Inorganic Chemistry of Life Principles & Properties

Prof. C. P. Rao
Department of Chemistry
Indian Institute of Technology, Bombay

Lecture – 47
Role of Mercury in the environment – Mercury reductase

Welcome you all to the next class on Inorganic Chemistry of Life Principles and Perspectives. In the previous class we have tried to complete the enzymes related to the molybdenum based enzymes.

Now, let us look at another enzyme it is not really for an enzyme function, but it is for something good that is mercury reductase. So, as you know very well the mercury ions are toxic to the body toxic to the cells and system. You know why? These are is because they can react with lot of proteins and many other biological molecules and make some kind of a cross links.

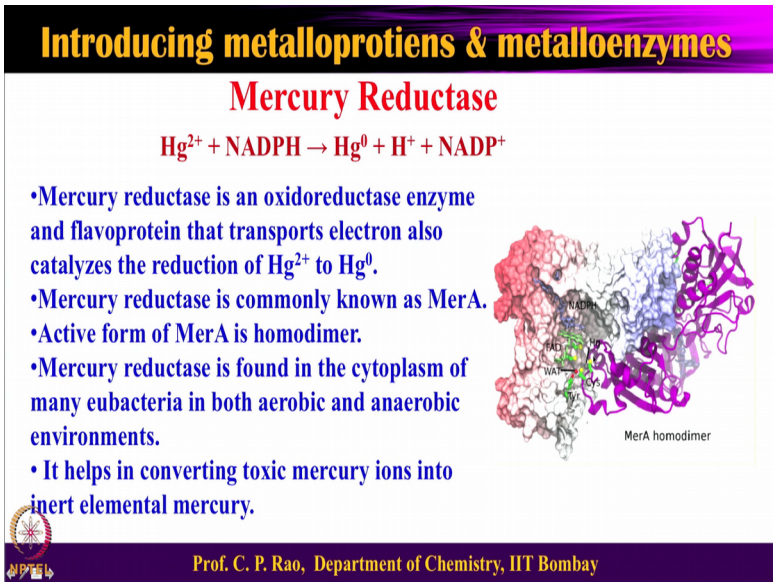
Why do they do that? How do they do that? I am sure you are aware that we have discussed in the beginning the mercury being a heavy ion it is a it is a soft Lewis acid as far as the HSAB principle is concerned. So, therefore, such kind of species are capable of binding very nicely, very efficiently to the systems having sulfhydryl functions. For example, in a protein you have or an enzyme you have a lot of cysteine functions so the mercury can attack those things too. So, therefore, the body needs to sort of remove such kind of things. So, one of such enzyme is mercury reductase.

So, mercury reductase means, reductase means it is reduces, it reduces what - mercury. So, it reduces mercury from mercury 2 plus to mercury 0. So, mercury 2 plus is more active therefore, more dangerous or in other words more toxic to even to human health. On the other hand mercury 0 is neutral not so much. So, binding incapacity therefore, it is less much less toxic we cannot say non toxic, but much less toxic, ok.

Now, coming to this story of the mercury reductase, mercury reductase obviously, ease and oxido reductase enzyme and this particular enzyme is supported by a flavoprotein. Generally what do we know about the flavoproteins? We know that the flavoproteins are electron transfer proteins, but here they also help in catalyzing the mercury to plus to mercury 0, ok.

So, therefore, the mercury reductase is in the short form is written as Mer A. So, this is important thing to say mercury reductase this is a short form is Mer A, ok. So, this is the short form of this enzyme. So, the active form of this enzyme consists of a a dimer kind of a structure. So, this is a homodimer that you have.

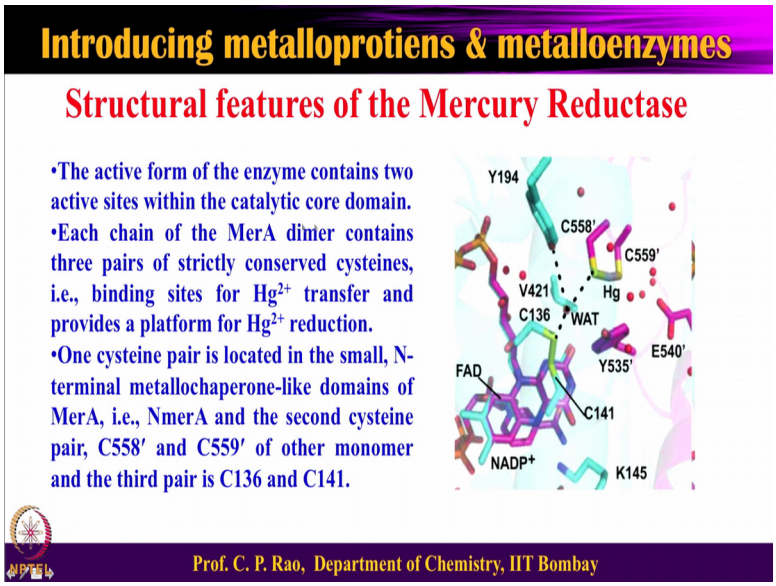
(Refer Slide Time: 03:18)



And this enzyme is found in the cytoplasm of many of the bacteria the bacteria can be the aerobic bacteria could be aerobic. So, both the aerobic and anaerobic bacteria contains in their cytoplasm this kind of a enzyme which is called the mercury reductase or Mer A this is referred to as.

So, what does this enzyme do? This helps in converting the toxic mercury ions to the non toxic or less toxic mercury 0. So, as you can see now on the top of this slide you can see mercury 2 plus plus NADPH giving mercury 0 plus H plus plus NAD plus NADP plus, so this is getting oxidized and mercury 2 plus is getting reduced. So, this is a redox. So, we will look at this particular thing again and of course, again later too ok.

(Refer Slide Time: 04:17)



Let us look at some important structural features of such an enzyme called mercury reductase. Mercury reductase has got a dimeric structure as I mentioned earlier as I mentioned based in the previous slide and it has therefore, it has two active center ok, and these are called the catalytic domains. So, therefore, mercury reductase enzyme or Mer A has two subunits, and O 2 polypeptides where each chain has an active center, and this active center; obviously, should be congenial to bind to mercury 2 plus also should be able to do the reduction process.

So, number one the there these active site should be such that it binds to mercury 2 plus and number 2 active site should be such that the electron transfer takes place in to the mercury 2 plus 2 in order to make the mercury 0. So, for this you have the flavin cofactor in the neighborhood and you have something called sulfhydryl functions also in the neighborhood either in the form of sulfhydryl function or in the form of a disulfide they are there.

So, this is what I talked to you. And in the beginning of this course I talked to you about the hearts of acid base concept where the heavy ions, cations or soft cations and soft acids. And similarly the sulfur is a soft base soft soft interactions very favorable and that is what we trying to look for.

Now, it is coming to the structure features of this particular enzyme this particular enzyme as I said it has two catalytic domains, each of these domain has 3 pairs of strictly

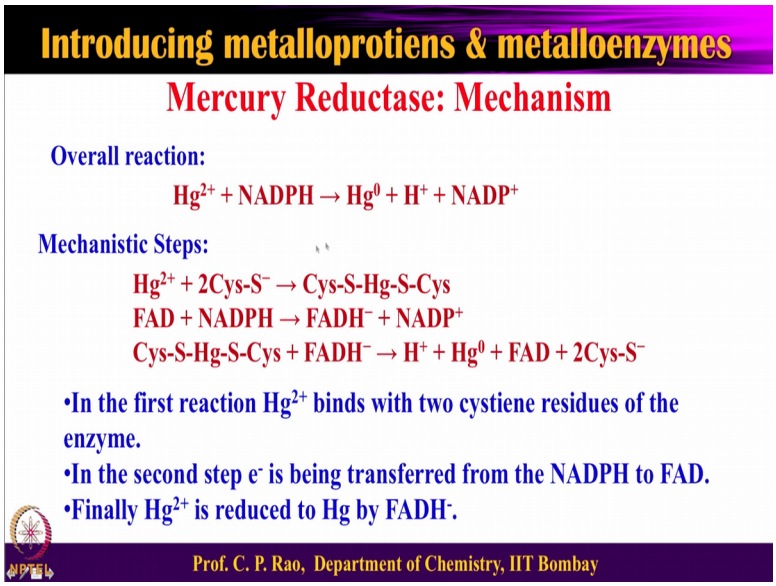
conserved cysteines pairs. So, why do you say pairs? The two cysteines can act like a I can stay as a disulfide also. So, that is why you talked about the pairs. Of which one of the cysteine pair is in the N terminal domain which is like a metallochopperone which means it carries the metal ion, basically it carries the metal ion and brings it.

And then you have in the vicinity of the active center you have another cysteine pair and this is in pain is the number as 558, 559 prime. As you can see over here in the structure this is the 558 and there is a 559 this is basically a disulfide otherwise. And then you also have another pair of the cysteines conserved which is here you can see 136 and 141 ok. So, that is very nice.

Now, you can see that now you have a region where you have two pairs of the cysteines have poist and where the mercury has to bind where the mercury has to get reduced. And then you also have the flavin kind of a the electron transfer part our cofactor we can called is a electron transfer cofactors. So, is this clear mercury reductase is an enzyme which reduces the mercury 2 plus to mercury 0 and the mercury 0 is of course, less toxic and then it will be thrown away by the excretion process that.

So, one of this is one of the ways by which you can remove heavy metal toxicity in particular the mercury toxicity. Of course, there are other kinds of proteins called metallothioneins they are also very well suited for heavy metal toxicity, but mostly they are very well suited for lead etcetera, but to some extent even the mercury 2, and the mercury reductase certainly does this particular kind of a job of reducing this one.

(Refer Slide Time: 08:24)



Now, let us come to the reaction. Coming to the reaction as you can see here mercury 2 plus NADPH this is the reducing equivalent as you know mercury 0 plus H plus plus NADP plus. So, you can see that and this is a redox reaction. While the metal goes from oxidized form to the reduced form the cofactor goes from the reduced form to the oxidized form.

Now, let us see in terms of the steps how it goes as I said initially the first thing is the mercury ions must bind to these sulfhydryl functions. The second thing is that the there should be an electron transfer to that you have an FAD FAD to NADPH will activate the FAD to FAD plus a sorry FADH minus, and this FADH will act on the bound complex and thereby remove the mercury ion reduce the iron to 0. So, there are 2 3 stages binding, then binding followed by the reducing factor activation, and the third part is the actual reducing the mercury to (Refer Time: 09:41) 0.

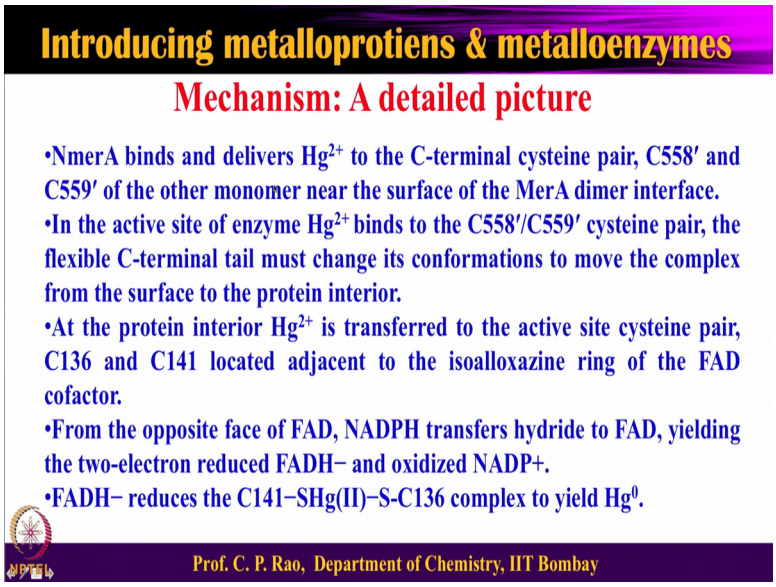
So, step one Hg 2 plus binds to one of these the pair of the cysteines, then FAD gets activated by the NADPH to the FADH minus, and then NAD plus, NADP plus then this FADH minus. Now, is good enough to transfer an electron to the mercury 2 plus to make into mercury 0. So, these are the 3 steps that we can find out, ok.

Let us look at for a while the mechanistic aspect. In the mechanistic aspect let us go for a while previous to previous slide you can see the structure. So, in this structure you have one disulfide pair, another disulfide pair and then one more somewhere in the exterior

part and this you are not shown in this particular picture. So, initially the mercury ion is bound by that N terminal pair ok, which is called N Mer A part of it and then comes to the C 558 to C 559. So, how it will you come to? Because once that binds there is a conformational change that will try to push this into this and then from here the mercury ion again further goes to the C 136 to 141 this particular pair.

So, and therefore, one more binding and the mercury here will make some more change in the conformation, and that will protrude or project this mercury towards inside it will allows to move inside and again it will get into the this one. Why? Because the fed factor is on this side, so because it is the effective factor which should reduce; so, if the mercury is sitting here how will this affinity factor will reduce know. So, that is where the things are.

(Refer Slide Time: 11:53)



So, now let us go further and so, first thing is the NMR a binds to Hg 2 plus. So, that is what I talked to you that we have the N terminal part of that. So, N terminal part of the a dimer of cysteine will bind first to Hg 2 plus and this will bring the mercury 2 plus 2 this to a corresponding conformational change, and because the N terminal 1 is at the interface or at the surface of the dimer it can easily pick up.

And then using the conformational change then it can transfer to the next one that is in the 558, 559 cysteine pair ok. And this again brings a kind of a conformational change and and now this particular complex of the mercury complex will slowly move from the

surface towards the interior and in fact, towards the C 136 to 141, ok. So, and this is located at the isoalloxazine ring of the FAD.

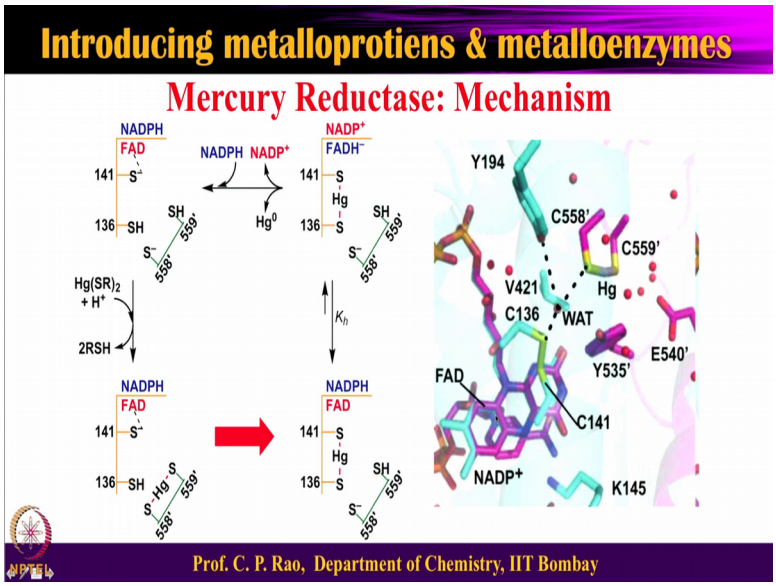
So, as I told you why it has to move, because it has to be close to the FAD; So, this by bringing the N Mer A mercury from the N terminal to that of the C 558 559 and from this C 558 to 559 to the C 136, 141. So, therefore, you have a mercury ions basically flowing from the exterior to surface to interior, so kind of things. So, these are all things where the mercury ion is taken up by the disulfide or cysteine pair we call it as.

And when it reaches the 136, 141 you have comfortably the isoalloxazine ring of the FAD is present there therefore, this particular part will indeed involved in the reduction reaction. So, that whole thing can we talk in terms of the step 1 or process 1. Then once it comes to the 136, 141 then this is close to the FAD and the FAD gets activated by the NADPH and then FAD is FADH minus.

And now this FADH minus will interact with the mercury that is present at the C 141 C 136 transfers the electron, and thereby makes the mercury to 0. And mercury 0 will come out of the complex and the FAD H minus will be basically the oxidized ok. So, these are the third process.

So, first process binding at the surface followed by flowing it to or moving to C 558, 559. Then further moving to C 136 141 you can call the whole thing as a process 1 or step 1, ok. And then next is the activation of the FAD, 2 FAD H minus, step 3 is FADH minus transferring the electrons to the mercury 2 plus and making to mercury 0, ok.

(Refer Slide Time: 15:10)



So, the same thing what I said in terms of the words let us see through a schematic means. So, the step is here and now if this is the actual protein where you have the 558, 559 dime and the disulfide part and the appear and the C 136, 141 pair in presence of the NADH NADPH this gets activated. So, this sulfur disulfides will get activated. And this is now ready to take up mercury ion. It can take simple mercury ion it can take mercury s or twice 2 either this. So, therefore, that will take the mercury ion comes to this particular thing.

So, the same as what I mentioned to you earlier it is initially bound at a pair which is close to the surface which is the N terminal kind of thing then moves into the 558, 559, then moves into the 136 141.

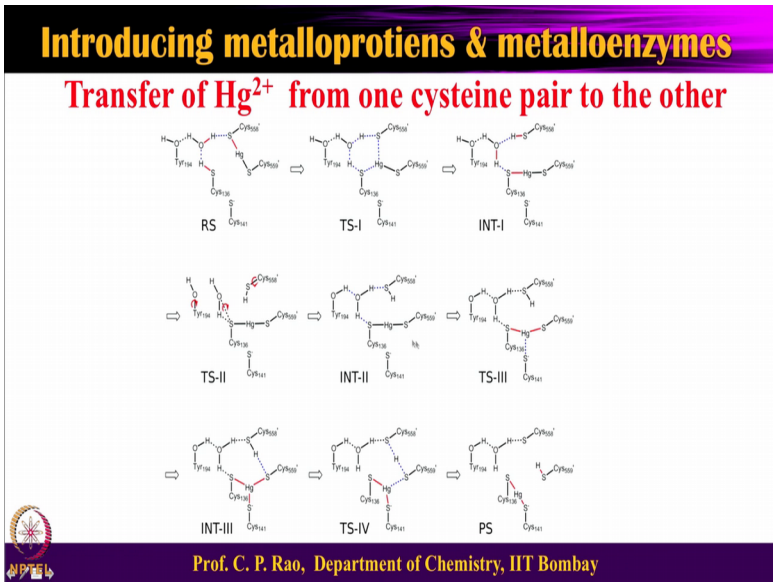
So, now you have an complexed form of it now this complexed form of the mercury moves from 558 559 to a 136 141. So, that is the mercury is moving from here to there, here to there, you see that here to there and that is what you are seen.

Now, NADPH once this mercury comes here the based on this kind of a conformational change that comes the FAD gets activated. So, the FAD gets activated and basically reduced by the NADPH to become FADH minus. Now, this is the reducing equivalents which will add electrons to the mercury which is bound and then throws out NAD P plus out and then and then the mercury 0 and that leads to the FAD bound. So, and NADPH

now this is the reduced form, this is in the normal form that you have. So, therefore, you have a redox. So, let us look at another way.

So, you have at the surface that is bound at another fair and then it is you the conformational change it is moved to 558, 559. Again when it binds here due to the conformational change it will flow the mercury ion through this particular thing to 136, 141. When it comes here the FAD plus is in the close proximity and gets activated by a NADPH NADPH will reduce the FAD to FAD H plus H minus and then NADP plus gets oxidized. Now, NADH plus is close proximity with this particular complex therefore, reduces this one and regenerates the FAD part of that, so from FADH.

(Refer Slide Time: 18:05)



Now, I hope that is very clear. Now, as I told you that you have the process or the step 1, step 2, step 3. In the step 2 itself initially you have a binding in the mercury at the surface and then transfer to the C 558 559 this is this one. So, let us look at each case what is happening.

So, this 558, 559 binds to the mercury and this one slowly moves towards one of these cysteine here that is this particular cysteine. So, therefore, it forms a kind of an intermediate with this one. So, this is transition state, and then try to give this mercury more towards this by the time this gets oriented because the conformational change and this will hold it.

Now, this particular species further because of the rotation over here and rotation over here and rotate over here it will go to this particular transition state. And this particular transition state will again transfer to this part of it. And as you can see once you have that you have the 136 and 141 and finally, binds both of them and this will become broken.

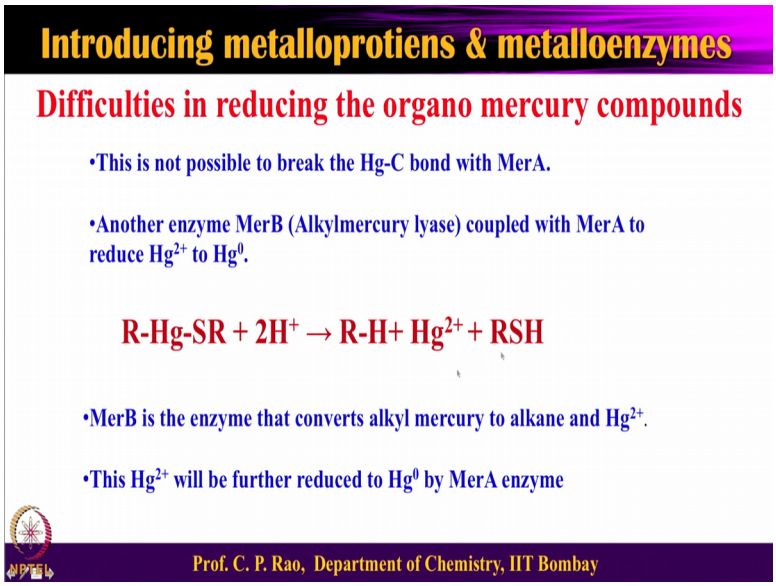
So, it start breaking from here itself the interaction one see there are two cysteines, first it will break another cysteine and next one it will break with another cysteine, then and then it makes a bond with the 136 then it will make a bond with 141. So, out of the 4 cysteine the cysteine 558 will break followed by 559 then they the mercury ion by the time would have bound to one of the 136 and 141. So, 136, 141, one of them will bind and then slowly once the other mercury contact is dissociated and the second cysteine center will also bind.

So, that mean thus the mercury ion is shifted from this pair to this pair. You see here on the top to the bottom. So, all these steps are visualization, you do not need to remember much there is a visualization. So, all this is because what are the reasons there are two major reasons, one is the affinity of the mercury 2 plus to bind to the sulphydral functions because of the soft character the mercury ion with it are the soft character the sulfur group, so therefore, soft soft combination.

The second thing is when the mercury comes in contact the kind of a conformational changes that happen. And as it binds to the this one as it goes to this so you have a continuously the conformational changes. So, it is a one is affinity driven as well as conformational driven. So, both the things are together functioning in this, ok.

Now, what we talked till now is the mercury 2 plus ions can bind as a mercury 2 plus ion or mercury 2 plus which is which is in contact with the with the organism as a mercury thiolate. So, in either of this case it can exchange because as you know thiolate can exchange reaction. So, thiolate exchange reaction is always feasible in this ok.

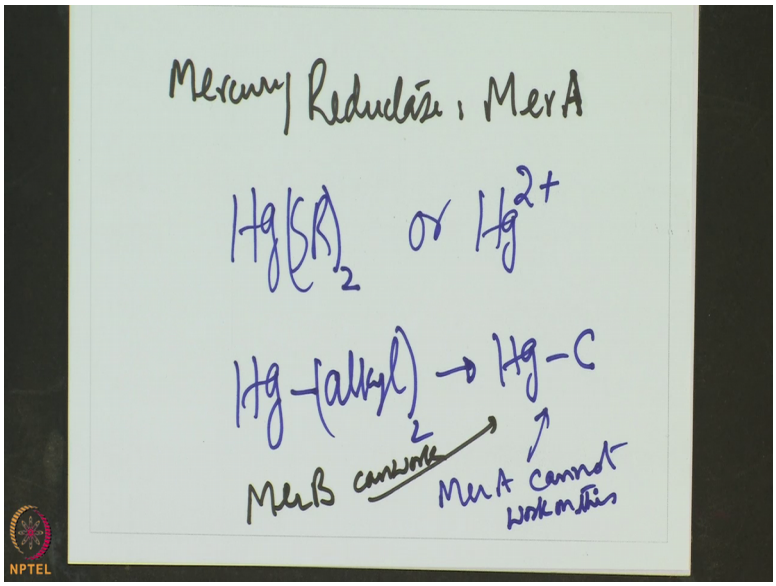
(Refer Slide Time: 21:36)



Now, instead you know very well that in the environment the mercury spillage is not left as it is, the mercury spillage is always contacted with some other kinds of moieties. One of the most common act is the mercury with making a bond with a alkyl group with the carbon alkyl groups of the carbon. And these are called mercury alkyls, it can be with one alkyl, give be with the two alkyls etcetera.

So, if you have such kind of environmental impurity can you expect that this particular enzyme works. So, what I explained to you earlier is it will work for Hg two plus ions it will also work for the Hg with the thiolated complex. So, that is Hg SR or you can call it as Hg SR complex or SR 2 complex, or Hg 2 plus ions, so all of this.

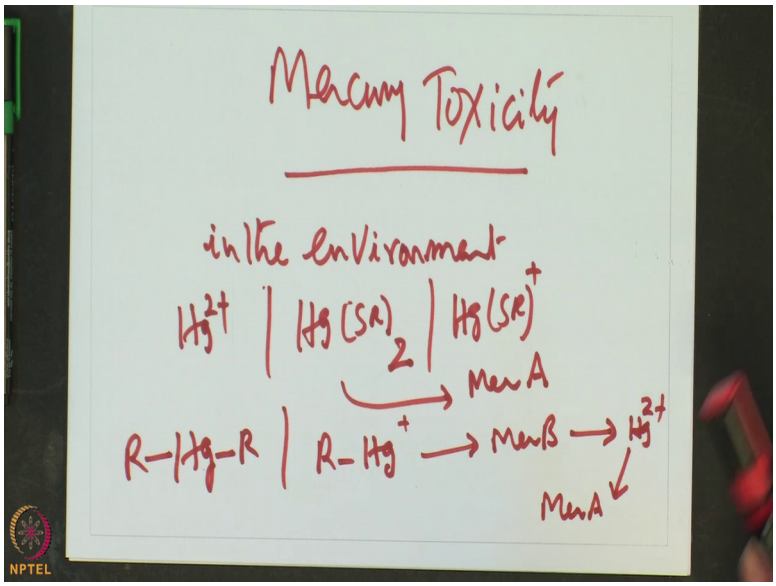
(Refer Slide Time: 22:33)



But a lot of the mercury in the environment is available with the Hg alkyl it could be twice also that means, ; that means, Hg C bond. So, this kind of a bond the Mer A cannot work on this. So, therefore, this enzyme is not suitable for a methyl mercury or dimethyl mercury or dialkyl mercury etcetera.

But there is another enzyme which is called the Mer B. So, there is another enzyme Mer B this can work on mercury alkyls. So, what will it do? First the this enzyme will take away this mercury alkyl bound and you as you can see from this one, it will break down to alkane part of it or the corresponding carbon part of it and then releases the Hg plus 2 plus. Even if you have both sides it will function. So, and once you are the Hg 2 plus or Hg 2 plus is RSH that can be worked out by the mercury Mer A.

(Refer Slide Time: 24:15)



So, therefore, the kind of a impurities or mercury toxicity in the environment can be mercury toxicity in the environment can be removed by by the enzyme Mer A or a combination of Mer B, Mer A. So, if the mercury is in the mercury 2 plus or mercury SR 2 etcetera or Hg SR 1 plus all of these can act directly with Mer A.

Now, on the other hand if you have mercury with alkyl or mercury with one alkyl all of these can be work first Mer B. So, this will release the the Hg 2 plus then this will be worked out by Mer A.

So, a combination of Mer A and Mer B in the reverse order that is Mer B will act first to cut the mercury alkyl bound and the and to reduce this and to convert this alkane plus mercury 2 plus. Now, mercury 2 plus will anyhow be reduced by Mer A enzyme as I explained to you earlier. So, you have all of these that.

So, thus what have we learned in this particular thing?. So, mercury reductase is an enzyme, redox kind of an enzyme reductive type of an enzyme which will reduce the mercury 2 plus to mercury 0. Mercury 2 plus is more toxic, mercury 0 is less toxic and this can function on a Mer A. But on the other hand if the mercury ions are not as a mercury ions are mercury thiolate complexes, then you need first if they are as a mercury alkyl compounds which is very commonly formed in the in the environment. So, mercury alkyl cannot be cannot be converted to mercury 0 by Mer A.

First the mercury alkyls have to be reduced have to be broken down, not reduced broken down to alkane and the mercury 2 plus and then and that part is done Mer B and the thus released mercury 2 plus is converted to mercury 0 by Mer A. So, it is a combination of things, ok. So, now, that is one thing.

Now, this is a kind of an enzyme where you have 3 pairs of you know cysteinyl pairs of there. One is at the surface which is the N chain part of it, and that is very which is exposed to the environment and that will first pick up the mercury ion and this mercury ion now is transferred to the 558, 559 pair by the conformational changes.

And this itself is not sufficient enough because this is far away from the reducing cofactor therefore, it has to move from 558, 559 to 136, 141. And this mercury moves in fact, very nicely through all this and the mercury is picked up by the 136, 141. I have shown all the steps as the mercury binding one of the cysteine break is, two cysteine break is, the next pair is started interacting, both the systems of the next pair started interacting and all these processes.

Next one I have also explained was the reaction the reaction is that binding part at the surface to the to the first level where we talked about the 558, 559 and then transferred to 136, 141. By that stage the conformational changes are good enough for the FAD to get activated FAD and NAD activated by NADPH.

So, therefore, NADPH it reduces the FAD to give FADH minus FADH minus is sufficiently good enough to reduce the Hg 2 plus 2 Hg 0 and reverse back the enzyme. So, the enzyme functions quite well with this thing. So, you have Mer A, Mer B, Mer A working on the mercury ions directly, Mer B working on the mercury alkyls breaking down to mercury ions and then followed by Mer A activity, ok.

So, this is basically the story one way you can say that the environmental protection or detoxification of mercury ions or mercury reductase you call whatever you want all of these. So, but remember that it is all because of the affinity to bind to the sulfhydryl functions for the mercury. And the second thing is the conformational changes that occurs here and the reducing equivalents which act on this between NADPH to NAFAD and then FADH minus to the mercury ions.

Thank you very much.