

Inorganic Chemistry of Life Principles & Properties
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Lecture – 48
Role of Selenium in life – Glutathione peroxidase

Welcome you all to the next class on Inorganic Chemistry of Life Principles and Perspectives. So, we were looking at the mercury reduction, from mercury to 2 plus mercury 0 by enzyme called mercury reductase. And in fact, this is the kind of the microorganisms which will act on the removal of the mercury from the environment. So, therefore, this is what we looked at in the previous class.

Now, let us look at some nonconventional ways of the metalloenzymes. Now, we cannot use the word metalloenzyme, but it is a metalloenzyme in a common sense in a common category. So, it is called based on the selenium.

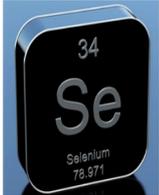
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Introducing metalloproteins & metalloenzymes

Selenium: An introduction

- Selenium was first discovered by Jons Jacob Berzelius in 1817.
- It is named on the Greek goddess of moon 'Selene'.
- It is one of the least abundant essential trace element.
- Position in the periodic table :

Block: p
Period : 4
Group: 16



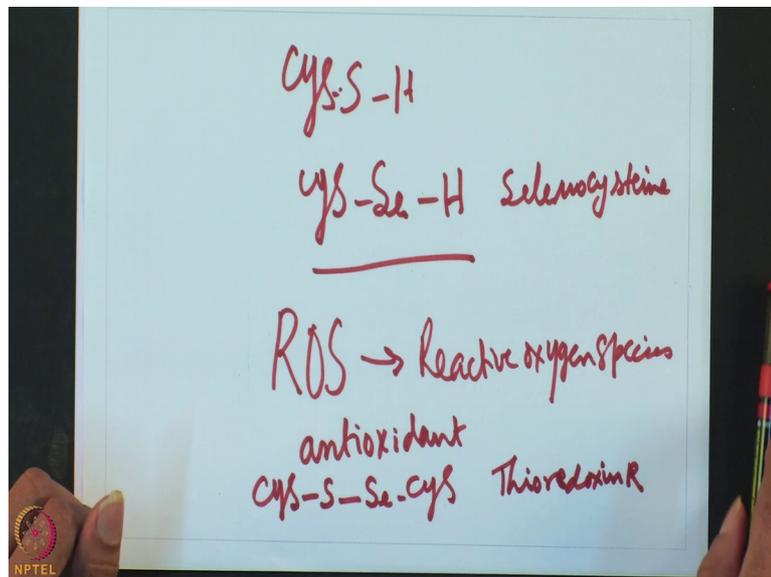
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So, let us look at the enzymes based on the based on selenium. So, before we go into that let us look at the selenium is in fact, is first discovered by Jons Jacob the Berzelius in 1817, and it is named on the Greek goddess of moon Selene. So, therefore, the name comes from the terms Selene.

And it is one of the least abundant essential trace elements as you could have seen the earlier my what I gave long ago in the early classes about the concentrations of these and their presence in the or human system etcetera. And this is present in the of course, there is a p block element with the period 4 and group 16.

So, most of the common ways that the selenium is present unlike the metal, ions, like iron, cobalt, nickel they are either directly attached to the protein side chains or there attached to some special compartments etcetera whereas, selenium is not like that. Selenium is basically involved as part of the amino acid like for example you know the cysteine sh instead of sulfur you will have a selenium.

(Refer Slide Time: 02:24)



So, the only way that you find the selenium is this is referred as selenocysteine. So, this is basically in the form of a selenocysteine. So, it is not like a metalloenzymes. So, it is with this.

(Refer Slide Time: 02:55)

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Function of Selenium in the body

- Selenium present in the form of selenocysteine is an essential component of the enzyme glutathione peroxidase (GPx).
- GPx is an antioxidant enzyme, that suppress the oxidative damage caused by the peroxide radicals.
- Selenium participates as a cofactor for the activation and deactivation of various thyroid hormones and their metabolites.
- Thioredoxin reductase is an enzyme uses a cysteine-selenocysteine pair (Cys-S-Se-Cys) to reduce the disulphide present in the thioredoxin.



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So, the selenium is present as the selenocysteine and it is an essential component of a number of enzymes particularly the really beautiful peroxidase.

So, what is glutathione peroxidase? Glutathione peroxidase is an enzyme which is which basically plays a role of removing radicals, toxic species. So, therefore, you can call it as an anti oxidant enzyme, ok. So, basically the cells or living on the oxygen therefore, oxygen rich portion in the cells would tend to convert the inner oxygen to various 1 electron reduced species called superoxide, 2 electron reduced species called peroxides and a broken species like a hydroxyl radicals.

So, a number of radical or active or reactive type of species are possible. So, these are called reactive oxygen species as I mentioned earlier too. So, these are reactive oxygen species. So, all of these pieces are dangerous to the tissue and so removing all this means acting as an antioxidant. So, therefore, the glutathione peroxidase enzyme acts like an antioxidant because it can remove all this, ok.

So, in all these cases the selenium part will be basically selenocysteine is a cofactor for the activation and deactivation of the number of thyroid hormones and some of their metabolites etcetera, ok. Another aspect you will find in the sequence of this selenocysteine thing is the thioredoxin reductase. So, thioredoxin reductase is an enzyme which uses a cysteine-selenocysteine pair. So, cysteine-selenocysteine pair is nothing but

this one. So, this is cysteine selenocysteine pair and this is from thioredoxin or reductase, ok.

So, this is the kind of a bond which will reduce the disulfide bond in the thioredoxin part of it, ok. So, therefore, the main role of the selenium in these enzymes is not like the conventional metal ions to form a metalloenzyme, but rather take up the position of the sulfur in the selenocysteine and involved in the number of enzymes including glutathione peroxidase.

(Refer Slide Time: 05:56)

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Optimum amount of selenium: Deficiency and toxicity

•Selenium should be present in the appropriate amount in the body for performing proper biological functions.

Deficiency	Toxicity
Growth retardation	hair loss
Cataract formation	sloughing of nails
Kashin-beck disease	Fatigue
Muscle inflammation	neurological damage
Enhanced skin pallor	garlic odor on the breath



Infected by Kashin-beck disease: Deficiency of Selenium

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Now, what happens? This as I said micronutrient, but very important nutrient and its deficiency its excess I have already mentioned earlier, but let me bring back those things again. So that means, the concentration of selenium must be maintained in the body in order to perform the biological functions in a proper way.

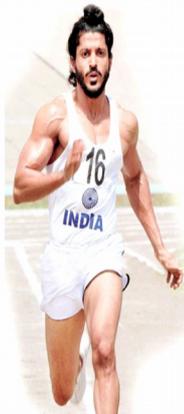
Suppose if the selenium concentration is low levels then the growth retardation is possible cataract kind of things are possible, this is a kashin-beck disease. So, kashin-beck disease is nothing but the kind of a (Refer Time: 06:27) bone shrinking kind of a problems. So, muscle inflammation enhance skin pallor. So, because of this kind of things you could see that the person looks at all these joint etcetera with this the bone and a basically everything shrinks kind of a situation.

So, there is a lot of problems of the definitions of selenium. If you are selenium access is also problem. So, excess selenium is toxic and less selenium is deficient, it shows a deficient syndromes, toxic syndromes, the hair loss, nail problems, fatty neurological, all kinds of things breath. So, several of these things are there.

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As a doctor would you suggest for selenium rich diet!!!!

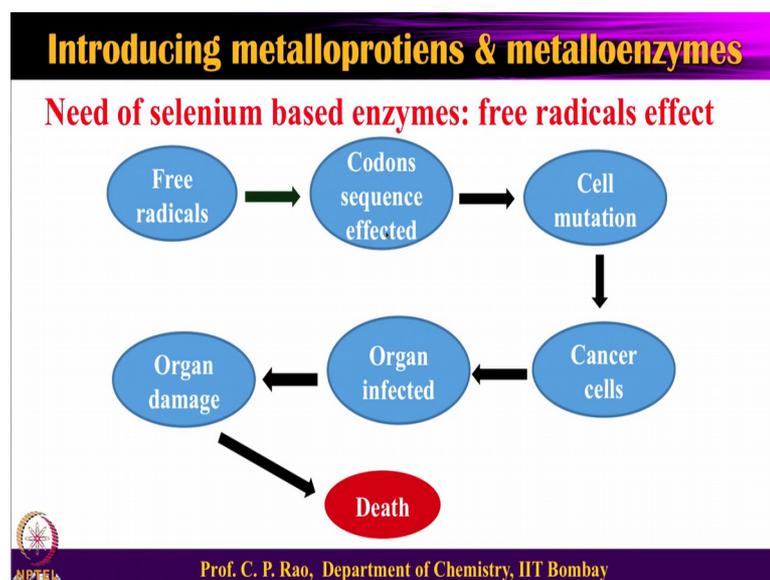
 Tuna (91 ug)	 Cheese (23 ug)	 Chicken (32 ug)	
 Sunflower seeds (53 ug)	 Mushroom (19 ug)		

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So, therefore, the selenium is an important micronutrient and it can be obtained from various food materials foodstuffs the tuna fish, cheese, chicken, mushrooms, sunflower seed etcetera etcetera. So, many of these things have got a good levels of selenium. So, therefore, these by eating these you will be able to get the selenium part of it.

So, what I was telling is that there is radicals which can which are generated in the body because of the oxidative reactions happening and therefore, the unless these radicals are reduced the tissue gets damaged. So, that is where you require the antioxidant kind of a property.

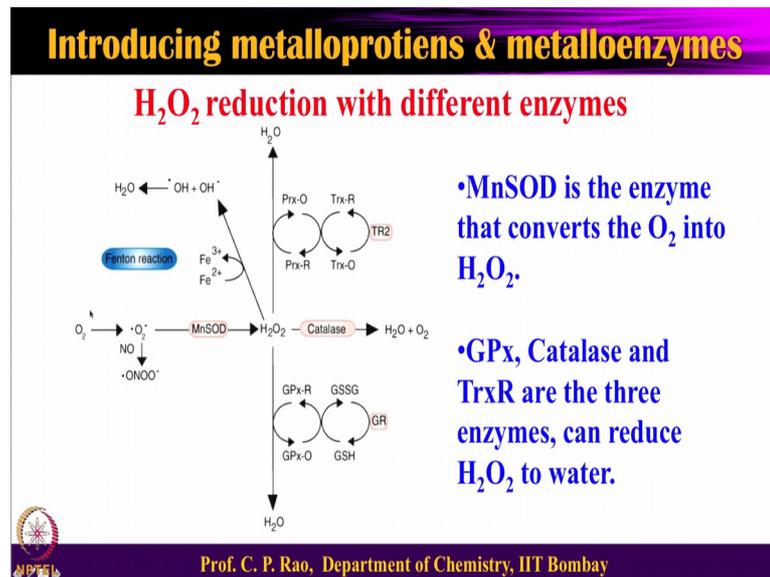
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So, the need of the therefore, the need of the selenium enzyme is very essential. The free radicals can act and codons they can act an act on cell mutation they can act on cancer cells, they can act an organs and in fact, they can damage the organs, it can even lead to the death of it. So, therefore, free radicals are very dangerous and therefore, these free radicals have to be removed by a system and in the in all systems the selenium based enzymes do take care of these ones.

So, we have already studied that couple of stages at the manganese, at the iron, at the copper zinc etcetera, the superoxide dismutase.

(Refer Slide Time: 08:46)



What does superoxide dismutase do? Superoxide this when this converts the O₂ minus dot radicals into H₂O and the H₂O₂ is further removed or disintegrated or disproportionated by a popular enzyme called catalase that we have seen.

And if not if not the metal ions present in the body like transition metal ions particularly iron these kind of things even copper some of these things can convert this into a hydroxy radical and therefore, this is the kind of reaction is called the fenton reaction. So, the hydroxyl radical is much more dangerous than even the H₂O₂. So, therefore, it has to be removed.

So, one of the method is by catalase other is by the glutathione peroxidase. So, so you have both these are involved. So, the SOD is for the superoxide dismutase and catalase is for the H₂O₂ disproportionation. So, and glutathione peroxidase also converts the H₂O₂ into water. So, these act for example, here thioredoxin r is also involved in such kind of a removal, ok.

So, the cofactors such as the Prx reduced will go to the Prx oxidized and that will bring T the thioredoxin reductase into the oxidized form and then this will act on the TR₂. So, this will also remove the H₂O₂ into water.

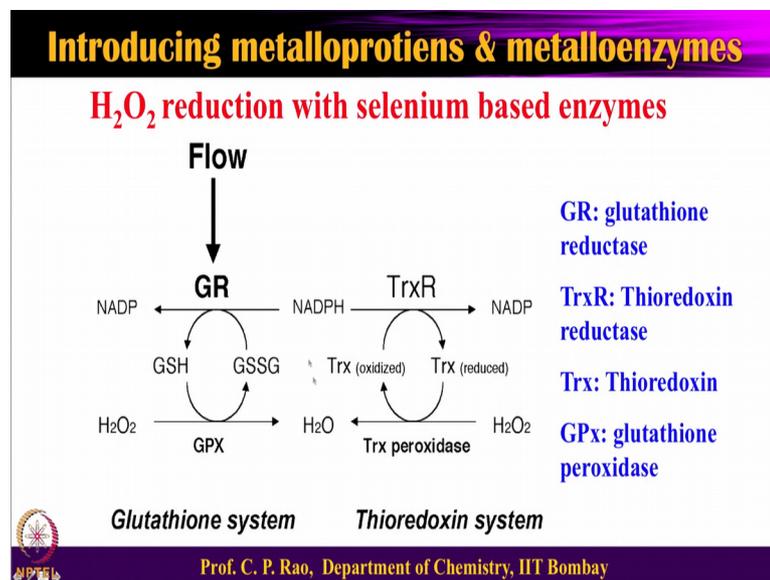
And the glutathione peroxidase again, glutathione peroxidase in the reduced state will act on the H₂O₂ and go to it oxidized form and and this is being reproduced. And then that

reduction will lead to the water and reduced GPx-R is gained back the oxidized, GPx is brought back to the reduced one by using GSH to GSSG cycle.

So, GSH to GSSG cycle will bring back the glutathione peroxidase oxidized to the glutathione peroxidase reduced form of it that means, cycle can work. So, the cycle can work when it couples with the GSH. GSSG is glutathione glutathione oxidized form. So, do two glutathiones joining together through sulfidehydal functions and forming a disulfide bond. So, the SOD is for the removing the superoxide and the catalase and glutathione peroxidases and the Trx reductase these are all the enzymes which will convert the H_2O_2 into water, ok.

We have already studied the catalyst part when we were in the manganese story, now we will study the glutathione part of it as a glutathione peroxidase. So, let us look at the glutathione. Where is the name glutathione peroxidase? Glutathione peroxidase is because the peroxide is being decomposed that is peroxidase, glutathione will return bring it back to the normal therefore, is a glutathione peroxidase.

(Refer Slide Time: 12:16)



Now, the same thing what I talked can be seen like in the form of a schematic representation and here you have let us say H_2O_2 the Trx reduced, thio reduction reduced will oxidize and reduce this one to water. And the oxidized Trx is brought back to the reduced by an enzyme called Trx reductase and this Trx reductase in turn takes

help of NADPH, ok. So, finally, it is the NADPH. So, NADPH to TrxR to the oxidized Trx and that is one cycle, ok.

Other cycle is glutathione peroxidase glutathione peroxidase again acts on H_2O_2 and reduces to give the H_2O and this is regenerated by the GSH GSSG cycle, ok. And that is glutathione you can call it as a glutathione reductase, this also helped by the NADPH, ok. So, therefore, glutathione peroxidase and this part is called glutathione reductase, thioredoxine peroxidase, thio redoxine reductase, ok. So, these are all what how the things are. So, as I repeat it to you a several times I am sure you will be able to understand without any difficulty in this.

Now, let us come to the for a while glutathione peroxidase. So, glutathione peroxidase is also not just one simple enzyme like that of the cytochromes you have huge family like even catalysis or any other reductases it is a family.

(Refer Slide Time: 14:20)

Introducing metalloproteins & metalloenzymes

Glutathione peroxidase (GPx)

- Glutathione peroxidase (GPx) is the family of enzymes exhibiting peroxidase like activity.
- It catalyzes hydroperoxide free radicals which are very reactive, to their corresponding alcohols and thereby protects from oxidative damage

$$2\text{GSH} + \text{R}_2\text{O}_2 \longrightarrow \text{GS-SG} + 2\text{ROH}$$

GSH: reduced monomeric glutathione
GS-SG: oxidized glutathione disulfide



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So, family of enzymes which exhibit peroxidase like activity. So, it catalyzes the hydroge hydro peroxide or hydro peroxide based radicals all these kinds of things which are very reactive to their corresponding alcohols, and by converting into alcohol now the danger has been reduced and it is thus it protects the oxidative damage.

Let us take an example here glutathione simple acting on the R_2O_2 this is R_2O_2 is what peroxide we can be alkyk peroxide or any kind of thing if it is both r is equal to H

gluhydrogen peroxide and that will lead to the oxidized form of GSH which is GSSG. So, SS you can see the bond and this is the disulfide bond and leads to the alcohol. So, GSH is a reduced form of the glutathione as a monomer GSH is oxidized form of glutathione disulfide which is a kind of a dimer, but not a simple dimer but it forms a bond between the two sulfur centers and the protons are lost, ok. So, now, you understand the glutathione.

(Refer Slide Time: 15:37)

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Glutathione peroxidase: Mechanism

$$\text{RSeH} + \text{H}_2\text{O}_2 \longrightarrow \text{RSeOH} + \text{H}_2\text{O}$$
$$\text{RSeOH} + \text{GSH} \longrightarrow \text{GS-SeR} + \text{H}_2\text{O}$$
$$\text{GS-SeR} + \text{GSH} \longrightarrow \text{GS-SG} + \text{RSeH}$$

In this particular enzyme, the selenium is present as selenocysteine residue (Se-Cys) in the enzyme.

First step: Selenol (RSeH) oxidised by the hydrogen peroxide to selenenic acid (RSeOH).

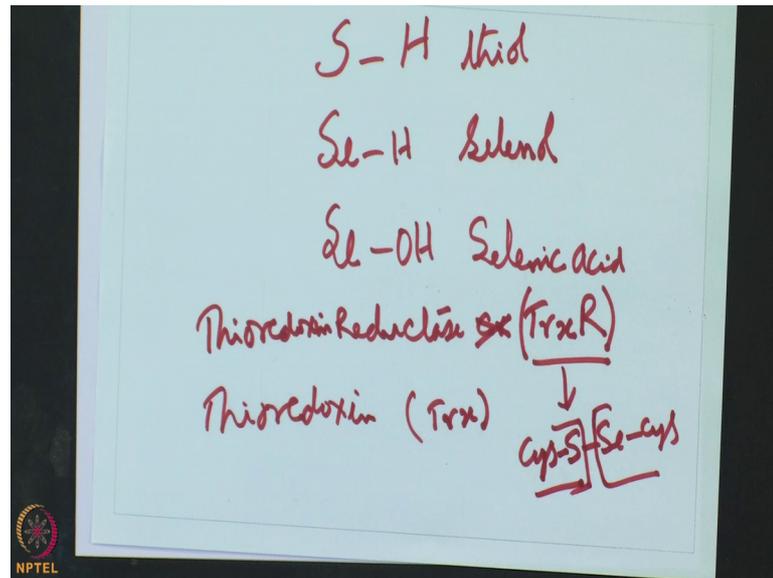
Second step: The selenenic acid is then converted to the GS-SeR and water by reacting with GSH.

Third step: GS-SeR further reacts with second molecule of GSH to form GS-SG and regenerate selenol

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So, how does this glutathione peroxidase function? So, glutathione peroxidase how it functions then you see that selenocysteine we are talking about and SeH we call although it can be called as a selenol just like SH is thiol, SH is thiol, SeH is selenol. So, this is not a difficult thing to understand.

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So, you have SH, SH you call it is a thiol and SeH you call it as a selenol, thiol and selenol, ok. Now, this reaction the selenol cysteine on this acts on the the selenocysteine is not a free simple amino acid we are talking about in the enzyme and this acts on the H₂O₂ and initially gets the oxidized to SeOH and this is selenic acid. So, this is the selenic acid. So, if you make Se into OH, this is selenic acid, so thiol selenol selenic selenic acid.

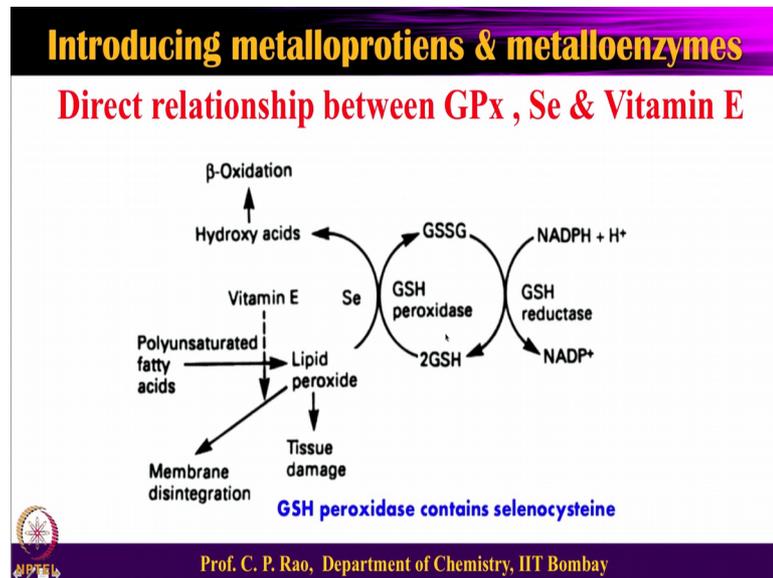
So, and this selenic acid further reacts with the glutathione reacts with the glutathione and then exchange will happen. So, the GS with the SeR, GS with SeR and loses the water, ok. So, it is a basically a kind of a condensation reaction into this. So, because this is acid and selenol and they here this oh and this will act like acid this will act like the base. So, therefore, you get you lose the water from here and then H⁺ and then here the OH⁻ both together will go as water and then you get GS SeR.

So, it is like a kind of its disulfide, but one of them is sulfur based other a selenium, so seleno seleno versus thio kind of a disulfide, disulfide form, ok. This kind of a selenium sulfur disulfide bond further reduced by GSH glutathione to form the GSSG and or SeH getting back to the enzyme is released relieved here.

So, it is basically enzymatic reaction and the step one as you can see that the selenocysteine part will be reacting with the H₂O₂ and the product of this selenic acid will again will react with one mole of the SH, and to form a SSE kind of a dimer or

oxidized dimer and this in turn again reacts with the GSH, GSH can reduce this one therefore, you form GSSG kind of thing and or SeH. So, you have all the things are present in this.

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Now, look at this particular cycle which will tell you the connectivity between the G Ph between the selenium and this is a part of the vitamin E there selenium and with that of the vitamin be E. So, the selenium center the lipid peroxides will always damaged, and this will convert selenium into hydroxy acid oxidation etcetera etcetera, and here the membrane disintegration will happen because you are using the lipid peroxide peroxidation of the lead.

And on this side which is more important to us not so much important this part of it, but this is what is important. The when the selenium acts on this and this is being coupled with the glutathione going to the oxidized form of the glutathione which is called GSSG and this GSSG is going back to the glutathione is activated by the NADPH going into the NAD plus, ok. So, this part is called basically the glutathione reductase part, ok. So, all these cases that you works.

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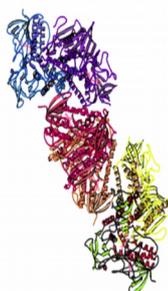
Introducing metalloproteins & metalloenzymes

Thioredoxin reductase

Thioredoxin reductase (TrxR) is the only enzyme that can reduce the thioredoxin (Trx).
It has been divided into two classes :

- In bacteria and some eukaryotes
- In animals
- Both the classes are homodimer
- FAD, NADPH and an active site (redox active disulfide bond) are present in both of the monomers.

Trx is the enzyme that facilitates the reduction of some different protein by cysteine thiol and disulfide exchange chemistry.



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Now, you have thioredoxin reductase also. So, as I have talked to you earlier there are two parts there. So, this is the thioredoxin reductase and this is glutathione peroxidase in the glutathione reductase, ok.

So, now let us look at for a while these thioredoxin reductase which is called the the the Trx R, these referred as Trx R. That is thio redoxine reductase, Tr, Trx, this is thioredox and R reductase. So, this will be thio redoxine and then you have the thio redoxine. So, thio redoxine is referred as Trx and this is referred as Trx R.

And so what we will do? The Trx R is thyroxine reductase will reduce the disulfide bond present in the thiredoxine and that will be coupled to the protein and the protein disulfide in turn is broken. And therefore, this Trx R has got one of the cysteine and one is the selenium disulfide. So, this is not a typical disulfide S Se one counterpart is sulfur other counterpart is the is the selenium type.

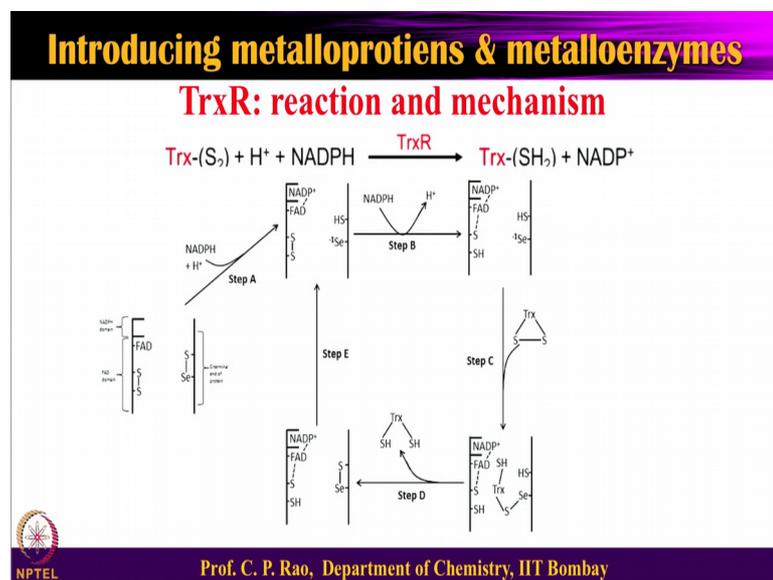
So, this is what is present in the thioredoxin reductase which is involved in reducing the disulfide present in the thioredoxin. And this in turn is coupled with the protein activity, the protein a disulfide bond breakage activity.

Now so this particular enzyme thioredoxin or and thioredoxin it has been divided in two clauses, one is that present in a bacteria and eukaryotes that is present in the animals both

the class or homodimer proteins; So, in these things as we have seen FAD NADPH like the other case like the case of the mercury reductase.

Here also we have the cofactors, the cofactors of NADH at the end, coupled with the FAD which in turn a little coupled with the center for this purpose; So, FAD, NADH, NADPH and an active site redox active disulfide or present in both the both the monomers. So, these are no these cases these are all dimers, but all these demers function function very well, both the dimers will have a catalytic species.

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Now let us look at the we have already looked at the glutathione peroxidase cycle, let us look at the thioredoxin reductase cycle, ok. See the enzyme here. So, in the negative form you have a disulfide you have a selenium sulfide cluster.

Now, this is activated by NADPH as a step A and that will bring activate to break down this bond between Se and S and give a open form of the Se and SH, this is the activation step. So, this is the activation step. So, the activation you have a NADPH involved, ok. And this NADPH directly does not activate goes via the FAD. Now, actual cycle start because it has come into the active form of it second equivalent of the NADPH will break the this side disulfide also it is broken.

And this now so you have a Se SH broken down, SS broken down, and this will act on thioredoxin thioredoxin has got its own disulfide bond. So, therefore, this disulfide bond

thioredoxin is the one which is activated by the kind of a complex we have with the thioredoxin part. And this in turn will reduce and give SH SH and correspondingly you have an oxidation part of it. So, which is coming over from here and you also have SS S Se bond formed and this one further act with the activity of NADP NAFAD thing then you will you can written back to the normal.

So, here normally is already activated step this particular step is already activated. So, therefore, that has broken the Se S bond and that is what you are seeing and those so therefore, the look both this way rather than this way you do not require.

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Thioredoxin reductase: Cellular role

Trx is the enzyme that facilitates the reduction of some different proteins by cysteine thiol and disulfide exchange chemistry

This particular schematic picture shows the reduction of disulfide bonds in the protein with usage of Trx and NADPH inside the cells.

Electrons has been transferred from NADPH via the enzyme TrxR to the active site of the enzyme Trx which will reduce the disulfide linkage of proteins.

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Once you activate the enzyme it can continuously work, unless there some, ok. Thioredoxin now what we do we said the NAD NADH and then thioredoxin reductase then redox then going to the thioredoxin then going to the protein and that is why.

So, let us look at. So, NADH gets oxidized and reduces the the thioredoxin thioredoxin or there is reductase the sulfhydryl function. So, this disulfide one will convert to the sulfide. So, what is this process? This is basically reduces, so this one. Now, it is reduced, but he does not come into the catalytic cycle until you put into this. So, this is coupled with because this one it gives this one, but this has to go back to this.

So, to go back to this and this the enzyme thioredoxin or will use it disulfide sorry, sulfur selenium sulfide bond and this selenium sulfide bond of this. So, with this process will

break down. So, as a selenium and SH and this is coupled with the with the Se S bond connected in the in the thioredoxin. So, again this will come to the Trx SH 2 and this particular reduced form will reduce the protein disulfide to protein thiol protein cysteine. Do you see that? This does not need to be activated by other things and this part and this part, this part and this part all of these are in connectivity.

Now you have seen glutathione peroxidase, we also have seen thio redoxin reductase. So, what did we study in this half an hour? We studied in this half an hour selenium is a a micronutrient, but cannot be ignored. Just because there are no enzymes no many enzymes which are bound like that of the metal ion, but we have an unconventional way where the cysteine is converted to the selenocysteine. The micronutrient deficiency will give you problems excess will do toxic problems.

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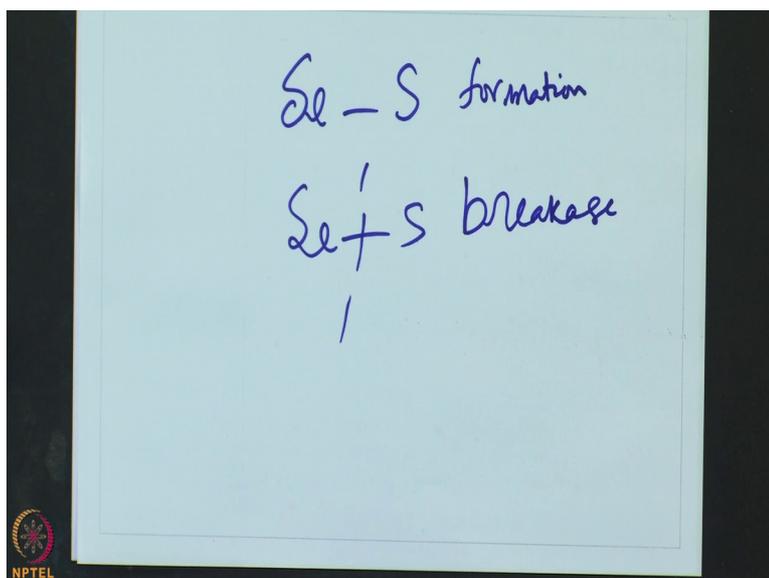
Conclusions

- Se: As micronutrient (deficiency & excess)
- Role of Se as SeCys in enzymes
- Glutathione peroxidase
- Thioredoxin reductase
- Thioredoxin in reducing the S-S in proteins

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So, and it is basically the selenium which is really in working in the selenocysteine enzymes, just by forming a disulfide kind of a bound or breakage a making a bond of Se Se S or breaking this bond, so formation and breakage.

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So, this is the main mantra for this particular entire enzyme.

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Conclusions

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So, that is where this the selenocysteine is working. And the glutathione peroxidase as I mentioned to you which will act on the H_2O_2 and then make into water and then I have talk to you that this is coupled with glutathione glutathione disulfide or dioxidized glutathione.

Then thioredoxin reductase or is coupled with the thioredoxin and thioredoxin reductase or has got 2 bond, 2 Se S bond which will break and which will be transferred into the

thioredoxin and thioredoxin in turn will reduce the disulfide bonds in the proteins, ok. And that is where the thioredoxin reductase thioredoxin in this.

So, thus we have seen an unconventional way of enzymes generally metalloenzymes are what a metal ion and lot of side chains are at of the proteins are attached or the heme is attached or a quorum is attached. Here it is not like that one of the amino acid itself is modified the cysteine is modified to selenocysteine.

So, therefore, nature can find variety of ways how it can it can survive how it can come back with toxicities and other things too. The mercury reductase is also a toxicity reducing enzyme similarly selenocysteine are also toxicity combating enzymes, ok. So, this is the which you call the glutathione peroxidase and that is the mercury reductase both are toxicity combating enzymes. So, with this I conclude this selenium containing part.

Thank you.