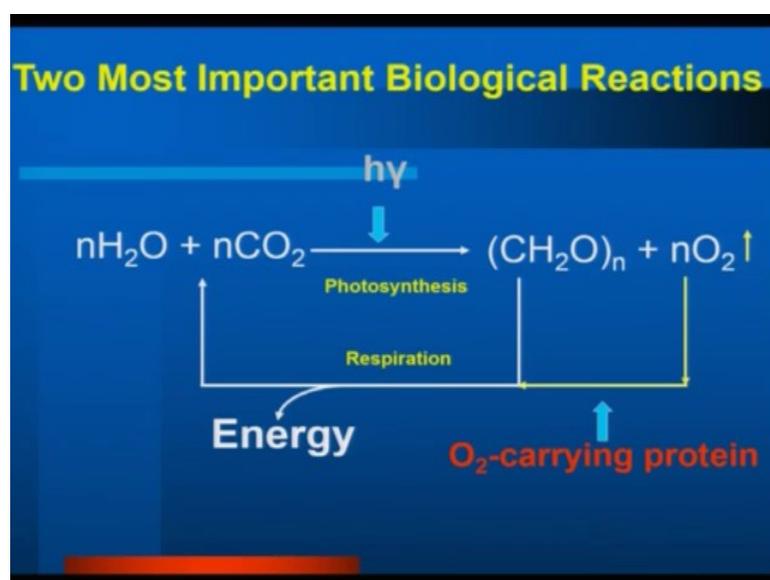


Bioinorganic Chemistry
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Lecture -12
Life with Oxygen: Superoxide dismutase activity

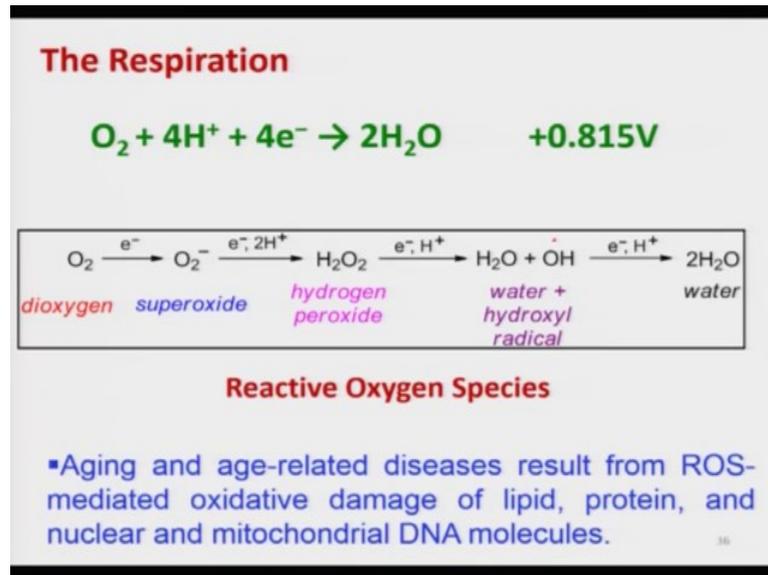
Hi everybody and welcome back to this short course of Bioinorganic Chemistry. We have been discussing about our life with dioxygen ok.

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Two most important biological reactions, as you all know; one is photosynthesis, another one is respiration. You know, in respiration you need oxygen which converts to energy and oxygen carrying proteins are responsible to carry this dioxygen and as one can see that this oxygen $+4\text{H}^+ + 4\text{electron}$ gives rise to 2 molecule of water at a potential of plus 0.815 volt.

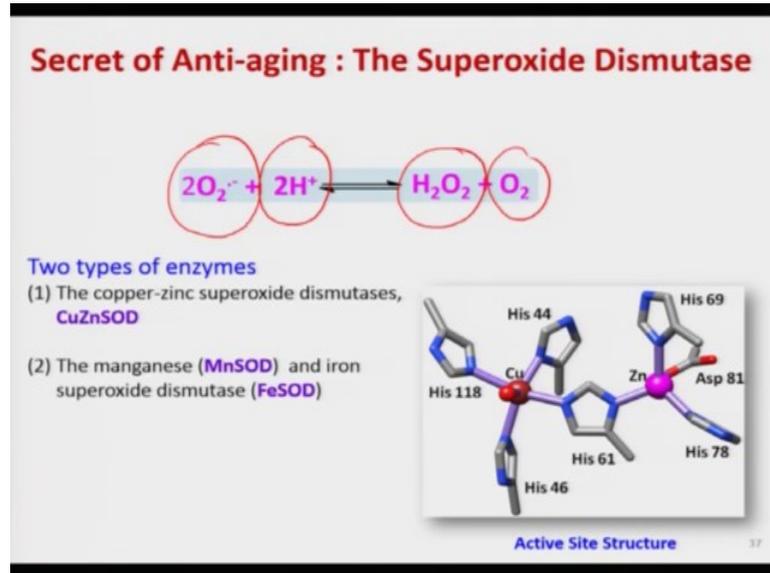
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So, oxygen converts to water which usually gives lots of energy and if you give 4 proton, 4 electron all at a time, then oxygen converted to water. As I have said earlier, the nature also do mistake. If this dioxygen reduced partially even, then it generates lots of intermediate. Those intermediates are harmful, for example, after 1 electron reduction O_2 converts to O_2^- super oxides, super oxide again, after 1 electron reduction converts to peroxide O_2^{2-} and O_2^{2-} converts to H_2O and OH^\bullet radical after 1 electron reduction and OH^\bullet radical again, after reduction converts to water.

So, unless you give 4 proton, 4 electron all at a time, it invariably produce, even if in small amount, some of these intermediate and these intermediates are highly poisonous in the biological system. They are called biological poison, because they produce uncontrolled reaction. You know that radical reactions are very-very dangerous, because it is difficult to stop and these radicals are the reduced species collectively called reactive oxygen species and aging and age related diseases results from this ROS mediated oxidative damage of lipid proteins and nuclear and mitochondrial DNA molecule. So, since this reactive species are produced, even if a small concentration so, nature has his solution for it.

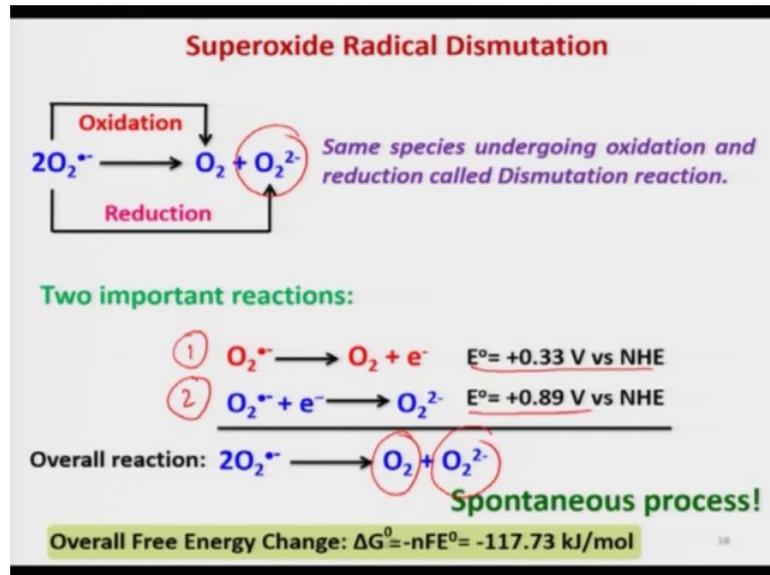
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Nature made a solution to get rid of this problem and this is what the nature designed super oxide dismutase enzyme to destroy the super oxides, H for this reaction, as you can see super oxides + 2 H⁺ produces peroxides and dioxygen. So, super oxide basically, converts to peroxides and dioxygen and the enzyme, which is responsible for this decomposition is super oxide dismutase.

There are two types of super oxide dismutase; one is the hetero metal copper zinc super oxide dismutase and another type is either manganese super oxide dismutase and iron super oxide dismutase also known in biology. You can see the active site structure of copper zinc super oxide dismutase. You see that there are copper unit, which is ligated with 4 histidine molecule and 1 water is coordinated at the fifth position and zinc is in tetrahedral geometry and 3 histidine and 1 aspartate is ligated to that, now, we will see in details in the coming slides.

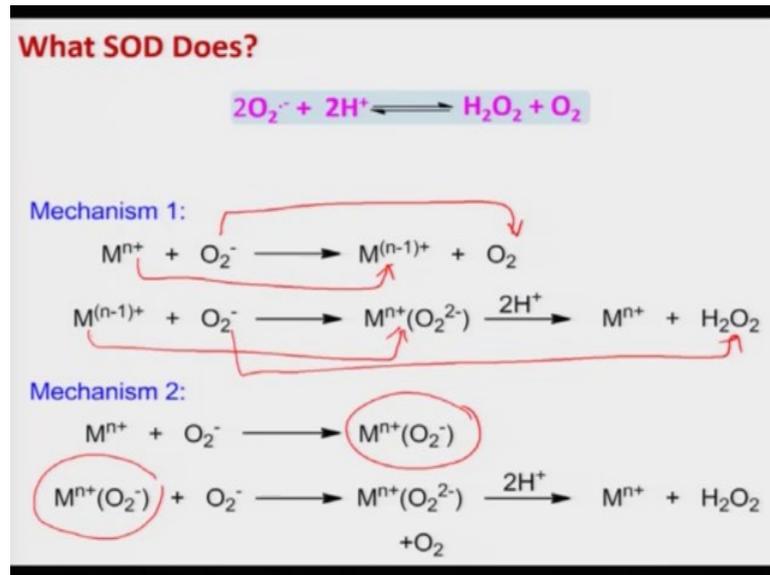
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Now, if we look at the super oxides, very-very interesting. Super oxides actually, converts to oxygen and peroxides, $\text{O}_2^{\bullet-}$ gives rise to O_2 plus O_2^{2-} ; that means, $\text{O}_2^{\bullet-}$ converts to O_2 , this is oxidation process, $\text{O}_2^{\bullet-}$ converted to O_2^{2-} , is a reduction process. The same species undergo oxidation and reduction at the same time, we call it dismutation reaction or disproportionation reaction and if we look at the thermo dynamics of this, the two redox potential; one is $\text{O}_2^{\bullet-}$ converting O_2 plus electron. It produce E° of +0.33 volt versus normal hydrogen electrode, this is reaction 1.

Second reaction, $\text{O}_2^{\bullet-}$ is reduced to O_2^{2-} peroxides where E° is plus 0.89 volt versus N H E. So, overall reaction is $2 \text{O}_2^{\bullet-}$ gives rise to $\text{O}_2 + \text{O}_2^{2-}$ and if you calculate the overall free energy change ΔG , it is -117.73 kJ/mol. Large negative free energy change; that means, the reaction is spontaneous process; that means, this super oxide spontaneously undergo decomposition giving rise to dioxygen and peroxide, spontaneously. This being so that why did nature design an enzyme called super oxide dismutase to do this decomposition process.

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So, this is although it is a spontaneous reaction as I has shown you the delta G is large negative and the spontaneous. So, this will always happen, but nature needs the super oxide to be decomposed as fast as possible, because super oxides are extremely poisonous in the biological system. So, it need to be decomposed almost immediately, if it can and that is the reason why super oxide dismutase enzyme has been designed. Now, how an enzyme actually accelerates this decomposition process?

Now, there are two types of mechanism; if the metal is an redox active metal ion, then what would happen? the mechanism one is shown with the redox actives metal ion, where the metal ion getting oxidized and reduced and thereby, oxidizing and reducing the super oxide also. For example, that in the first reaction the metal get reduced whereas, super oxide get oxidized to dioxygen, whereas, in the second reaction, the reduced metal further get oxidized and super oxide get reduced to peroxides.

So, same metal getting reduced and oxidized also forcing the super oxide to oxidize and reduce ok; in mechanism 2 the metal ion is non-redox active. So, if that is so, what would happen in that case? As you can see that M^{n+} metal oxygen state does not change during these reactions. So, what would happen? Metal and super oxides forms a complex or adopt this metal super oxide adduct, reacts with another super oxides in presence of proton, it converts to peroxides and dioxygen.

So, here the beauty over here is that metal ion did not change. It is oxidation states rather super oxides converts to oxygen and peroxides; that means, oxidation and reduction. In mechanism 1, the metal also getting oxidized and reduced, because metal ion is a redox active metal ion; it has been seen mechanism one is actually in operational. this being so, what kind of metal is suitable for this purpose.

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The Choice of Metal Ions in Superoxide Dismutase

The redox potential for

- O_2/O_2^- couple is -0.33 V
- O_2^-/H_2O_2 couple is +0.89 V

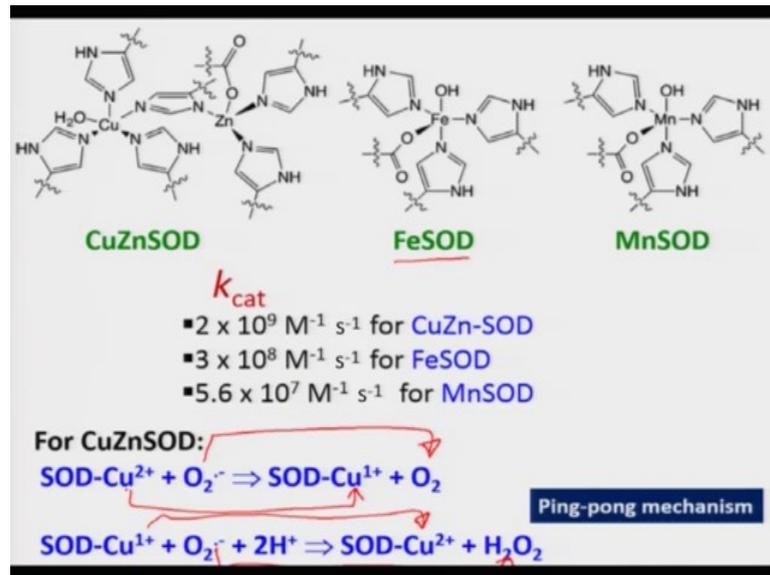
Thus any metal atom capable of one electron redox chemistry between M^{n+} and $M^{(n+1)+}$ states that has a potential between the limits $-0.3 \leq E^\circ \leq +0.9$ V will be useful.

	E° (V)
$Cu^{2+} + e \rightleftharpoons Cu^+$	+0.153
$Fe^{3+} + e \rightleftharpoons Fe^{2+}$	+0.771

Now, let us see that that why did nature choose some metal ion. Now, what is exactly happening in super oxide dismutase, that it is that super oxides, this is there are two couple one is O_2/O_2^- couple, it's redox potential is -0.33 volt and another couple is O_2^- to O_2^{2-} at the redox potential is +0.89 volt. Thus, any metal, which are capable of 1 electron redox chemistry between M^{n+} and M^{n+1} state, that has a potential between the limits of -0.3 to + 0.9 volt ok.

So, any way redox active metal ion, which have a potential window of -0.3 to +0.9 volt is in principle capable of doing this dismutation reaction and there is no surprise that why nature use copper, iron metal ion. As you can see, there redox potential indeed come in between these two extreme limit of -0.3 to +0.9 volt.

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As, I have already mentioned that there are basically copper zinc SOD is present and there are some evidence that iron SOD and manganese SOD also known in the literature. You can see their structure, in copper zinc SOD as I have already said that there are 2 metals, 1 is copper center and zinc center and a water molecule, which is ligated at the fifth position which present with copper, in case of iron also there are three histidine, one aspartate is ligated to the fourth position and a OH group is look at the fifth position which is in iron SOD, in manganese SOD also very similar to iron SOD. If you look at the catalytic conversion k_{cat} , what you find?

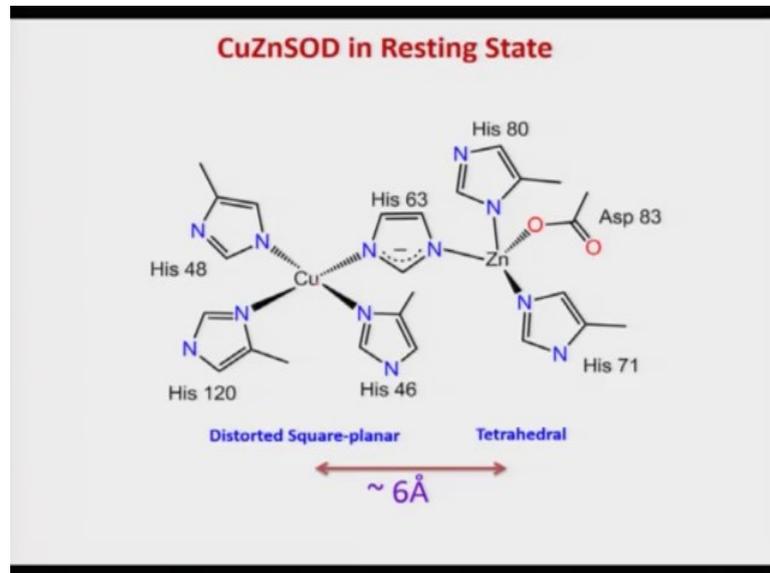
You find that for copper zinc SOD, rate of catalysis is $2 \times 10^9 \text{ M}^{-1} \text{ S}^{-1}$, for iron SOD the rate is 3×10^8 , for manganese SOD 5.6×10^7 . You see that the conversion rate is very high, very high 10^9 whereas, uncatalyzed reaction is very low and nature has used this enzyme to speed up this rate of conversion. Copper zinc SOD what is happening?

The proposed mechanism is as follows that copper goes to the mechanism 1, as I have already told you that copper $2+$ undergo a reduction to copper $+1$ while, O_2^- super oxide undergo an oxidation to O_2 . In the next reaction, this copper(I) again reacts with another molecule of super oxides and converts to is an oxidation. So, copper (I) is getting oxidized to copper(II) plus while, O_2^- is reduced to O_2^{2-} .

So, super oxide converts to peroxides and copper getting oxidized to copper $2+$. If you see what is happening that copper $2+$ converts to copper (I) and Cu(I) converts back to

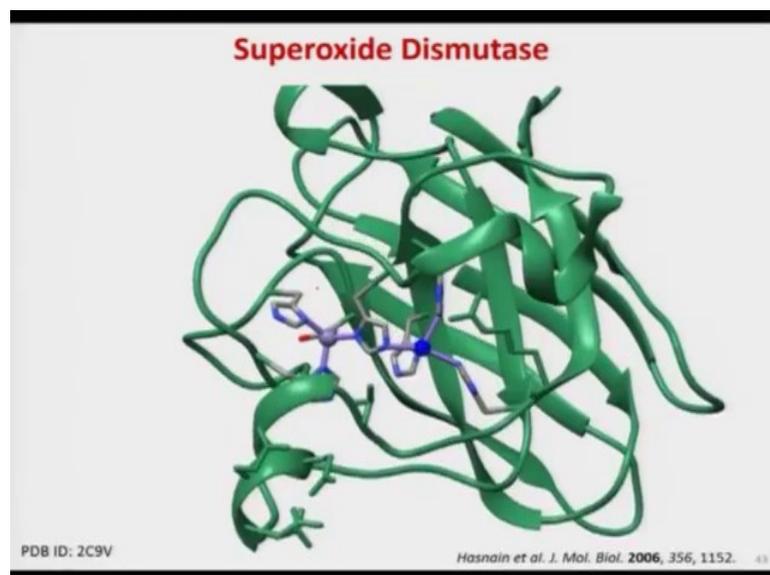
Cu(II) This kind of shuttling back and forth of copper between two common oxidation states is popularly known as ping pong mechanism and this has been believed to be happening for copper zinc super oxide dismutase reaction.

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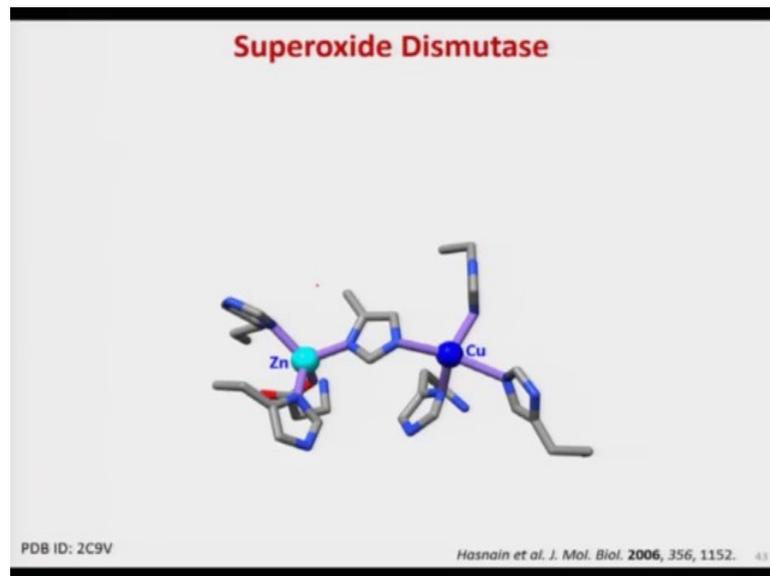
So, if you look at this copper zinc super oxide dismutase in the resting state and you see that this copper is in distorted square planar structure in the resting state whereas, zinc is in tetrahedral structure and the difference between copper and zinc is or more than 6 angstrom in the resting state.

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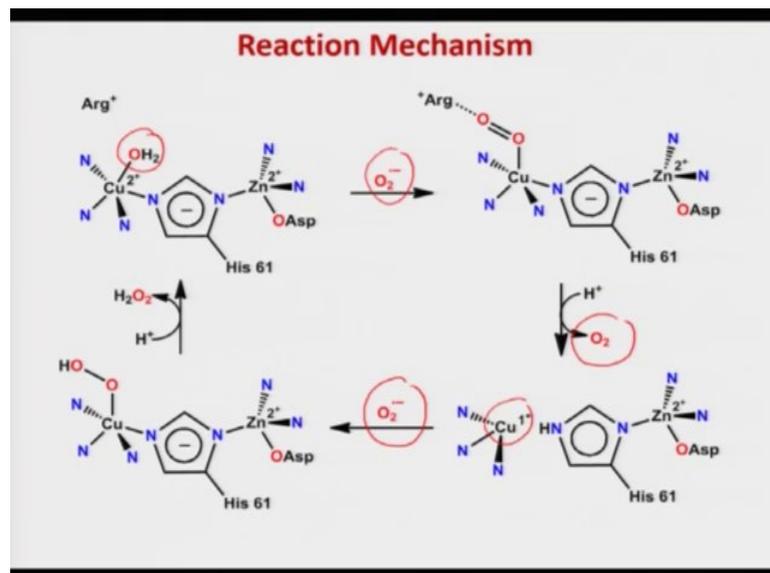


So, this is the X-ray structure of super oxide dismutase, as you can see there is a huge protein, which are wrapping this copper zinc SOD molecule and you see that if I remove this protein, this copper zinc SOD, which is actually the active site structure, I have already shown you, it can be seen whereas, this copper and zinc is bridged to a histidine group and in order to see that what kind of mechanism is happening in copper zinc SOD case and you can see that copper ion, which is ligated to a weak axial ligand.

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Like; water molecule is replaced in first step by a super oxide O_2^- and the super oxide is getting stabilized with a amino acid arginine + and this super oxide, then undergo oxidation and Cu^{2+} undergo reduction simultaneously. So, super oxide converts to oxygen and Cu^{2+} converge to Cu^{1+} . Now, once it forms a Cu^{1+} , there is no bond between copper and this histidine nitrogen, which was bridging between copper and zinc.

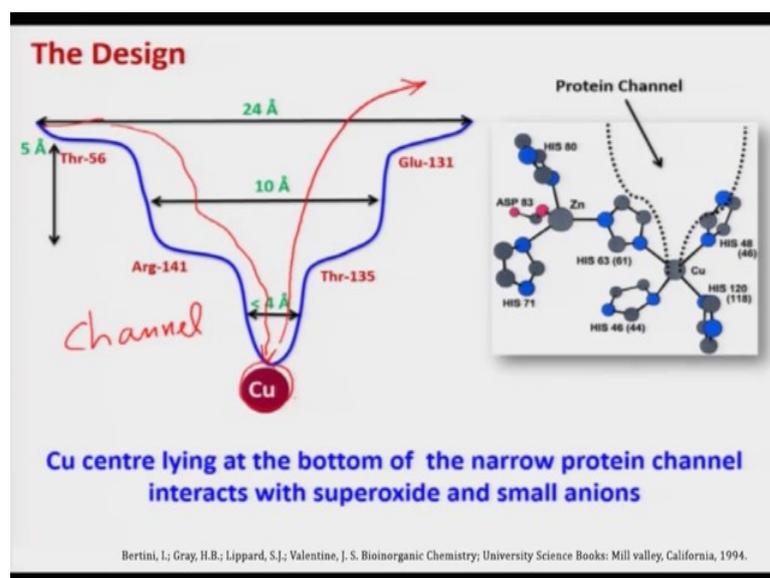
Now, this once again binds to another super oxide molecule, this Cu^{1+} is oxidized to Cu^{2+} while, super oxide is reduced to peroxides, O_2^{2-} and as you can see that this peroxides is very weakly coordinated and a water molecule immediately, replace this peroxides and gives rise to hydrogen peroxides and water comes back and this catalytic cycle starts its action once again. So, this is a proposed mechanism how a super oxide getting oxidized and reduced while, the metal copper is getting reduced and oxidized and they are helping each other.

They are coupled, both the redox process are coupled in such a fashion that rate of these reactions becomes very-very high so, that disproportionation of super oxide by redox active metal ions. It generally, thought to occur in 2 1 electron steps like as I have shown the ping pong mechanism. Cu^{2+} getting reduced to Cu^{1+} while super oxide oxidized to oxygen; again, Cu^{1+} is getting oxidized to Cu^{2+} and super oxide getting reduced to peroxides.

So, if you look at this rate constant value uncatalyzed rate constant is something like $2.5 \times 10^5 \text{ M}^{-1}\text{S}^{-1}$ at pH 7, while if we use free Cu^{2+} ion, the rate is $8 \times 10^9 \text{ M}^{-1}\text{S}^{-1}$ and. However, the free ion has never been used, because as I have said earlier, the free ion is like a poisonous if it is not good. So, nature never use any free metal ion, rather complexed ion they use it. Now, the problem is that once the copper is complexed, then rate of this catalytic reactions getting reduced. Now question is that how in copper zinc SOD the rate is so high.

So, in order to achieve the very high catalytic efficiency, the active site must provide four things; one a high rate of access of super oxide to the site and rapid release of products here it is dioxygen. Number 2 is an open or exchangeable coordination position at the metal ion, if an inner sphere electron transfer mechanism is involved. Third is rapid protection of the peroxide anion and fourth is appropriate thermodynamic driving force.

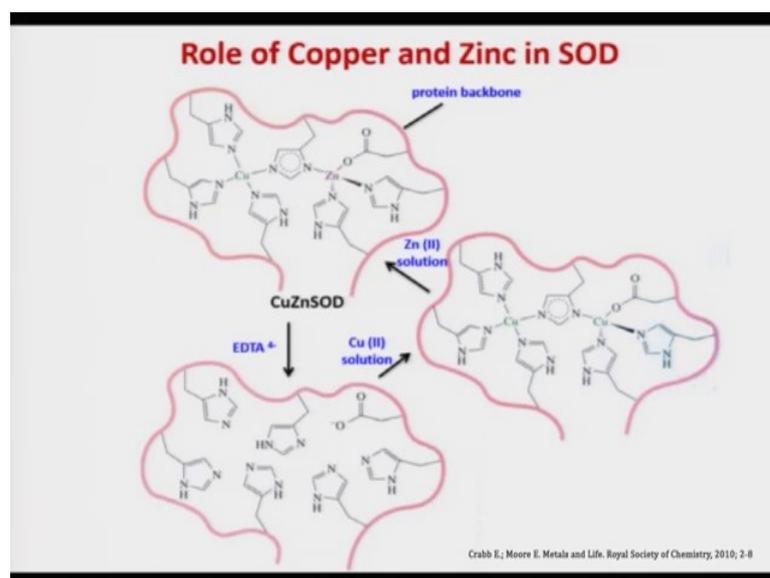
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And this is what is shown in the design. If you look at the design, very nice design, it is a channel like structure and copper site is clearly the site of primary interactions of super oxides, from the X-ray structure it has been shown that copper ion lies at the bottom of the narrow channel that is large enough to admit only water or some small anions or similar small ligands. In the lining of the channel there are positively charged arginine residue, which are actually gives a direction to the super oxide to reach to copper center and near the mouth of the channel, at the surface of the protein, which is two positively charged lies in each residue is present, which are believed to play a key role in attracting the anions and grinding them into the channels.

So, that it can reach to copper ion and then it can be released again after formation of either oxygen or peroxides. So, in this way this dismutation reaction become so fast. So, copper center lying at the bottom of this narrow protein channels and interacts with super oxides and all small anions and the design is such that only small anions can passes through and reach to copper, no big anions or no other molecules can reach copper centers ok.

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Now, we try to understand, what is the role of copper and zinc in copper zinc SOD? Whether copper center is important or zinc center is important or both the center is equally important. Now, in order to do that so, this is what is the copper zinc SOD, you can see the protein backbone is shown over here. Now, if one use is strong chelating ligand like EDTA what would happen? It will immediately remove copper and zinc and you get a native enzyme without any metal and which is completely inactive molecule.

Now, once you put copper to solution into this demetallated species what would happen? This two copper ion is sitting and ligated to the histidine molecule and it has been found that this dicopper unit also is active towards super oxide dismutase activity; however, they are not thermally very stable. Now, once you put zinc to solution, then one of this copper which is ligated to aspartate group will be replaced by the zinc and this copper zinc SOD is reproduced. The enzymatic activity is there as well as is thermally very stable.

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Role of Copper and Zinc in SOD

What does Zinc do?

- Primarily maintaining structural integrity.
- No evidence for the binding of water/anions/potential ligands
- Superoxide also cannot bind here.
- Removal of Zn doesn't significantly diminishes SOD activity.
- Zinc depleted protein denatures at lower temperature
- Zinc provides thermal stability.

What does Cu do?

- Site of superoxide binding.
- Responsible for enzymatic activity.
- Removal of the metal stop the SOD activity completely

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Now, so what does zinc ion do? Zinc is primary maintaining the structural integrity of this enzyme. Zinc cannot bind super oxides or any anions and removal of zinc does not significantly diminishes super oxide dismutase activity. Zinc actually, provides the thermal stability of the enzyme. Now, what copper center is doing? Copper center is responsible for all this activity, what we have seen that simultaneous oxidation and reduction of the metal as well as the super oxide.

So, this is the site of super oxide binding is responsible for all this enzymatic activity and removal of this copper ion completely stop the super oxide dismutase activity, which suggests that copper center is the key catalytic center for copper-zinc, super oxide dismutase. The great oxidizing power of dioxygen is utilized in respiration to produce huge amount of energy that we enjoy every day, during such respiration process O_2 is fully reduced to water which would require 4 protons and 4 electrons that need to be added in a concerted manner.

However, incomplete reduction of dioxygen produce reactive oxygen species such as super oxide, peroxide and radicals which are extremely poisonous for our body. Aging and age related diseases are result from reactive oxygen species mediated oxidative damage of lipid protein and nuclear and mitochondrial DNA molecules. I have discussed today, how our life is fully protected from such reactive oxygen species; although,

dismutation of super oxide is a thermodynamically favorable and also a spontaneous process.

However, in order to destroy the extremely harmful super oxide almost immediately, our mother nature has designed such super oxide dismutase enzyme. I have shown here, the enzymatic structure, roles of the metal ions involved, and also the mechanism of their actions in great details. Although, super oxide dismutase decomposes extremely harmful super oxide, it also produce peroxide which is another harmful product in biology. In my next lecture, I will talk about catalase and peroxidase enzymes, which not only destroy these harmful peroxides, but also utilize them to something extremely useful for us.

Thank you.