Bioinorganic Chemistry
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Lecture - 14
Life With Oxygen: Oxygenase activity

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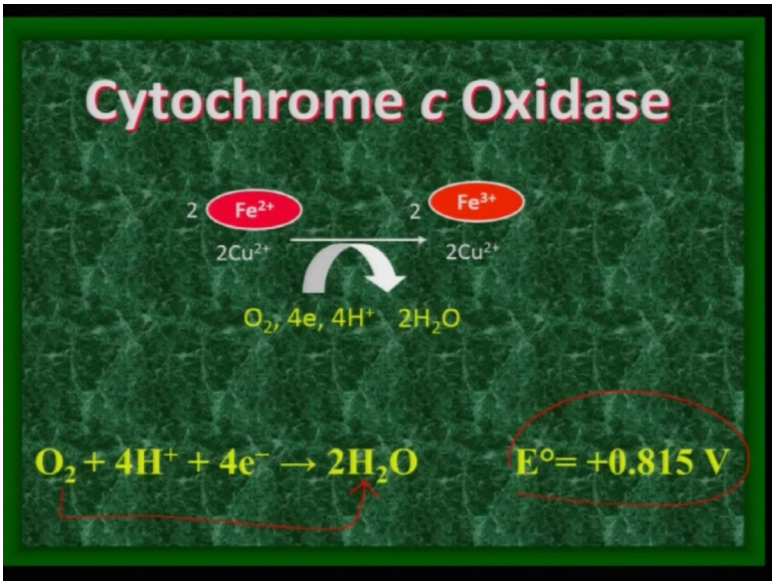

Hi everybody and welcome back to the short course of Bioinorganic Chemistry. We have been discussing about our life with dioxygen. The great oxidizing power of dioxygen is utilized in respiration to produce huge amount of energy and Cytochrome *c* Oxidase is the terminal member of this respiratory chain in which this dioxygen converts to water with the help of 4 protons and 4 electrons. This is what is the reaction, so, O_2 converts to water with a potential of 0.815 volt.

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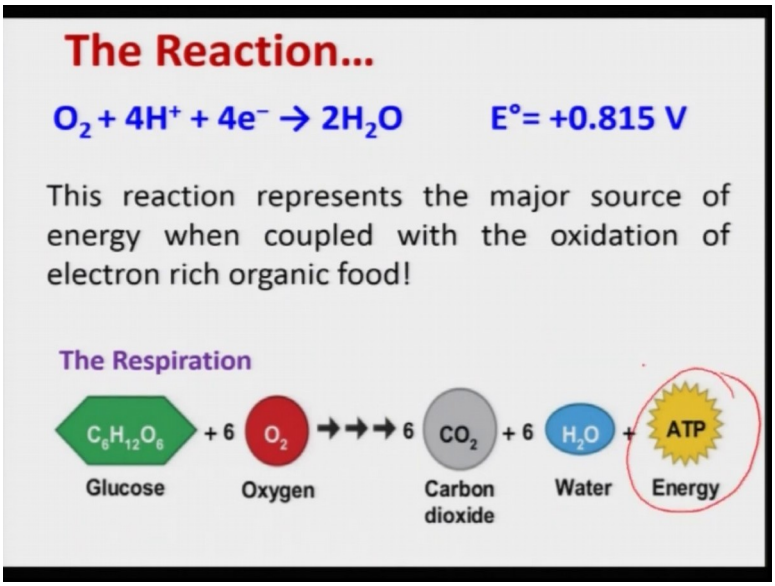

This reaction of dioxygen converted to water with the help of 4 protons and 4 electrons represents the major source of energy when coupled with the oxidation of electron rich organic food, and as you know that respiration produces huge amount of energy in the form of ATP. I have discussed all this in details in my last lecture.

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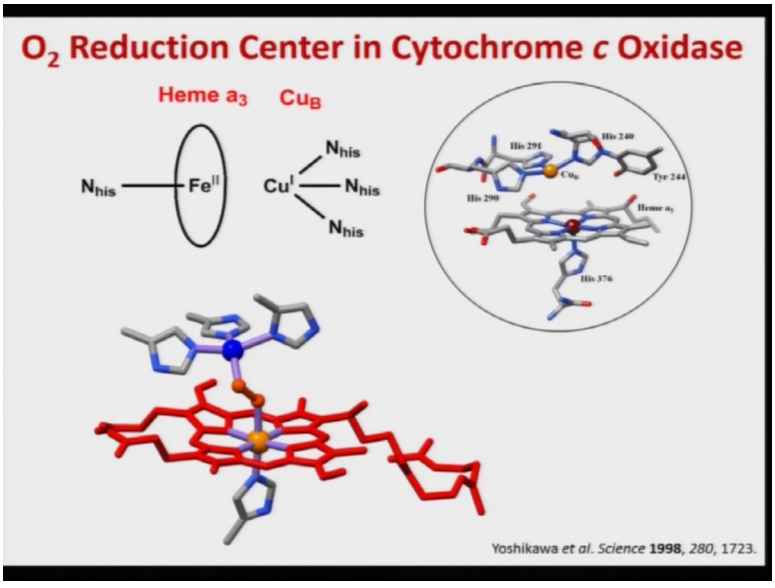

Dioxygen reduction center in cytochrome *c* oxidase is shown over here once again and as one see that this heme which is 5 coordinated andCu_B, which is 3 coordinated can indeed bind dioxygen and X-ray structure is shown here. And when it binds to dioxygen the X-

ray structure also been reported and one can see that dioxygen binds between iron and copper and thereby, this O-O bond get cleaved and it eventually converts to water. Unless one gives 4 protons and 4 electrons all at a time, dioxygen will not be converted to water.

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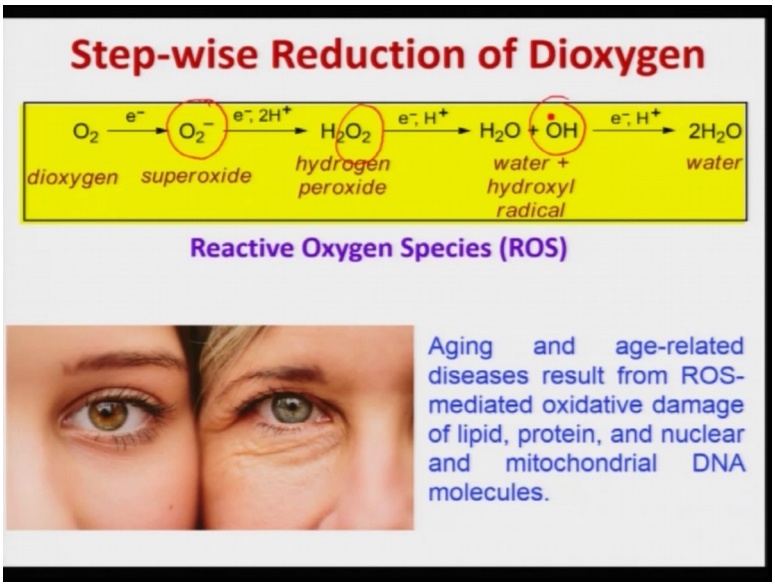

Indeed, if one do stepwise reduction then, dioxygen produces superoxide, peroxide and OH radical which are indeed extremely harmful for our life. These are called reactive oxygen species ROS and which are actually responsible for aging and age related diseases in our body. So, I have discussed all this in my last lecture.

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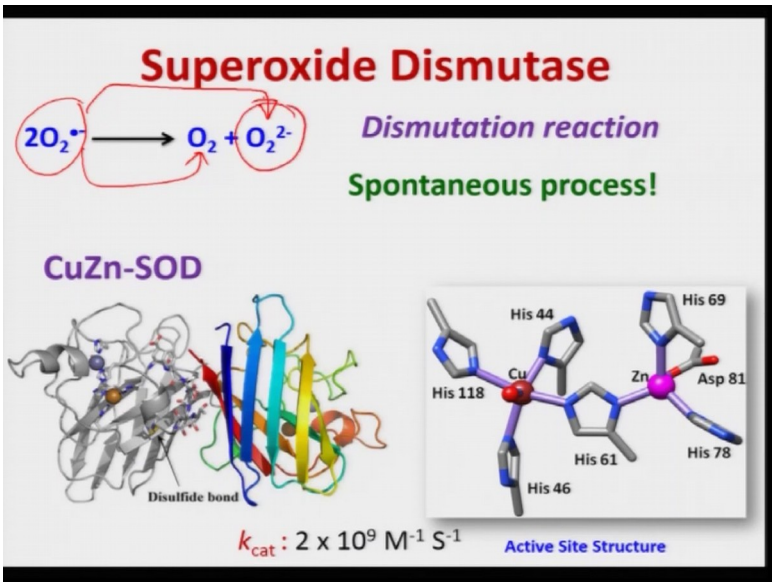

Now, superoxide dismutase which actually convert superoxide into oxygen and peroxides, as you can see that this is a disproportionation reaction or dismutation reaction and spontaneous. So, superoxide getting oxidized to dioxygen and also another molecule of superoxide getting reduced. And although it is a spontaneous process, nature need to design an enzyme so that the superoxide can be destroyed almost immediately, and as one can see that k_{cat} is $2x10^9$ M^{-1} S^{-1} ,very high values. So tthis has been designed by our mother nature to destroy superoxide almost immediately produced in our body. The superoxide as you also can see produce peroxide which is another harmful product.

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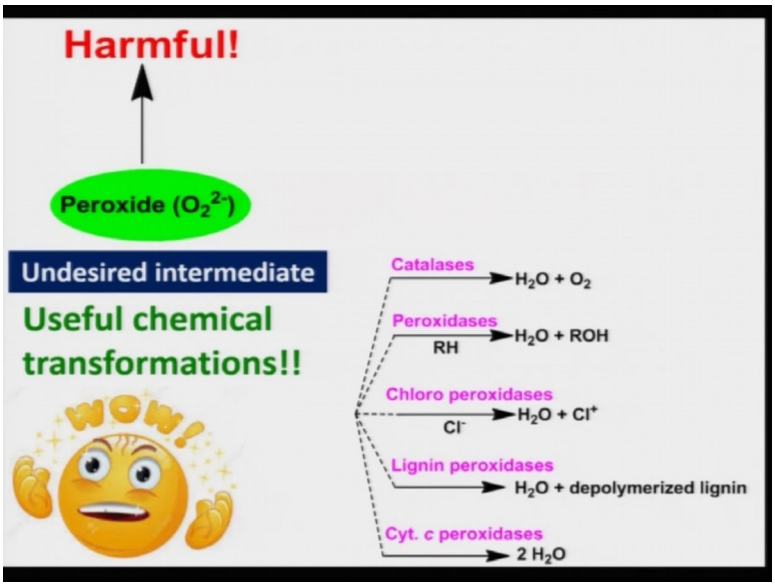

This peroxides is an undesired intermediate and extremely harmful for biology and nature cleverly make use of these peroxides to produce lots of very important chemical transformations, which is otherwise impossible to do. And as you can see that catalase, peroxidase, chloro peroxidase, lignin peroxidase and cytochrome *c* peroxidases are designed which eventually convert these peroxides to something very useful products which is otherwise impossible. I also have discussed in details the action of catalase and peroxidase in my last lecture.

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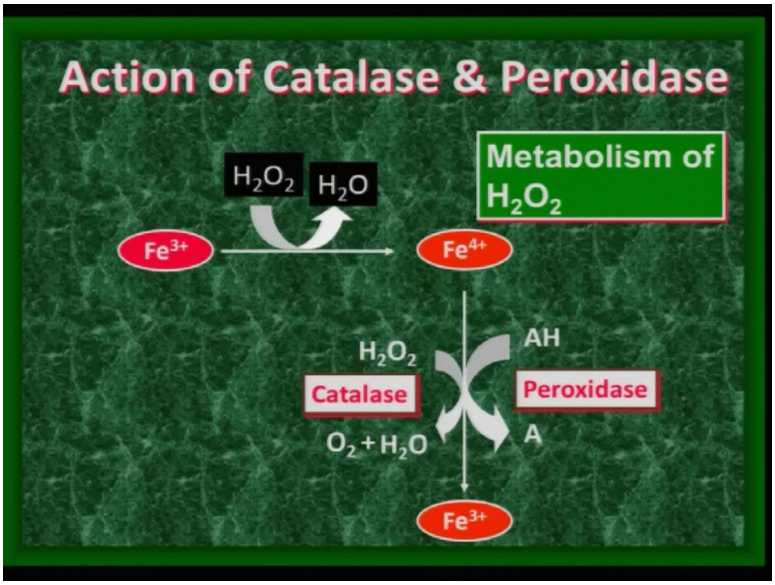

As one can see that hydrogen peroxide, oxidize Fe(III) centers toFe(IV) oxo cation radical, which then converts to Fe^{3+} heme centers, in case of catalase enzyme, the hydrogen peroxide is the substrate which is converted to oxygen and$2H^{+}$. In case of peroxidase enzyme the organic substrate is getting oxidized to form A.

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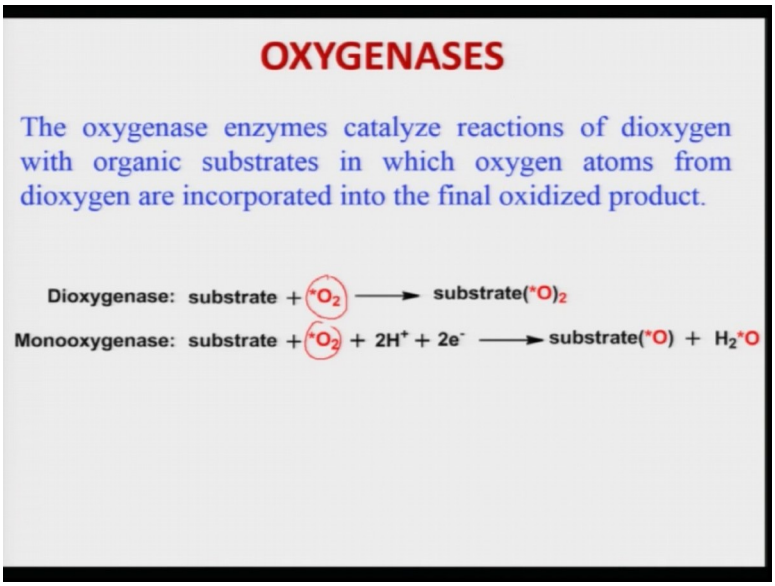

Now I will talk about oxygenase enzymatic system. The oxygenase enzymes catalyze reactions of dioxygen with organic substrates in which oxygen atom from dioxygen are incorporated into the final oxidized product. There are two types of oxygenase reactions. One is dioxygenase reaction in which both oxygen atoms of dioxygen are incorporated into a single substrate. The other is monooxygenase reaction in which only one oxygen atom of dioxygen is incorporated into the major substrate. So, you see that this labled oxygen which is red in color in mono and di, they are actually incorporated inside the substrate and that is what dioxygenase and monooxygenase names come from.

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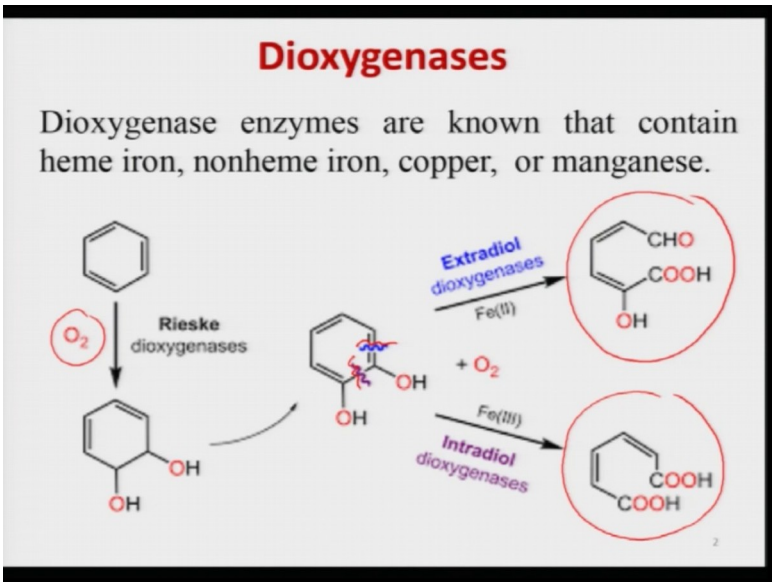

Let us talk little bit about dioxygenase. Dioxygenase enzymes are known that contains heme, non-heme iron, copper or manganese. Here is a typical example of Rieske dioxygenase, as you can see that the benzene converted to catechol in presence of dioxygen and ; so, this catechol dioxygenase is responsible for cleaving this catechol. It can cleave in two ways,one is Extradiol-outside the diol over here Extradiol dioxygenase, which is a product you can see that the ring is getting cleaved completely in presence of oxygen and Fe(II) is involved in the enzymatic system. Whereas, Intradiol dioxygenase- it cleaved between two diol group and Fe(III) ion is involved in the enzymatic system and the products are quite different. So, based on the position of the cleavage, the products are different whether it is an extradiol or intradiol.

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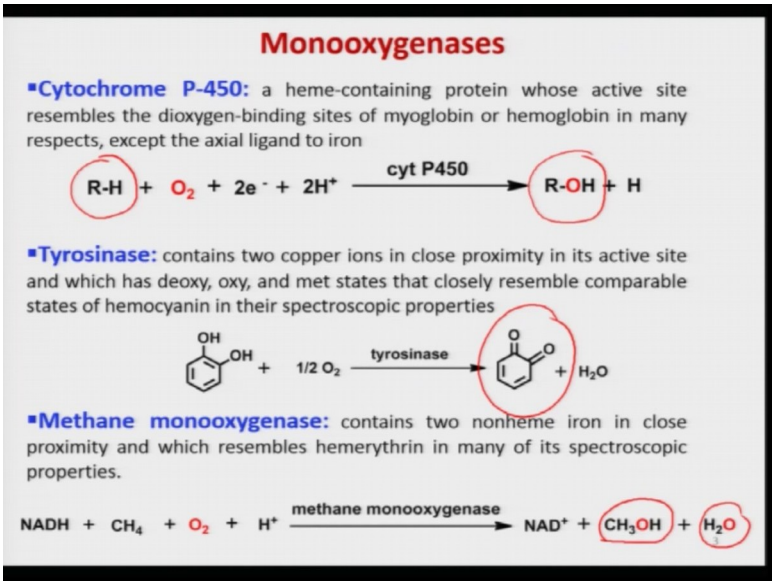

Now, I will talk about monooxygenase enzymatic system. For example, cytochrome P450, a typical example of monooxygenase, it is an heme containing enzyme whose active site resembles the dioxygen binding sites of myoglobin or hemoglobin in many aspects, except the axial ligand to iron. Indeed, in cytochrome P450 cysteine sulfur involved rather than an imidazole side chain from histidine which is involved in hemoglobin or myoglobin.

Now, the typical reaction is shown over here where you can see R-H substrate converted to R-OH and which is water soluble. And next is tyrosinase the another monooxygenase enzyme which contains 2 copper ions in close proximity in its active site, which has

deoxy, oxy and met that closely resemble comparable state of hemocyanin in their spectroscopic properties. And as you can see the typical reactions like that catechol converts to quinone and typically tyrosinase does this kind of conversion.

Next monooxygenase is methane monooxygenase. There are many monooxygenase known in the literature now. Now methane monooxygenase contains 2 non-heme iron in close proximity and resembles closely to hemerythrin in many of its spectroscopic properties and the typical reactions that methane monooxygenase does is NADH+ methane+dioxygen and in the help of proton NAD^+ and methanol and water. So, methane converts to methanol that is why it is called it methane monooxygenase.

In addition to these three, there are also a large number of monooxygenase enzyme known today, which contains nonheme iron or copper ions sometimes even manganese. So, I am not going to discuss all in details, because of time shortage and today I am going to discuss one of this very popular monooxygenase enzyme cytochrome P450.

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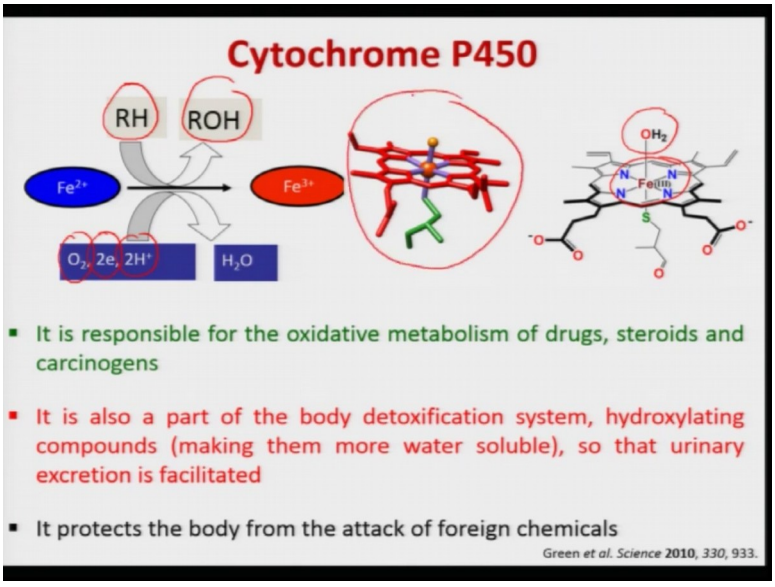

Cytochrome P450 as I have discussed already that RH, the substrates converts to ROH with the help of oxygen, 2 electrons and 2 protons which eventually forms water. And this is a very important enzyme for our body, for our life. It is responsible for the oxidative metabolism of drugs, steroids and carcinogen. It is also a part of the body's detoxification system, hydroxylating compounds making them water soluble so, that it can easily excrete through urine.

In other word it protects our body from the attack of foreign chemicals. So, this is very very important for our day-to-day life. And the active sites structure of the cytochrome P 450 in the resting state is like this, as you can see a porphyrin group is ligated with Fe(III) state and fifth position is cysteine sulfur and the sixth position is weakly coordinated water molecule. TheX-ray structure is also shown over here.

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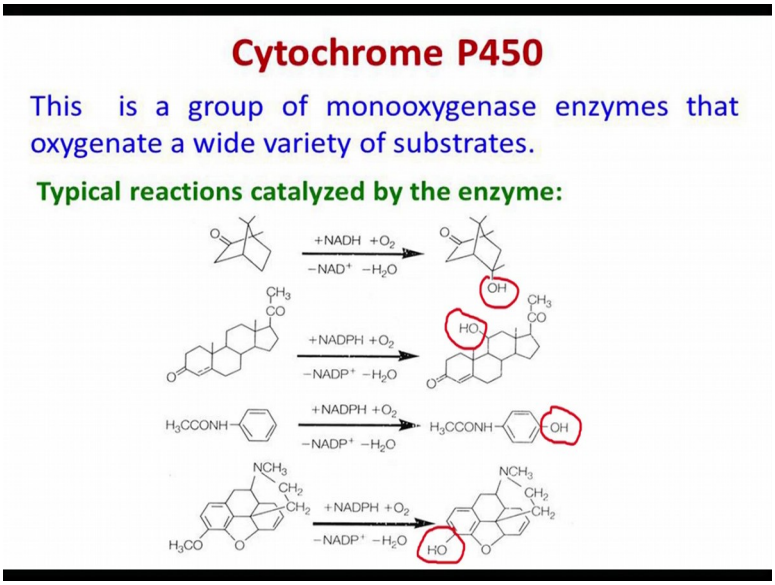

Why we call the enzyme as cytochrome P450? This unusual name is given to it, the reason is that it exhibits an intense (Ref. Time 13:46) band at unusual 450 nm for its ferrous carbon monoxide derivative. Typically for normal heme centers this generally comes around 420. So, since it is an unusual position so, when it was discovered it was given a peculiar name cytochrome P 450; P is a pigment.

So, this is the way it has been named since it was discovered; however, this enzyme does lots of chemical transformations in our body. As I have said already, this is a group of monooxygenase enzyme and a varieties of substrate being hydroxylated, for example, this camphor is hydroxylated over here. The next steroid is hydroxylated over here, this has been hydroxylated here. So, a typical position very selective not random, please note that this hydroxylation is stereo specific, not random and that is the reason why this so special.

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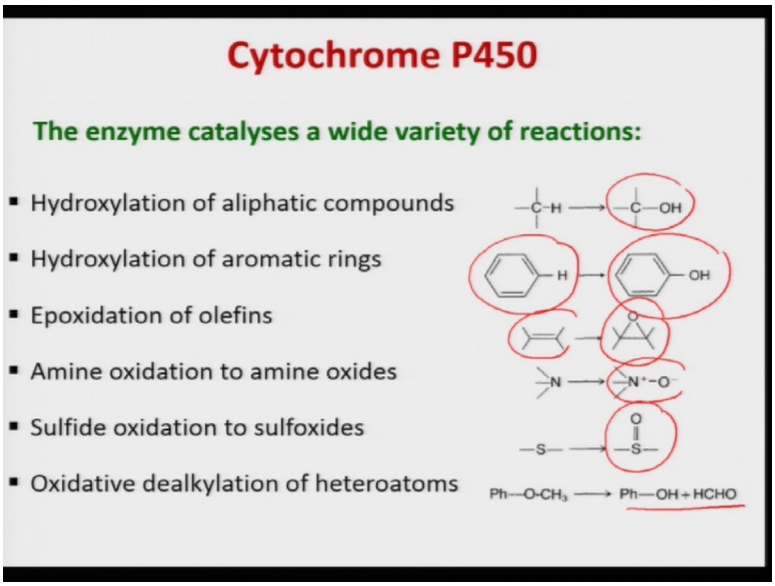

The enzyme catalyzes a wide varieties of reaction. For example, some of these reactions are listed over here, like hydroxylation of aliphatic compound, C-H converted to C-OH, hydroxylation of aromatic rings like if you have benzene, it converts to phenol. Epoxidation of olefins, the olefin get converted to epoxides as shown, amine oxidations to amine oxides N to N-O, sulfide oxidations to sulfoxides S-O and oxidative de-alkylation of heteroatoms; for example, if it is Ph Ph-OH plus HCHO. So, like there are many such reaction I can write which is basically catalyzed by cytochrome P450 in our body.

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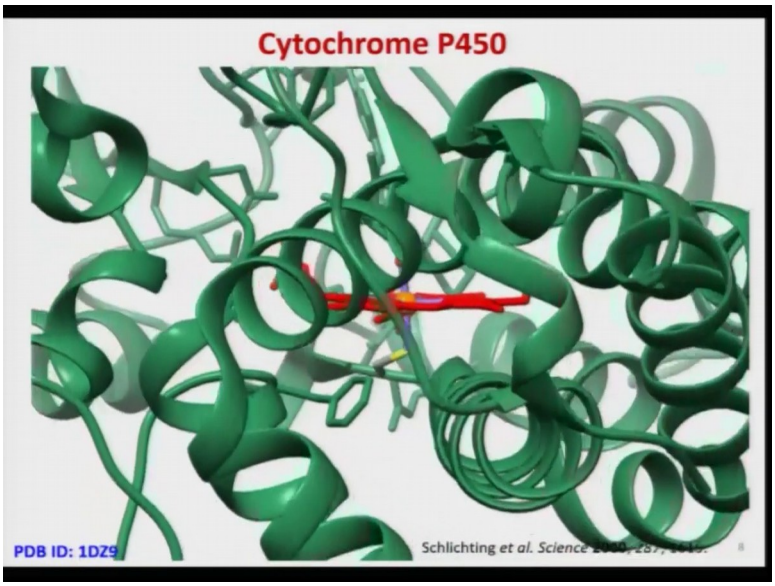

This is what is the protein structure of cytochrome P450. As you can see that at the center there is a heme group and the fifth position is ligated with cysteine sulfur and sixth position is ligated with a water molecule. What is interesting to note over here is a huge protein chain is wrapping around this molecule. And what this protein chains are doing, the protein chains are actually dictating the reactivity and also the specificity of the substrate hydroxylation, the position of the hydroxylation and other things which I will soon discuss.

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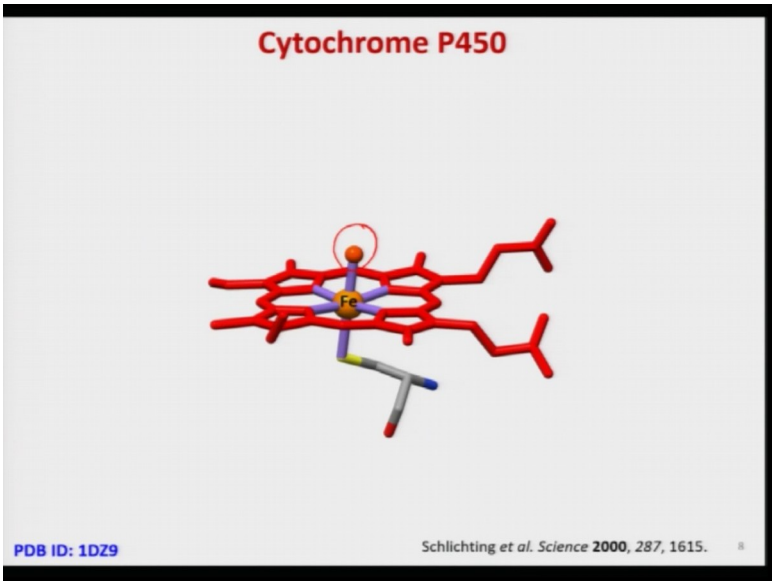

How are the basic chemical transformations taking place at the iron centre that hydroxylation and thus this iron is so, important. If we remove this iron, this enzyme is completely inactive, that shows the importance of having iron in cytochrome P450. Now, once I remove this protein chain what you see that, you see this molecule that heme centers, iron is sitting at the center and there is a weak coordination of a water molecule and the cysteine residue is over here, and this is the molecule which is actually responsible for so many organic transformation.

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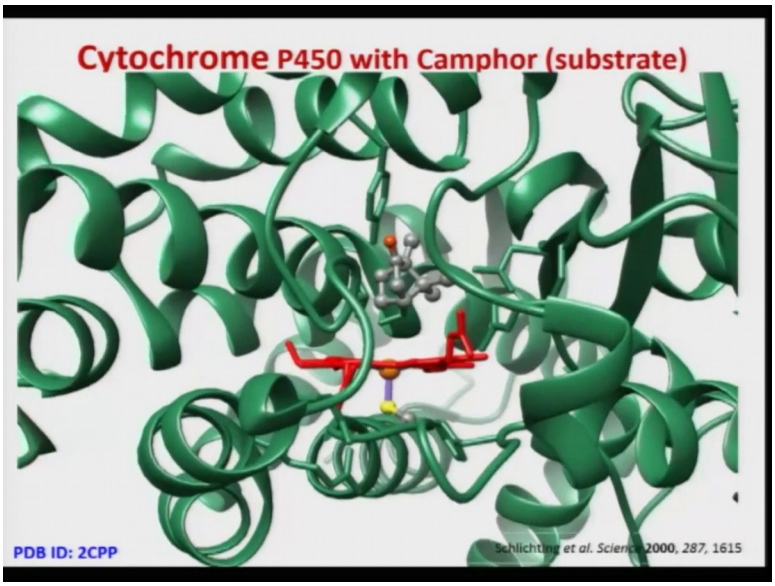

Let us look at that the cytochrome P450 with camphor as a substrate. Now, you see that this molecule is crystallized with camphor which is substrate and camphor sits just on the top of this iron center and very close to that and also there is no water molecule which was earlier present. So, this camphor replaces this water and place a particular position so that it can be hydroxylated at a particular position. And this is what is happening here, you see for when I remove this protein chain this camphor is sitting at the top of this iron center and this carbon is actually hydroxylated which we will see soon.

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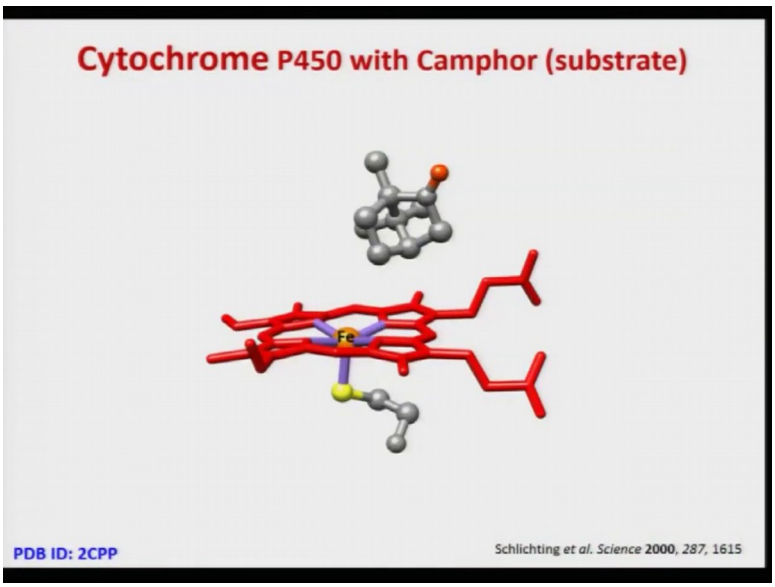

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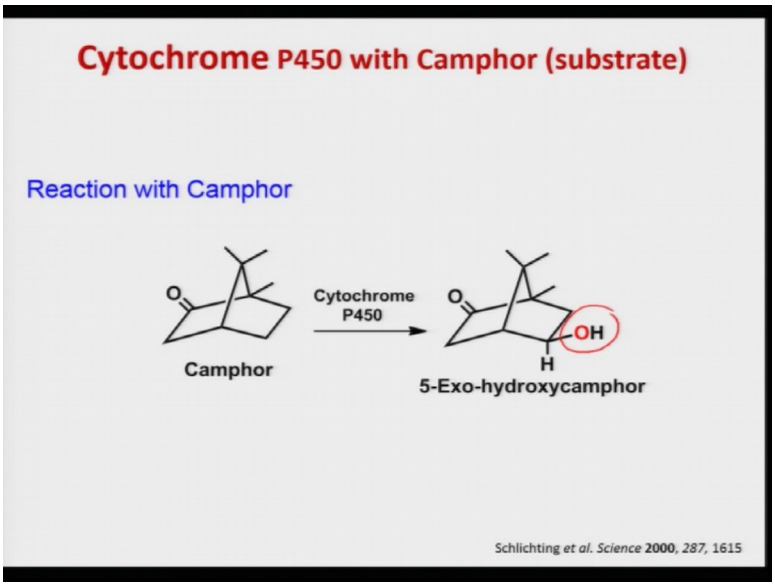

So, this is the camphor and this position is hydroxylated and also it is very stereo specific and that is what the entire protein chains are doing. So, protein chains are responsible for the stereo specificity and also controlling the rate of this transformations. So, we will see in next few slides more details about it.

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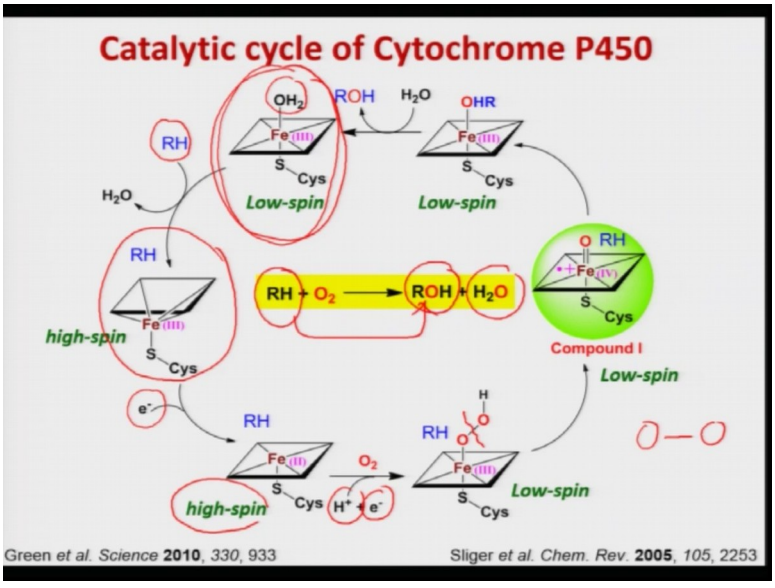

So, a catalytic cycle of this enzyme is shown over here. Now in the resting state this is what it is in the resting state, thisFe(III)center and cysteine sulfur is at the fifth position and sixth position is a weak water molecule. indeed a low spin species, S is equal to1/2.

Now, when this substrate RH comes close to this molecule, then what would happen, it replaces this water and it forms an enzyme-substrate complex as it is shown.

However, there is a remarkable change in the spin state. The iron center, which was low spin in the resting state, now becomes high spin since it is a 5 coordinated iron center, although oxidation states remains theFe(III). Now in the next step this Fe(III) undergo a quick reduction in presence of an electron and this Fe(III) transformed to Fe(II) and this also another high spin complex.

Now once it forms thisFe(II), it is ready to bind dioxygen as we have seen in case of hemoglobin and myoglobin. Indeed this is what is happening so, Fe(II) binds dioxygen and as you know that then it will becomeFe(III)O_2^-, but in presence of one more electron and proton this becomes O_2^{2-}ok, it is peroxides. And as I have discussed earlier that if O-O bond need to be cleaved peroxide is the best state, because the O-O bond dissociation energy is lowest. So, immediately the O-O bond get cleaved and it forms Fe(IV)oxo cation radical, which is called compound 1, please note that this is a very reactive and unstable intermediate and this also forms with RH. It immediately transformed to ROH and Fe(IV)is reduced toFe(III).

And in the next steps once it forms ROH, then water molecule replaces that ROH and this comes back to the original molecule that Fe(III)with an axial ligand of water and this is low spin complex which is in the resting state and the catalytic cycle starts once again. During this transformation what is exactly happening, this RH the substrate is getting hydroxylated, converting to ROH in presence of oxygen and proton and electron and oxygen getting converted to also water along with ROH. So, this is monooxygenase reaction and this is very important.

So, all this molecule getting hydroxylated and we can use this Fe(IV)oxo cation radicals for various catalytic applications in the laboratory as well, since this is a highly reactive and very strong oxidant. So, many people, many research group utilize this molecule for transforming lots of organic substrate into various species in the laboratory.

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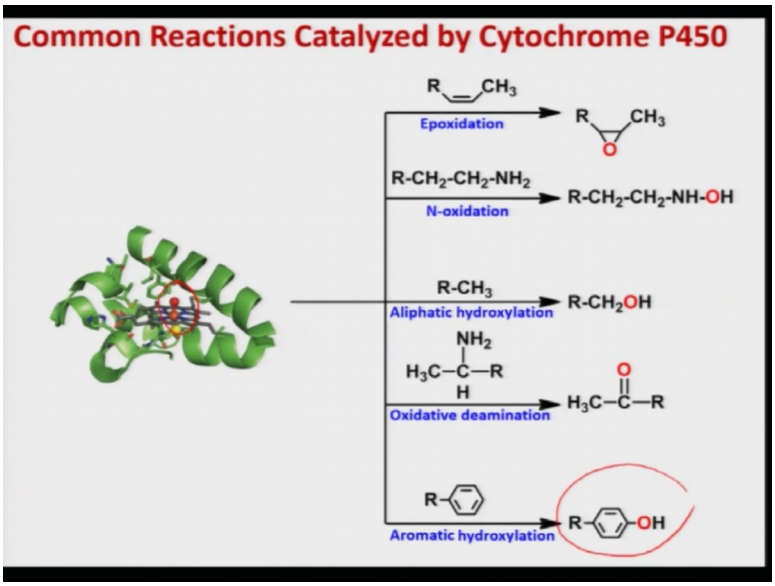

So, key step is this Fe(IV)oxo and you can see that lots of substrate can be hydroxylated and this oxo group can be inserted, whether it is an epoxidation, whether it is N-oxidation whether it is aliphatic hydroxylation, whether it is oxidative de-amination, whether it is aromatic hydroxylation; large number of substrate can indeed be converted to a various important and interesting molecules which is otherwise very very difficult to make ok. So, basically the organic C-H substrate converted to C-OH during this transformations.

So, now, I will show you a beautiful design principle that is adopted in biology.

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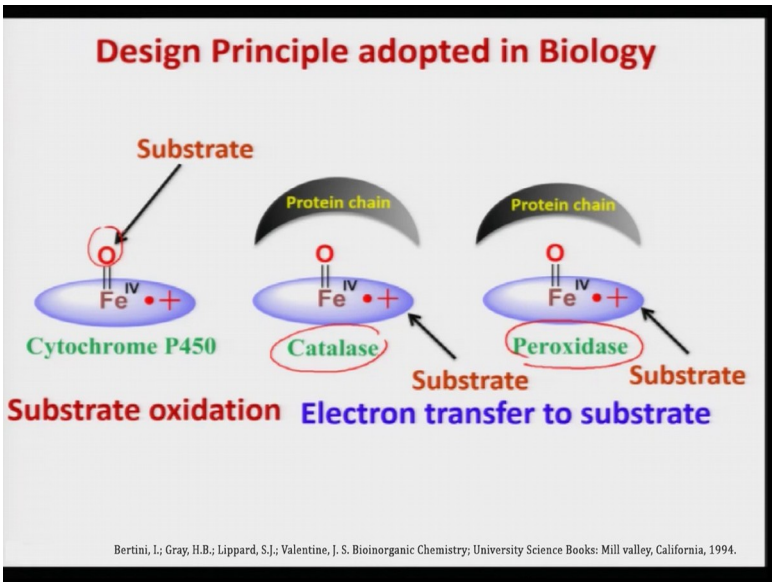

Now we already discussed catalase, peroxidase and, cytochrome P450 and their catalytic transformations and now I will show you that although the catalytic process are very similar, they are doing completely different job in biology. As you know that catalase which actually catalyze the conversion of hydrogen peroxide to oxygen and water. Peroxidase, they actually oxidize the substrate, but all these reactions are not oxo transfer reactions, they are basically the electron transfer reaction.

However, cytochrome P450 we have just seen that substrate oxidation is taking place, this is due to the design principle. Although in all three enzymes the common active intermediate is Fe(IV)oxo cation radical, but they are doing completely different reactions in the biological system. The design is such that in cytochrome P450 this Fe(IV)oxo is exposed to the substrate; so, that substrate can come close to the oxo group which is highly reactive and transformed to hydroxylated compound.

Now this protein chains which are actually wrapping around this cytochrome P450 indeed allowing the substrate to comes close to oxo group, thereby this oxo transfer is possible. In contrast in case of catalase and peroxidase, the protein chains which are actually blocking this oxo group so, the substrate cannot come close to the oxo group; however, substrate can come through the porphyrin periphery. It can come close to the porphyrin periphery and as you can see that the porphyrin is cation radical.

So, this radical transfer reactions are actually possible in case of catalase and peroxidase and that is the reason why these two are doing a completely different reactions, not oxo transfer reaction; whereas, cytochrome P450 undergo oxo transfer reaction. So, you see that the beauty of the design in biology here, although they are very similar or they are almost identical in their reactive intermediate, but they are doing completely different jobs in biological system. And here you see that how the protein chains which are wrapping around this molecule are actually controlling the reaction and specificity of the reaction.

I have discussed today about the reactions of dioxygen with organic substrates catalyzed by oxygenase enzymes, in which oxygen atoms from dioxygen are incorporated into the final oxidized products that are very useful for our daily life. Dioxygen gets activated and inserted into the substrates which fall into two categories depending upon whether both or only one of the atoms of dioxygen molecule ends up in the substrate.

Although, a large number of monooxygenase and dioxygenase are known today, we could only able to discuss one such monooxygenase cytochrome P450 here today due to short duration of this course. In my next lecture I will discuss various dioxygen carrying proteins in biology.

Thank you.