Bioinorganic Chemistry
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Lecture - 13
Life with Oxygen: Catalase and Peroxidase Activities

Hi everybody. Welcome back to the short course of Bioinorganic Chemistry. We have been discussing our life with dioxygen. Let us now go back to see what I have discussed in my previous lectures.

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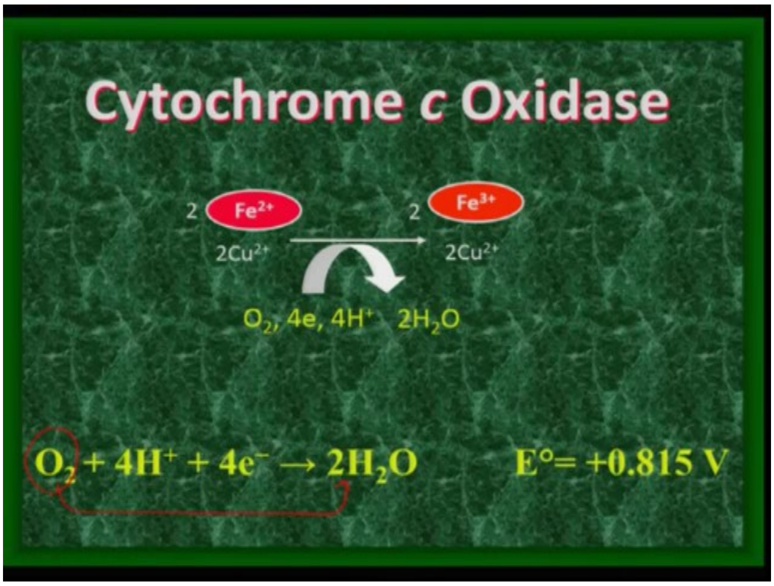

Cytochrome *c* Oxidase

$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$ $E° = +0.815$ V

I have discusse how dioxygen is reduced to water by the use of 4 protons and 4 electrons in cytochrome *c* oxidase, which is the terminal member of the respiratory chains. And you see that the important reactions is O_2 converted 2 molecule of H_2O and you see that 4 protons and 4 electron shave to be added all at a time with an E^0 value of 0.815 volt.

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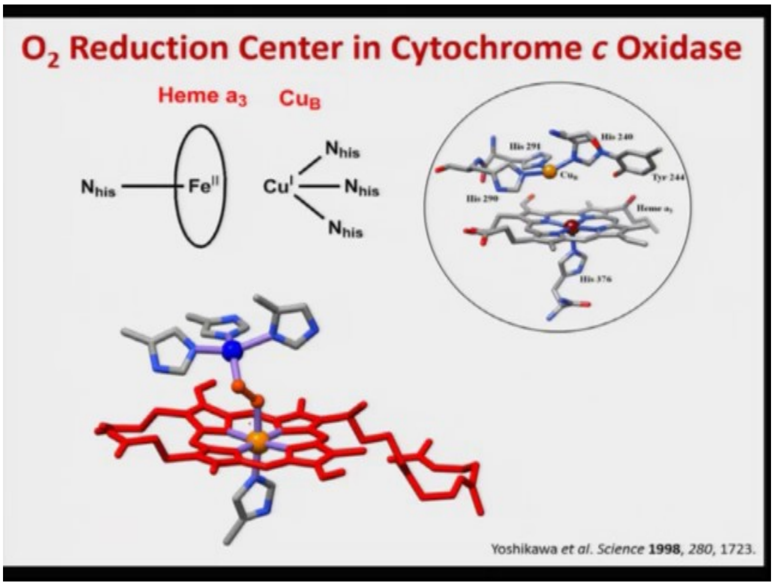

O_2 Reduction Center in Cytochrome *c* Oxidase

Yoshikawa *et al. Science* **1998**, *280*, 1723.

The dioxygen getting reduced in cytochrome *c* oxidase as I have just shown and the reduction center is shown over here. as one can see that this Fe(II) heme centers of this heme a_3 and Cu_B And heme $a_3$is 5 coordinated Cu_B is 3 coordinated and the structure is shown here where dioxygen comes and binds perfectly between these two metal centre. the X-ray structure is displayed over here. You can see yourself how dioxygen appears perfectly between iron and copper centers.

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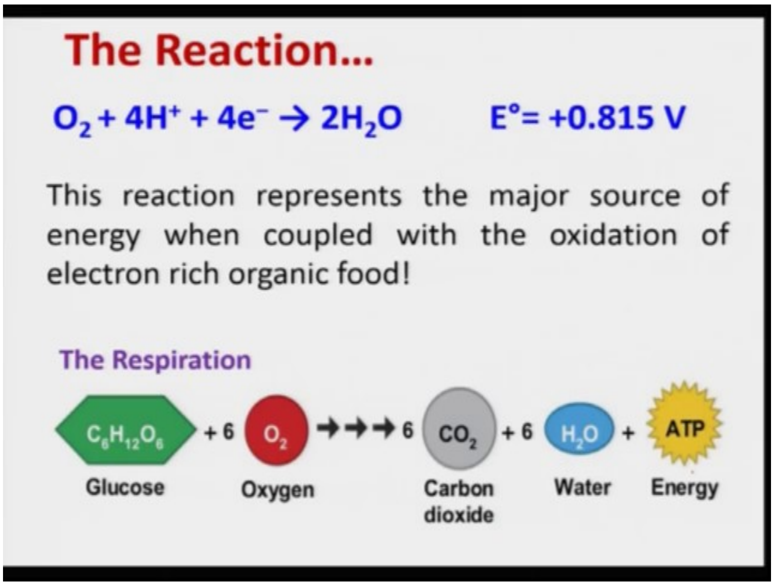

The Reaction...

$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$ $E° = +0.815$ V

This reaction represents the major source of energy when coupled with the oxidation of electron rich organic food!

The Respiration

Now, this converting oxygen to water is very important reaction. this reaction represents the major source of energy when coupled with the oxidation of organic food. And in the respiration as I have discussed that, the glucose and 6 molecule of dioxygen leads to 6 molecule of carbon dioxide, 6 molecule of water and releases huge amount of energy which we use in our day to day activity.

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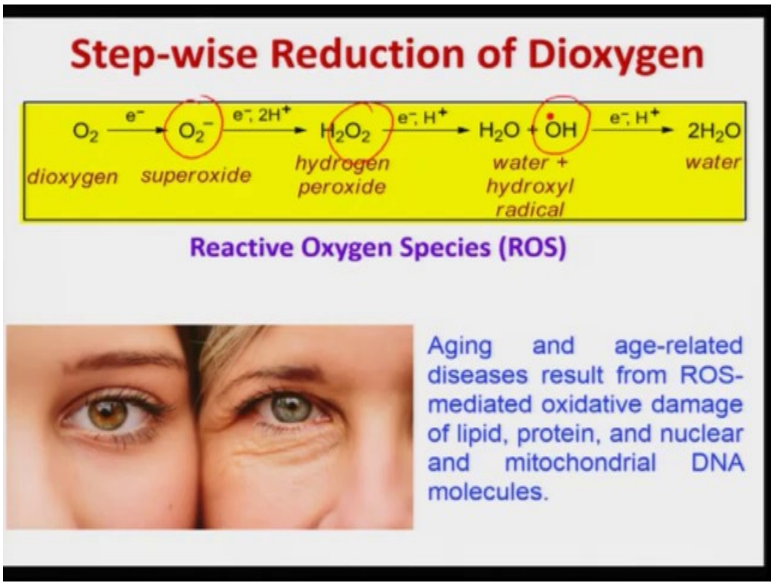

Step-wise Reduction of Dioxygen

$$O_2 \xrightarrow{e^-} O_2^- \xrightarrow{e^-, 2H^+} H_2O_2 \xrightarrow{e^-, H^+} H_2O + \dot{O}H \xrightarrow{e^-, H^+} 2H_2O$$

dioxygen — *superoxide* — *hydrogen peroxide* — *water + hydroxyl radical* — *water*

Reactive Oxygen Species (ROS)

Aging and age-related diseases result from ROS-mediated oxidative damage of lipid, protein, and nuclear and mitochondrial DNA molecules.

If the reduction takes place in a stepwise fashion, then dioxygen would produce superoxide, peroxide and hydroxyl, radical. Then, I have discussed in details that these are called reactive oxygen species, and this reactive oxygen species are responsible for various age related diseases in our body. And we have discussed these in details in my previous lectures.

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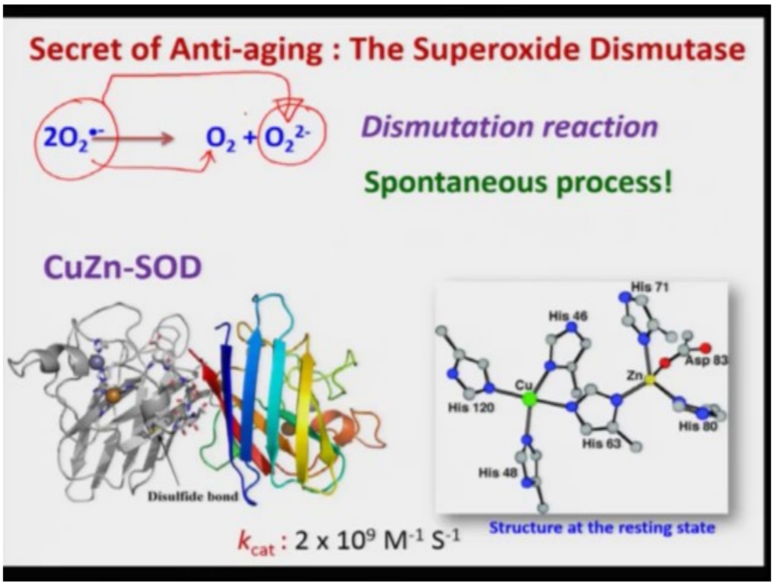

Secret of Anti-aging : The Superoxide Dismutase

$2O_2^{\bullet-} \rightarrow O_2 + O_2^{2-}$

Dismutation reaction

Spontaneous process!

CuZn-SOD

k_{cat} : 2 x 10^9 M^{-1} S^{-1}

Structure at the resting state

Now, this superoxides, which is extremely poisonous in our body converted to dioxygen and also the peroxides. So, one molecule of superoxide oxidized to oxygen, one molecule of superoxide getting reduced to peroxides. And as you all know this reaction is a spontaneous reaction, it is called dismutation or disproportionation reaction. And although this is a spontaneous process, nature design an enzyme called superoxide dismutase which indeed immediately decompose these super oxides with a k_{cat} value of $2x10^9$ M^{-1} $S^{-1}$and destroy this superoxides to peroxides and dioxygen.

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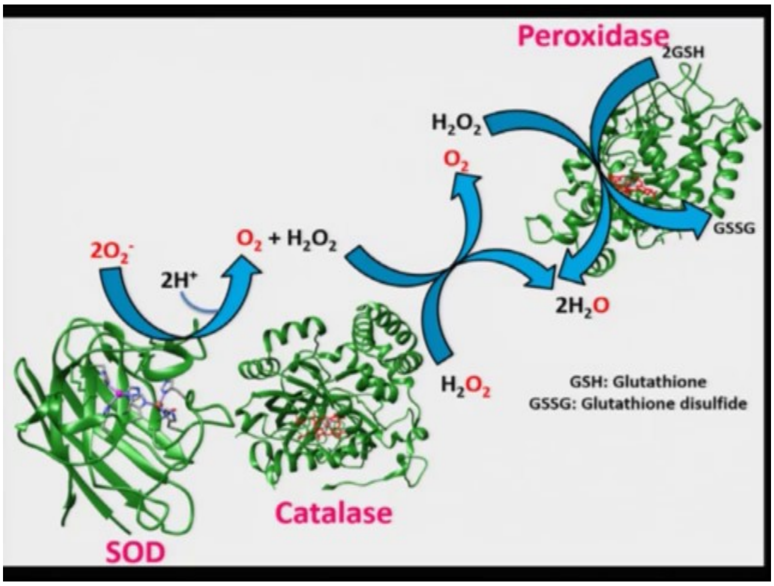

GSH: Glutathione

GSSG: Glutathione disulfide

Indeed, this peroxide which is produced in this reaction is also a highly poisonous and nature has designed catalase and peroxidase, in such a way that this peroxides convert to water and dioxygen. I will discuss these things in details.

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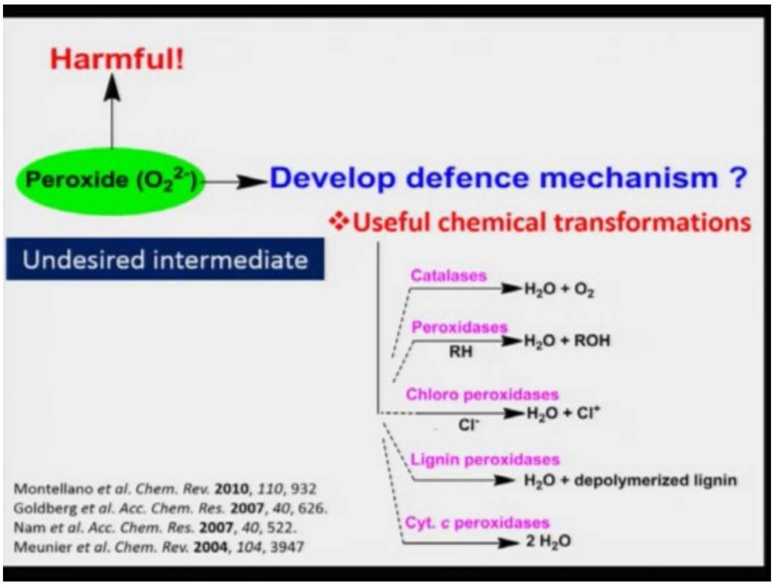

Harmful!

Peroxide (O_2^{2-}) → Develop defence mechanism ?

❖Useful chemical transformations

Undesired intermediate

$\xrightarrow{\text{Catalases}} H_2O + O_2$

$\xrightarrow[\text{RH}]{\text{Peroxidases}} H_2O + ROH$

$\xrightarrow[\text{Cl}^-]{\text{Chloro peroxidases}} H_2O + Cl^+$

$\xrightarrow{\text{Lignin peroxidases}} H_2O$ + depolymerized lignin

$\xrightarrow{\text{Cyt. c peroxidases}} 2\ H_2O$

Montellano *et al. Chem. Rev.* **2010**, *110*, 932
Goldberg *et al. Acc. Chem. Res.* **2007**, *40*, 626.
Nam *et al. Acc. Chem. Res.* **2007**, *40*, 522.
Meunier *et al. Chem. Rev.* **2004**, *104*, 3947

Now, this peroxides is extremely harmful and it is an undesired intermediate in the dismutation reaction. Now, nature need to develop a defense mechanism to protect from peroxides; indeed, nature has utilized this undesired intermediate to an extremely useful products using various enzymes such as catalase peroxidase, chloro peroxidase, lignin peroxidase and cytochrome c peroxidase.

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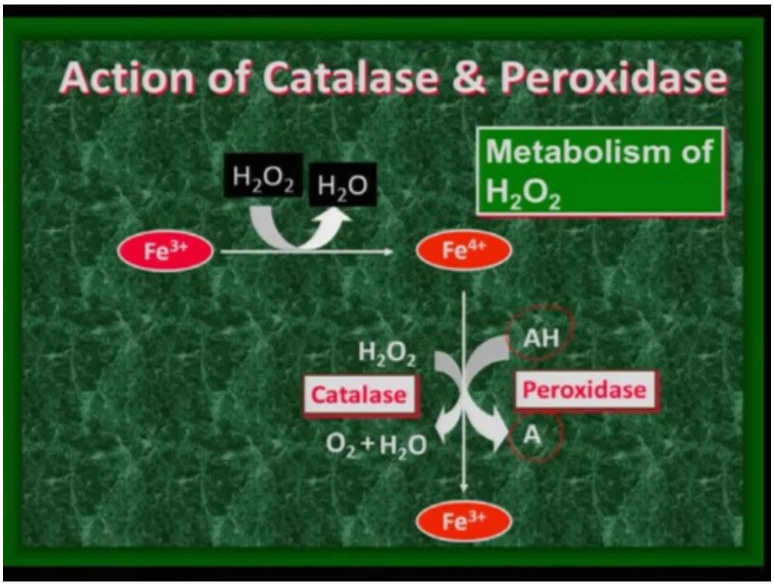

Action of Catalase & Peroxidase

Metabolism of H_2O_2

H_2O_2 H_2O

Fe^{3+} Fe^{4+}

H_2O_2

Catalase

O_2+H_2O

AH

Peroxidase

A

Fe^{3+}

Today I will be talking about catalase and peroxidase in details. Now, as one can see this catalase and peroxidase going through a common intermediate Fe(IV) oxo cation radical over here. Indeed, hydrogen peroxides oxidize this heme center, Fe(III) heme center to Fe(IV) and which is a highly reactive intermediate. And in case of catalase this hydrogen peroxides is a substrate, which converts to oxygen and water. Whereas, in peroxidase the substrate is getting oxidized to convert something very useful oxidized product and converts back to Fe(III) heme center, which again getting oxidized to Fe(IV) and goes back toFe(III). So, as one can see that Fe(IV) is a common intermediate for both catalase and peroxidase.

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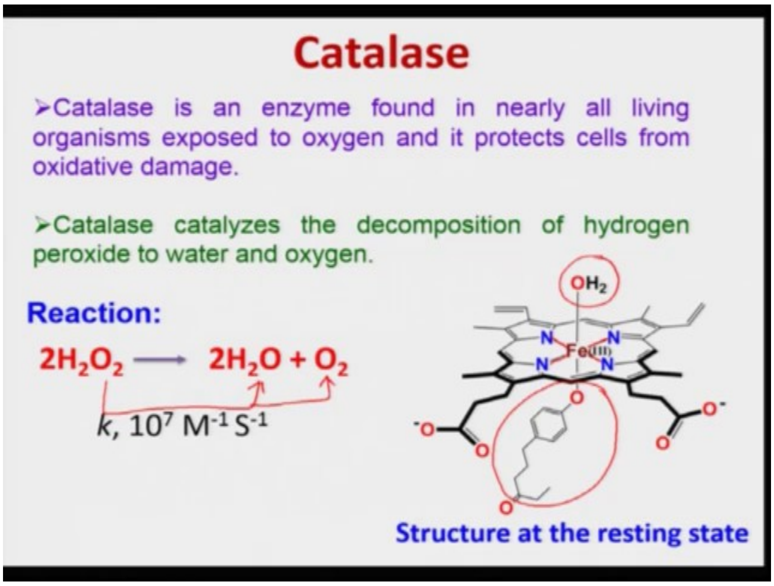

Catalase

- Catalase is an enzyme found in nearly all living organisms exposed to oxygen and it protects cells from oxidative damage.
- Catalase catalyzes the decomposition of hydrogen peroxide to water and oxygen.

Reaction:

$$2H_2O_2 \longrightarrow 2H_2O + O_2$$

k, 10^7 M^{-1} S^{-1}

Structure at the resting state

Now, let us discussed one by one in great details. Now, catalase is an enzyme found in nearly all living organisms exposed to oxygen; and it protects cell from oxidative damage. catalase also catalyze the decomposition of hydrogen peroxide to water and oxygen. As one can see hydrogen peroxides is converting to water and also dioxygen. And the enzyme catalase which is responsible for this transformation is shown here. this is a structure of the enzyme at the resting state. And you see this iron is sitting inside the porphyrin ring and there is 4 Fe-N bond and fifth position is as one can see that this is the tyrosinate. And the 6th position coordinated with a water molecule and iron is in Fe(III) oxidation state.

So, this is basically the structure at the resting state of the enzyme. In presence of this catalyst this transformation becomes highly efficient and $k_{cabt}$is 10^7 M^{-1} S^{-1}

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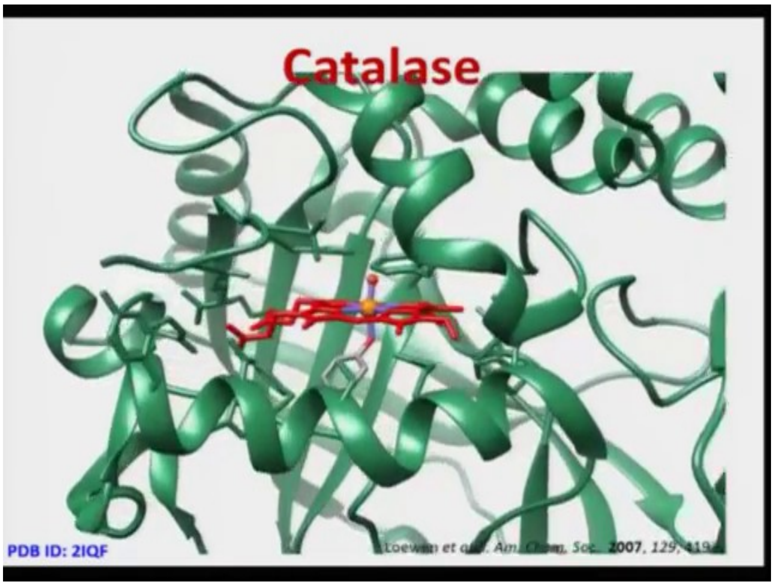

Catalase

PDB ID: 2IQF

Loewen et al. J. Am. Chem. Soc. 2007, 129, 419

And X-ray structure of this catalase enzyme is shown over here as one can see that heme center is sitting at the center in red in color.

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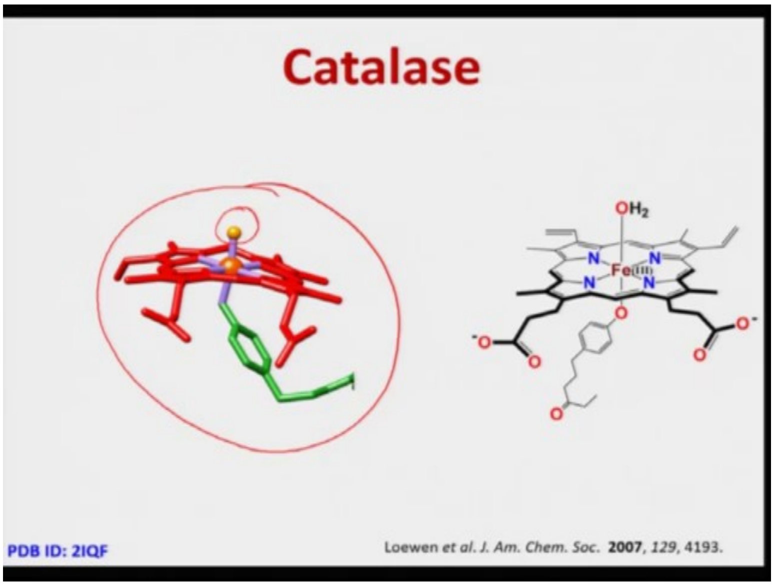

Catalase

PDB ID: 2IQF

Loewen *et al. J. Am. Chem. Soc.* **2007**, *129*, 4193.

And you see that a huge amount of protein, which are indeed wrapping the molecule; and if I remove this protein chain. What you can see that you can see only heme center is shown over here and this is iron is sitting on the plane of the porphyrin ring and a water molecule is weakly ligated at the sixth position. So, this is what is the resting stage structure of the catalase enzyme.

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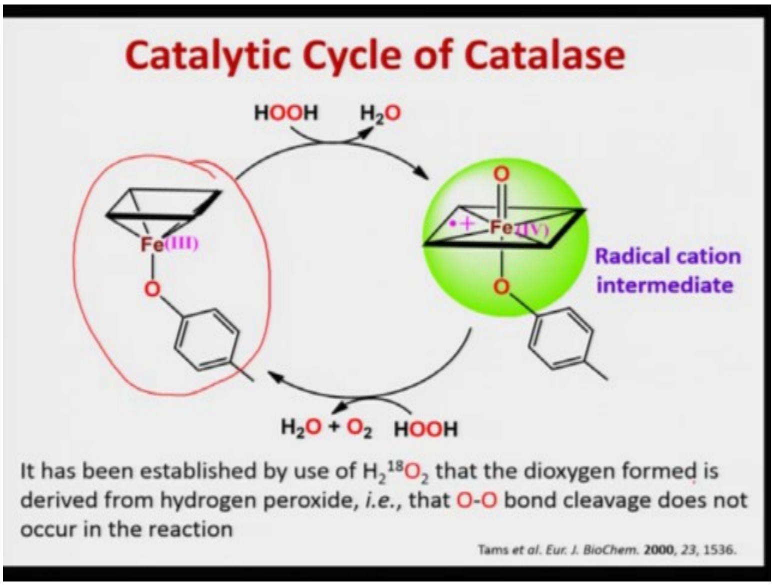

Catalytic Cycle of Catalase

It has been established by use of $H_2{}^{18}O_2$ that the dioxygen formed is derived from hydrogen peroxide, *i.e.*, that O-O bond cleavage does not occur in the reaction

Tams *et al. Eur. J. BioChem.* **2000**, *23*, 1536.

Let us look at the catalytic cycle of this enzyme. Now, this is what is Fe(III) center at the resting state and hydrogen peroxides oxidize this Fe(III) center to Fe(IV) oxo cation radical. It is a two electron oxidation; as you can see that Fe(III) converts to Fe(IV) and porphyrin ring also getting oxidized to porphyrin pi cation radical.

Now, these two electron oxidized species is highly reactive and it takes another molecule of hydrogen peroxides as a substrate converts to water and dioxygen and goes back to the resting state, which again getting oxidized by using hydrogen peroxide to Fe(IV) oxo cation radical. So, this is what is happening in the catalytic cycle it has been established by use of leveled hydrogen peroxides where oxygen is leveled that the dioxygen formed is derived from hydrogen peroxides. That means, that O-O bond cleavage does not occur during this catalysis process. So, hydrogen peroxide this O-O bond remains intact.

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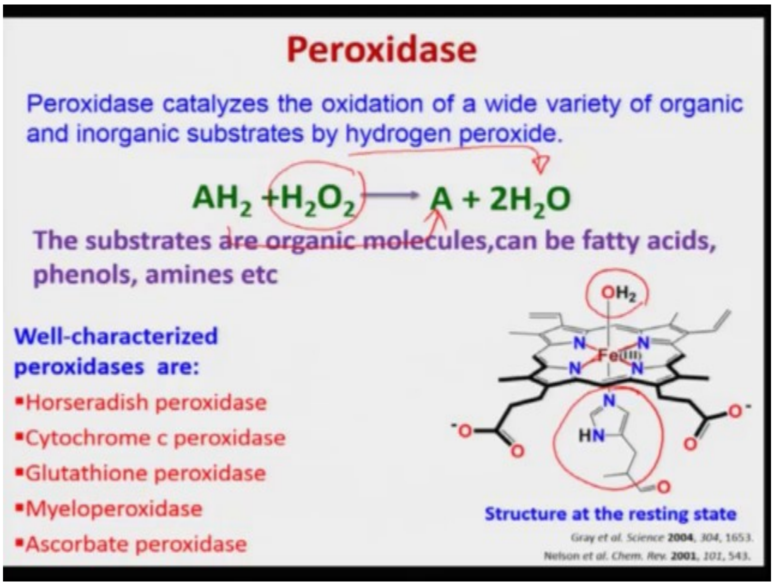

Peroxidase

Peroxidase catalyzes the oxidation of a wide variety of organic and inorganic substrates by hydrogen peroxide.

$AH_2 + H_2O_2 \longrightarrow A + 2H_2O$

The substrates are organic molecules,can be fatty acids, phenols, amines etc

Well-characterized peroxidases are:

- Horseradish peroxidase
- Cytochrome c peroxidase
- Glutathione peroxidase
- Myeloperoxidase
- Ascorbate peroxidase

Structure at the resting state

Gray et al. Science **2004**, 304, 1653.
Nelson et al. Chem. Rev. **2001**, 101, 543.

Now, let us talk about another enzyme called peroxidase. Peroxidase catalyzes, the oxidation of a wide varieties of organic and inorganic substrates by hydrogen peroxides. Overall reaction as one can see that this is what is the substrate AH_2 and in presence of hydrogen peroxides and in presence of peroxidase enzyme converts to water while substrate is getting oxidized to A.

So, in presence of this peroxidase enzyme, the substrate is getting oxidized to A; while hydrogen peroxides which is extremely poisonous converts to a water molecule. So, the substrates are generally organic molecules and can be fatty acids, phenols, amines and other species. Now, let us look at the structure of peroxidase enzyme at the resting state. As you have seen in case of catalase here also this iron is sitting at the center of the porphyrin ring. And as you can see there are 4 Fe-N bonds and fifth position is an histidine residue while 6th position is occupied by a water molecule which is weakly coordinated to iron.

Now, there are several peroxidase enzymes which are well characterized now, and some of them are shown over here- horseradish peroxidase, cytochrome *c* peroxidase, glutathione peroxidase, myeloperoxidase and ascorbate peroxidase, these are all well known peroxidase in the literature today.

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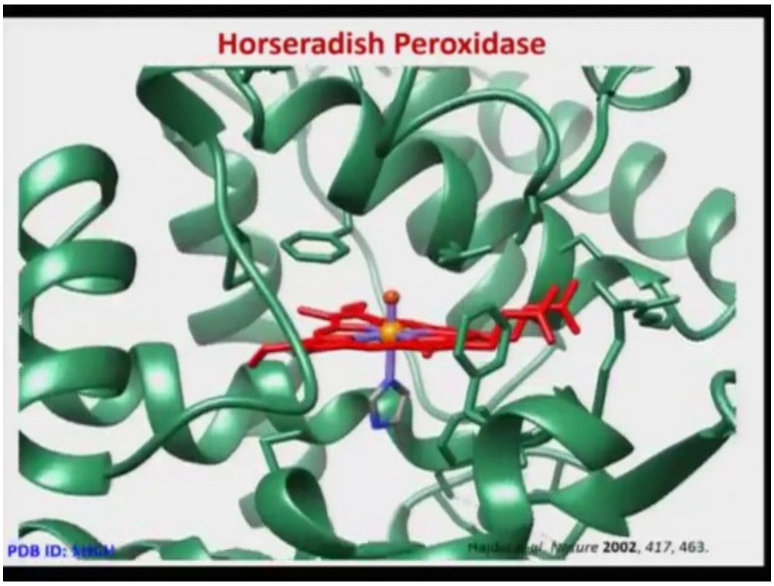

Horseradish Peroxidase

PDB ID: 1HCH

Hajdu et al. Nature **2002**, *417*, 463.

Now, horseradish peroxidase is well characterized and X-ray structure is shown over here. As one can see this heme center just like we have seen in case of catalase sitting at the centre and the huge protein chains which are surrounding that heme centers. Indeed, this protein chains actually controls the specificity of the reaction like the type of substrate that would be allowed to come in close to the heme center, I will discuss this in details.

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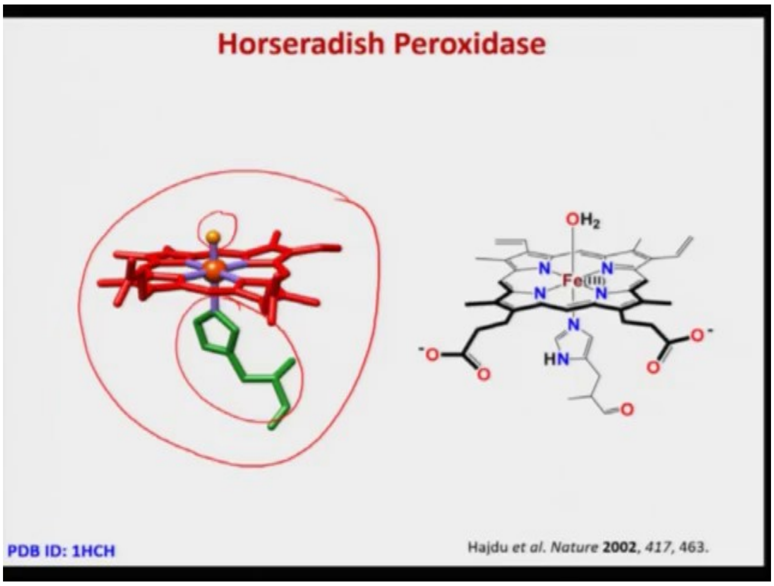

Horseradish Peroxidase

PDB ID: 1HCH

Hajdu et al. Nature **2002**, *417*, 463.

And if I remove this protein, you see that this is what is the heme centers and this is iron which are sitting on the plane of the porphyrin ring, and this is histidine moiety and the 6th position as I have said that it is weakly coordinated to a water molecule.

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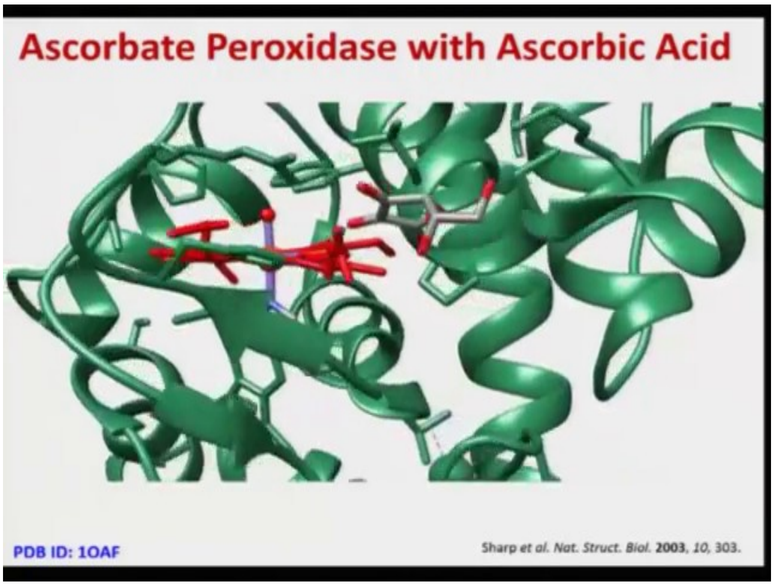

Ascorbate Peroxidase with Ascorbic Acid

PDB ID: 1OAF

Sharp *et al.* *Nat. Struct. Biol.* **2003**, *10*, 303.

And this is another x structure of ascorbate peroxidase, with ascorbic acid as a substrate. You can see yourself this heme center is sitting at the center and you also can see the ascorbic acid is also placed within this protein chain.

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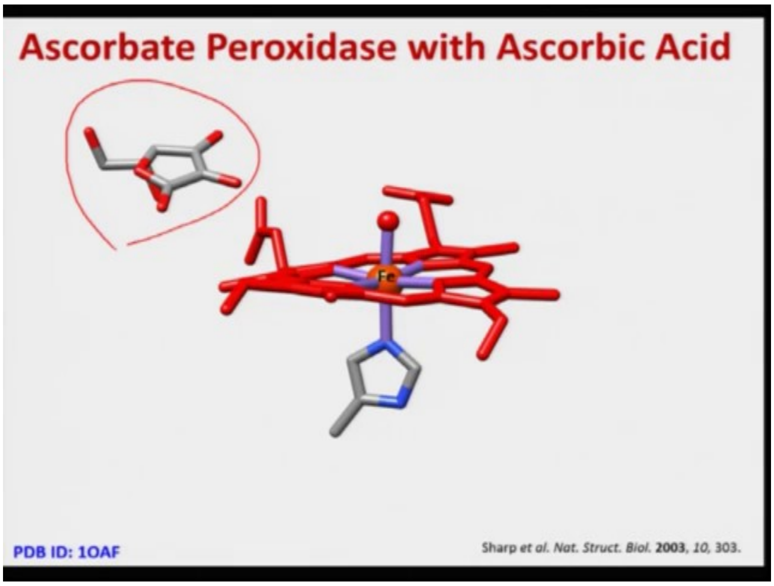

Ascorbate Peroxidase with Ascorbic Acid

PDB ID: 1OAF

Sharp *et al.* *Nat. Struct. Biol.* **2003**, *10*, 303.

And if I remove this protein, what you can see that this is the ascorbic acid if this ascorbic acid is used as a substrate.

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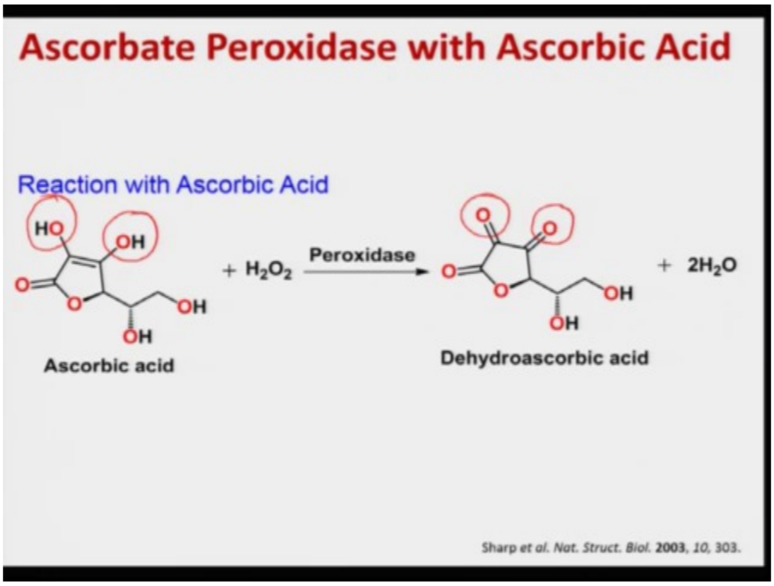

Ascorbate Peroxidase with Ascorbic Acid

Reaction with Ascorbic Acid

Sharp *et al. Nat. Struct. Biol.* **2003**, *10*, 303.

And this is the reaction with ascorbic acid, you see that this is the ascorbic acid and in presence of peroxidase enzyme. And with the help of hydrogen peroxides this ascorbic acid actually oxidized to dehydro ascorbic acid, as one can see that this 2 OH group is getting oxidized to the CO group. And this is the ascorbate peroxidase which is responsible for this transformation of ascorbic acid to dehydro ascorbic acid.

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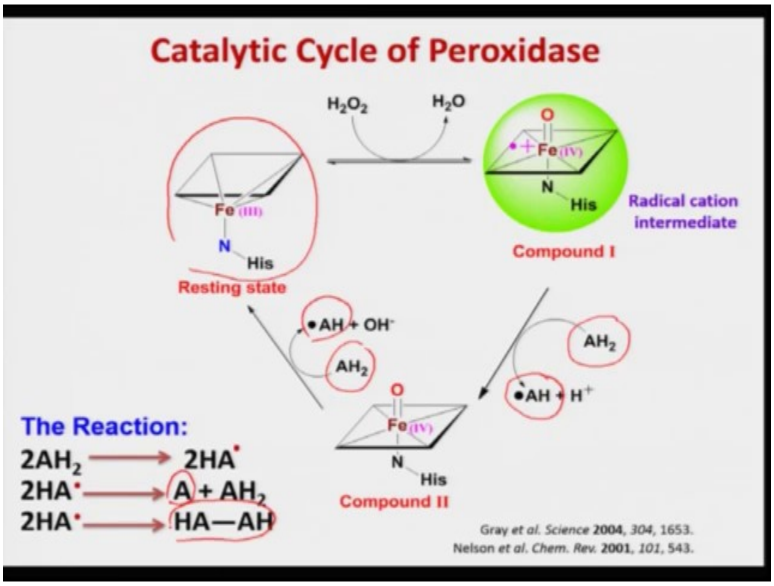

Catalytic Cycle of Peroxidase

The Reaction:

$2AH_2 \longrightarrow 2HA^\bullet$
$2HA^\bullet \longrightarrow A + AH_2$
$2HA^\bullet \longrightarrow HA—AH$

Gray *et al. Science* **2004**, *304*, 1653.
Nelson *et al. Chem. Rev.* **2001**, *101*, 543.

Now, let us look at the catalytic cycle into more details. As one can see that this is the resting state of this enzyme, Fe(III) heme centers which oxidized by hydrogen peroxides converts to F e(IV) oxo cation radical. This is two electron oxidation where Fe(III) is oxidized to Fe(IV) and this porphyrin ring is also oxidized by one electron to convert into a pi around radical. So, this is 2 electron oxidized species called compound I and this Fe(IV) oxo cation radical is highly reactive intermediate; and also very similar as to the intermediate observed in case of catalase enzyme also. Now, this compound I highly reactive oxidized speciestransfer one electron to the AH_2substrate and it forms $AH^{\cdot}$ radical and converts to Fe(IV) oxo species which is also highly reactive it is known as compound II. Now, this reactive Fe(IV) oxo takes another molecule of substrate AH_2 and converts to this AH radical, another oxidized product, and goes back to this Fe(III) heme which is present at the resting state.

And this molecule again oxidized by hydrogen peroxides to form compound I and compound I convert to compound II, and compound II goes back to the resting state. And during this catalytic cycle as one can see two molecules of radical is being formed; and this radical actually can convert to the oxidized species A or A-A coupling can be there because these two radical can form a C-C bond to one another and form a dimeric molecule like this.

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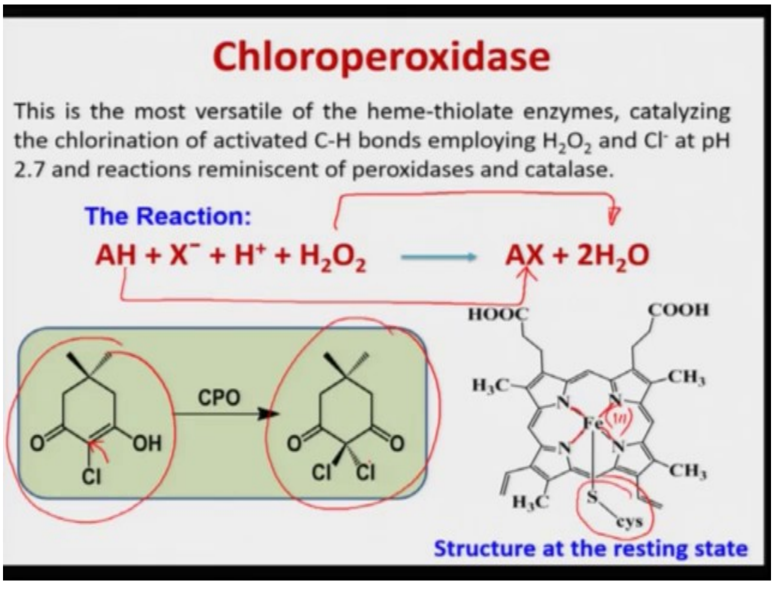

Chloroperoxidase

This is the most versatile of the heme-thiolate enzymes, catalyzing the chlorination of activated C-H bonds employing H_2O_2 and Cl^- at pH 2.7 and reactions reminiscent of peroxidases and catalase.

The Reaction:

$$AH + X^- + H^+ + H_2O_2 \longrightarrow AX + 2H_2O$$

Structure at the resting state

And so now, I will be talking about another very important enzyme called chloro peroxidase. This is the most versatile of the heme thiolate enzymes catalyzing the chlorination of the activated C-H bonds employing hydrogen peroxides and Cl^- at pH around 2.7, and the reaction is similar to peroxidase and catalase as we have just discussed in our previous slides. So, overall reaction is shown here that AH+ X^-, X is the chloride. So, AH+ X^-+ H^+ and with the help of hydrogen peroxides what is happening. So, AH converts to AX and hydrogen peroxides converts to water.

So, while the substrate getting chlorinated is something very useful product in biology. hydrogen peroxide, which is extremely poisonous it converts to water, very useful product for our environment. This is what is the structure of chloro peroxidase at the resting state, as one can see here also a iron metal is sitting at the center of this porphyrin ring and there are 4 Fe-N bond taking place and 5th position is cysteine sulfur, you can see iron is iron is in Fe(III) oxidation state. So, this is the structure of chloro peroxidase at the resting state.

And one can see that this chloro peroxidase undergo lots of transformations in biology. Say, once such example is shown over here as you can see this molecul,e this mono chloro dimedone converts to 2,2-dichloro dimedone. You see that this chlorination is taking place at the two position and this is highly stereo specific.

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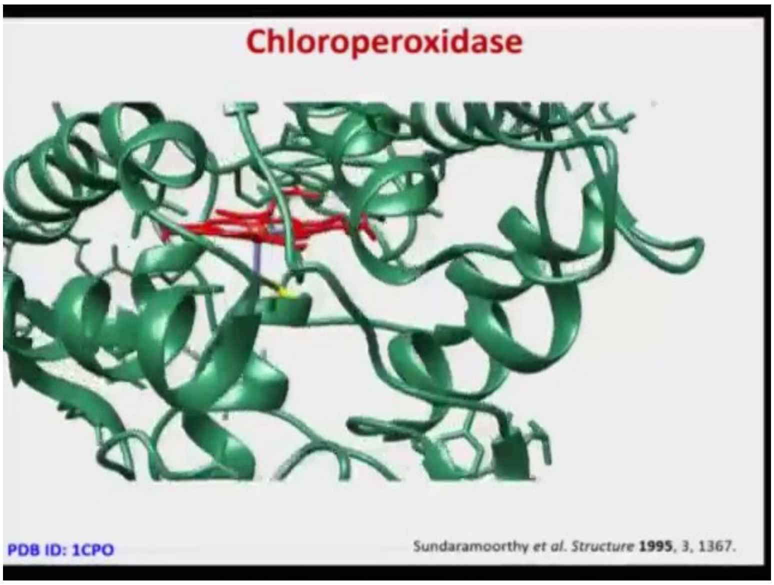

Chloroperoxidase

PDB ID: 1CPO

Sundaramoorthy *et al.* *Structure* **1995**, 3, 1367.

X-ray structure of chloro peroxidase is shown over here, as you can see once again that this molecule, thehat heme center is sitting at the center of huge protein chains. This protein chains indeed responsible for its specificity and why you know it is so stereo specific.

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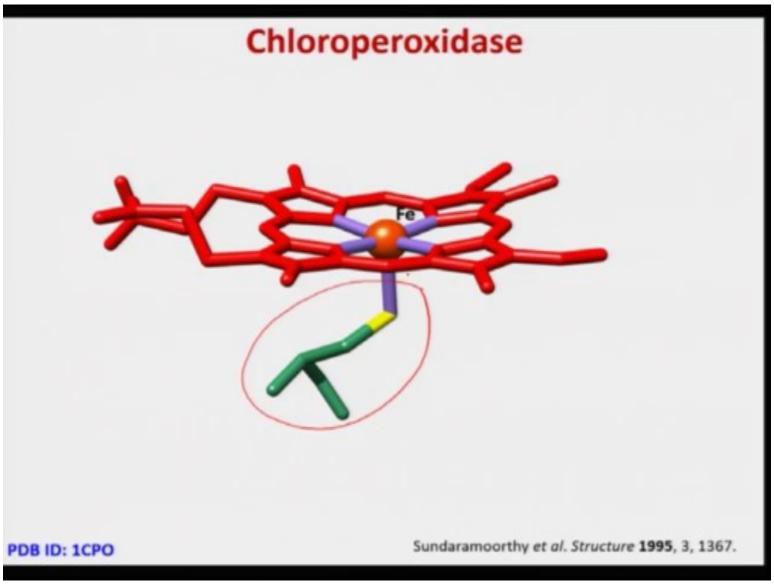

Chloroperoxidase

PDB ID: 1CPO

Sundaramoorthy *et al. Structure* **1995**, 3, 1367.

Now, once I remove this protein chain you can see these heme centers, this is the iron and cysteine sulfur is this and this is the active site structure of the enzyme.

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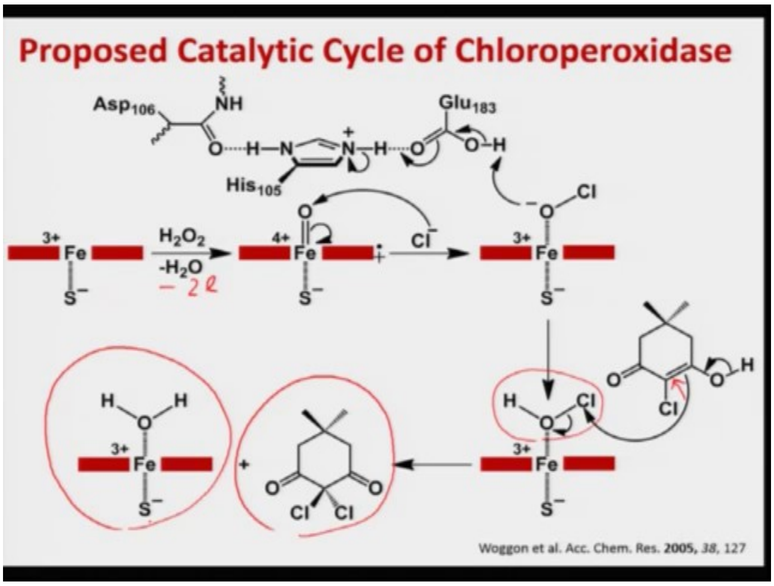

Proposed Catalytic Cycle of Chloroperoxidase

Woggon et al. Acc. Chem. Res. **2005**, *38*, 127

Now, let us look at the catalytic cycle of chloro peroxidase. Now, as we have observed in case of peroxidase, their mechanism is very very similar. So, what is happening, this the resting state of the enzyme is shown over here, which is getting oxidized to Fe(IV) oxo cation radical by hydrogen peroxides, it is 2 electron oxidized species and as you all know that this intermediate is highly reactive. It takes a chloride ion and it forms that O-C l bond and where Fe(IV)converts toFe(III).

Now, this O-Cl abstract 1 proton and then it converts to OH-Cl and Fe(III) remains asFe(III). And this Cl actually chlorinate at this particular carbon and converts to 2,2-dichloro dimedone and goes back to the original species which are present in the resting state. So, this is the way this enzyme chlorinate stereo specifically the substrate.

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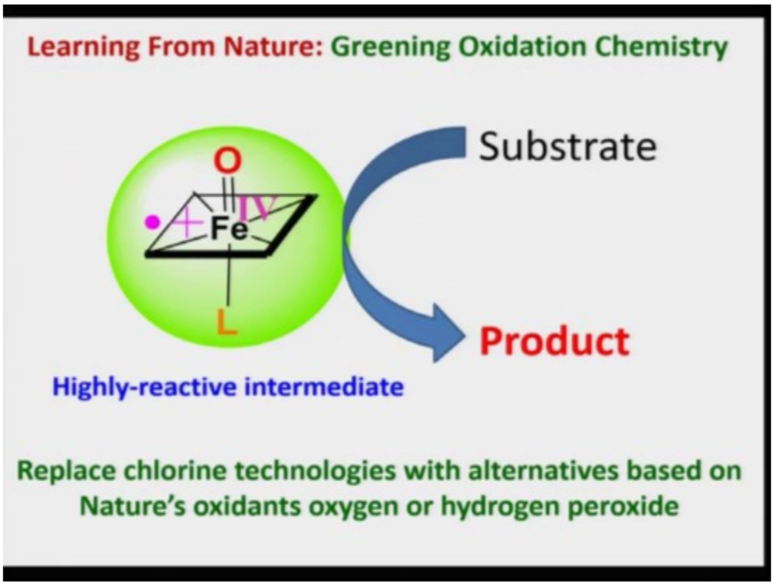

Learning From Nature: Greening Oxidation Chemistry

Substrate

Product

Highly-reactive intermediate

Replace chlorine technologies with alternatives based on Nature's oxidants oxygen or hydrogen peroxide

Now, we can learn lot of things from nature. So, as you have seen that in case of catalase, peroxidase and chloro peroxidase, the reactive intermediate like compound I is this Fe(IV) oxo cation radical, only difference was this axial ligand L, ok. And this is being formed using hydrogen peroxides; one can utilize this highly reactive intermediate and substrate can convert to a variety of products. Like, sometimes it is an oxo transfer reaction can take place, sometimes it is an electron transfer reaction can take place, sometimes if it is a like a chloro peroxidase ,it can chlorinate at a particular position in a stereo specific manner.

Now, we can make use of this process, we also can learn from such catalytic activation of hydrogen peroxide for a large variety of transformations that the enzymes have been doing. Forever the process has immense potential for various practical utilizations in the laboratory and industry, such as clean up applications for eliminating toxic and polluting substances and undesirable organisms.

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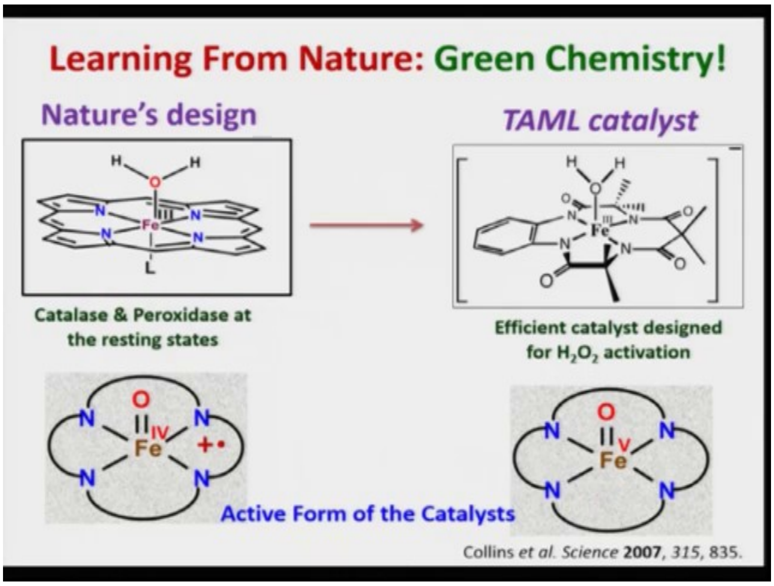

Learning From Nature: Green Chemistry!

Nature's design

TAML catalyst

Catalase & Peroxidase at the resting states

Efficient catalyst designed for H_2O_2 activation

Active Form of the Catalysts

Collins *et al.* *Science* **2007**, *315*, 835.

Now, what you will learn from nature? This is the nature’s design in catalase and peroxidase at the resting state. Now, the search for useful peroxidase, catalyst has involved numerous group for many decades, and important catalyzed processes have been developed in such field. The central mission of such green chemistry is to invent new processes that do not pollute our environment, improve the quality of our life and promote sustainability.

One such commercially successful synthetic catalyst TAML is shown over here whose design is fully inspired by catalase, peroxidase enzymes present in our mother nature. This can replace chlorine technology with alternatives based on natures’oxidant oxygen or hydrogen peroxides. Indeed, their active forms are also very similar. In enzyme it is Fe(IV)oxo pi cation radical while in TAML catalyst it is Fe(V)oxo species. As one can see the design is fully inspired by catalase and peroxidase enzymes present in our mother nature.

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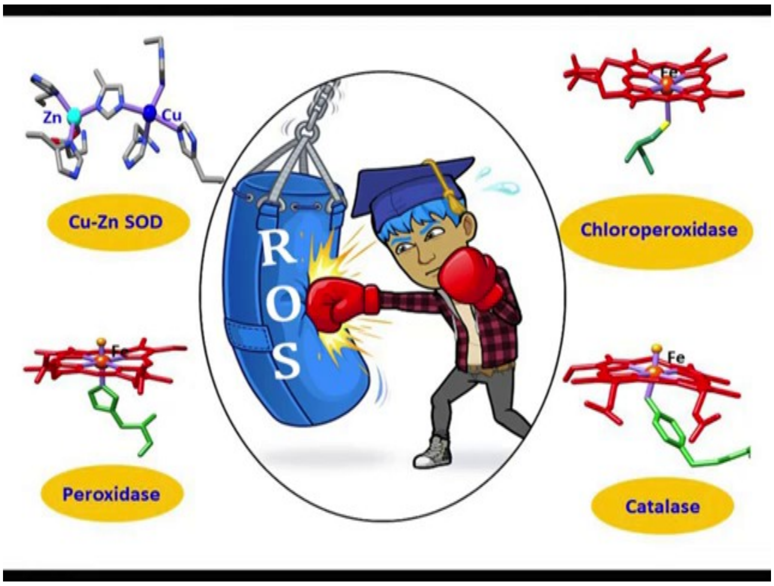

In conclusion, our mother nature has designed superoxide dismutase, catalase and peroxidase which not only destroy those reactive oxygen species but, also converts them to something extremely useful for us. I have showed the enzymatic structure of catalase and peroxidase today and discuss about the mechanism of their actions in great details. I have also highlighted here, how the beautiful design principle adopted by nature control all these processes in our life.

In my next lecture, I will talk 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; indeed dioxygen gets activated first and then inserted into the substrate which we would be talking in great details in my next lecture.

Thank you.