carrying proteins in biology.

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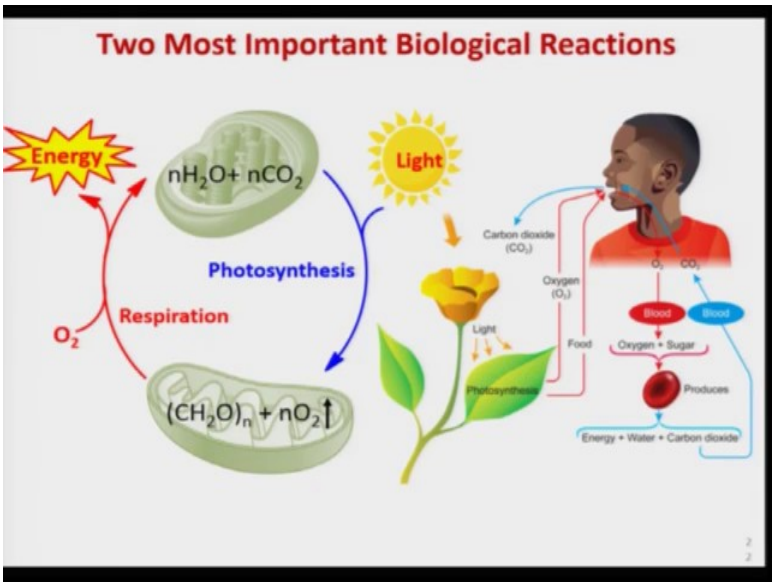

As you all know, that the oxygen is the source of all energy. We take oxygen from the environment and this oxygen goes through the blood and responsible to generate energy, water and carbon dioxide and then also this carbon dioxide has to brought back and release to the environment. So, this is what is happening in our body.

So, inhale oxygen and we release carbon dioxides. We will now discuss, that how this oxygen is getting circulated or getting transported in our body whereas, how this carbon dioxide also getting released.

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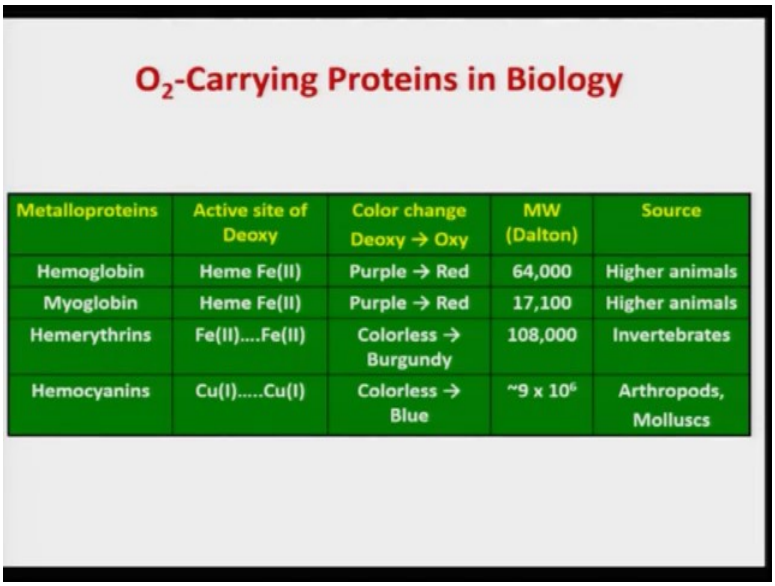

Now, before going to discuss let us look at what are the dioxygen carrying proteins are available in biology. So, these are all tabulated over here, these are all called metalloproteins like hemoglobin and myoglobin. There active site structure of deoxy-hemoglobin, myoglobin contains Fe(II) heme centre. However, after oxygen binding they convert to oxy form red in colour and Fe(II) converted to Fe(III) we will discuss in details. Now hemoglobin, that the molecular weight is very large, because of each tetrameric nature 64,000 Da and higher animals have this hemoglobin in their body.

Myoglobin also heme protein and the colour change from deoxy to oxy is purple to red, the molecular weight is around 17,100 Da higher animals have this myoglobin in their body. Now, in contrast hemerythrin is also iron containing protein but non heme proteins and in deoxy form it is Fe(II)-Fe(II) diiron proteins, we will discuss in details. Now, colour change from deoxy to oxy, colorless to burgundy molecular weight is very high 1,08,000 and invertebrates have hemerythrin in their body. Hemocyanin however, is a copper containing proteins.

So, two copper centre is present here, we will discuss in details and color changes from colorless to blue from deoxy to oxy form and having a very large molecular weight around $9\text{-}10^6$ Da. Arthropods have hemocyanin in their body. So, let us first talk about the hemocyanin.

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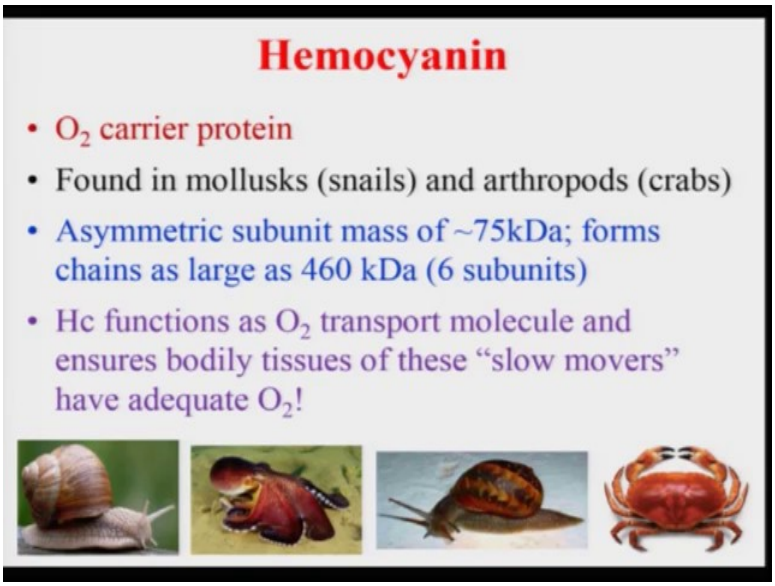

Now, hemocyanin it is an oxygen carrying protein found in Mollusks and Arthropods. The asymmetric subunit mass of around 75 kDa forms chains as large as 460 kDa for six subunits. Hemocyanin functions as oxygen transport molecule and ensures bodily tissues have those slow movers have adequate dioxygen.

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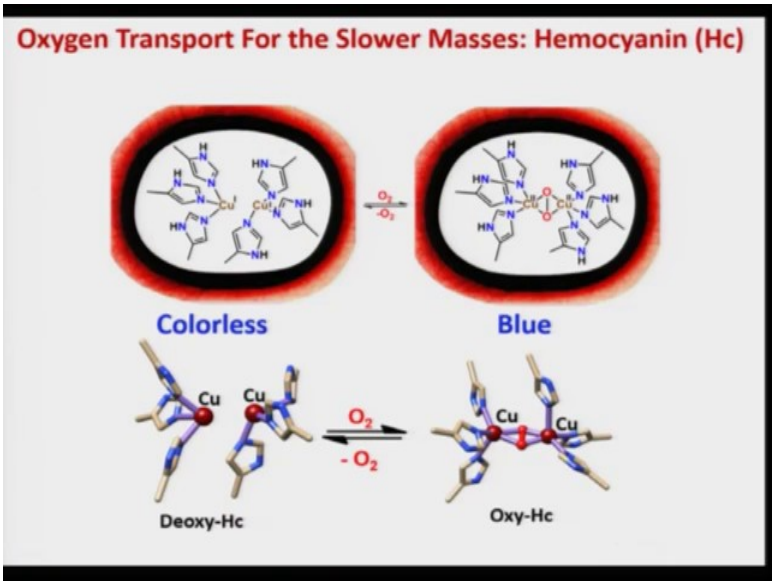

This is a schematic representation of hemocyanin deoxy and oxy and you can see the structure (Refer Time: 04:40) structure of deoxy-hemocyanin and oxy-hemocyanin you see dioxygen bridges between two copper centre and Cu(I) convert to Cu(II), that is a

oxidation and dioxygen getting reduced to peroxides. So, metal getting oxidize and oxygen getting reduced and they are reversible.

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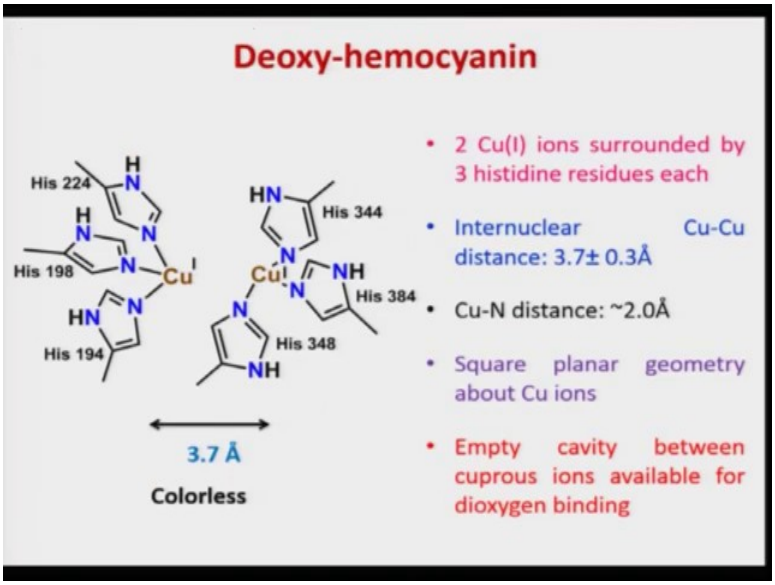

Now, deoxy-hemocyanin active size structure is shown over here, as you can see that copper ion surrounded by three histidine moiety and the inter nuclear Cu-Cu distance is around 3.7±0.3Å, Cu-N distances are nearly around 2Å. This is square planar geometry around copper and there is an empty cavity sites available for possible dioxygen binding.

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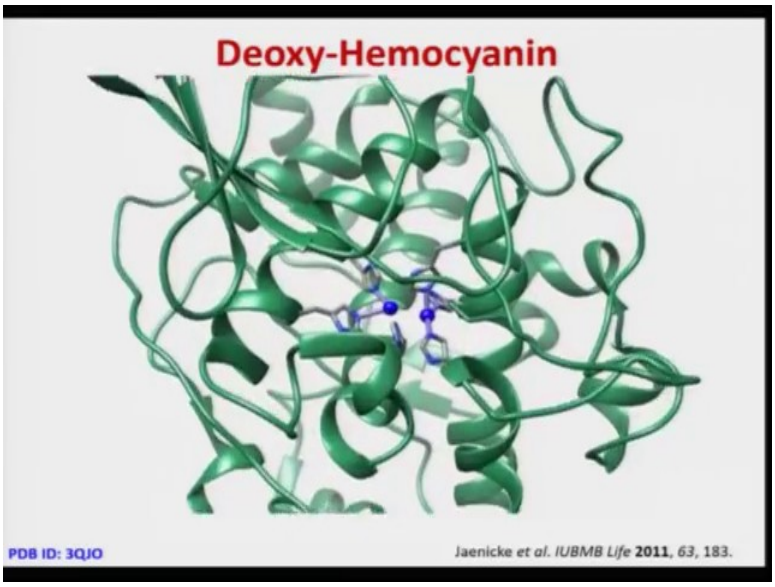

This is the X-ray structure of deoxy-hemocyanin. As you can see that there is a huge protein chains wrapping the dicopper units, which are sitting at the centre. And once you remove this protein chain, you can see that clearly two copper unit they are separated around 3.7Å distance and as I have just said that this three histidine groups coordinated to the copper centre.

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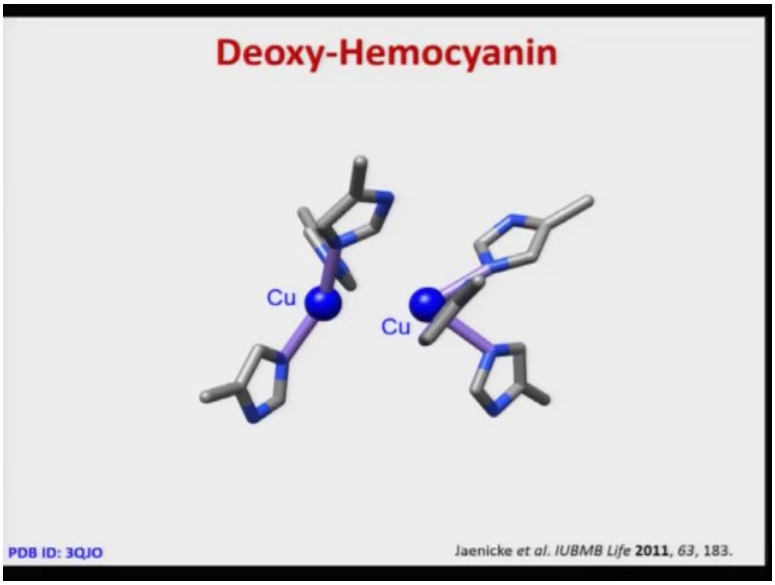

So, there is a empty site, where dioxygen possibly can bind. Let us see what is happen? See in oxy-hemocyanin, the color change from colorless to blue colour, because it is Cu(II).

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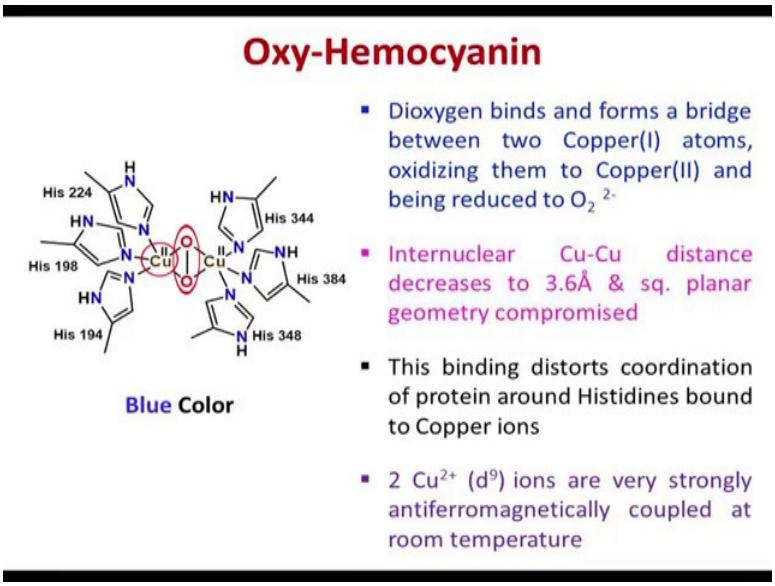

So, dioxygen binds and forms a bridge between two Cu(I) atoms oxidizing them to two Cu(II) and dioxygen getting reduced to O_2^{2-} the inter nuclear Cu-Cu distance was decrease from deoxy, it was 3.7 Ånow, it become 3.6 Å and square planar geometry is compromised around copper. This binding distorts coordination of the protein around histidine bound to copper ions and two Cu(II) ions are very strongly antiferromagnetically coupled at a room temperature.

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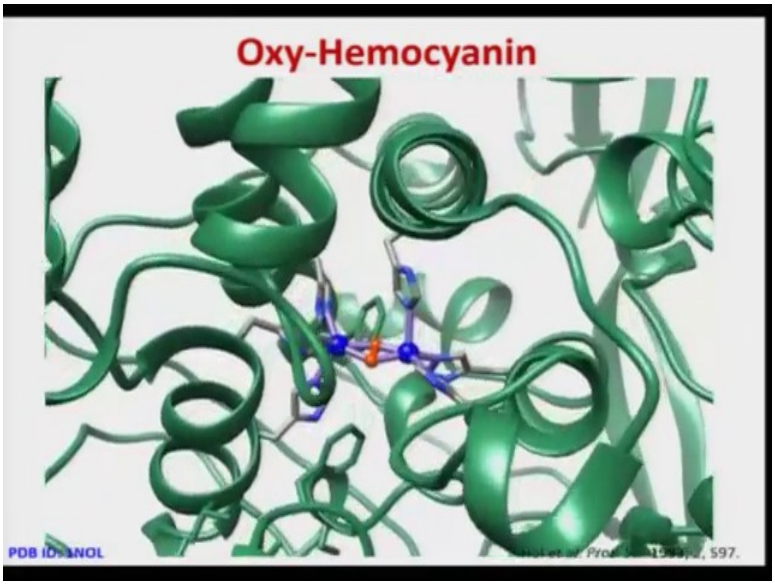

So, this is the protein structure of oxy-hemocyanin. As you see that again the huge protein chains wrapping this molecule. However, dioxygen comes and binds between these two copper centre, which you can see very clearly and if I remove this huge protein chains, then you see that that inorganic molecules, where this dioxygen binds in O_2^{2-} the dioxygen is bridging between these two copper unit and bring two centers close enough so that it can properly binds dioxygen.

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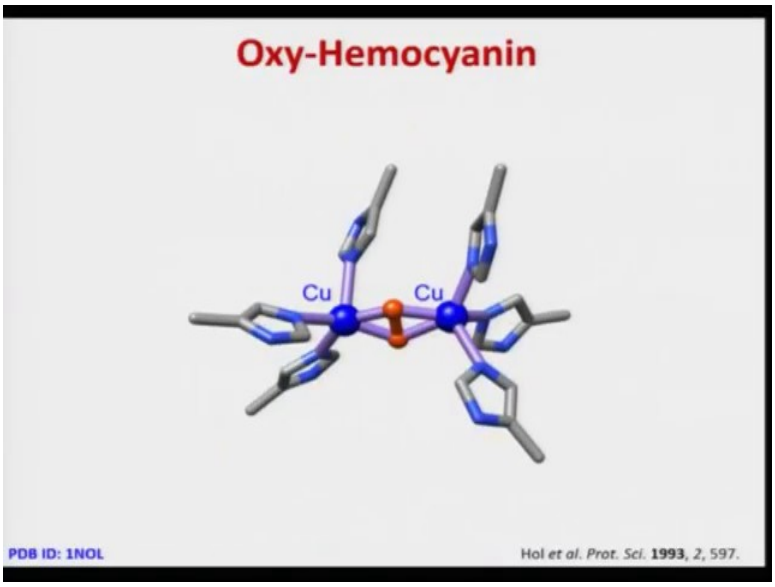

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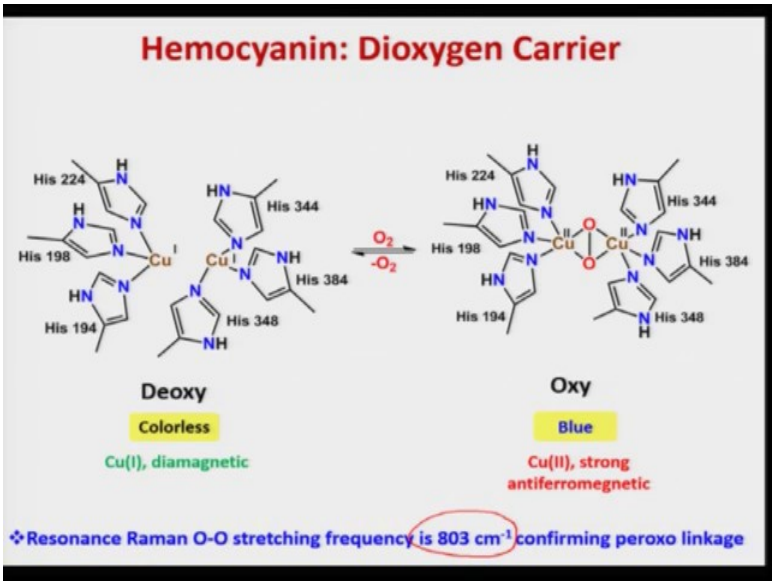

Now, this is this schematic presentation between deoxy and oxy.As I have said that the colour is getting changed from colorless to blue from deoxy to oxy and Cu(I) getting oxidized to Cu(II), whereas dioxygen getting reduced to O_2^{2-} that means peroxide. How we know that this is peroxide because Resonance Raman stretch of O-O is around 803 cm^{-1} which confirms the peroxo linkage.

Also, this two copper centres in oxy-hemocyanin are strongly antiferromegnectically coupled, where as these two copper ions here, in deoxy the Cu(I) is a d^{10} system so, it is diamagnetic.

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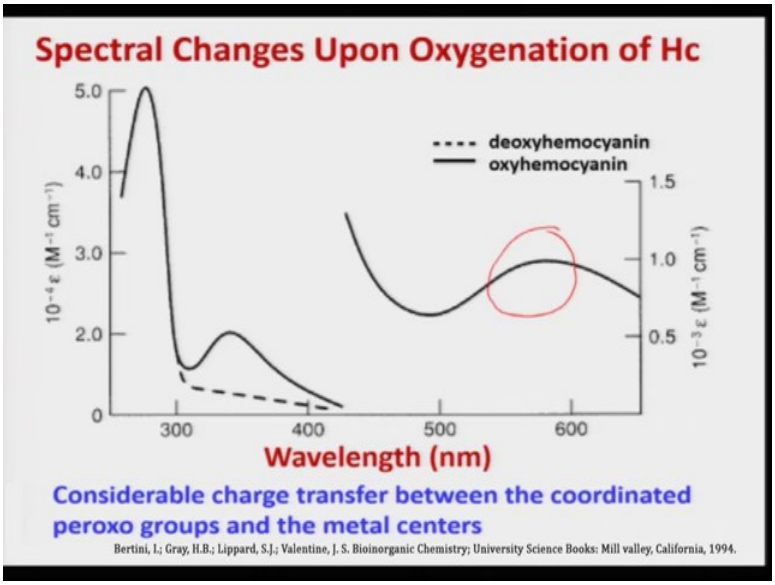

Now, this is what is the spectral change taking place often oxygenation to hemocyaninso, this dotted lines are deoxy-hemocyanin as you can see over here. However, after oxygenation, there is a huge change in the absorption spectroscopy and you see that there is a band around580 nm and this is, because of the considerable charge transfer between the coordinated peroxo group and a metal center. So, this confirms again the binding of peroxo within this to two Cu(II) centres.

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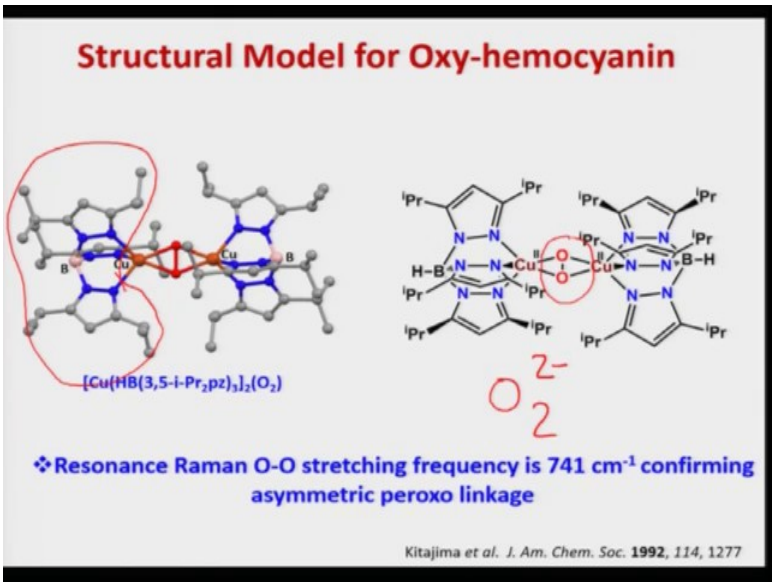

Now, many people have tried to model this hemocyanin and try to understand that how actually dioxygen binds reversibly and in order to confirm that they have used different tridentate ligands. I am showing one such examples having a pyrazolylborate ligand. You see this tridentate pyrazolylborate and which actually is resembles to three histidine group and this binds dioxygen and this is the X-ray structure shown over here.

This is a schematic representation, what is being seen that yes this is the Cu(II) centre and also the Resonance Raman O-O stretching frequency is around 741 cm^{-1} further confirming the peroxo linkage. So, this is further validated by the model study, which confirms that the dioxygen is in O_2^{2-} in oxy-hemocyanin. We will talk about hemerythrin, another oxygen carrying proteins in biology.

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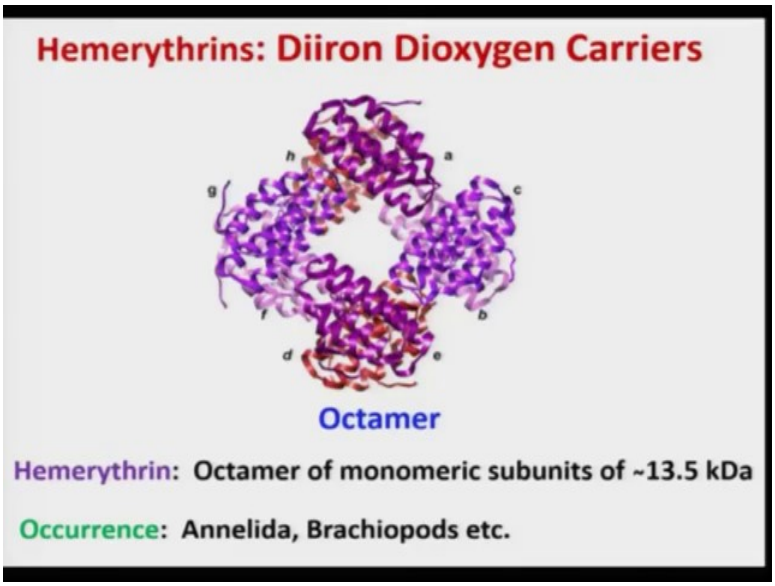

Now, this is the octameric structure, hemerythrin octamer of monomeric subunit of around 13.5 kDa and as I have said already that it has been present also in many organisms.

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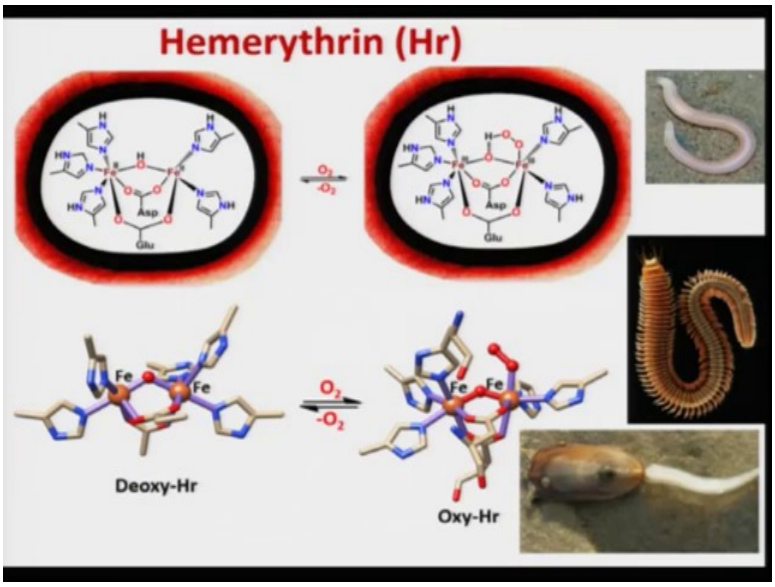

Now, this is a schematic representation between deoxy and oxy-hemerythrin. So, in the deoxy-hemerythrin here, again this is the centre. It is a non-heme proteins where there is two iron centre having Fe(II) state and there are one asparate and one glutamate bridges between these two iron centre and also a hydroxo group bridges between these two iron.

In each iron there are three histidine moieties in one iron centre however, remarkably in another iron centre, it is only two histidine.

So, one iron centre is six coordinated where as another iron center is five coordinated 1.e., there is a vacancy in the coordination site where dioxygen can possibly bind. This indeed happens and as you can see in deoxy-hemerythrin, the dioxygen comes and binds over there and I will show you soon, this dioxygen again converts to O_2^{2-} that means, peroxides and Fe(II) converts to Fe(III).

So, metal is getting oxidized and dioxygen getting reduced ok. The X-ray structure is shown over here, as you can see. Now, this is in case of oxy-hemerythrin, it becomes oxo-bridged indeed, it is converted from μ-hydroxo to μ-oxo. This is also a remarkable change in the structure and magnetic properties, we will see all this in details.

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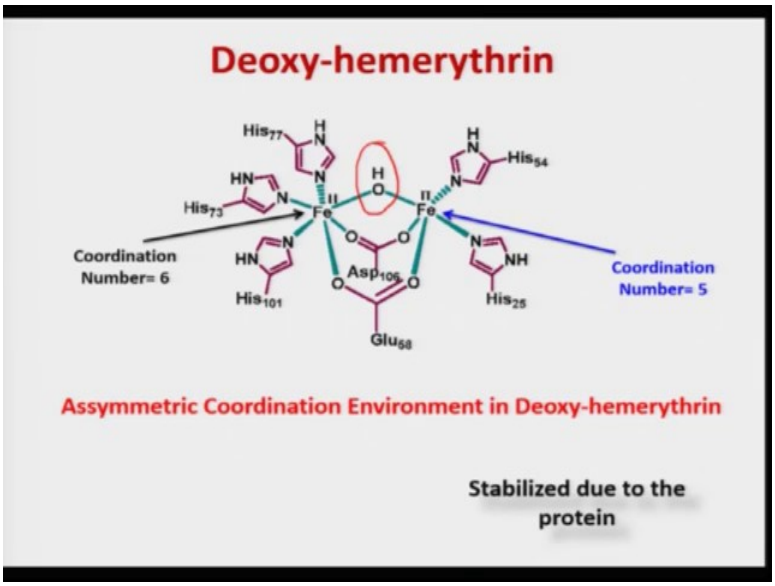

So, this is what is deoxy-hemerythrin, let us look closely. As I have said that three histidine moieties legated to the one iron in contrast in the anotheriron centre only two histidine moieties bind to that and this is aspartate and glutamate bridging between these two iron centre and both the centers are Fe(II) and also, there is a hydroxo group, which bridges between these two iron centre.

Now, this types of asymmetric coordination environment is possible in biology in presence of protein. If you like to make similar molecule in the laboratory, it would be

extremely difficult. I will come into a little bit more details about that however, interestingly as I have already said that one iron centre is six coordinated, where as another iron centre is five coordinated, where dioxygen actually comes and binds. So, this kind of situation, this asymmetric coordination environment is stabilized due to the huge protein change around this molecule.

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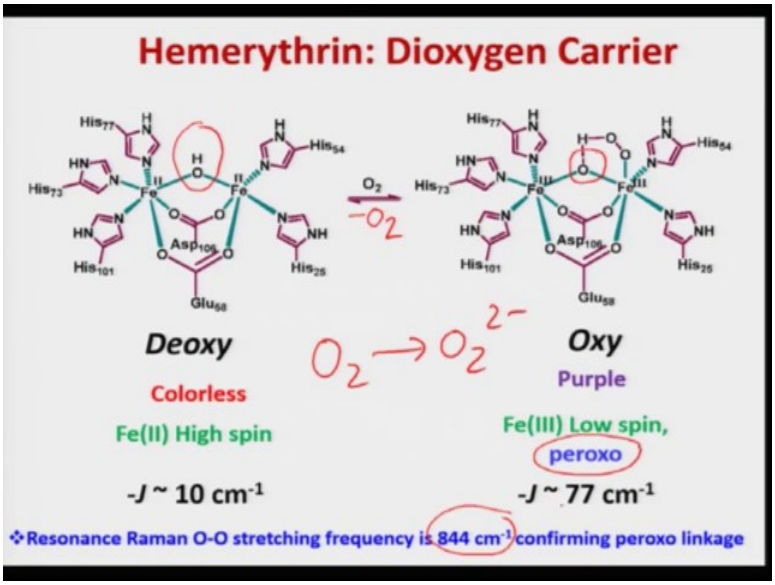

This is a schematic presentation where deoxy-hemerythrin converted to oxy-hemerythrin in presence of oxygen and oxy-hemerythrin goes back to deoxy releasing the dioxygen. So, they are reversibly inter converting between each other and as I have said that this Fe(II) center binds dioxygen and this dioxygen is no more in neutral form it O_2 converted to O_2^{2-} and you see that this proton is there which is actually hydrogen bonded to the μ-oxo group, which is present in oxy-hemerythrin.

Also, this results from deoxy to oxy is a huge colour change from colorless to purple and Fe(II) centre, which is high spin in the deoxy state becomes low spin and also Fe(III). So, this peroxo coordination in oxy-hemerythrin is further confirmed using the Resonance Raman stretching frequency of dioxygen, which gives a value of 844 cm^{-1} and does confirming the peroxo linkage between two diiron centre in oxy-hemerythrin. You can see that in deoxy a hydroxo group, which bridges between these two diiron center where as in case of oxy, this hydroxo converted to just an oxo group which bridges between just we call it μ- oxo.

So, μ-hydroxo converted to μ-oxo. So, now how we know that and the magnetic study indeed confirms that yes this is μ-hydroxo getting converted to oxo, because if it is μ-hydroxo then the coupling between two iron centre would be less. This indeed happens that antiferromagnetic coupling of course, two iron centres undergo antiferromagnetic coupling and the value is much less like -10 cm^{-1} in case of deoxy state where as, in case of oxy-hemerythrin the values has changed remarkably.

This coupling between two iron centre undergo antiferromagnetic coupling and the values is much larger -77 cm^{-1}. So, this coupling is much stronger and this also confirms that yes, this is indeed μ-oxo, because we have seen in model study that if this is converting from μ-hydroxo to oxo there is a huge change in the coupling constant values also.

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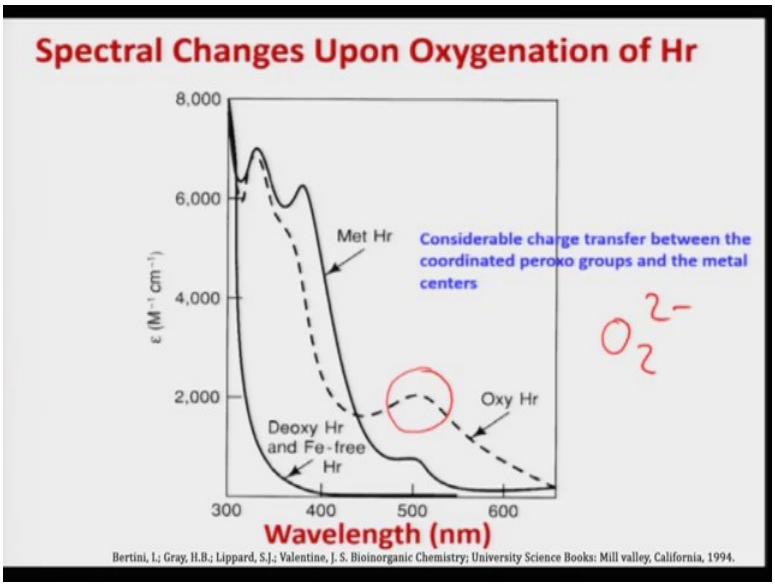

Here, I am showing the spectral change often oxygen is often hemerythrin. So, you see that net hemerythrin means oxidized means in Fe(III) form. however, without dioxygen coordination in oxy-hemerythrin it is also Fe(III), but with oxygen bond form of course, oxygen bound in O_2^{2-}. So, this gives a remarkable shift of it's UV visible spectrum. As you can see there is a band around 500 nm which is due to a considerable charge transfer between the coordinated peroxo group and the metal centre. This also suggests that dioxygen binds to this iron centre.

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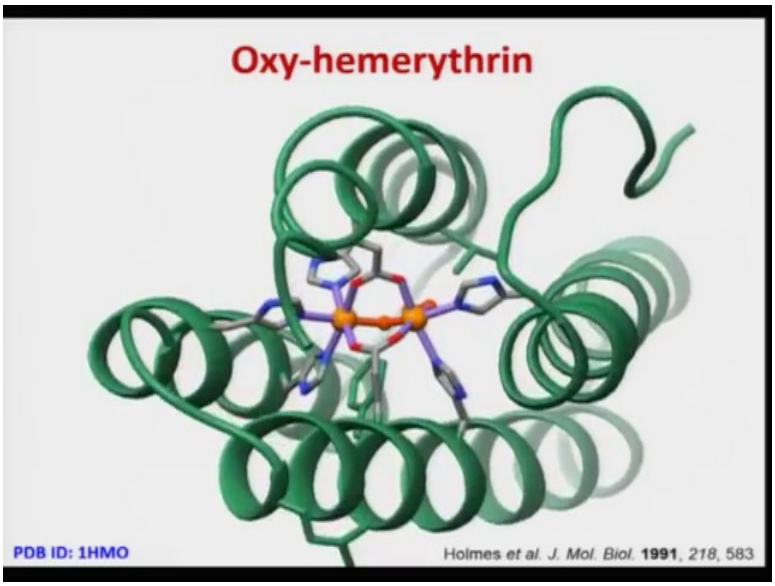

X-ray structure of this oxy-hemerythrin is shown over here, as you have seen in case of hemocyanin, there is a huge protein chains are wrapping around this diheme centre and the diheme unit sitting at the centre where actually dioxygen comes and binds.

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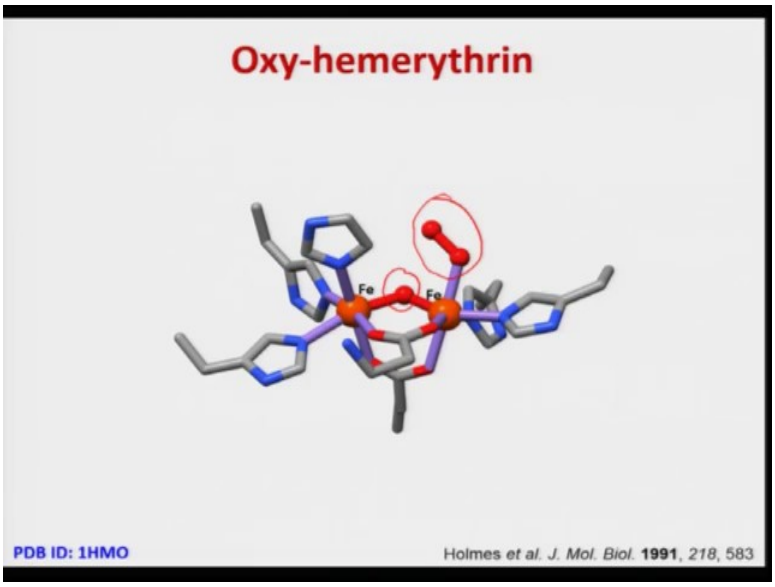

So, let us remove this protein chains and see that how actually that is and binds you can see that dioxygen binds like this in O_2^{2-} form and two iron centres are bridged by a μ-oxo group. As you have seen that glutamate and aspartate are bridging between these two iron center and this three histidine group for one iron centre where as, another iron centre

coordinated to two histidine, which we have just looked at in the previous slides. So, X-ray structure also confirms that all this coordination environment around with their angles, distance and we now, know exactly how dioxygen binds to which centre, what is the geometric, if you have a protein structure and then you know all details of that.

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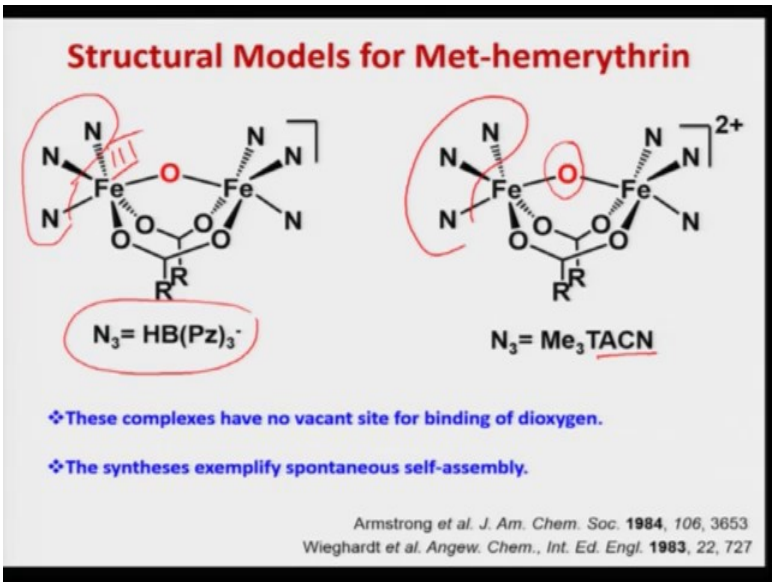

Now, if you try to model this hemerythrin in the laboratory, lots of people around the globe has tried to model this hemerythrin. And here, I have given to very famous example as you can see that this iron centre is no longer asymmetric, they are symmetric. Now, it is impossible to make asymmetric iron centre, which we have observe in case of hemerythrin without protein ok, because protein makes them possible two different from centers.

This three histidine group is replaced by pyrazolylborate, a tridentate chelating agent and it is the in the met hemerythrin means, it is in Fe(III) state and you can see that two iron centre is bridged to the oxo group and there is another examples also when the tridentate ligand is a different triazacyclononane, then also it was reproduced this met-hemerythrin, this it also μ-oxo bridged between two iron centres.

So, interestingly these complexes have no vacant site for dioxygen binding and the synthesis exemplify the spontaneous help is assembly. So, it is very easy to make this kind of molecule, spontaneously they found this diironunit. However, since there is no vacant site around iron so, dioxygen cannot bind and the synthetic model could not

reproduce the two different iron centre one is six coordinated, another is five coordinated. So, that dioxygen comes and binds in at the five coordinated site and which possibly mimic the hemerythrin as observed in biology.

So, we have been discussing various dioxygen carrying proteins in biology. Today, we have discussed two such oxygen carrying proteins hemocyanin and hemerythrin in great details. The content dicopper and diiron centers in their active sites. Once, dioxygen binds to the bimetal core, metal centers get oxidized by one unit each while, dioxygen gets reduced by two electrons to form peroxide. Also, to metal centre undergo strong antiferromagnetically coupled in their oxy form. I also have highlighted, how the beautiful design principle adopted by our mother nature, control reversible binding of dioxygen in these proteins.

In my next lecture; however, I will talk about the dioxygen transport and storage in our own body. Nature has designed hemoglobin and myoglobin, hemoglobin selected for transporting oxygen while myoglobin stores oxygen. I will discuss how protein chains are actually responsible for reversible dioxygen binding in hemoglobin and myoglobin. I will also highlight how the beautiful design principle makes all these processes possible in sustaining our life.

Thank you.