

Inorganic Chemistry of Life Principles & Properties
Prof. C. P. Rao
Department of Chemistry
Indian Institute of Technology, Bombay

Lecture - 31
Role of Iron in life - Reductases

Welcome you all to the next class on Inorganic Chemistry of Life Principles and Perspectives. Just in the previous class we have discussed a few enzymes of iron particularly those of a oxygenase type, in that we have looked at the mono oxygenase and then the dioxygenase. Mono oxygenase based on a heme also non heme, which is methane mono oxygenase and then based on the dioxygenase based on the non heme which is protocatechuate. So, all of these we have looked at. I think now it is the time to move into the topic on the iron enzymes as little different particularly those on the reductase properties.

So, to get into this reductase properties, we I would like to make is some kind of a subclass of the iron proteins or an enzymes, which are based on the binuclear iron center.

(Refer Slide Time: 01:14)

Introducing metalloproteins & metalloenzymes

Enzymes and proteins containing binuclear iron centre

Enzyme	Reaction
Hemerythrin	$\text{Hr} + \text{O}_2 \rightleftharpoons \text{Hr} \cdot (\text{O}_2)$
Acid phosphatase	$\text{RCH}_2\text{OPO}_3^{2-} + \text{H}_2\text{O} \rightarrow \text{RCH}_2\text{OH} + \text{HPO}_4^{2-}$
Methane monoxygenase ^a	$\text{CH}_4 + \text{O}_2 + \text{NAD(P)H} + \text{H}^+ \rightarrow \text{CH}_3\text{OH} + \text{NAD(P)}^+ + \text{H}_2\text{O}$
Ribonucleotide reductase ^b	$\text{NDP} + \text{R}(\text{SH})_2 \rightarrow \text{dNDP} + \text{RS}_2 + \text{H}_2\text{O}$
Rubrethrin	$\text{H}_2\text{O}_2 + 2\text{e}^- + 2\text{H}^+ \rightarrow 2\text{H}_2\text{O}$

Di-iron Stearoyl-ACP desaturase

Prof. C. P. Rao, Department of Chemistry, IIT Bombay

Let us look at this particular table first, and I will tell you something which are familiar already and something which is not familiar already. So, we can look at those ones. So, all of these given in this or by nuclear iron enzymes, number 1 we can see that the hemerythrin. Hemerythrin we have already been aware, where did we study the

hemerythrin. I am sure if you go back and look at the oxygen transport more detailed if we studied for the myoglobin and a hemoglobin.

But then we compare with another hemerythrin and hemocyanin because these are found in other kinds of organisms. So therefore, we are familiar with the hemerythrin, hemerythrin is the oxygen transport protein. Then we are also familiar recently with the methane mono oxygenase again and iron containing enzyme, which converts methane into methanol of course, plus water because in mono oxygenase you use only one oxygen for a oxidation and the second oxygen goes as a water.

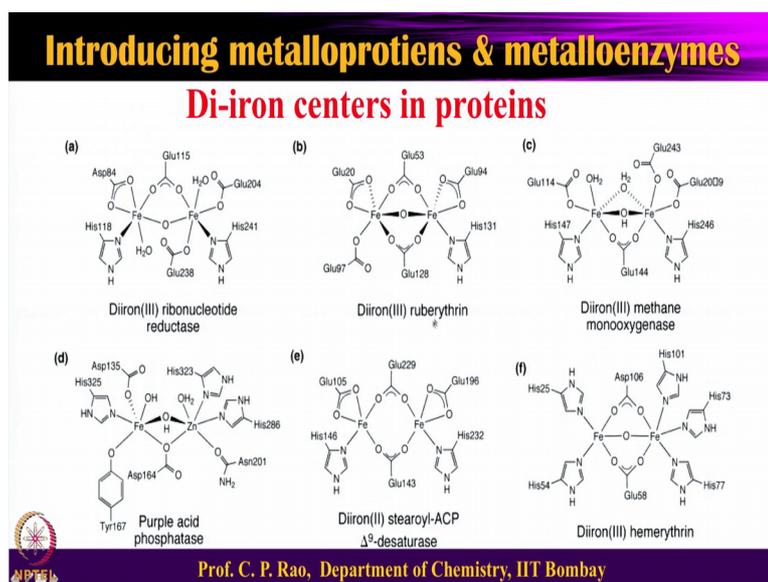
Now, let us look at something newer ones, the newer ones one of them is ribonucleotide reductase. So, it is a reductase family and it does some reduction. So, what it does? It does generate the nucleotide which are the deoxy type from the ribonucleotides. So therefore, you have a ribonucleotides to deoxyribonucleotides, because to synthesize DNA you need deoxyribonucleotides and deoxy deribonucleotides are not entry to the body therefore, ribonucleo tides have to be reduced to the deoxyribonucleotides and more details we will discuss bit later and so therefore one more enzyme in that.

And then let us look at a rubrethryin. Rubrethryin is the one which is you know catalyzes the breakdown on the hydrogen peroxide and you know hydrogen peroxide is generated from the enzyme of superoxide dismutase, one of it is water other of it one of it is oxygen other is a H_2O_2 and the H_2O_2 is further reduced further broken down to water and you can also call as a catalyze. So, rubrethryin gives a catalyst kind of an activity and this is also di-iron we will look at the details. Then having looked at the reductase and then the H_2O_2 breakage let us look at some acid phosphatase.

So, acid phosphatase is the one which basically breaks down the phosphodiester bond. So, in other words hydrolyzes this one and so, these 3 cases we are going to study a bit more detail, but in addition there are some other di-iron stearyl ACP de saturase, it is because there is a double bond the saturase desaturase. So, it creates a kind of unsaturation in the in the saturated thing and this is the kind of a conversion.

But we are not going to look at the details in this particular enzyme because these are not so much very well known. So, having said that these are the kinds of things and I talked about these are all binuclear iron enzymes, let us look at their structures and the known ones and then known unknown ones.

(Refer Slide Time: 04:42)



So, we know already methane mono oxygenase as you can see here and we also know which is the hemerythrin these are the things and the ones which are ribonucleotide reductase. Which we are going to study now ruberythrin which we are going to study now purple acid phosphatase again we are going to study now.

So, and all of these see di-iron; all the di-irons always are connected always are bridged by the carboxylic group. Most of the times I would say more or less always more or less always you take even other di-iron centers or di metallic centers, most of the times di metallic centers are all or bridged by carboxylates and the same thing is true even the di-iron centers, you can see a carboxylate with. Carboxylate comes from where comes from this side chain of the only 2 amino acids, which are those amino acids which is aspartic acid and glutamic acid.

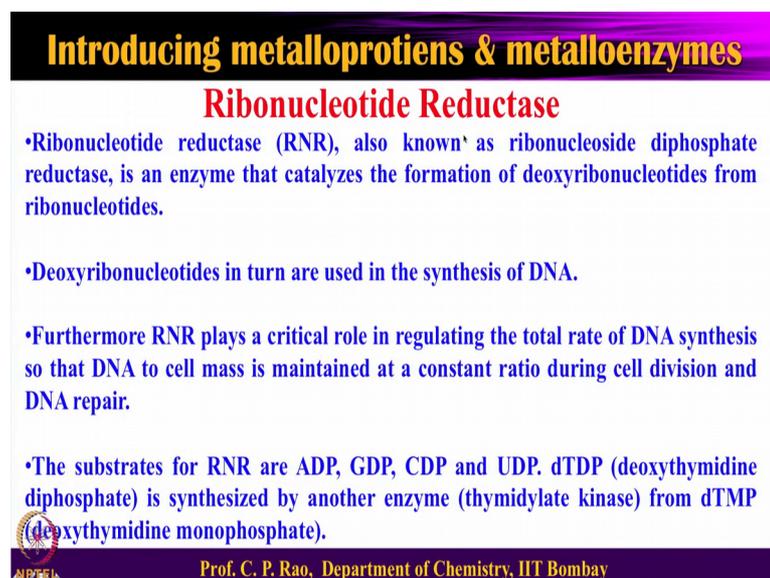
The only difference is one methylene group difference between the aspartic and glutamic ok. So therefore, but the, but there is an end the side chain end carboxylic group and which is involved. So, you can see a bridging here, you can see a bridging here carboxylic bridging carboxylic and the only case here is bridging, but it a little differently between the 2 and the bridging is there and the bridging is there. So, more or less we can say among the di-iron and many other di metallic centers and this two metal centers are connected by the carboxylate bridge and the carboxylate being coming from

the either from the aspartic often the glutamic and majority is in the case of the glutamic case.

Now, see the other differences; the other differences is here you have one histidine on either side again one histidine on either side and then in histidine and tyrosine and two histidines etcetera and then. So, there is a little bit of coordination differences on all these things. What you cannot see from the slide is that the protein structural variation is also expected. So, in each of this case protein is different. So, protein structure is different that is not reflected in this slide I would tell you that you need to take as a granted in that and when we look at the some of the proteins you will see that among all these the maximum the histidines is in the hemerythrin, you have a 3 histidines and 2 histidines on the other side and you have a 6 and 5 coordinated kind of thing where the o 2 gets bound etcetera and then gets stabilized as HO 2 minus species in this.

And the other case; yes. So, this is an important then you have all other cases have got some histidines or the other, the ruberythrin are having the least number of histidines in always. So, let us try to look at some case for example, let us look at start with the some reductase, ribonucleotide reductase one in more detail ok.

(Refer Slide Time: 08:06)



Introducing metalloproteins & metalloenzymes

Ribonucleotide Reductase

- Ribonucleotide reductase (RNR), also known as ribonucleoside diphosphate reductase, is an enzyme that catalyzes the formation of deoxyribonucleotides from ribonucleotides.
- Deoxyribonucleotides in turn are used in the synthesis of DNA.
- Furthermore RNR plays a critical role in regulating the total rate of DNA synthesis so that DNA to cell mass is maintained at a constant ratio during cell division and DNA repair.
- The substrates for RNR are ADP, GDP, CDP and UDP. dTDP (deoxythymidine diphosphate) is synthesized by another enzyme (thymidylate kinase) from dTMP (deoxythymidine monophosphate).

Prof. C. P. Rao, Department of Chemistry, IIT Bombay

Ribonucleotide reductase that is I told you and let me repeat once again is involved in the synthesis of deoxyribonucleotides and the body entry is ribonucleoside and you need a deoxyribonucleoside within the cell has to synthesize the DNA, a cell requires the de

deoxyribonucleotides. And you know cell progress and the cell division, when the cell division takes place you know the nucleus material DNA material also gets divided So therefore, you need to synthesize the DNA.

So, this is synthesize the DNA you need the deoxyribonucleotides and the entry to the body is a ribonucleotides. So, conversion from the ribonucleotide the deoxyribonucleotide is in that proportion, one of the first and foremost is that ribose to deoxyribose and that is converted by this enzyme. So, this enzyme is very important for the cell division cell growth etcetera; so the substrates for such an enzyme or the diphosphates, adenosine diphosphate, guanine diphosphate, cytidine diphosphate, uridine diphosphate and some triphosphates as well.

(Refer Slide Time: 09:36)

Introducing metalloproteins & metalloenzymes

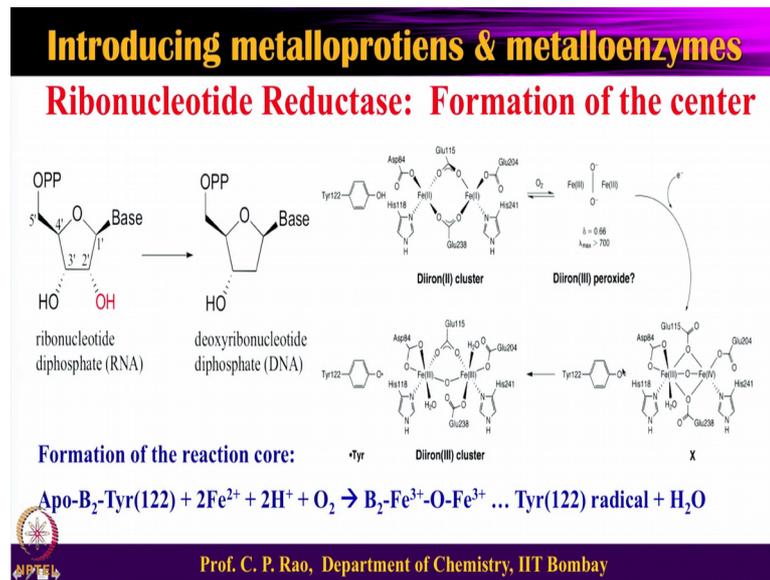
Ribonucleotide Reductase

- The iron-dependent enzyme, ribonucleotide reductase (RNR), is essential for DNA synthesis. Class I RNR enzymes are constructed from large RNR1 and small RNR2 subunits which associate to form an active heterodimeric tetramer.

 Prof. C. P. Rao, Department of Chemistry, IIT Bombay

So, therefore, from the ribosyl to the deoxy ribosyl that we need to look at that. So, in this case if we look at the enzyme is a bit like complicated, it is a dimeric structure and the dimeric structure and each of the it has got a unit the RNR 1, ribonucleotide reductase is in a short form we refer it as a RNR do not get confused with anything else and in this case you have a situation with 1 and RNR 2; two sub unit and this 2 heterodimer heterodimeric subunit and this is twice to that. So, it is a heterodimeric tetramer you have. So, that is where the thing is.

(Refer Slide Time: 10:18)



So, if you look at this further interior, now let us look at for a while reaction. So, this is the ribose you can see that and this is the dioxribose. So, what is the difference this part is same, this part is same only this part is. So, 2 prime which has a hydroxyl becomes deoxy. So, de hydroxy you can say either way to and then this is replaced by this OH is replaced by a hydrogen. Therefore, this is a two two prime deoxyribonucleotide ribonucleotide in this because the base is there and the phosphate is there it is a nucleotide ok.

So, this is the reaction that happens and then let us look at what is the iron center, you take the ribonucleotide reductase as I said there are 2 parts of that either one is called the RNR 1 part and the other one is the RNR 2 part. So therefore, and in this you have a the enzyme, where the reaction occurs how the reaction occurs that we will see in a while and one of them is the iron center di-iron center. So, you take a protein with the apoprotein; that means, the irons are removed and start adding the iron to salts you will start seeing the iron 2 core being built in.

Now, this is when it is exposed to oxygen, it will go to in the iron 3 kind of a form of course, it is not very well understood in terms of the product, but iron 3 is very well understood because of from the EPR and other studies. So, at this stage an electron reduction will activate this part of it and will generate the tyrosyl kind of a species, which is involved and that will make the oxidized form further intra redox form will lead

to the tyrosinyl radical. Of course, this tyrosine radical is also stabilized by various factors, which we will see in a while and there is also a hydrophobic core, which keeps this tyrosine radical intact.

Let me tell you one point never forget, with this if this radical is not formed this is not reactive. So, this iron center is active catalytically only when the tyrosinyl radical is there. So therefore, these 2 are basically interconnected. So, the existence of this the catalytic phenomenon of this protein is existing only when you have both of these are 2 together ok. So therefore, you are oxidizing and when you oxidize the iron, then I will mention the oxo bridge will also come ok. So therefore, this oxo bridge is already is already is there and this oxo bridge and the oxidized iron center.

Now, is sufficient enough with the with the electron reduction to take to a radical of the tyrosyl radical. So, tyrosyl radical is somewhere around within about 4 to 5 angstroms from the di-iron center, and it is stabilized by some residues side chains as well as a hydrophobic compartment. And as I told you and I repeat to say that the radical and this center oxidase center both are essential for the catalytic activity otherwise there is no activity. So, whatever I said in this let me put in a little different format, I take an apoprotein and then to that you add the iron 2 plus and then you get into the oxygen exposed, then you get a iron 3 oxo iron 3 kind of a thing and this when the in the in the electron reduction presence will give there tyrosyl radical.

So, therefore, this whole unit is very very essential to that, and in the oxygen there is added one O is used for bridging, other O will go as a water that as you can see here and then excellent kind of thing. So, whole system is required let us look at a little bit more details.

(Refer Slide Time: 14:50)

Introducing metalloproteins & metalloenzymes

RNR: RNR1 & RNR2 subunits

- RNR2 contains a diferric iron center and a stable tyrosyl radical.
- The tyrosyl radical is deeply buried inside the protein in a hydrophobic environment, located close to the iron center that is used in the stabilization of a tyrosyl radical.
- The structure of two μ -oxo-linked irons is dominated by ligands that serve as iron binding sites: four carboxylates [aspartate (D146), glutamate (E177, E240, and E274)] and two histidines (H180 and H277).
- Association occurs between the C-terminus of RNR2 and the C-terminus of RNR1.
- Enzymatic activity is dependent on association of the RNR1 and RNR2 subunits.
- The active site consists of the active dithiol groups from the RNR1 as well as the diferric center and the tyrosyl radical from the RNR2 subunit.



Prof. C. P. Rao, Department of Chemistry, IIT Bombay

So, I have talked to you the di-iron center and the di-iron center is stabilized by as a di-iron diferric or di-iron 3 center stabilize with the tyrosyl radical this all thing is present in the RNR 2 ok. So, this radical is further stabilized and there is a hydrophobic core and as you can see in the previous slide itself, then there is a lot of glutamate residues which also stabilize this one the iron center and that is also important. And now this RNR 2 and the second part or second subunit is a RNR 1 as labeled, and these 2 are in close proximity and the C terminal of RNR 2 is in close proximity which with the c and C terminal of the RNR 1.

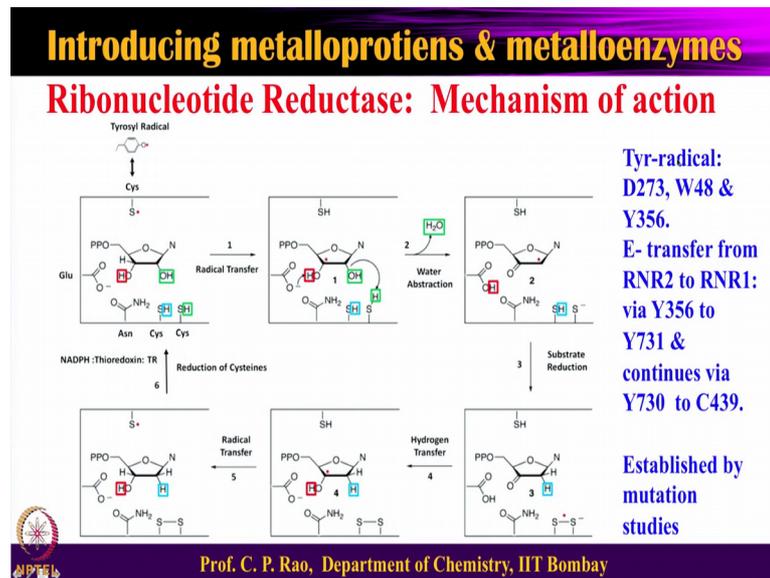
So, with that you can understand; that means, there must be some kind of a communication going on between the RNR 2 with the RNR 1, where the C terminal of these 2 proteins are interconnected or very close by I am not do not say that interconnect, but they are close by. That means, certain residues at the C terminal of a RNR 2 and the certain residues of the C terminal of the RNR 1, they are the ones which are mediating the connectivity or mediating the enzymatic reaction ok.

So, therefore, the enzyme activity is one thing is dependent on the stability of the radical and the di I diferric center and also is dependent on a how these 2 units are associated RNR 1 and a RNR 2 or associated closely at the C terminals ok. So, this is absolutely essential. So, and what is there in the in that RNR 1 there is a dithiol function we will see how is it important. In the RNR 2 it is a di-iron center diferric center with a tyrosyl

radical, that is important in the RNR 1 it is the dithiol group which is equally important how that is important, then we can look at in the next slide.

So, having talked about the di-iron center, tyrosyl radical stabilization in the RNR 2, dithiol center in the RNR 1 and these 2 are connected through at the C terminal and there are certain residues coming from the RNR 2, the certain residues which are coming from the RNR 1 there is a communication in these ones.

(Refer Slide Time: 17:27)



And let us look at those which the residues are associated. In the tyrosyl radical as I said radical is placed with some a residue side chains, they are also placed with certain the hydrophobic core and the kind of the residues are there aspartic 273 and this is the tryptophan 48 and tyrosine 356.

So, these are from the RNR 2 side; that means, this radical is just not shown this resin the core, there is a core the protein core which is protecting this with a with a hydrophobic as well as the aspartic tryptophan and tyrosine. And coming to the other part which is the RNR 1, there is an electron transfer there is a or radical transfer going from the RNR 2 to the RNR 1 this whole region is a RNR 1. So, here the RNR 1 is going through via some residues here that radical transfer or electron transfer goes through them a tyrosine 356 and tyrosine 731 and this continues via tyrosine 730 to the cysteine 439.

So, going from one tyrosine to the other tyrosine and then other tyrosine and then to the cysteine. So, that is how this whole thing happens. One another aspect I have forgotten to tell you in this aspect is that, RNR 1 is also having a compartment for binding of the substrate. So, what are the substrates here? The substrates here are the ribonucleotides the product is deoxyribonucleotides. So therefore, and I have to show you earlier, there are different kinds of ribonucleotides the ADP, GDP, CDP, UDP etcetera etcetera all of these are recognized and bound all of these are recognized and bound by the RNR 1.

So, RNR 2 has only one component, that is the diferric iron with the radical center and the RNR 1 has got the compartment for binding to the substrate and substrate specificity, substrate is being recognized anything else come will not fit here, anything comes here will not be recognized by the enzyme. So, I have told in the beginning of these courses that and for an enzyme action substrate should be recognized. So, substrate specificity is important. So, substrate specificity is also there in the ribonucleotide reductase, but in the RNR 1 compartment.

So, now RNR 1 compartment has a substrate binding, substrate specificity and dithiol as you can see here dithiol; these 2 cysteines of these. And there are some residues in this vicinity there are residues in this vicinity which I have not shown here for clarity. Now let us look at the mechanism of a such a kind of a enzyme. So, as I said there is a radical at the tyrosyl and the C terminal is interfaced with the C terminal of the RNR 1, this radical is basically generates a radical at the cysteine at this interface into the into the RNR 1 and in turn this radical is being transferred to the ribosyl compartment, because ribosyl is recognized and fixed exactly at this particular position and it is a selective.

So, therefore, the radical is contrast for not the C 2, but the C 3 prime and this radical converts by this to the position of this 2 prime and in this process the oh is combined with the hydrogen dot and that will leave as water. So, the water is lost and so therefore, now the radical is being generated over at this 2 prime position and this 2 prime position radical will pick up the hydrogen dot radical and make the C 2 H.

So, now C 2 prime has got a hydrogen; that means, deoxy and that results in the partial oxidation of the disulf hydral functions or dicysteine and this will transfer one more electron to this and therefore, that becomes a disulfide. So, the partial disulfide to disulfide and that will result in the hydrogen shift over to this and then to the carboxylic

this carboxylic group, that you can get back to that. So therefore, you have a different kinds of reactivity.

So, therefore, this radical, induces the radical formation at the interface and this radical is transferred to the to the 3 prime not the 2 prime and there is a radical resmization takes place or radical transformation taking place to 2 prime and then the 2 prime picking up the hydrogen and 2 prime H and that is reflected in the sulfidal functions into the semi and to the full disulfide formation, and that leads to the to the hydrogen transfer over here and the radical make and this is further reduced and this is reduced by some other the a thioredoxin nadph thioredoxin.

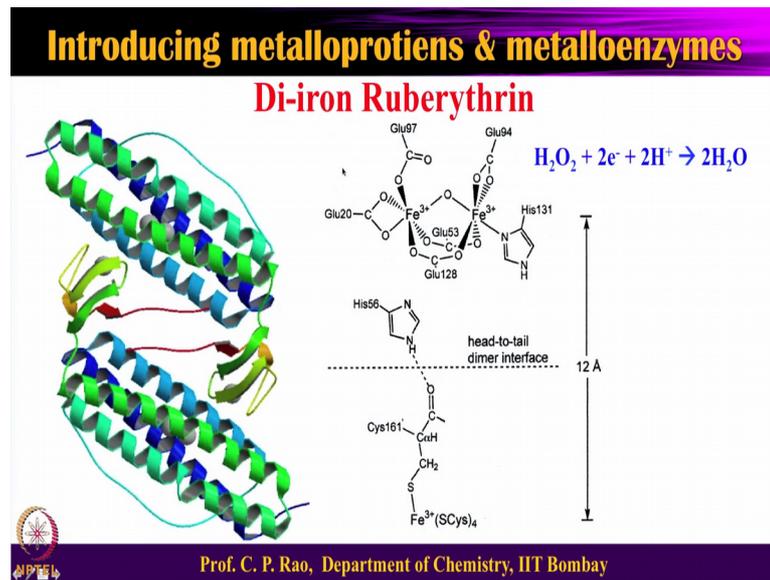
. So, the thioredoxin is an another other enzyme that enzyme gets and brings back to the normal and this is how it is. So, what are the evens that we have learned in this ribonucleotide, reductase ribosyl to the deoxyribosyl it was absolutely essential for a DNA synthesis of this. So, all that you need is the all what you need.

You need to remove this OH and put hydrogen that is all you need. So, that is going through the following mechanism because of this di-iron. So, there is the reaction though this is the di-iron center with the tyrosyl radical is important direct reaction does not directly occur at this as you can see, because the substrate is bound away from it not in the RNR 2 compartment, but in the RNR 1 compartment.

So, substrates binding substrate recognition this is one of the cases where you have a bit of deviation that direct a substrate binding at the catalytic center is not happening. So, here we can call as a catalytic center or an inducing center and that will induce the radical into this particular sulfhydryl function cysteine, which is transferred to the C 3 prime converted to the C 2 prime and then pick up a hydrogen and they are release of this to form this S S semi oxidized to the fully oxidized form will be back to that and this disulfide which is formed is further reduced back to the some hydrogen by the thioredoxin process. See very nice kind of a ribonucleotide reductase kind of thing; so ribose to deoxyribonucleotide.

So, we understand there are 2 parts ribose 1 RNR 1 containing the diferric iron tyrosyl radical and the RNR sorry RNR 2 containing the diferric iron center with tyrosyl radical, RNR 1 containing the compartment for the substrate binding as well as the di sulfhydryl function. So, we have seen the total role of this enzyme the 2 compartments.

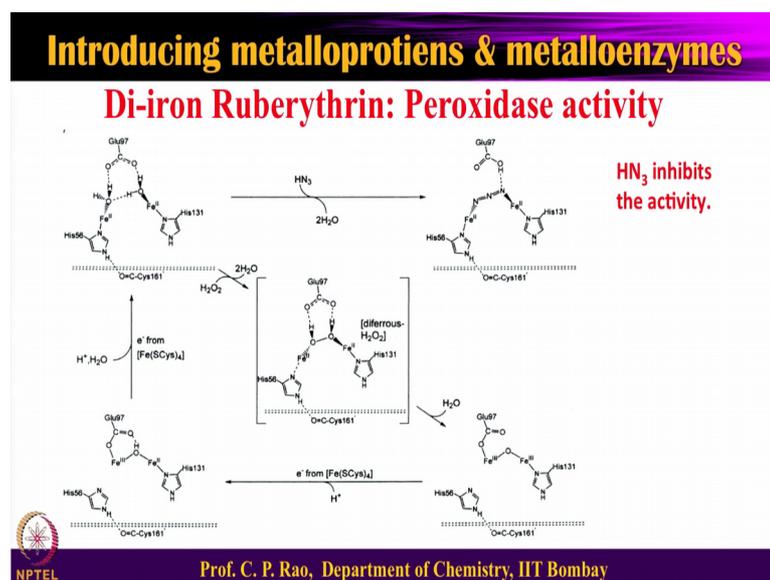
(Refer Slide Time: 25:31)



Having seen this, let us move to the next enzyme of the di-iron which is a ruberythrin. As I mentioned already it catalyzes the breakdown of the H_2O_2 to water so; that means, it is a catalyst activity it is essentially a catalyze kind of an activity.

So, you can see again this is the kind of an enzyme structure that huge you know alpha helical structures the 2 interfaces, and this is the how the interface is connected head to tail dimer interface. So, the center difference between this distance between this is about twelve angstroms. So, what are the electron transfer takes place its takes place through this kind of thing.

(Refer Slide Time: 26:14)



So, this reaction is a very well known reaction to us by now and how does this happen. This is the di-iron center and the di-iron center when the substrate here the substrate is a H_2O_2 , and then H_2O_2 binds this is now proven species and. So therefore, that reaction goes from the redox process that is occurring at the iron centers and that cleaves and loses 1 water and forms the oxo bridge. Whenever the iron 2 goes into the iron 3 both the irons go into iron 3 always in the oxo bridge that we have seen that.

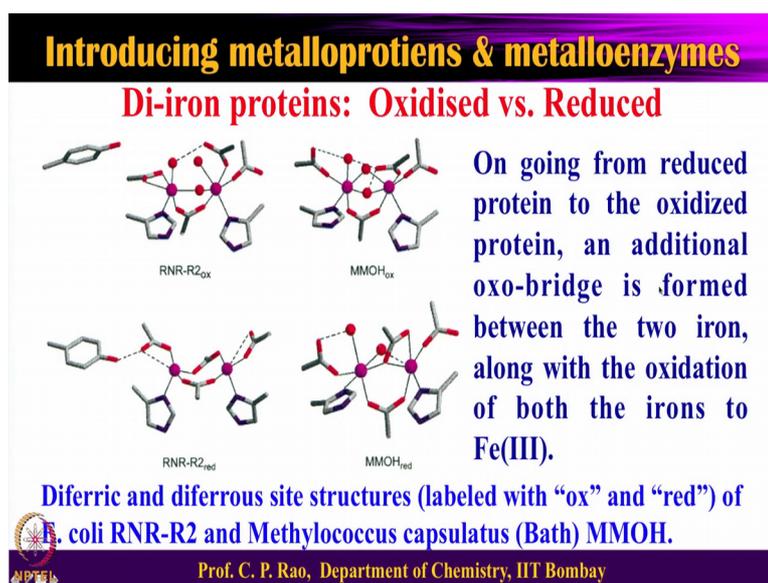
And this is further reduced by the iron sulfur protein called the rubrydoxin. So, that is why it is called ruberythrin rubrydoxin will reduce back this one and the protonation also takes place. So, at this stage one more oxygen is reduced to water and that will go back and again rubrydoxin further reduces and brings back to this. So, that they are iron center how do we know this is the really center that is responsible for the reactivity. Now you use react with the H_2 . So, the H_2 will in will bind in this region where otherwise the hydro H_2O_2 binds. So, H_2O_2 binding here in the same place H_2 will bind n 3 minus will bind.

So, when the H_2 minus will bind, this will inhibit the reaction. So, this clearly tells this is the reaction center ok. So, now, we have a di-iron center, the H_2O_2 binding, leading to the oxidation of this breakage of the H_2O_2 one water and one oxo bridge further reduction by the rubrydoxin and reducing the iron center further reduction leading to the

protonation leading to the one more water generation and that lead to the starting part of that enzyme.

So, we know this is the region where di-iron center is the one where the hydrogen peroxide is bound and this has been shown by this particular aside binding an 3 minus binding.

(Refer Slide Time: 28:35)



So, what we learned in all these is whenever you have the iron di-iron centers particularly when the 2 irons are 2 plus and going from 2 plus to the 3 plus from a reduced protein called 2 di-iron 2 and oxidize protein called di-iron 3, whenever you go from reduced to the oxidized protein you get an oxo bridged. This can be coming even from O₂ breakage mostly and then the other one will go in the water and this is where you can see this is for the oxidized the ribonuclear reductase reduced ribonuclear reductase you can see.

Similarly, here the o bridge extra here. Then in this case methane mono oxygenase the oxidize there is a oxygen that that oxo bridge is lost over there. So, you can see in all these di-iron centers, what you have is essentially what you have essentially is that the 2 iron tools which are in reduced form will go to the oxidized form and that will pick up an a o and then oxo bridged. So, what we have learned that we have learn look at the 2 enzymes, one is a ribonucleated reductase, which converts a ribo ribo nucleotides into deoxyribonucleotides and the second one is H₂O₂ catalyze breakdown of this.

So, in one case the di-iron center is not a directly catalytic center because the substrate binds somewhere else, but it induces what is required what is required? The cysteine radical and this radical further transforms as I explained to you and then finally, the this converts this 2 prime OH into 2 prime H and I have told you all the steps going through that. And there is a dithiol in the RNR 1 which undergoes oxidation and then at the end it will be reduction analogous and in the case of the ruberythrin the H_2O_2 is the substrate is directly bond bonded to that how do we know, because the assign n 3 minus binds here and inhibits the reaction.

So, we have looked at that too. So, both the di-iron enzymes when they are reduced they have a oxo bridges not there when they are oxidized their oxo bridge is there. I hope you keep these things in mind. And we will look at in the next class on something on the phosphatases kind of thing in other words, some kind of a phosphate bond hydrolysis of this.

Thank you very much.