Bioinorganic Chemistry
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Lecture – 11
Life with Oxygen: Cytochrome c Oxidase

Hello everybody and welcome back to the short course of Bioinorganic Chemistry, we have been discussing about our life with dioxygen.

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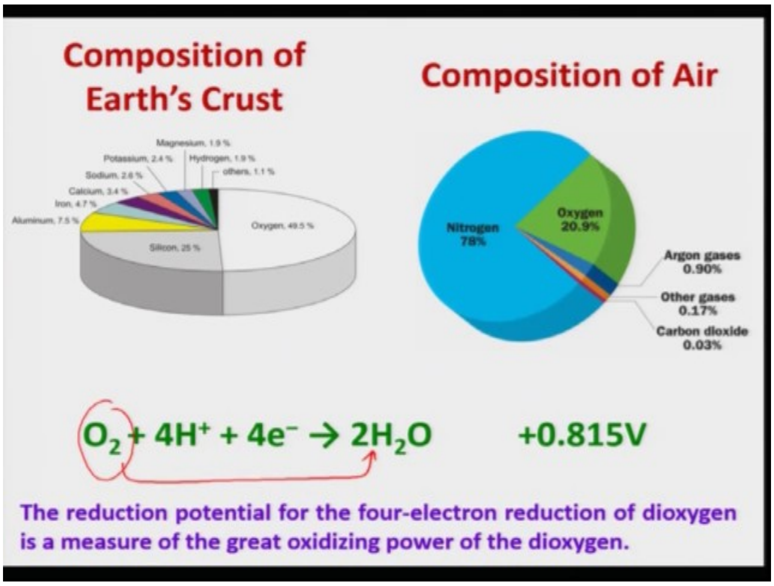

As one can see that the composition of earth crust where this dioxygen is the most abundant and composition of air, where this dioxygen concentration is around 20.9 percent, the huge dioxygen concentration. And most of the oxygen consumed by aerobic organisms is used to produce energy in a process referred to as oxidative phosphorylation.

A series of reaction in which electron transport is coupled to the synthesis of ATP and in which the driving force for the reaction is provided by the four electron oxidizing power

$$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$$

of dioxygen. As yoy can see that with a E^0 value of 0.815 volt is a measure of great oxidizing power of the dioxygen.

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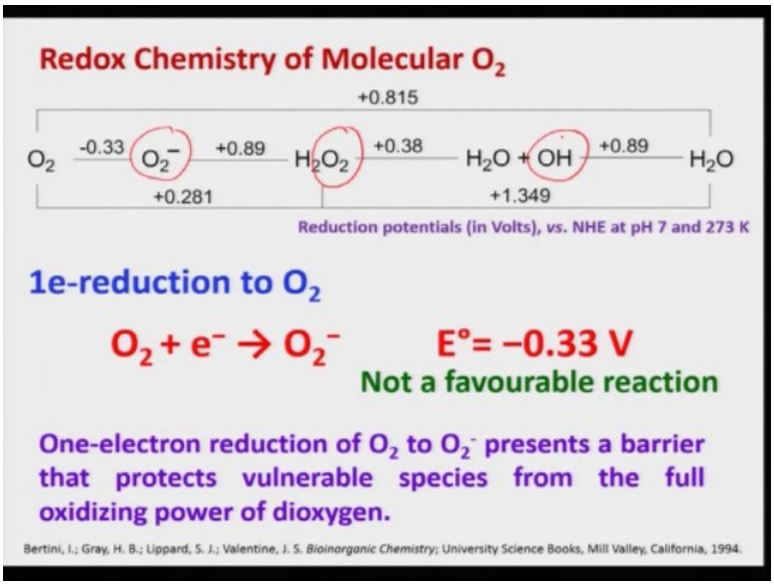

Once you look at the redox chemistry of molecular oxygen, if the four protons and four electrons is not added all at a time then, you can get the stepwise reduction which eventually leads to O_2^-, O_2^{2-} and OH radical which of course, converts to water eventually. So, we have discussed all these in details in my last lecture.

However, if you look at reduction potential of all the steps then what you can find that first reduction of dioxygen to produce O_2^- is a very unfavorable reaction. E^0 is -0.33 volt and this one electron reduction of dioxygen to O_2^- presents a barrier that protects vulnerable species from the full oxidizing power of dioxygen. So, this is the thermodynamic barrier.

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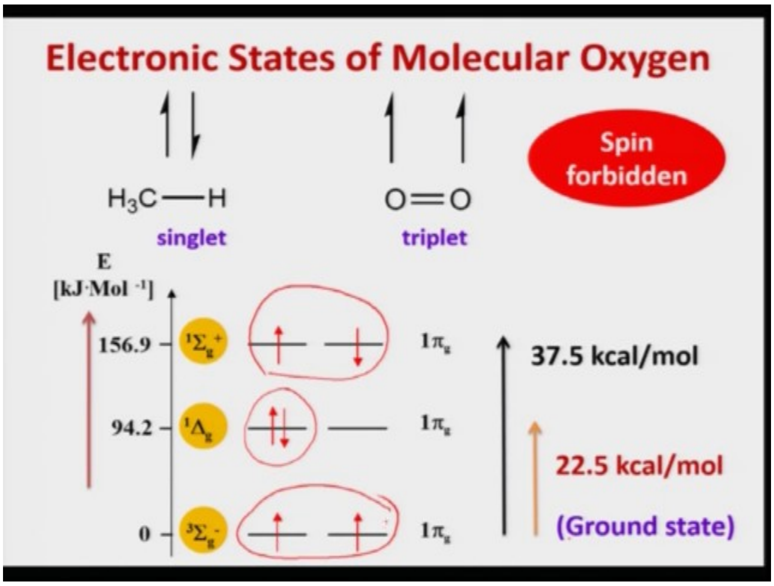

As I discussed in my last lecture dioxygen is in triplet state, while the organic molecule this is in singlet state. So, it will not react and this is because of spin forbidden. So, triplet state or dioxygen has to convert to singlet state to react with an organic molecule and if you look at this diagram as you can see that this is the ground state of dioxygen which is shown over here as a triplet state.

And this goes to singular state which is in first excited state which is in Δ state, with the help of 22.5 kcal/ mol and then the second excited state it is a σ state, which equal 37.5 kcal/mol. So, once you provide energy then and then only dioxygen can convert from triplet to singlet and thereby it can react any organic molecule. So, this is a kinetic barrier which dioxygen need to cross for it's reactivity.

Now, we will talk about the survival of life all about bioinorganic chemistry. Actually two most important bioinorganic reactions or rather in other word two most important reactions in biology is one is as you know photosynthesis, another one is respiration.

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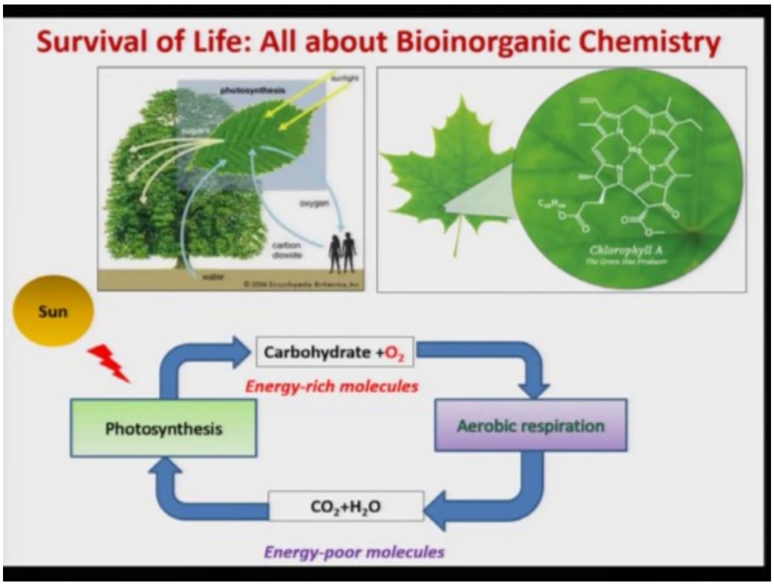

In photosynthesis, what is happening in presence of light? This carbon dioxide and water forming carbohydrate, this carbohydrate during the respiration in presence of oxygen convert to that huge amount of energy, which we use in our day to day activity.

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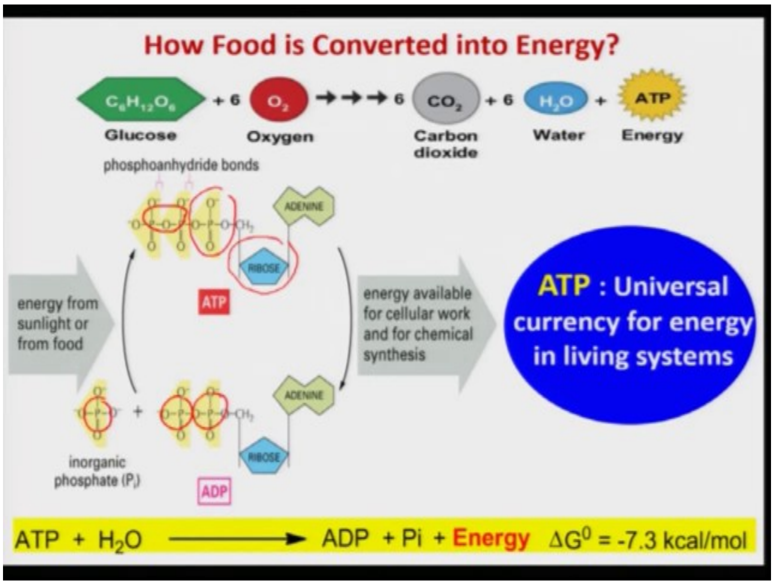

For example, how this food is converted into energy?

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + ATP$$

ATP is adenosine triphosphate. As you can see that this is adenine, this is ribose sugar and there are three phosphate group. This is one phosphate, second phosphate and third phosphate that we call it triphosphate. Now, this phosphate to phosphate is actually linked with P-O-P bond and these bonds when it breaks releases energy. Actually when you need energy ATP convert to ADP, adenosine diphosphate. When you see that two phosphate; phosphate 1, phosphate 2 and another phosphate Pi inorganic phosphate and releases energy which we use for various activity all the time.

And then again when we generate energy, then it will be stored by converting ADP to ATP, adenosine diphosphate to triphosphate. When we have more energy to store, what is happening that P-O-P bond will be formed just like ATM; if you have ATM card, and you can store your excess money you have, or you can take out some of this money when you need it.

So, ATP is universal currency for energy in living system and when you need it ATP converts to ADP, plus inorganic phosphate Pi and releases energy. And ΔG^0 is -7.3 kcal/mol, which is lot of energy and this energy is utilized for our day to day activity say what I am talking in front of you also I am spending energy.

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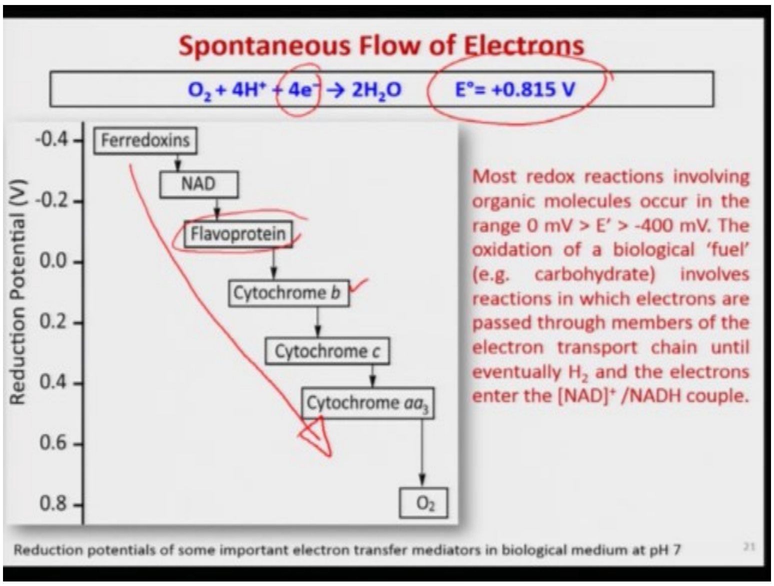

Most redox reactions involving organic molecules occur in the range of 0 to -400 millivolt, the oxidation of the biological fuel like carbohydrate involves reactions in which electrons are passed through members of the electron transport chain, until eventually hydrogen and electron enters the NAD^+/NADH couple. You can see that this is what is chain reactions. So, electrons comes to ferredoxin and this is has a potential -0.4 volt and ferredoxin gives the electron to NAD.

And once it gives to electron to NAD, ferredoxin get oxidized and can take another electron from the food source. Now, NAD when it gets one electron, it becomes reduced. And once it becomes reduced, it spontaneously gives electron to the flavoprotein and flavoprotein get reduced and NAD get oxidized. Flavoprotein gives electron to cytochrome *b*, then cytochrome *b* will be reduced flavoprotein get oxidized. Cytochrome *b* gives electron to cytochrome *c*, then cytochrome *c* get reduced and cytochrome *b* get oxidized, cytochrome c then gives electron to cytochrome aa_3 and where this all this electrons comes to O_2 you know that,

$$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$$

It requires a potential of plus 0.815 volt. So, this potential is equal and biology gets this potential and also this all these electrons like this spontaneously without any problem, you need four electrons all at a time. And ofcourse, protons should be there, and this dioxygen converted to water. So, this is what is happening, that dioxygen at this potential of 0.815 volt with the help of four proton and four electron converts to water releases energy.

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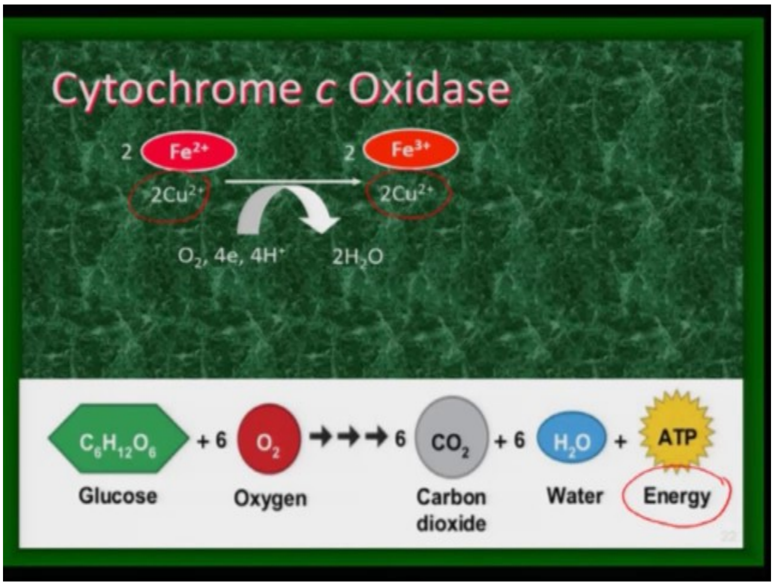

And, this is what is happening in Cytochrome *c* Oxidase and as you can see two Fe(II) heme center and two Cu(II) center convert to two Fe(III) heme and two Cu^{2+} and the dioxygen takes four proton, four electron and converts to water and this religious lots of energy, this is the terminal step of this oxidation process.

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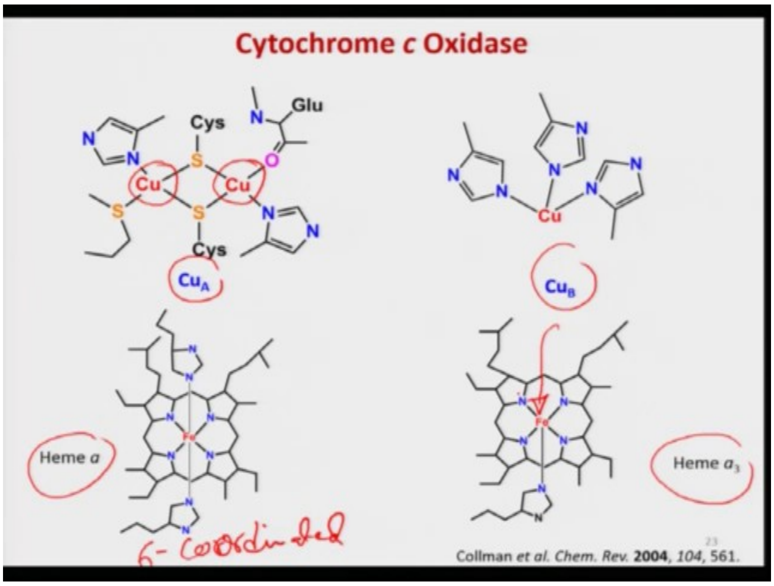

If we focus on this heme and copper unit. So, what is there? There are two types of copper, as I have said. Cu_A, which is a dimeric copper; as you can see two copper unit are here, this is one copper this is one copper and cysteine sulphur bridges between these two copper. Now, Cu_B as you can see the 3 histidine are ligated to this metal and also 2 types of heme; one is called heme *a* and heme a_3, two histidine moieties are there, in heme *a* which is 6 coordinated. And here in heme a_3, it is 5 coordinated.

So, heme *a* actually responsible for electron transfer because, it is already coordination number 6. Dioxygen cannot bind over here. But, heme a_3 it is 5 coordinate iron center where dioxygen or other species can comes in binds in.

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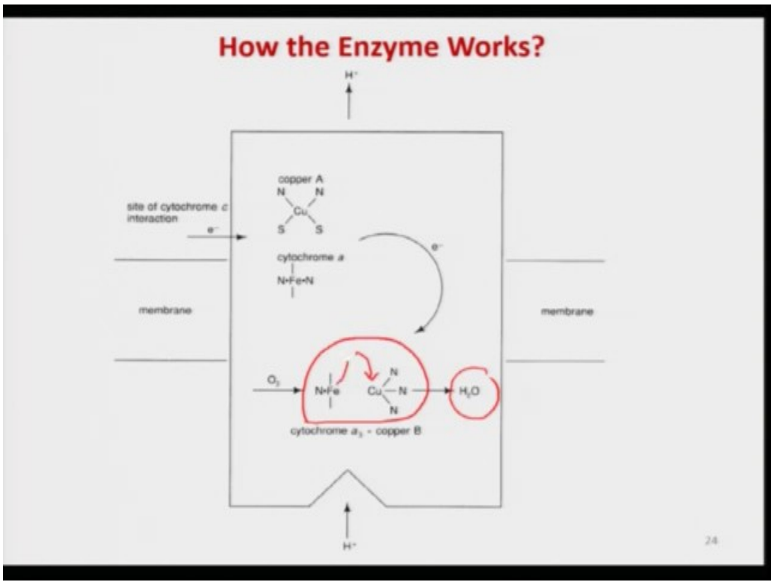

So, let us look very carefully that how this enzyme works, cytochrome *c* gives electron to copper A and cytochrome *a*, they get reduced and they transfer this electron to cytochrome a_3 and copper B, and then this copper A and cytochrome *a* get oxidized. And this is the place cytochrome a_3 and copper B; where actually dioxygen binds and converted to water molecule, in presence of H^+ ion.

If we look at that cytochrome *a*, which is already 6 coordinated. So, there is no place for dioxygen to coordinate. Whereas, here you see dioxygen can coordinate to iron center and dioxygen can coordinate to copper center as well.

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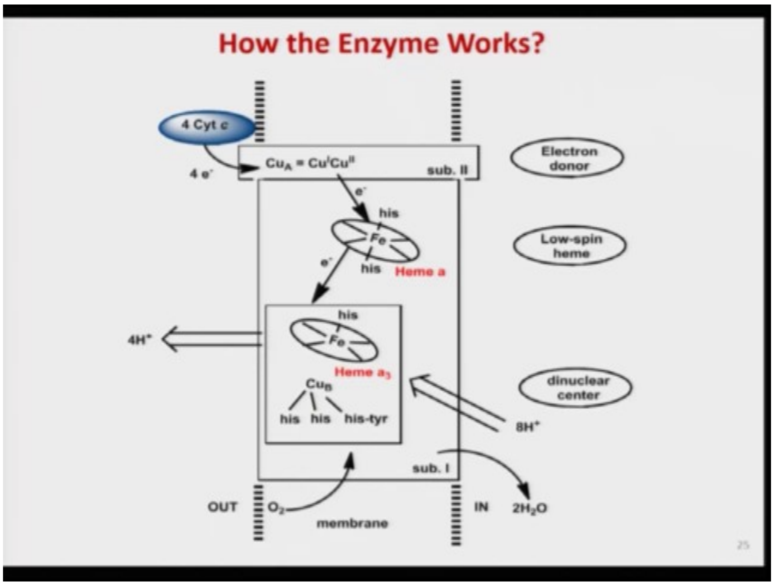

So, this is what is the flowchart; like, four electron. So, this comes to first copper A and copper A getting reduced, then copper A transfer electron to heme *a* and heme *a* get reduced Fe(III) becomes Fe(II) and this Cu(I) gets oxidized to Cu(II), which takes another electron. So, all these electron passes heme *a* to this heme a_3 and copper B; this region where actually dioxygen binds, where dioxygen needs four proton, four electron to reduce it completely to water.

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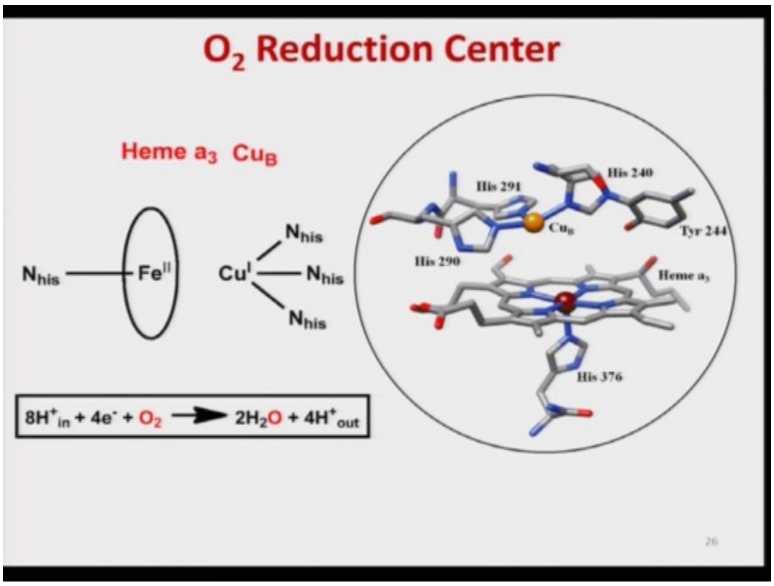

Indeed, this is what is happening. This is what is the dioxygen reduction center, and this is what is the chemical reaction

$$8H^+_{in} + 4e^- \rightarrow 2H_2O + 4H^+_{out}$$

And as you can see the X-ray structure of iron copper center is shown over here. A heme porphyrin ring is there and this is histidine, and it is a 5 coordinated species. Where a similarly copper, it is 3 coordination, three histidine is ligated to copper B. So, there are empty back sides where, dioxygen indeed can comes and binds. Now, we will see that how it binds dioxygen.

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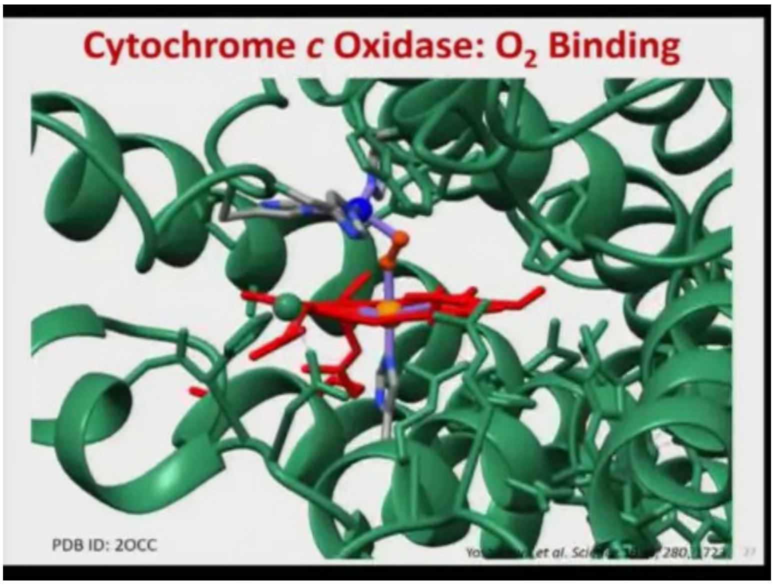

So, this is the structure of Cytochrome *c* Oxidase, where it binds dioxygen you can see that, this iron and copper binds a dioxygen and there is a huge protein chains which is wrapping this small iron copper unit.

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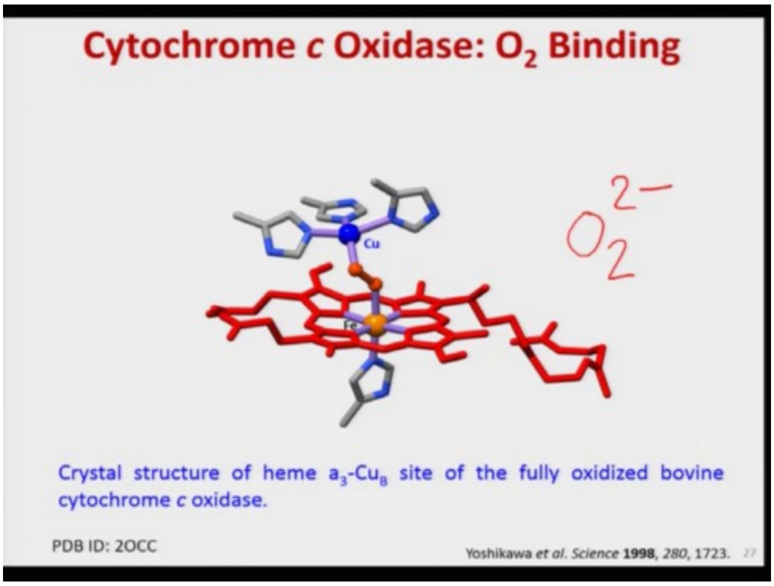

And if I remove this protein chains, you see now that this iron copper unit binds dioxygen like this and this dioxygen is O_2^{2-} resonance Raman stretches confirms that this binds in O_2^{2-}. So, this is the crystal structure of heme a_3-copper B sites of the fully oxidized Cytochrome *c* oxidase.

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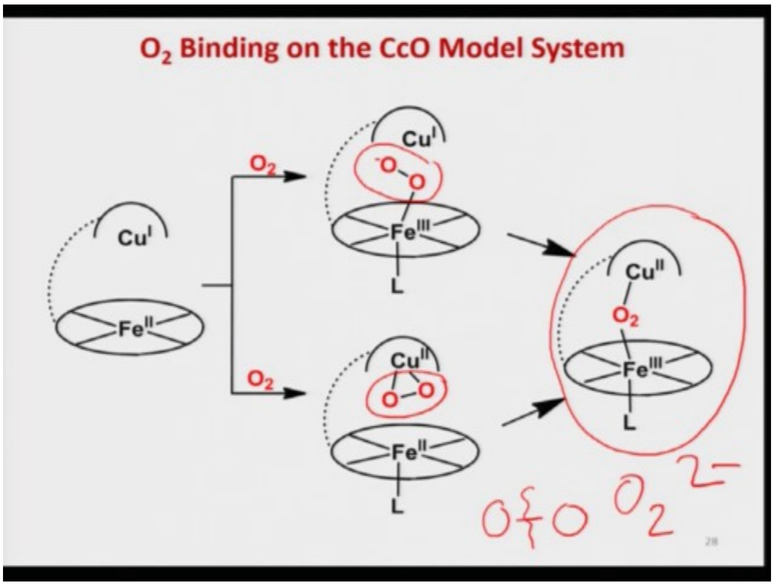

Now, question is that how dioxygen can binds? Whether dioxygen binds first to copper then, what would happen that it can forms like this O-O bond or dioxygen first can bind to iron center as well. And it forms this Fe(III)-O_2^- as we already studied. So, how both these forms gives rise to this copper iron O_2^{2-} and this is the product which is forming. It does not matter whether it first binds to copper or first binds to iron this is this question is still debatable most probably dioxygen first binds to iron center and convert to this species.

And as I have said that this superoxides, this O-O bond dissociation energy is lowest where this O-O bond easily breaks and converts to water we will see the mechanism right now.

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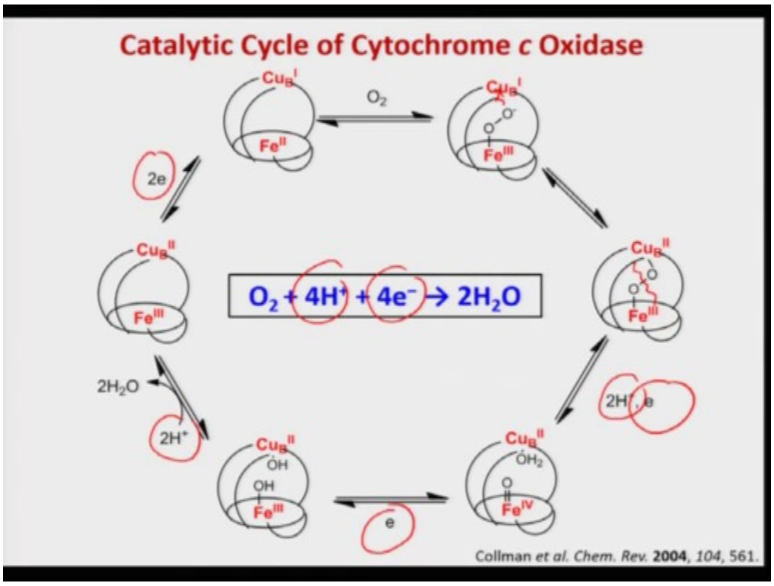

So, the overall chemical reactions is:

$$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$$

Now this is what is iron copper unit in the resting state where it is Fe(II) and Cu(I) and dioxygen probably will bind to iron center. As I have said in previous slide that it also can bind to copper center as well. But possibly if the iron center is preferable to bind dioxygen and you know that Fe(II) will be converted to Fe(III) and O_2 will be converted to O_2^-.

And once it forms O_2^-, then O_2^- this also binds to copper and then Cu(I) will convert, it will oxidize to Cu(II). So, iron copper peroxide complex is being formed and with the help of two proton and one electron this forms Fe(IV)-oxo and Cu(II)-OH_2 and then after one electron reduction this Fe(IV)-oxo converted to Fe(III)-OH. And this then react with $2H^+$ which would convert to two molecule of water and gives rise to Fe(III)-Cu(II) fully oxidized species which will reduce by two electron gives rise to this Fe(II)-Cu(I).

And which is now again ready to bind dioxygen and start the catalytic process once again. So, overall reaction as you can see that here one electron, two electron and 2 electrons. So, four electron and four proton, 2 H plus 2 H plus 4 proton gives rise to two H_2O and releases huge amount of energy and which we store it. The mechanism is so full proof that you will not get any partially reduced species. However, nature also can do mistake.

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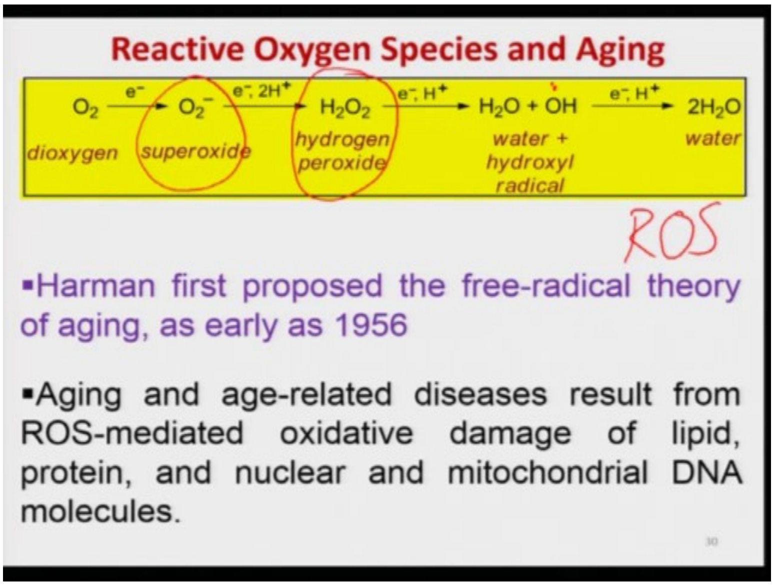

So, instead of getting water, there is a chance that you can generate the partially reduced species like it can form O_2^- after one electron reduction which is as you know that it is called superoxides. After another electron reduction it also forms peroxides O_2^{2-} they also can form OH radical. So, all these species are highly poisonous for our body and in biological system, they are called biological poisons.

And these are collectively known as reactive oxygen species ROS. Reactive oxygen species O_2^-, O_2^{2-}, OH radical all these are actually responsible for various problems related to aging. Now, let us little bit talk about on that topic. Now Harman first proposed this free radical theory of aging as early as 1956. Now, aging and its related diseases result from ROS mediated oxidative damage of lipids, protein and nuclear and mitochondrial DNA molecules ok.

These are all results of the radicals, you know radicals are very dangerous in biology radical reactions are indeed very very difficult to control.

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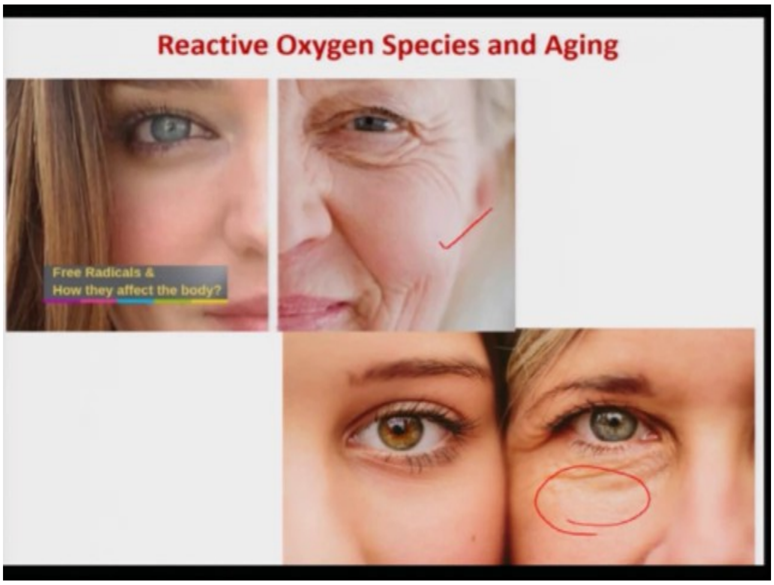

You can see - effect of reactive oxygen species and aging in this two picture and you see that this sides, as you can see that this is clear effect of free radical and aging problem. And you can see this part and also very clearly. So, without aging problem another one is once you have this reactive oxygen species and how it creates. The problem on your face, on your neck, on your eye, on your body there are many many examples just two examples I have shown you to attract your attention over here.

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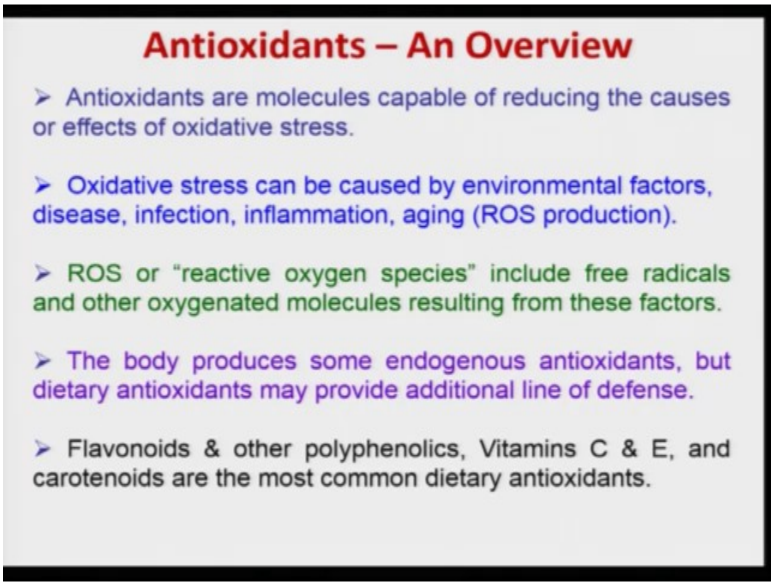

Now, let me talk a little bit about antioxidant. Now antioxidants are molecule, capable of reducing the cause or effects of oxidative stress; oxidative stress can be caused by environmental factors diseases, infection, inflammation, aging as I have just shown that ROS production reactive oxygen species production ROS. Or reactive oxygen species includes free radicals and other oxygenated molecule resulting from these factors.

The body produces some endogenous antioxidants but, dietary antioxidants may provide additional line of defense. Flavonoids and other polyphenolics vitamins C and E, and carotenoids are the most common dietary antioxidants. And these days there are many herbs and botanicals also contain antioxidants.

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Now, these are the fruits have you have antioxidants ok. Here is a amount of antioxidants like green tea, onion and this oranges, tomatoes and all sorts of things there are full of antioxidants. And we can use those antioxidants to get rid of age related problems also.

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As you can see one can protect the skin from the effects of such reactive oxygen species, just by applying these antioxidants time to time. And thus, one can stay teen even at very old age this has been very popular therapeutic techniques at home as displayed in this slide. In summary, I have discussed today how dioxygen is reduced to water by the use of four protons and four electrons in Cytochrome *c* Oxidase, which is the terminal member of the respiratory chains.

The reaction eventually responsible to produce huge amount of energy, that we use every day. However, incomplete reduction of dioxygen produce reactive oxygen species such as superoxide, peroxide and OH radicals which are indeed extremely poisonous for our body. Aging and age related diseases also result from reactive oxygen species mediated oxidative damage of lipid, proteins and nuclear and mitochondrial DNA molecules. I will discuss in my next lecture, how our life is fully protected from such reactive oxygen species.

Thank you.