Fundamentals of Protein Chemistry
Prof. Swagata Dasgupta
Department of Chemistry
Indian Institute of Technology, Kharagpur

Module - 08
Motor Proteins and Metalloproteins
Lecture - 38
Metalloproteins - I

In our continuation of module 8 about motor proteins, we will now be understanding metalloproteins. Now as the name implies, these are proteins that have metal ions coordinated with them. We will look at some specific examples and see how important they are in the functioning of specific biochemical processes.

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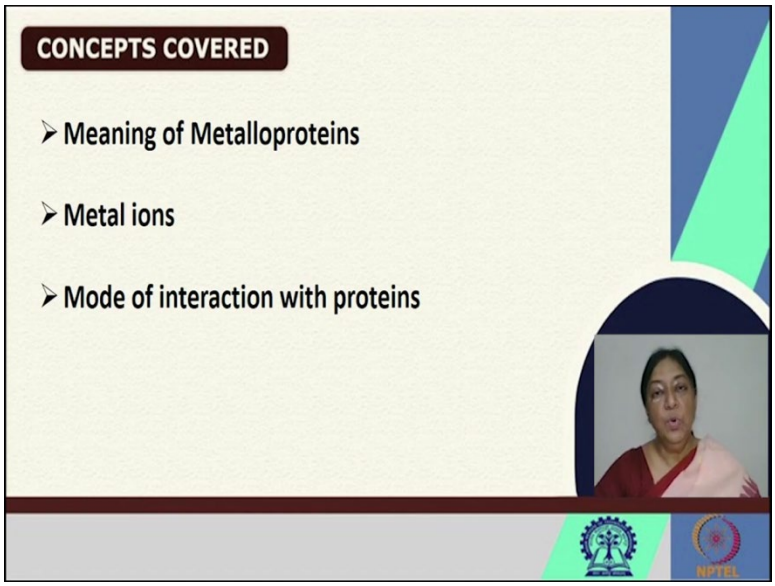

When we look at these metalloproteins, we will see which metal ions are involved and their modes of interaction with the proteins.

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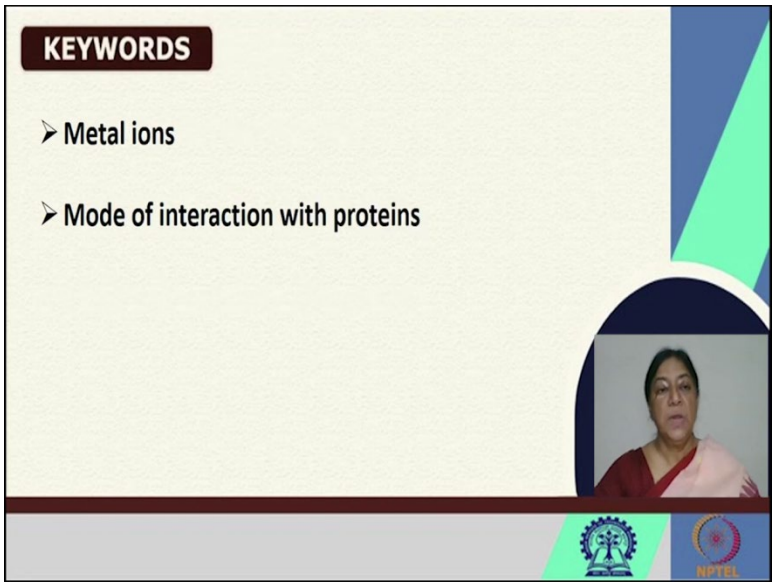

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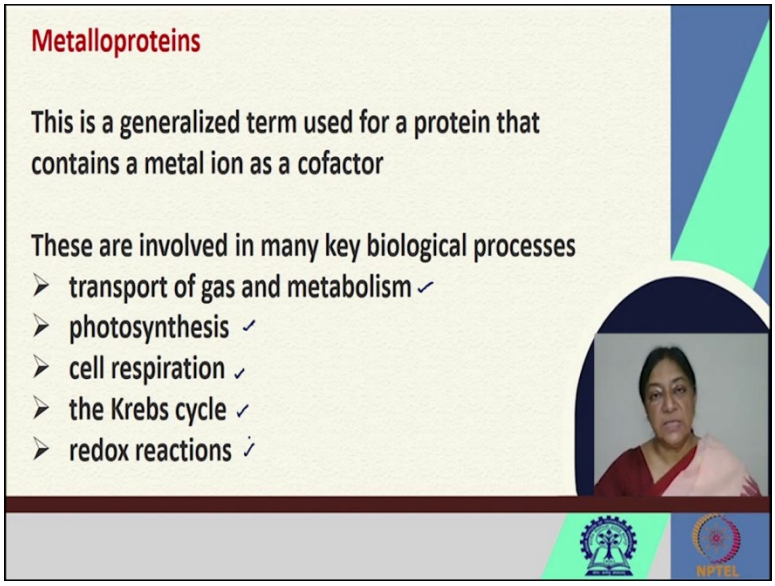

The metal ions that we have and the specific modes of interaction, indicate that this is the way the coordination of a metal ion would occur in a protein. So, the term metalloproteins is a generalized term that is used for a protein that contains a metal ion as a cofactor.

These are involved in many biological processes, as in the transport of gas and metabolism, in photosynthesis, in cell respiration, in the Krebs cycle and specific redox reactions. Some of which we have looked at in our discussion on enzyme and enzyme mechanisms.

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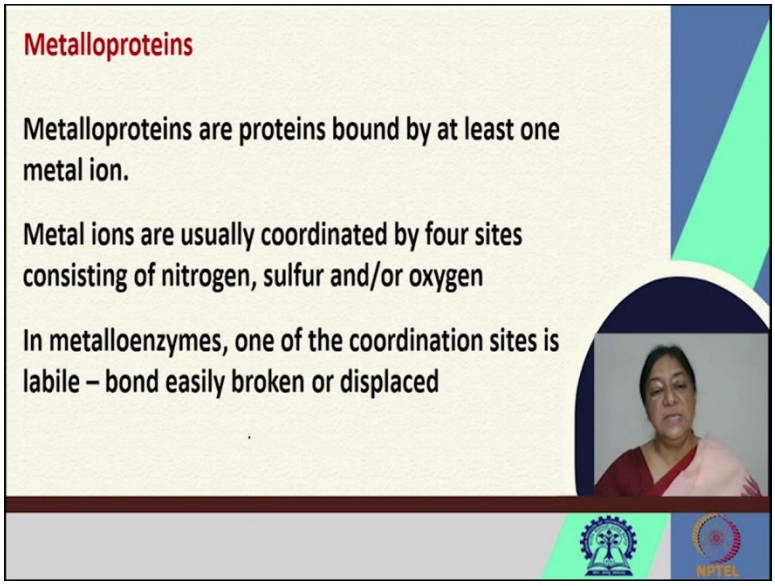

When we look at metalloproteins in general, we try and understand how these metal ions are bound in the protein. Metalloproteins can contain one or more than one metal ions and these are usually coordinated by four sites that consist of nitrogen, sulfur and/or oxygen.

This nitrogen or the sulfur or the specific moieties by which they are coordinated, could come from the protein backbone chain or could come from other atoms that are associated with other molecules. In metalloenzymes, one of the coordination sites is labile, which means that this particular bond is easily broken or displaced for an enzymatic reaction to occur.

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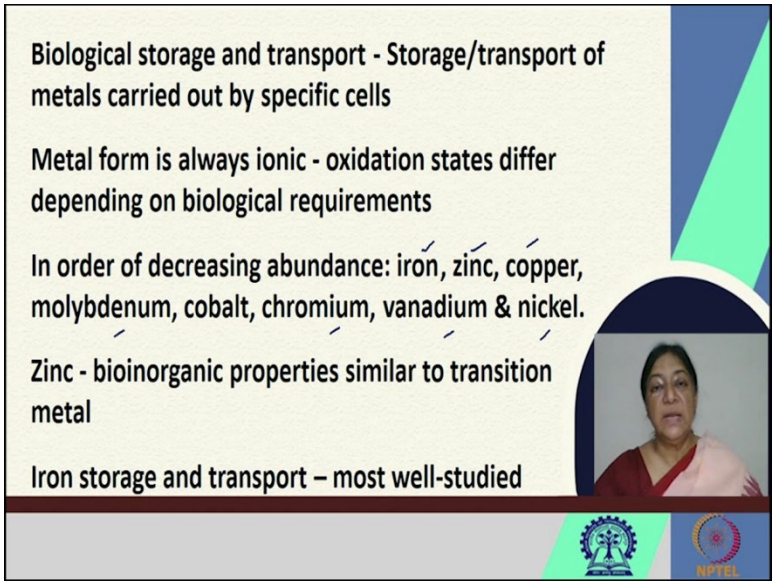

When we look at biological storage and transport in general, there are specific cells that would be accommodated or mediated to do this. The metal ions are always ionic in nature and the oxidation states that we see, differ depending upon the biological requirements.

In order of decreasing abundance we have iron, zinc, copper, molybdenum, cobalt, chromium, vanadium, nickel, in addition to some other trace elements that are present in the body.

Zinc is also present in the body. We have usually those that are transition metals, so that we have differing oxidation states in the specific redox type reactions that they are involved in. But zinc has bioinorganic properties similar to transition metals. So, they are included in this specific metalloproteins and the way they occur, in their functionalities as we saw in carbonic anhydrase.

Iron storage and transport is the most well studied of these, as we will be discussing hemoglobin and myoglobin, and looking at how important they are in their structural activities.

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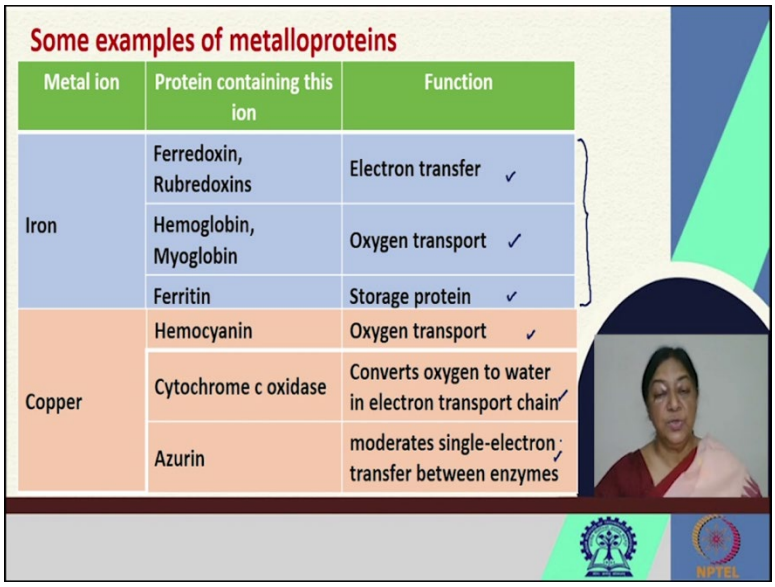

These [refer to slide] are some examples of metalloproteins, where we have the metal ion, the protein that contains this ion and the specific function involved. As we can see most of these are involved in electron transfer.

Where electron transfer in different biochemical processes could be involved; for example when we look at the iron type we have hemoglobin and myoglobin involved in oxygen transfer, rubredoxins and ferredoxins involved in electron transfer, and ferritin is an important storage protein. And knowing that this is the most abundant metal that we have in the body, there would be different ways in which this will occur.

For copper we have hemocyanin, cytochrome c oxidase and azurin. These are typical examples of these proteins that have this corresponding metal ion present. Which again here, we have oxygen transport specific examples and specific reactions that occur due to these methods.

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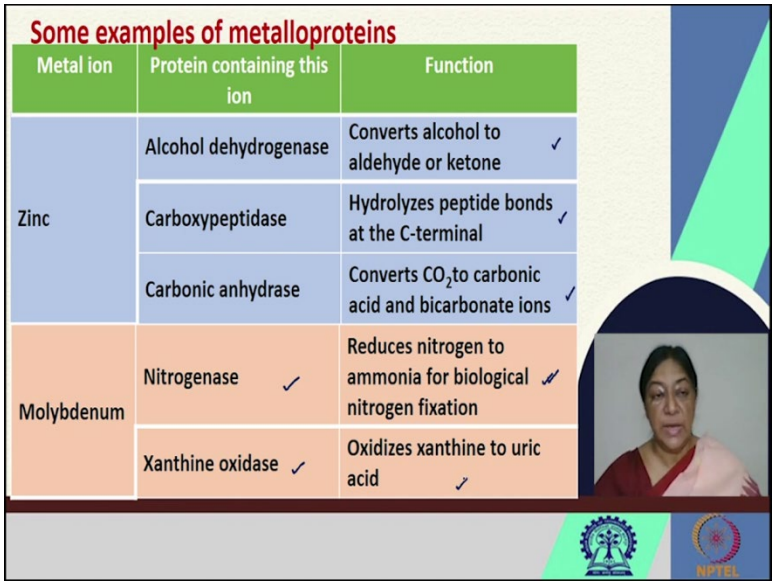

In the case of zinc and molybdenum, we have these [refer to slide] types of interactions. We have looked at the type of interaction of carbonic anhydrase and carboxypeptidase, which hydrolyzes peptide bonds at the C terminal and alcohol dehydrogenase that converts alcohol to aldehyde or ketone in the body.

Molybdenum metal ion is involved in nitrogenase and xanthine oxidase. This is an important reaction that is involved in biological nitrogen fixation, that reduces the nitrogen to ammonia in the body. And here we have the oxidation of xanthine to uric acid.

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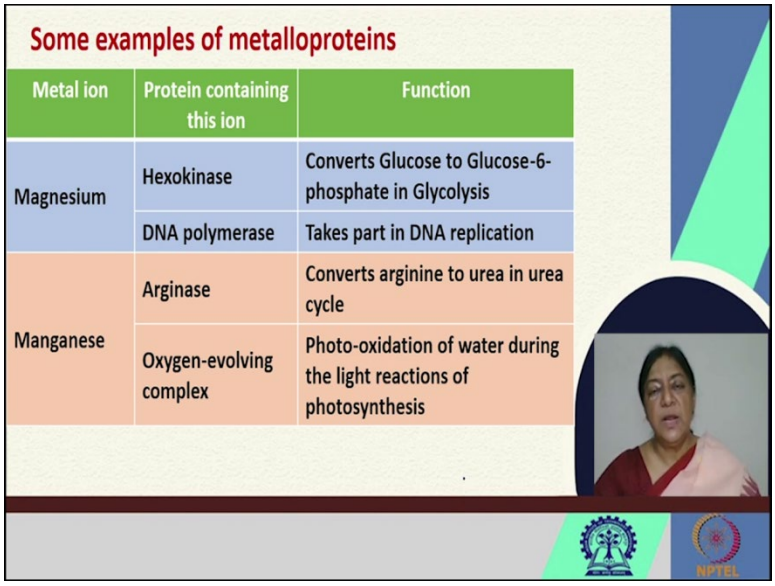

Other examples are magnesium and manganese. Magnesium in hexokinase and DNA polymerase taking part in DNA replication, where we have the Mg^{2+} ion involved in the sugar phosphate backbone. And hexokinase involved in the transfer of the phosphate moiety from ATP to

glucose, to form glucose-6-phosphate in the glycolysis reaction. Manganese has arginase that converts arginine to urea in the urea cycle and an oxygen evolving complex that includes manganese.

So when we look at these different types of metals, we will be looking at some specific examples, just to look at their properties and see how they coordinate in the proteins and what overall reactions occur.

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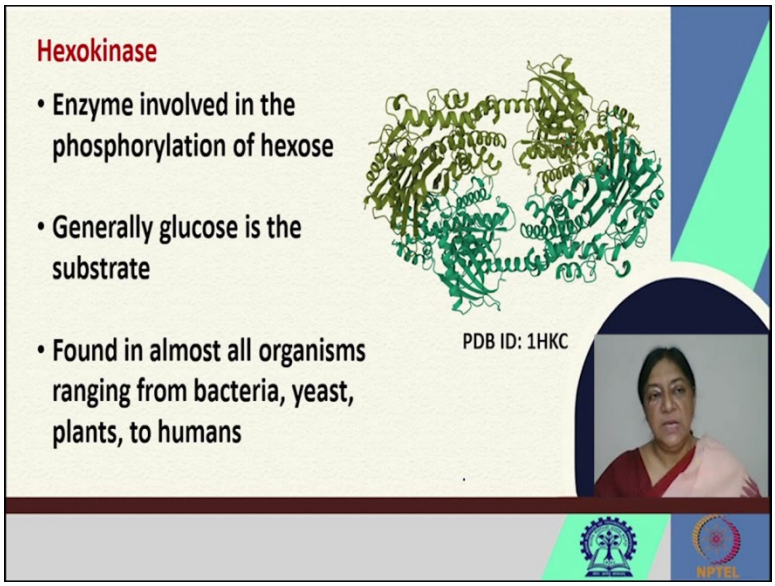

For example hexokinase. In hexokinase we have this as the protein molecule. It is an enzyme that is involved in the phosphorylation of hexose and it generally glucoses the substrate, where we have glucose to glucose-6-phosphate in the kinase reaction. And this is found in almost all organisms ranging from bacteria to yeast to plants to humans because of the specific activity that this enzyme is involved in the formation of glucose-6-phosphate from glucose.

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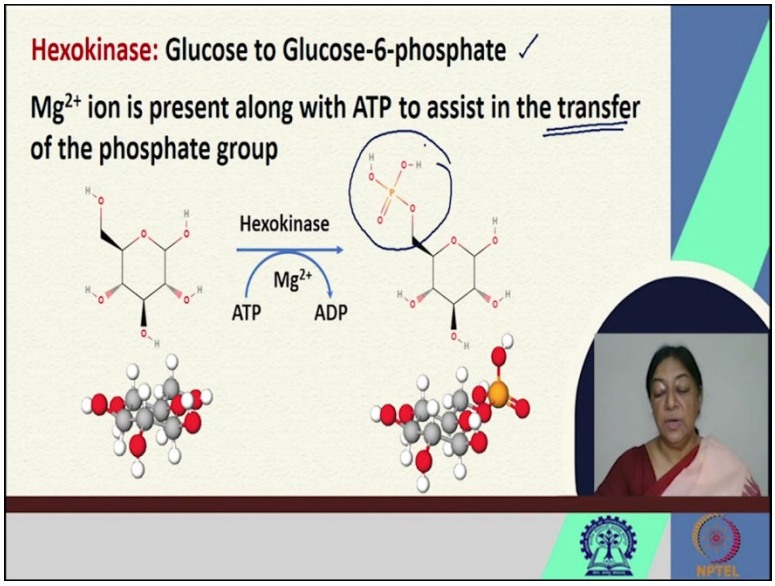

Here, Mg^{2+} ion is present along with ATP that is going to be assisting in the transfer of the phosphate group, in the reaction that converts glucose to glucose-6-phosphate. If we have the representation of glucose, we have hexokinase, we have the magnesium, the ATP going to ATP + Pi and in the process we have a phosphate transfer to the molecule of glucose forming glucose-6-phosphate.

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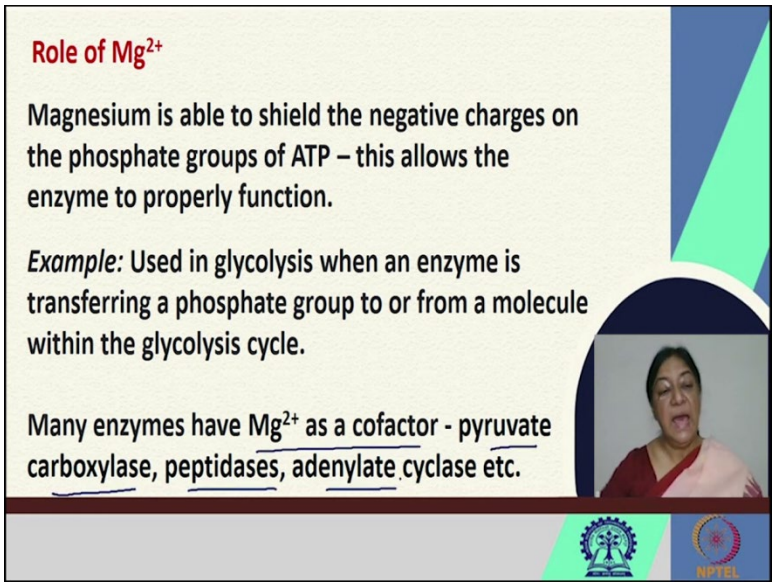

The role of magnesium lies in shielding the negative charges on the phosphate groups of the ATP, that allows the enzyme to function properly. And the example of this reaction where we have it in glycolysis, when an enzyme is transferring a phosphate group to or from a molecule within the glycolysis cycle. Many other enzymes also have Mg^{2+} as a cofactor, where we have pyruvate, carboxylase, peptidases and adenylate cyclases.

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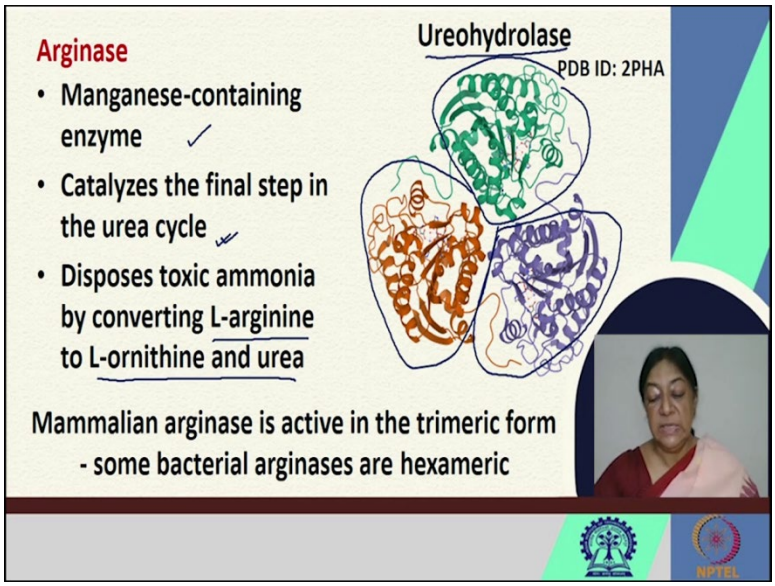

Arginase is a manganese containing enzyme and this catalyzes the final step in the urea cycle. It is a ureohydrolase. What this does is, it disposes toxic ammonia by converting L-arginine to L-ornithine and urea. Mammalian arginase is active in the trimeric form, but some of the bacterial ones are hexameric in nature.

So we can see [refer to slide] distinctly the three subunits that are involved in this arginase protein, making it a trimeric protein for its activity.

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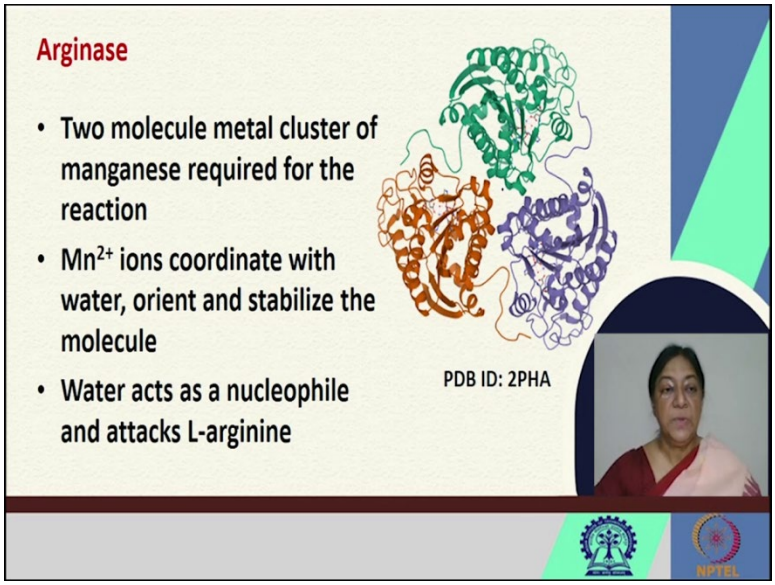

What happens here is, there is a two molecule metal cluster of the manganese that is required for the reaction and the manganese ions coordinate with the water, orient and stabilize the molecule. Then water acts as a nucleophile and attacks L-arginine.

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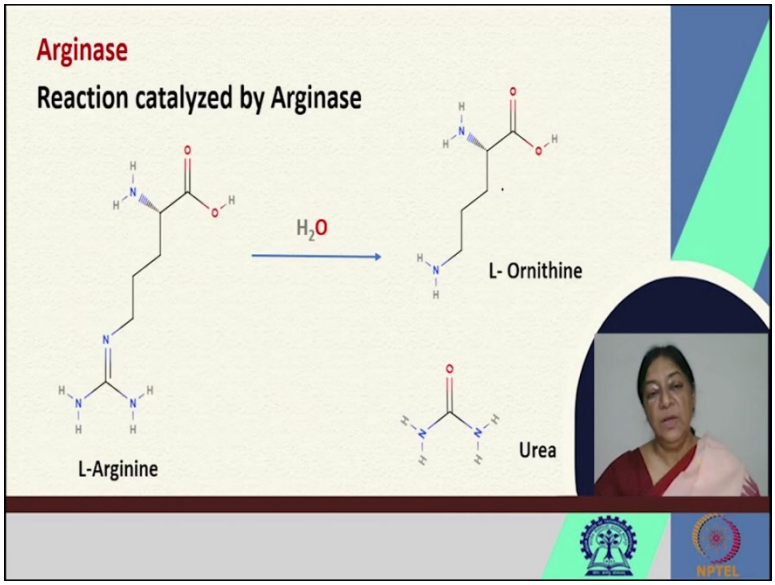

The reaction that is overall catalyzed by arginase is L-arginine, that takes the water molecule where we have the split to L-ornithine and urea.

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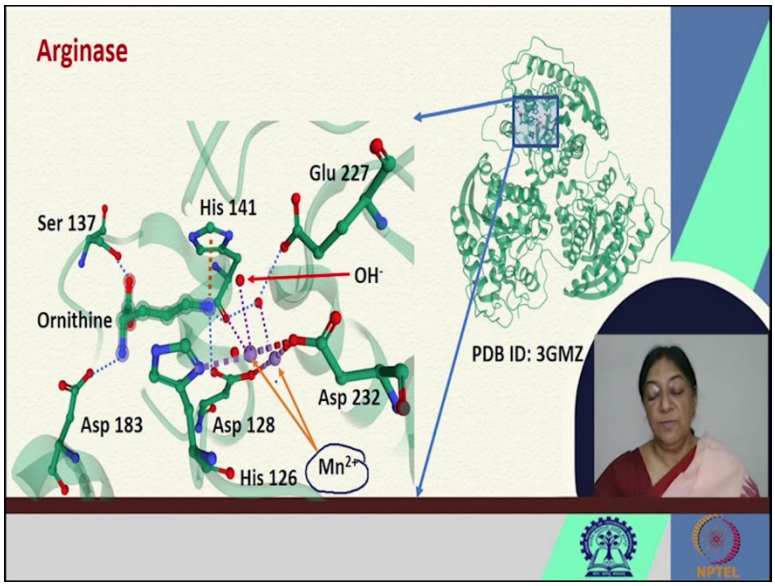

In this case, we can see [refer to slide] that there are two of the manganese ions present in the coordination. The interesting part that we will see in metalloproteins is the coordinating atoms from the side chains or the backbone of the protein.

We will usually see cysteine providing the sulfur, histidine with its imidazole group providing the nitrogen and acidic groups of amino acids providing the oxygen. In addition to water that is present to create the coordination sites for the specific metal ions.

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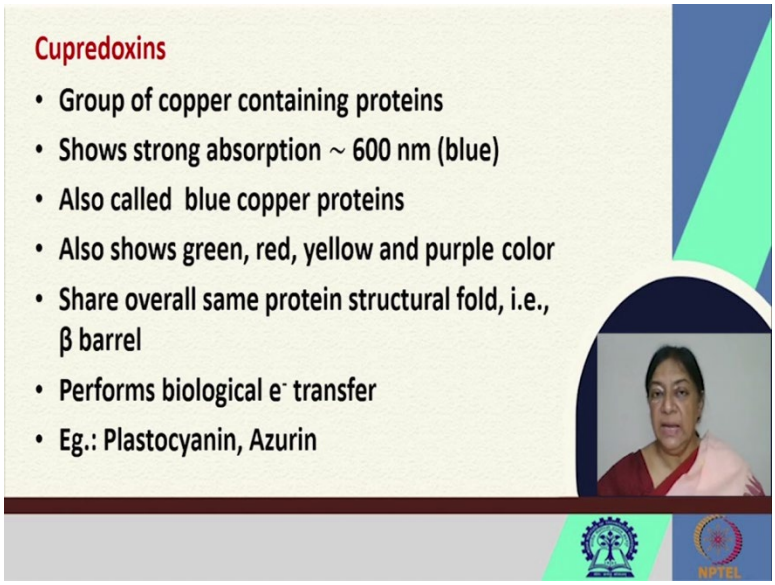

In cupredoxins, we see these are a group of copper containing proteins. They show a strong absorption at around 600 nm giving a blue color, which is why they are also known as blue copper proteins. In addition, they could also show other colorations depending upon the coordination and their conjugation that could change the absorption characteristics.

They share an overall β barrel type structure in a specific protein structural fold. And they all perform a biological electron transfer. Examples of such proteins are plastocyanin and azurin.

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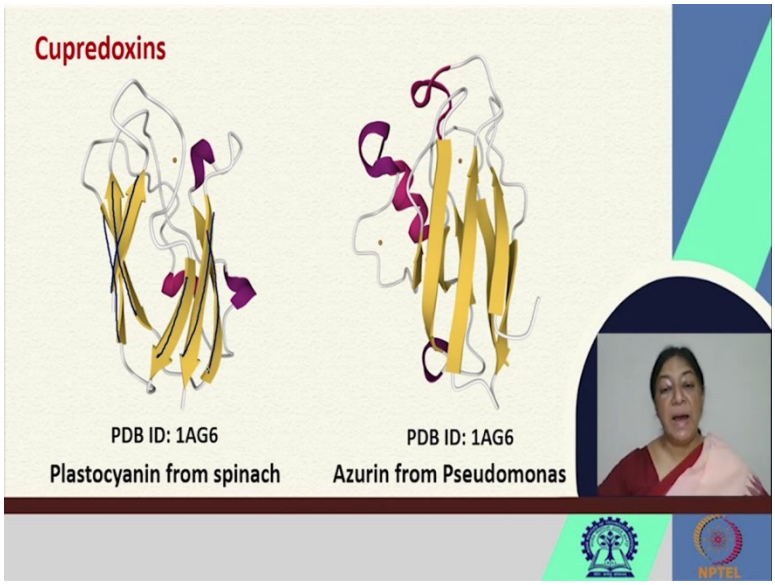

This [refer to slide] is a structure of the plastocyanin from spinach and we have the structure of azurin from pseudomonas. As you can see, there are specific strands; the β strands that we see with the specific proteins that show the two structural aspects here. So we have the β strands that are in this fashion, where we have a typical β barrel formation because of the β strands.

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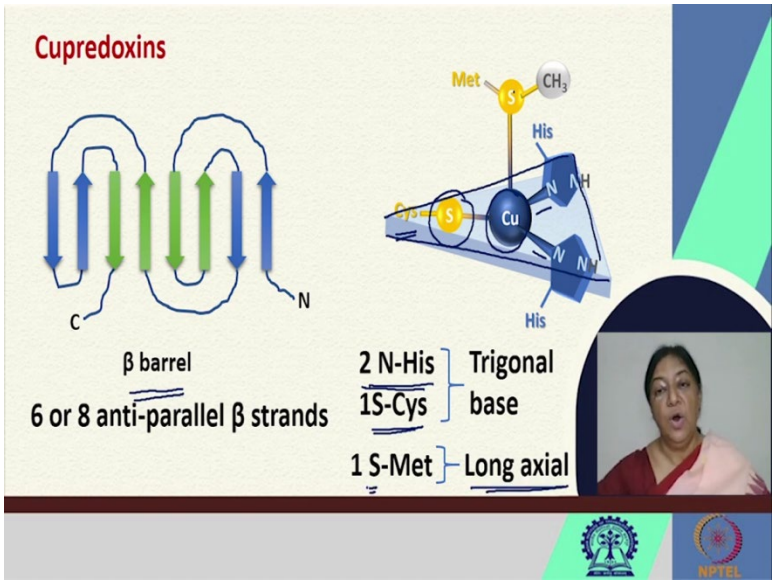

So when we look at this β barrel formation, we have typically 6 to 8 anti-parallel β strands in this β barrel. The structure of cupredoxins sees the formation of a trigonal base. The coordination that we see [refer to slide], is a sulfur from cysteine and nitrogens from 2 histidine imidazole moieties. We see therefore, a trigonal base. In addition, we have a coordination with a sulfur atom from methionine, giving us the long axial coordination of the sulfur of the methionine group.

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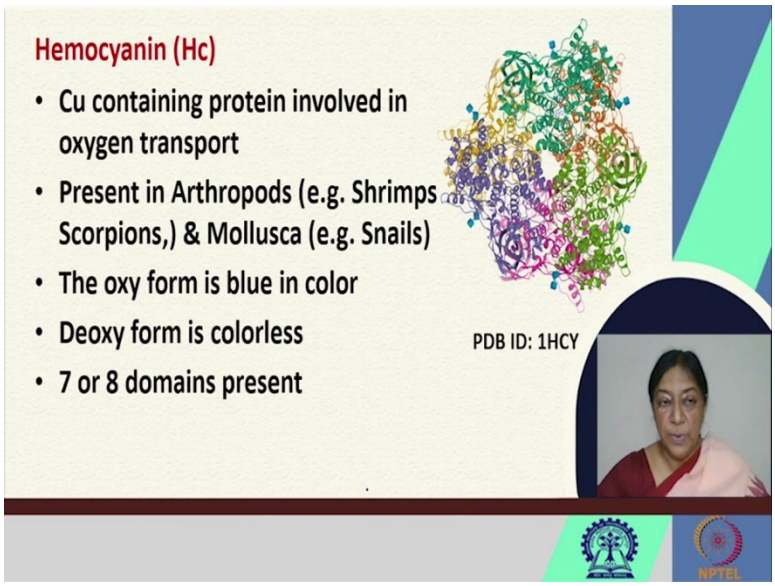

The hemocyanin is another protein that also contains copper and it is involved in oxygen transport, much like hemoglobin. The difference being that hemoglobin has the heme groups that have the iron that render a red color to the red blood cells. This hemocyanin is present in arthropods and molluscsa and the oxy form is blue in color. However, the deoxy form is colorless. It has 7 or 8 domains present and is involved in oxygen transport.

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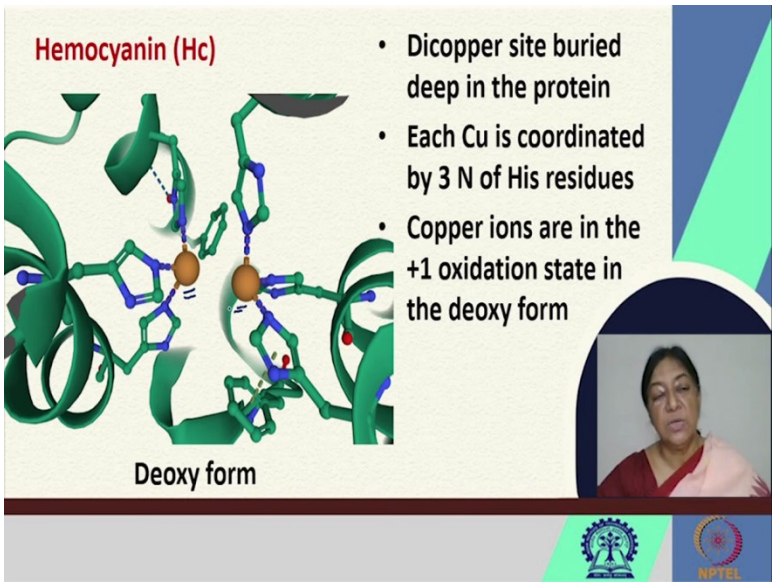

When we look at the deoxy form that is colorless, it has a dicopper site; this [refer to slide] is the dicopper site that is buried deeply in the protein. Again, if we look at the coordination we see that the coordination is through the nitrogen atoms present in the imidazole moiety of histidine residues. So, each copper is coordinated by 3 nitrogen of the imidazole group, that is the side

chain of histidine residues and the copper ions are in the +1 oxidation state in this deoxy form, that is colorless.

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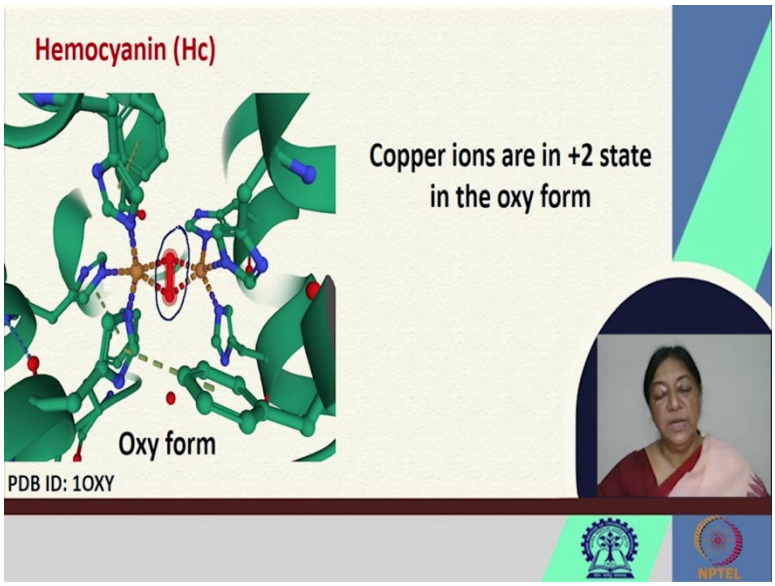

When we look at the oxy form, the copper ions here are in the +2 state. This is blue in color and we have a special coordination where we have the oxygen molecule bound to the copper ions present that are there in the +2 state, rendering it blue in color

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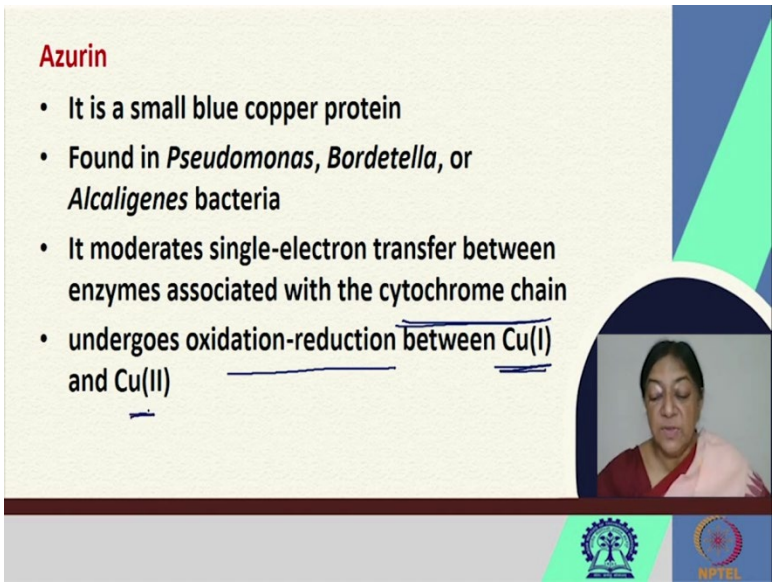

Azurin is another small blue copper protein found in Pseudomonas, Bordetella or Alcaligenes bacteria. It moderates the single electron transfer between enzymes associated with the

cytochrome chain. And again it undergoes oxygen reduction between the +1 and the +2 states. So we have the Cu(I) and the Cu(II) states.

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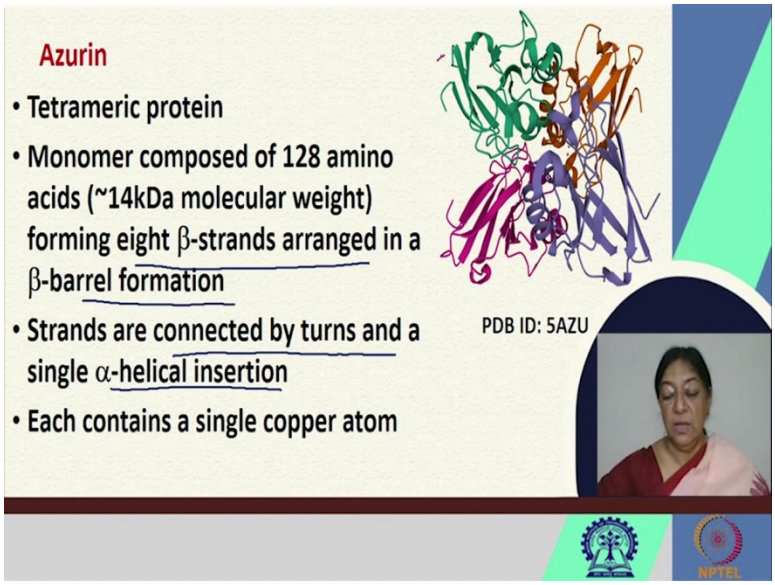

It is a tetrameric protein and the monomer is composed of 128 amino acids. Again we see the formation of a β barrel from 8 β strands. These strands are connected by turns and a single α-helical insertion that holds this barrel together and each of these contains a single copper atom.

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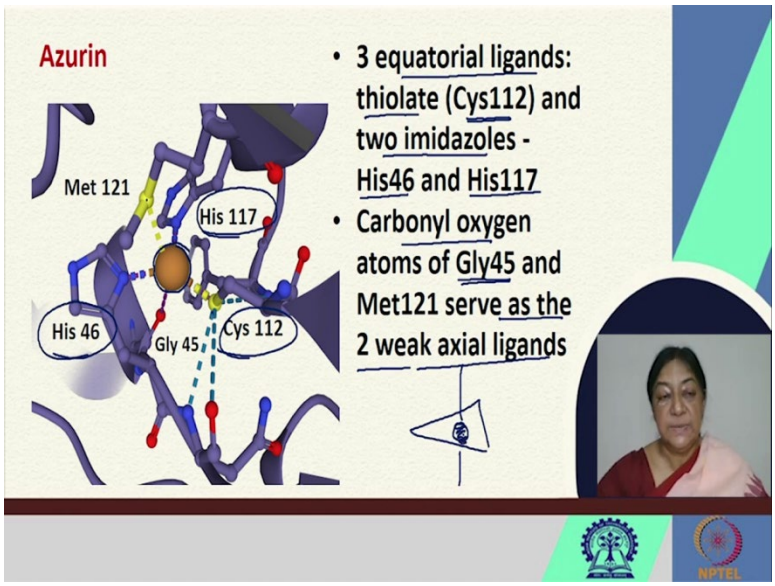

So, here [refer to slide] we see the copper atom. There are 3 equatorial ligands; thiolate that is the sulfur from cysteine 112, two imidazoles that is the histine 46 and histidine 117, here is histidine 46 and histidine 117. This is the thiolate group from cysteine, the sulfur that we see. The

carbonyl oxygen of atoms of glycine 45 and the methionine sulfur, serve as the 2 weak axial ligands. So again we 3 equatorial ligands and we have 2 axial ligands that hold our copper atom in place.

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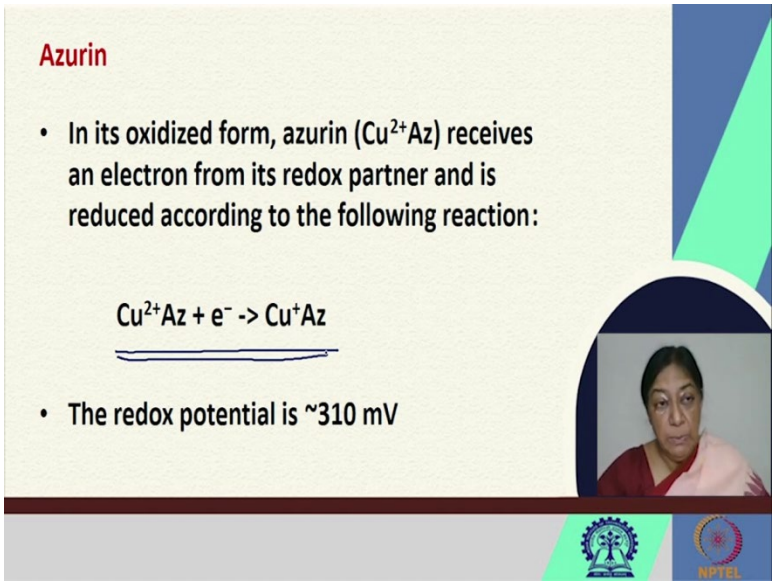

In its oxidized form, Cu^{2+} azurine receives an electron from its redox partner and it is reduced in this specific reaction $Cu^{2+}Az + e^- \rightarrow Cu^{+}Az$, where the redox potential is approximately 310 mV.

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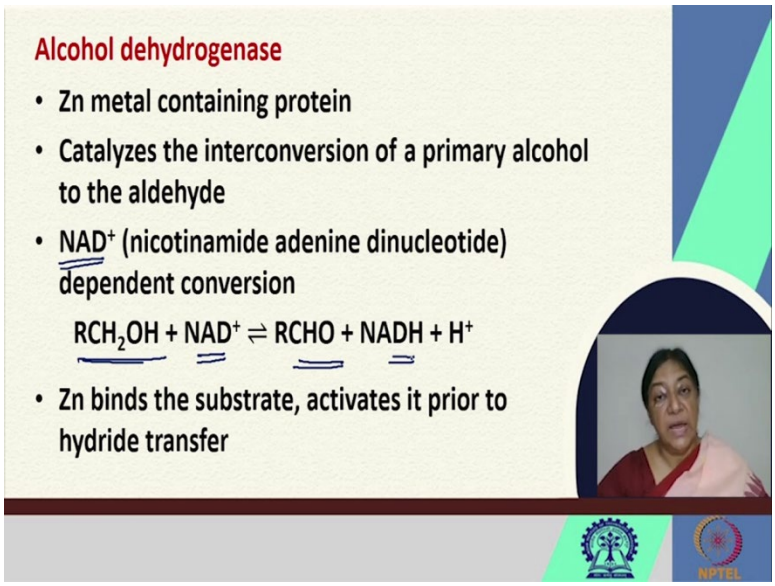

Alcohol dehydrogenase; another example that we will be looking at, contains zinc. It is a zinc metalloprotein and this catalyzes the interconversion of a primary alcohol to the aldehyde. It has

a dependency on NAD^+, that is part of the enzymatic reaction: $RCH_2OH + NAD^+ \rightleftharpoons RCHO + NADH + H^+$ The zinc binds the substrate and it activates it prior to hydride transfer.

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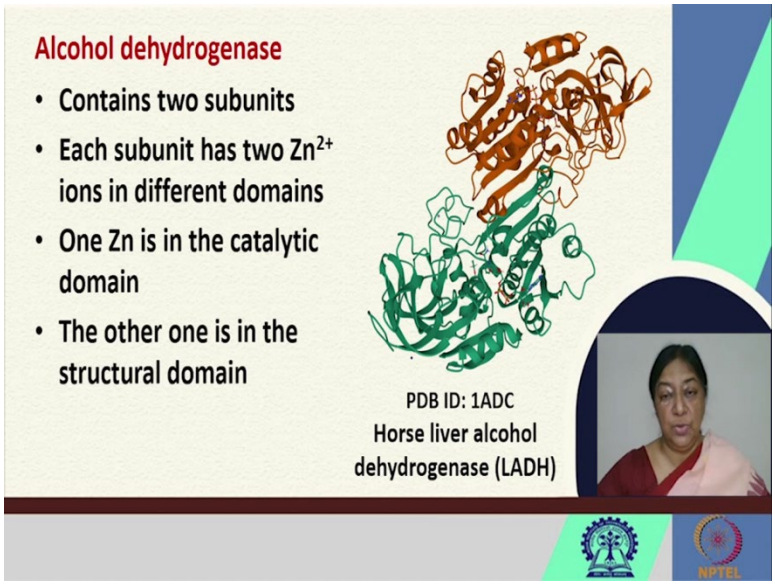

The reaction is such that the protein molecule contains two subunits and each subunit has two zinc ions in different domains. One zinc is in the catalytic domain and another zinc is in the structural domain.

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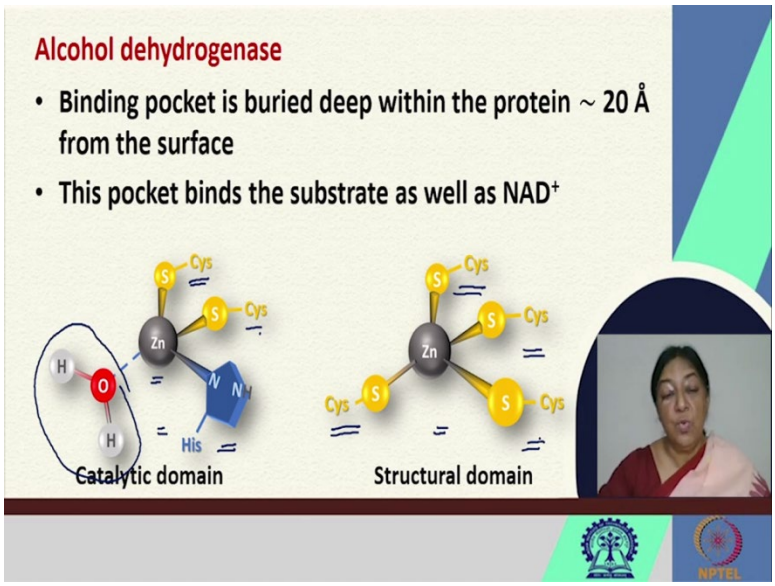

The binding pocket is buried deep within the protein approximately 20 Å from the surface of the protein. The pocket binds the substrate that is the alcohol, as well as NAD^+. So this [refer to slide] is where we have our zinc metal ion. We have the coordination through the sulfur of the

cysteine residues and the histidine nitrogen atom from the imidazole moiety. We have a water molecule coordinated in a four coordinated structure, a tetrahedral structure.

So we have a catalytic domain and we have a structural domain zinc There. are two zinc molecules and in the structural domain we have four cysteines that provide their sulfur for the coordination of the zinc.

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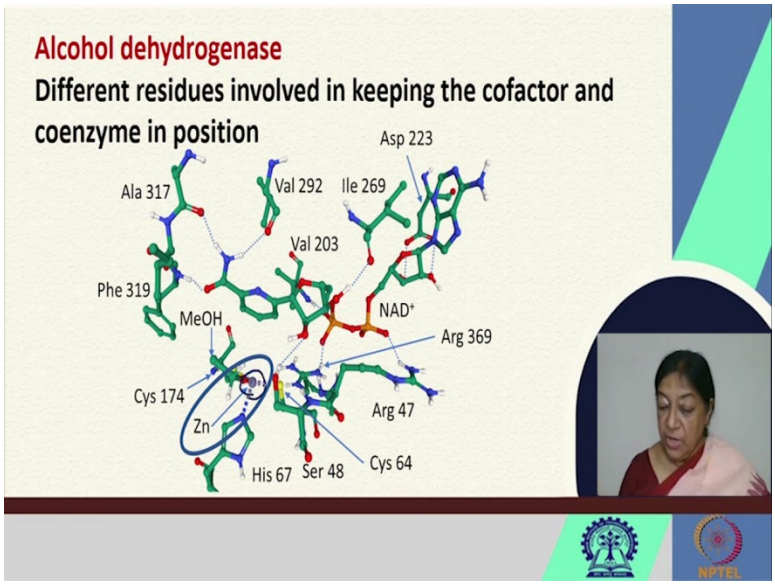

The different residues are involved in keeping the cofactor and the coenzyme in position and we can see there is one catalytic zinc and one zinc in the structural domain.

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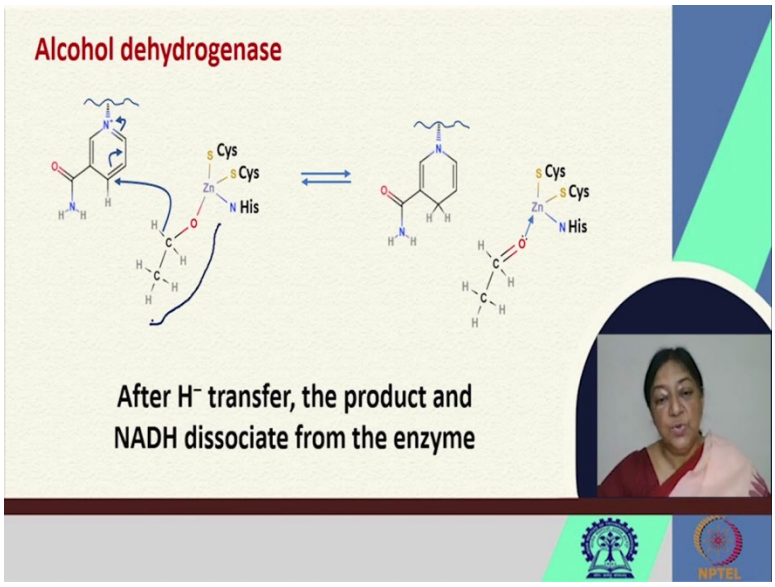

The overall reaction is such that we have our association here [refer to slide] with the primary, where the location is with the primary alcohol. We have now a transfer of a hydride ion, and after hydride transfer the product and the NADH dissociate from the enzyme.

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The role of the zinc here is to facilitate the deprotonation of the alcohol in the initial step and it positions the substrate in a manner, that minimizes the stereo-electronic factor that leads to the direct hydride transfer to the coenzyme.

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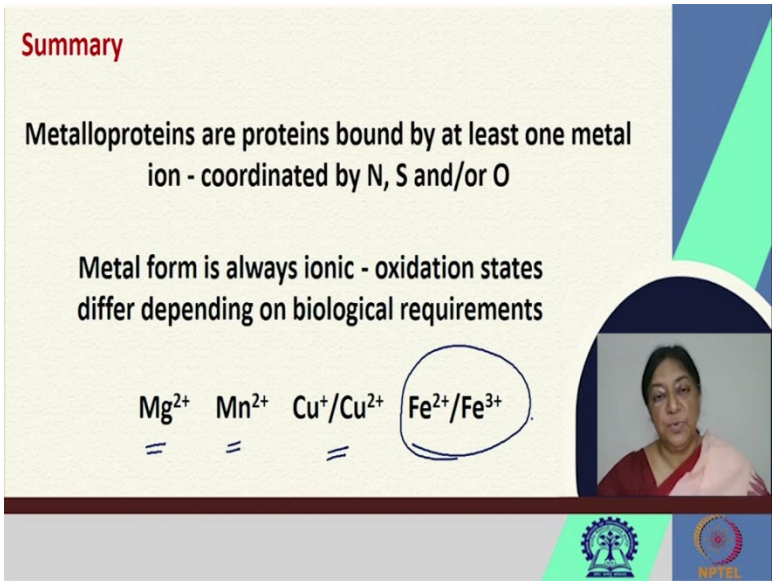

So what we have seen in this 1st lecture on metalloproteins, is an understanding that these metalloproteins are proteins that are bound by at least one metal ion, that is coordinated by

nitrogen, sulfur and/or oxygen. The metal form is always ionic and the oxidation states differ depending upon the biological requirements. For example, if a specific oxy-deoxy form were to exist, we would have different oxidation states associated with this.

We have looked at magnesium, manganese, copper and zinc examples. In our next lecture, we will be looking at specifically iron examples, the most well studied metalloproteins.

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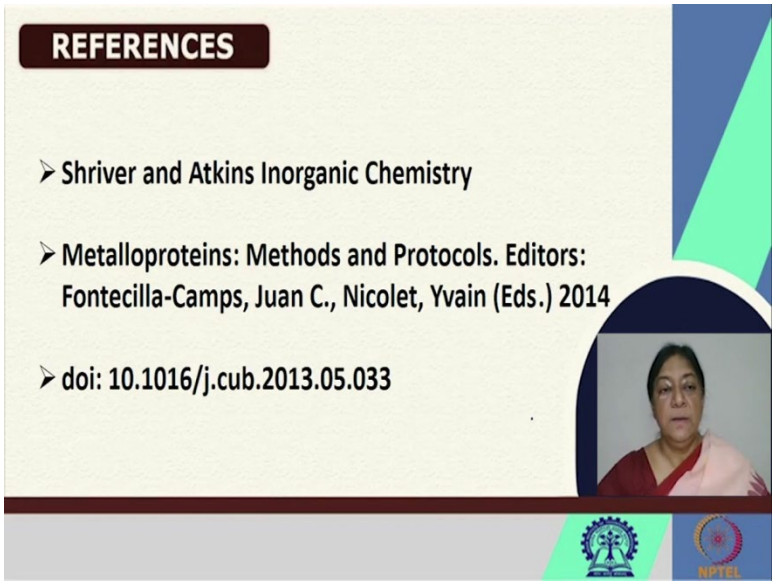

These [refer to slide] are the references that have been followed.

Thank you.