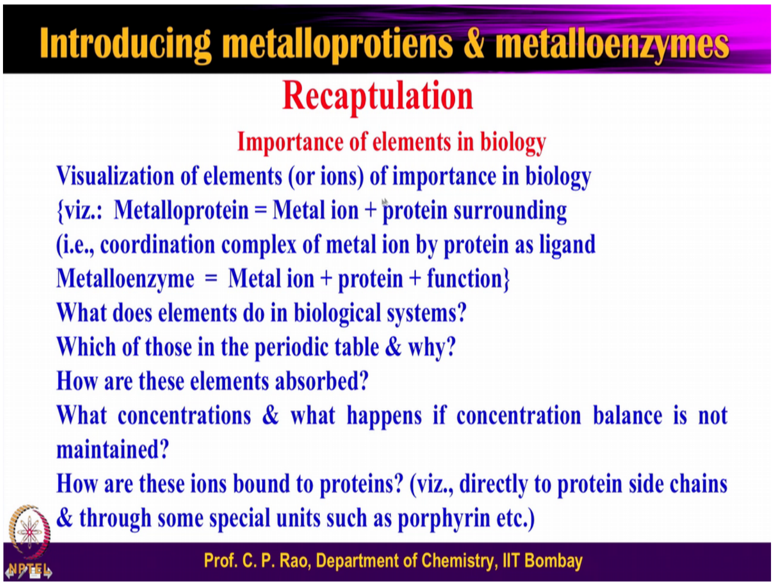
Inorganic Chemistry of Life Principles & Properties
Prof. C. P. Rao
Department of Chemistry
Indian Institute of Technology, Bombay

Lecture – 15
Recap on metalloenzymes

Good morning, welcome you all to the next lecture on Inorganic Chemistry of Life Principles and Perspectives. Let us first initially have a little recapitulation of what that in the past several lectures. During the past several lectures I tried to give you basics of the elements of their importance in the biological systems.

(Refer Slide Time: 00:42)



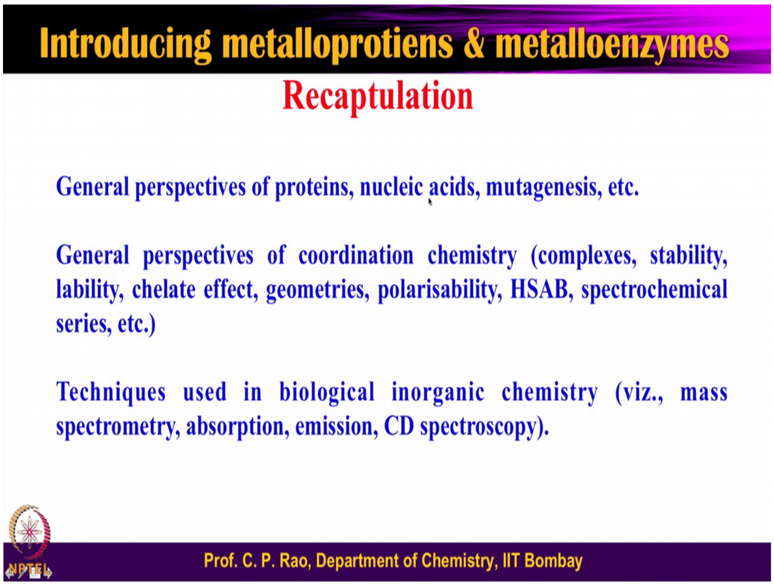
First of all metalloprotein; I have try to give you a feel a metal ion surrounded by the protein, a metal ion bound by the protein side chains etcetera and, the protein surrounding the next. So, it is basically a kind of a coordination complex of metal ion by a protein as a ligand and, then followed by that so you can also define metalloenzyme so, metalloenzyme will be the metal ion the proteins surrounding and the function.

So, therefore, you have a function for that and then I have also have given what does this elements do in biological systems, which of these in the periodic table are important are essential for the biological process, why are they essential to the biological process, how these elements have been absorbed? All of these we have looked at what concentrations, what will happen, if the concentration is imbalanced in the body.

When we look at some of these concentrations can be greater, can be lower than what you what will happen when it is lower, what will happen when it is a higher. So, the kind of a syndromes that are expected out of these concentrations, we have all looked at. So; that means, a particular concentration it should be balanced and the balance should be maintained. So, the lower will be a problem, the higher will be a problem. So, those kinds of things I have already highlighted and how are these ions bound.

When you say that they are in the protein how they are bound? So, they are directly go to the side chains, or they are bound to the sub special units like porphyrins etcetera. So, various kinds of things are possible. So, having completed that then I went into explaining you the basic very basic things of proteins, nucleic acids, mutagenesis all those kinds of things, protein structures, protein synthesis, nucleic acids structures and mutagenesis (Refer Time: 02:38) mutagenesis all these then utility in the context of inorganic chemistry of life.

(Refer Slide Time: 02:31)



So, then followed by that, I also try to explain certain basic aspects of coordination chemistry because, this is a biological inorganic chemistry, where the inorganic chemistry of life. So, inorganic chemistry of life is inorganic chemistry in the presence of the biological systems. So, therefore, then one need to look at how these inorganic ions bind, interact, coordinate with the biological systems.

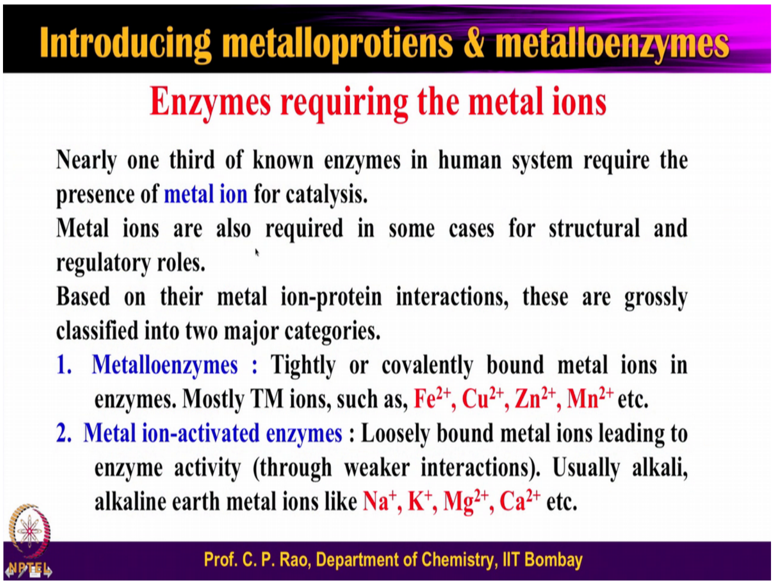
So, in that context the coordination chemistry comes in to picture therefore, I have tried to give some very basic things like. What is a complex, how the complex is found, what is the stability, complexation stability, chelate effect, geometries theirpolarisability, hot soft acid base concepts, spectrochemical series all of these I have explained and all of these have a direct relevance in this particular course.

If somebody require a little additional you know support, the books that I have given you can please go through that, little bit refresh your 12th standard, or first year BSC coordination chemistry is good enough, if you are knowing that much is good enough, but you need to know much. After that I have talked about their techniques used in the biological inorganic chemistry, various techniques like mass spectrometry, absorption spectroscopy, emission spectroscopy, CD spectroscopy, EPR spectroscopy, mass boir spectroscopy very of electronic type, nuclear type all this kinds of things that we look.

We also looked at some life time measurements; we have also looked at the microscopy techniques. So, variety of techniques which are used as a biological inorganic chemistry or inorganic chemistry, which is playing a role in life, where one needs to map the molecules, or look at the processes that are happening by spectroscopy, what kind of a species are generated, different oxidation states of the species, their electronic properties their magnetic properties. So, all of these I have already talked at a very basic level, which is just sufficient for this particular course.

Now, having completed let us now start with the next topic basically on the enzymes, where the metal ions play a role; before that let us look at a few carbon aspects.

(Refer Slide Time: 05:12)



So, enzymes; obviously, we are talking about the enzymes containing, requiring the metal ions; in other words the enzymes which we are going to talk in this particular course, those where the activity is coming from the metal ion.

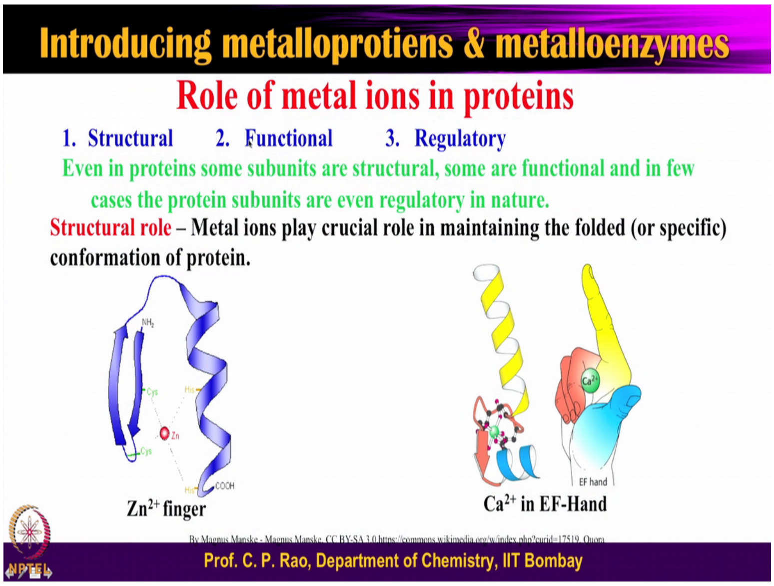
So, unless the metal ion is present that a particular enzymes is not active that is a first and foremost that you should keep in your mind, to the throughout the course entire course this one ok. So, therefore, the metal ions are required, not only for the enzyme activity enzyme activity is also known as catalysis. So, it becomes enzymes act like a catalyst, I will come back to the catalysis part after a couple of slides, just hold on that part of it. In a mean while let us see they are required in these enzymes not only for catalysis, but also for substation roles, as well as for the regulatory roles; we will understand this as we keep going through this slide and next slide much more better ok.

So that means, these metal ions interact with their proteins and give their function, or induced function functional aspects. So, what are the ways by which this metal ions can interact with the proteins? One of them is referred as the kind of an interaction which is like a covalent or tightly bound. So, covalent and tightly bound to the proteins, such kind of things are referred as metalloenzymes like transition metals like, iron, then copper, the cobalt, nickel all these kinds of things manganese, zinc all of these kinds of ions, which are transition metal ions can directly bind to the metalloenzymes very much similar like that of a covalent situation.

So, therefore, they form covalent complexes, with the protein and result in the form of the metalloenzymes. This is another class where the metal ions are not so strongly bound, but interact; interacts about weekly probably by the ion with the dipole ion it is a ion. So, mostly ion dipole is the once which are important. So, this is all weaker interactions. So, we are talking about the weak interactions, which will play a important role and these are generally seen with the alkali, alkaline earth ions, like sodium, potassium, magnesium, calcium these are the ones which are important in the biological systems.

So, generally we can classify the interaction of the metal ions leading to metalloproteins metalloenzymes as metalloenzymes, where the metal ions are bound by covalent metal ion activated enzymes, where the metal ions are not covalently bound, but weakly interacting with the system and there by activating the protein to form an enzymatic activity of this.

(Refer Slide Time: 08:12)



Let us look at as I said that there are different kinds of roles played by the metals ions in proteins, metal ions can be played a catalytic role which is called functional.

Sometimes some of the metal ions not necessarily show any function, not necessarily show any catalysis, but can show some kind of a structural integrity, structural rigidity, structural integrity. So, therefore, these kind of a structural rigidity and integrity is also possible by some ions, some ions can be showing a catalysis or functions, some of them may be regulating the functions of the protein 2.

So, we have the metal ions as a structural, metal ions as a functional, metal ions as a regulatory. Similarly even in the protein, we do that I have already showed you in the protein primary structure, secondary structure, tertiary structure and quarterly structure in the past. So, if you look at the proteins may have some subunits and some of the subunits may have a structural component, some of the subunits may have a functional component, some of the subunits may have a regulatory component, all three are not required in all proteins all enzymes.

Some enzymes may not have any structural necessity, functional is enough some proteins may have both functional structure, some proteins may have functional and regulatory and, some proteins may have structural, regulatory and functional all three. So, you can have either functional alone, or structural alone, or structural function together, or structure and function and regulatory together.

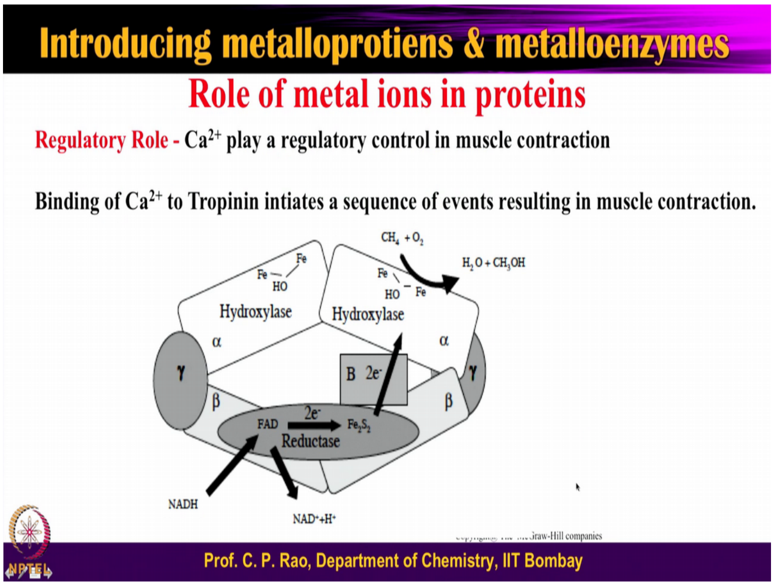
So, all these kinds of combinations are possible in the metalloproteins and the metalloenzymes. Look at one example here, this is called zinc finger; I will come to the characteristics of this, when I come to the zinc story. So, because I am going to have a separate chapter on zinc, how this zinc can also play a role in the biological systems, but till then what is important to be here is on this right side, you can see there is a helical component.

On the left side you can see a ribbon like. So, this is a beta sheet this is alpha helix and, these two are tied together through anion zinc through certain amino acid residues side chains, cystine, cystine and histidine and then histidine. So, these kind of thing so, you have so, what this called? This is a structural rigidity. So, it brings the two components of the structure one is the beta sheet, other is a alpha helical these two structures are held together by the zinc ion.

So, therefore, in this particular case zinc is referred as a structural element in this. Similarly you can see an example here, there is a alpha helical component, which is shown in the yellow color, there is a another alpha helical, which is shown as the bluish kind of color and, there is some other region which is open kind of a region and that is shown in this kind of a cyan color and there is some beta sheet also.

And in this region you have a zinc; so zinc ion is holding this region. So, that the alpha helix alpha of this and this alpha helix are more or less perpendicular like this thumb role of this. So, what is the role of this one? The role of this one is a structural role. So, structurally it is holding this part of the protein, with this part of the protein together with respect to the metal center. So, you could see very well this structural component of this.

(Refer Slide Time: 11:49)



And let us look at the role of the metal ions, even beyond the structure you can look at even the regulatory.

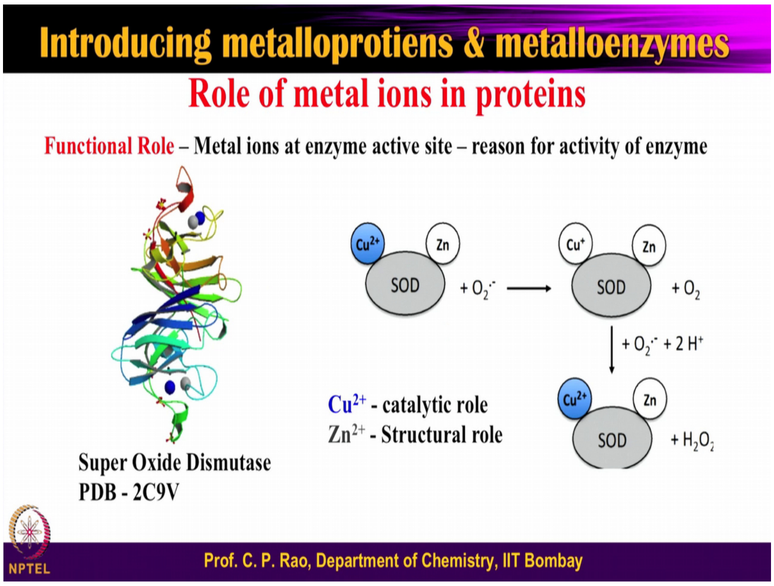
For example calcium 2 plus plays a regulatory role in the muscle contraction ok. So, binding of calcium to tropinin is one of the protein, which initiates a sequence of events and all this sequence of the events will result in the muscle contractions. So, after several slides I will be explaining the muscle contraction, but right now let us take it as granted, then the calcium plays a regulatory role in the muscle contraction, how it plays a regulatory role we are going to see later stage.

I have given another enzyme here, which I will talk to you when we come to the iron story is called methane monooxygenase; methane monooxygenase is a protein, or an enzyme which converts the methane to methanol. So, this enzyme converts the methane to methanol and containing higher, but there are the three possible kind of a units are there, there is one unit where is the hydroxylase property showed this particular unit, there is that what alpha and this is a unit which is called reductase; where the electrode transfer takes place one of these. And then finally, your reaction takes place over there.

But you require coupling of all these things together, but this coupling of all this together is done by this protein, which is called the regulatory protein and that is called gamma here, gamma here, gamma here, this will regulate the alpha and beta, when should alpha should function when should beta should function. The beta is of one which functions to the electron transfer alpha is a one where the reactivity occurs and finally, the methane goes to methanol.

And out of the 2 oxygen, 1 oxygen is added to methane to become methanol, second oxygen goes as water. These details are explained not now, when we come to the individual metal ion stories of all these for example, iron case. Now, you understand from the previous one structure here regulatory.

(Refer Slide Time: 14:07)



Let us look at something on the activity or functional. So, as I said the metal ions also play a functional role; obviously, that is the most important one; that is a most primary thing of this metalloenzymes. Metal ions act active enzyme active site reason for the activity of the enzyme. Let us look at one particular example here; the details of functioning will be given later stage right now you can see one example of the enzyme. This enzyme is called superoxide dismutase. It has 2 metal ion centers, one is the copper, other is the zinc; there are two ions here, you can see there is a two ions are here because this is a dimeric protein.

So, in each monomer there is a bimetallic centre, the copper center, the zinc center both the copper center and zinc center. So, the copper is shown in the blue form, zinc is shown in the non blue form so these are the ones. So; that means, this enzyme can react from this part of the subunit, as well as this part of the subunit; what will it do? It converts the O 2 minus dot, which is called superoxide radical into the form of at the end to H 2 O 2.

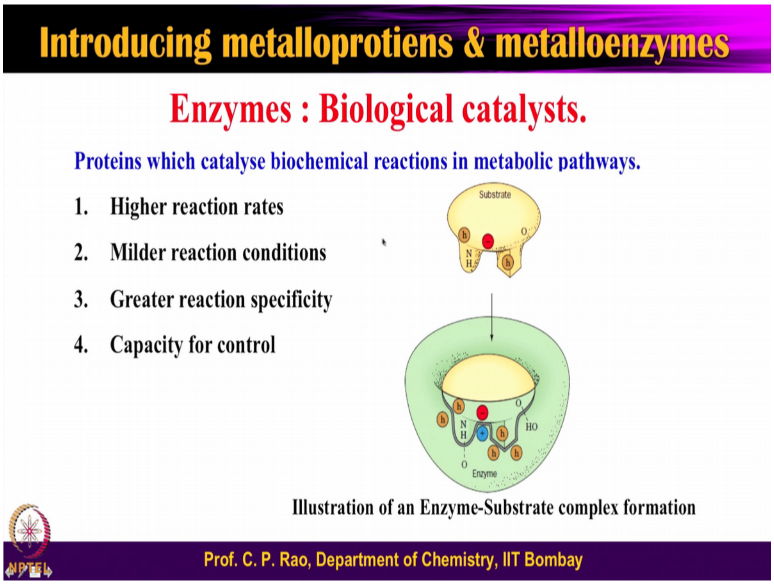
So, O 2 to O 2 minus dot is a very reactive because, the radical. It can react with the tissue, it can spoil the tissue. And therefore, the body has a mechanism of diverting this particular species, basically this mutane this kind of a species to less harmful species which is H 2 O 2; H 2 O 2 is also harmful, but much less level of harmness as compared to the O 2 dot minus.

So, that is superoxide radical. Now, let me come to the explanation of the 2 metal enzymes, 2 metals ions containing in these present in enzymes 1 is copper 1 is zinc. Actually the zinc is also present in the zinc 2 plus form and zinc 2 plus is required for a holding the structure of this unit regime in a particular conformation.

So, the conformation is frozen by the presence of zinc 2 plus. If I remove the zinc 2 plus conformation is collapsed. And the copper two plus is actually the centre, where the reactivity occurs. Suppose I remove the zinc 2 plus and allow only copper to be their function will not be seen because, the structure (Refer Time: 16:40) is important, or alternatively I remove the copper 2 plus allow the zinc 2 plus to be there and look for the activity that will not be there, because the functional unit is not there. Therefore, copper 2 plus is a functional, zinc 2 plus is a structural and in the first step, it will it will convert into O 2 and second mole of additional the O 2 dot will make it H 2 O 2.

This mechanism will be explained to you later on in several cases, in case of iron in case of manganese, in case of copper zinc, I will be explaining later. So, you will understand this much better later stage not now right. Now, I wanted you to understand, there is a copper 2 plus center which is a catalytic role, zinc 2 plus center which as a structural role. That is all the information that you require from this particular slide at this stage, but later stage I will give more details of this two ok.

(Refer Slide Time: 17:36)



Now, we are taking about the enzyme you know is a biological catalyst. So, any biological catalyst; what will a catalyst do? It will enhanced the reaction rates, it will work at a much normal conditions not necessary any abnormal conditions like the chemical reactions that you require, reactions that require any industry not like that it is a much more milder, much more easier, much more safer kind of a conditions. And it will also give greater specificity towards the reaction centers, or towards the substrates etcetera it will also have a capacity to control the reaction.

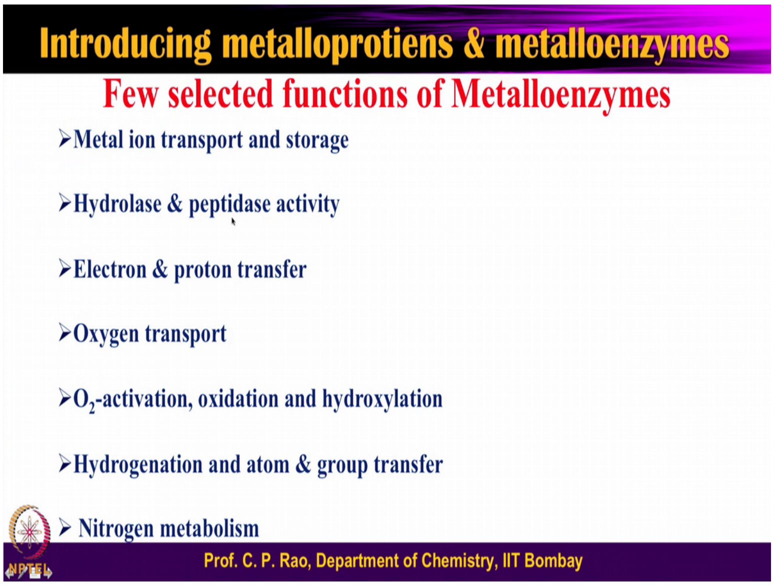
So, specificity and control all of these are shown. So, to have an idea of what an enzyme you see if you take this an enzyme and, you consider this is an active site. So, therefore, obviously, at the active site you expect the substrate to interact. So, substrate come to this and interact with this and this is like a substrate, it is a enzyme this kind of a lock, this kind of key and lock and key kind of a mechanism.

So, that is not necessarily true because later stage this was a very preliminary model later stage models have talked about the flexibility in the protein, the flexibility in the in the substrate as well and also people have talked about the induced fit all those kinds of things. So, it is not necessary step to know at this stage all that, we need to know is the enzymes of biological catalyst, in our case the enzymes are metalloenzymes.

So, the reactivity is happening at the metal ion center and at the enzymes can do a higher rate of reaction, they can work at low at simpler conditions, easier conditions, normal conditions, at not any harsh conditions at all, they can show greater specificity they can show greater selectivity, they can show greater control of all this.

So, therefore, we are going to work with such kind of a units in the entire course. So, you should understand when I say in enzyme ok, have you at looked at different kinds of the metal ions, they are the metal ions which form the rules of the covalent kind of a bonding.

(Refer Slide Time: 19:57)



And they are there are kind of a metal ions which know from the covalent bonding, but they interact with the metal ions with through some through proteins through some weak interactions and activate the proteins to convert it to enzyme.

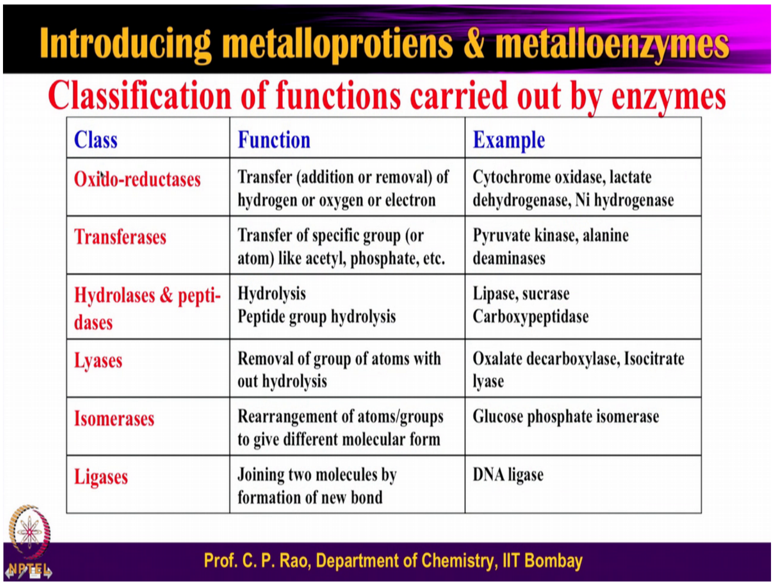
So, what kind of reactions are we talking about, what kind of functions are we talking about? Something like the oxygen transport, we know very well metal ion transport we know very well, their storages. We can also very important thing is hydrolases of many condensed modes, they we know that there are lot of peptidase in the body, lateral proteins in the body, the proteins have to be broken to make into the innovative amino acids because, they are required for the synthesis of the protein.

So, therefore, this peptidase kind of thing, we need for all of these we need some electron transfer, we need also proton transfer and there are some cases both electron and proton will occur together the in the protein together as a coupled kind of a system.

So, you also not sufficient enough to have hydrolysis peptidase, you also need the oxidation reactions, to get the oxygen attached to the substrate you need oxygen to be activated O 2 to be activated, this can bring oxidation, this can bring hydroxylation. And it is only not oxidation you can also have a reduction, reduction can come into the from hydrogenation, reduction can be like removal of oxygen it can be reduction, addition of a hydrogen is also reduction all of these kind of things hydrogenation removal of oxygen.

So, therefore, these are called oxidoreductases and atom and group transfers across the different substrate to the product, substrate to the product kind of a group transfers, all of these atom transfer group transfer and metabolism, metabolism of carbon, metabolism of nitrogen, metabolism of oxygen, metabolism of sulphur; so, many kind of metabolisms. So, in almost all these kind of functions the metal ions are involved, metalloenzymes are involved in all of these things. Let us look at a little bit more closer or a specificity of these.

(Refer Slide Time: 22:13)

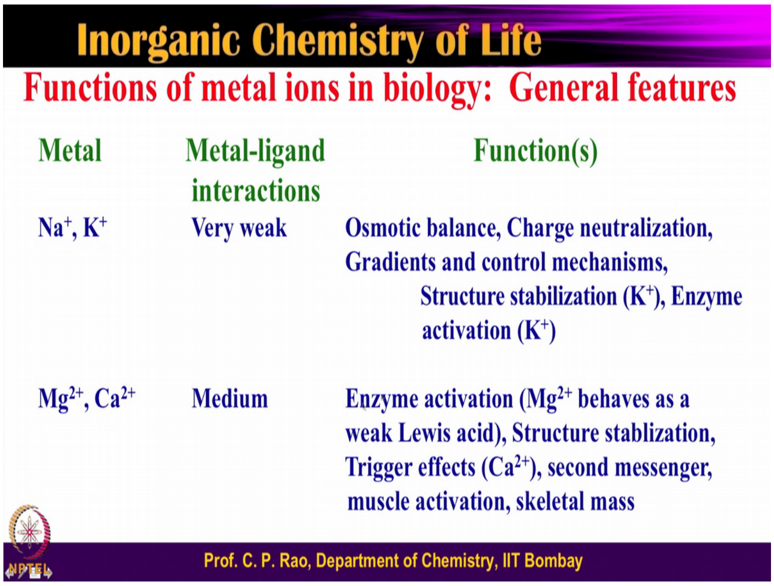


So, let us look at the classification. So, the all these functions can be classified into oxidoreductase; that means, they are involved in oxidation, they are also involved reduction transferases, they are involved in transferring from one to the other, there hydrolysis, they are involved in the hydrolysis peptidases, they are involved in the peptide bond hydrolysis peptide bond breaking, lyases they are involved in removal of some groups atoms without the hydrolysis atoms of groups without the hydrolyses system; isomerases you know the isomerization, it can be an atom, it can be a group from one to the other, their molecular formula will be the same, their structure will be different.

So, therefore their functions is also going to be different, ligases you can join and form a bond 2. So, in general the metalloenzymes can be classified as a oxido reductases, as transferases, as a hydrolases, as peptidases, as lyases, as isomerases, as ligases ok. So, there are some examples are given many of these examples are we are going to deal with as we keep going to the each individual metal ion storing in that.

So, right now I am not going to read all of this, I just told you oxidation process reduction process, transferring a group, hydrolyzing a condensed bond, or peptide bond hydrolases, or removal of subgroups, or isomerising the things, or adding things together to form a bond etcetera. So, all of these are important classes of enzyme functions.

(Refer Slide Time: 23:48)

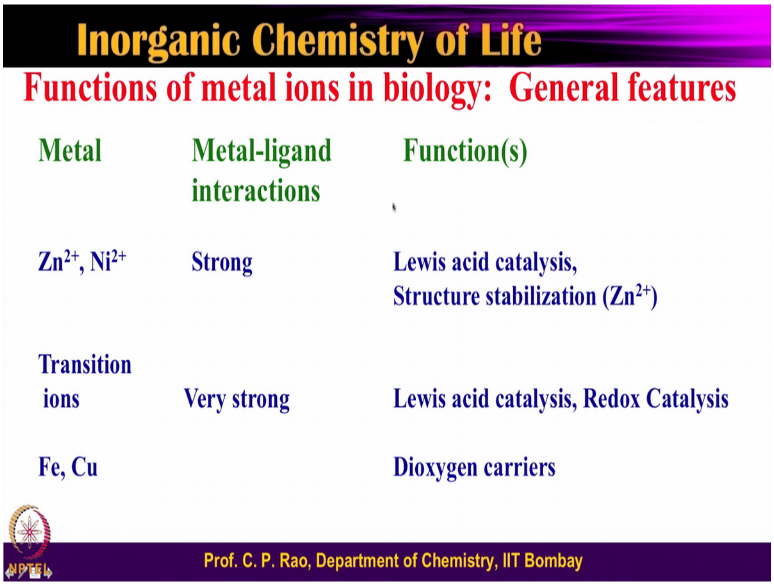


Let us look at how these are monitored or benured by the metal ions. So, we see that the sodium, potassium their interaction with the proteins is very weak, they are involved in osmotic balancing, osmotic pressure balancing, they also are involved in charge neutralization, they can bring gradient in the charge, control because of a gradient the potential is vary therefore, they will controls and mechanisms, structure stabilizations and enzyme activations. So, some of these kind of activities are showed by the weekly interacting ions such as alkaline ions sodium and potassium ions.

Let us look at next set of ions, magnesium and calcium; magnesium and calcium their interaction with the proteins a bit more stronger than the sodium and potassium, that is why I written as a weak medium kind of a interaction. So, we talked about very weak the medium kind of an interaction. So, with this kind of an interaction the magnesium and calcium able to activate the enzymes because, you know the magnesium is Lewis acid calcium is also a Lewis acid 2 plus, we have talk about all of these.

They can stabilize a structure calcium can be even trigger the functions, calcium can acts as a secondary messenger, I will explain after sometime in some lecture, secondary messenger, muscle activation, as I told you skeletal mass. So, all of these different kinds of functions are generally done by the sodium potassium, alkaline ions magnesium calcium, alkaline earth ions.

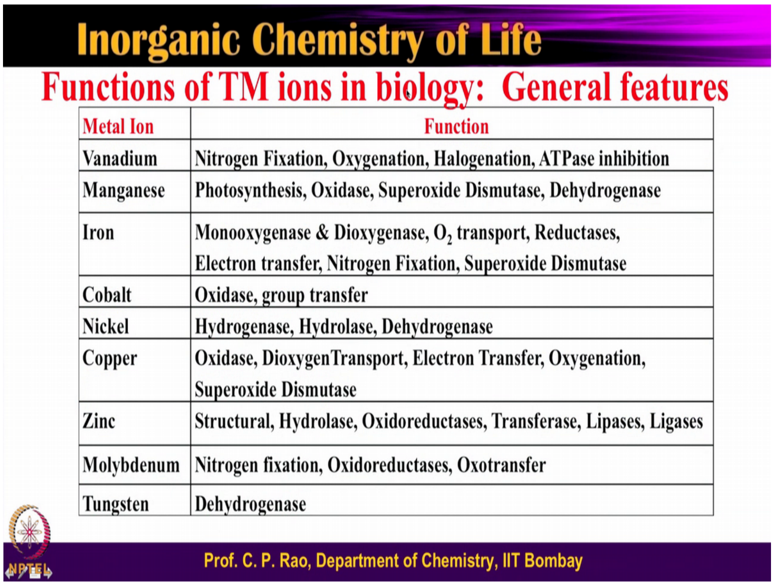
(Refer Slide Time: 25:32)



And we have a bunch of transition metal ions. The transition metal ions as you can see let us take zinc and nickel, they can bind very strongly. I told you a few slides prior the transition metal ions bind to the protein like a covalent bonds. So, those coordination complexes are basically covalent complexes. So, therefore, their interaction they with the proteins instead of saying the ligand, I would say proteins is strong of course, there are Lewis acid. And they can also go through redox nickel, but zinc. What about the other transitions ions like iron, copper, manganese many things and they can bind even stronger than these so we use the word very strong, they can do the Lewis acid catalysis they can also do redox catalysis ok

And many iron and copper is involved in oxygen transport, I will explain you once I come to the story of iron, where I take one subsection as the oxygen transport category. So, what I told in the previous to this is that we have a sodium potassium very weak interaction and magnesium calcium somewhat better interaction, zinc and nickel strong and other transitions metals ions are very strong. So, all of these are involved in activating the proteins to form enzymes to convert it to enzymes, to behave like enzymes. Let us look at bit more closely what are the kinds of functions this transition metal ions, can play while sitting in enzymes some very general.

(Refer Slide Time: 27:11)



Metal Ion	Function
Vanadium	Nitrogen Fixation, Oxygenation, Halogenation, ATPase inhibition
Manganese	Photosynthesis, Oxidase, Superoxide Dismutase, Dehydrogenase
Iron	Monooxygenase & Dioxygenase, O_2 transport, Reductases, Electron transfer, Nitrogen Fixation, Superoxide Dismutase
Cobalt	Oxidase, group transfer
Nickel	Hydrogenase, Hydrolase, Dehydrogenase
Copper	Oxidase, DioxygenTransport, Electron Transfer, Oxygenation, Superoxide Dismutase
Zinc	Structural, Hydrolase, Oxidoreductases, Transferase, Lipases, Ligases
Molybdenum	Nitrogen fixation, Oxidoreductases, Oxotransfer
Tungsten	Dehydrogenase

There is no specific, because specific things will come later stage. We will take a vanadium, vanadium is involved in the nitrogen fixation, you know the nitrogen is not directly utilized therefore, nitrogen should be converted and this kind of conversion is called nitrogen fixation into ammonia, you know we will be explaining not in the vanadium, I will explain you when it comes to the molybdenum story, but vanadium also is found, where in some microorganisms where the molybdenum is not available, vanadium enzymes do the same function as that of the molybdenum enzymes.

Vanadium is also involved in oxygenation, oxidative kind of a reaction halogenation and it can also inhibit ATPase activity I will be explaining all of these manganese is involved in photosynthesis, you in photo synthetic reaction photo system 2, there is a oxide evolution, is oxide evolution it comes from the manganese, I will explain all of those. Oxidase property, superoxide dismutase, I explained a while ago and dehydrogenases is again means removing hydrogen which means basically oxidation.

Look at the iron, iron can be involved in oxygenase property both mono, di; iron is involved oxygen transport, iron is involved in reductases, iron is involved in electron transfer, of course nitrogen fixation and superoxide dismutase, they are many different kind of a functions are possible by iron. Because iron is present both as the heme, type of enzymes non heme type more details will come up bit later, when I come to the story of iron cobalt, cobalt is very limited and it is found only in one case that is as a vitamin B 12 is a co enzyme only that case, but that co enzyme coupled with your large number of enzymes in function to give oxidase property group transfer.

The majority of the functions in the case of cobalt, or group transfer reactions which you will come to know when you come to the story of the cobalt, nickel is involved in hydrogenase means hydrogenation kind of thing, hydrolase means hydrolysis kind of thing and dehydrogenase which means removing the hydrogen means oxidation. So, nickel is involved hydrolases bond, oxidative bond, reductive bond all of these are there; that means, different nickel enzymes will show different kinds of properties, not properties functions let us say to be precise functions ok.

Copper, so copper is involved in oxidation, copper is involved in electron transfer, copper is involved in oxidization means oxidase the substrate copper is involved in superoxide dismutase, copper is also involved in oxygen transport, variety of reactions functions are exhibited by copper containing enzymes. We are talking about the enzyme functions containing these metal ions, the enzyme functions containing these, come to the story of this zinc.

Zinc is involved in hydrolase property ok, zinc is involved also in structural property; that means, that particular zinc which is in the form of the structural will not show any function, but it can show only the structural rigidity there are some zinc, where the catalysis the catalysis they can be hydrolase, it can be reductase also it can be oxidoreductases, it can be transferase, it can be lipases, it can be ligase a few slides prior to that I have explained you all these classes of this.

Hydrolase is the one which is involved in hydrolysis, oxidoreductases is the one which is involved in oxidizing substrate, reducing the substrate and the other categories is transferase either the atom, or the group is transferred from one to the other and we have also explained lipases and, then we also explained the ligases to ok. The removal of a groups without the hydrolysis making a new bonds in the ligases all of these have been explained already, it just mentioned already to you earlier, is talking about. Now molybdenum can be involved in a nitrogen fixation, I will explaining this very clearly when you come to the story of the molybdenum, then it is oxidoreductase property and it is oxido transfer properties.

So, molybdenum conatining enzymes are involved in nitrogen fixation, they are involved oxidation, reduction of the substrate, it can reduce a substrate, it can oxidize a substrate. They can transfer the oxygen from the protein to the substrate, from the substrate to the protein reversible and we can show all of these when we come to the story of the molybdenum. Then tungsten is not found in the normal systems, this is found only in high temperature lavas, in the high temperature lavas, there are there are some micro bonds which are stable and at that temperature like 300, 400 degree Celsius with the tungsten is present and, this is involved in the dehydrogenase.

So, overall what I tried to tell you in this particular part of the class is that, we there are general functions of the metal ion, metal ion containing enzymes, metal ions can bound to the proteins, in a covalent fashion and activate to get various function, metal ions can also be interacting with weekly and they are metal activated enzymes. So, metalloenzymes and metallo activated enzymes all both I have brought to your notice, also I have brought to your notice the metal ions can act as a structural type role, functional role as well as regulatory role.

And I have explained to you there are combination of these roles, not only for the metal ion point of view even from the protein point view, you have a structural part of the subunit, functional part of the subunit and as well as the regulatory part of the subunit I have explained all these.

Then I have come to the general class of functions the functions like hydrolysis, oxidoreductases, transferases, isomerases, lipases, ligases all these kinds of things are involved in these enzymes and then I explained to you the interaction of sodium potassium is very weak. So, and when you go to the calcium and magnesium, they are bit more stronger and, they can even activate the enzyme, they can even trigger the functions which I am going to comes very soon, then we will come to the transition metals, they might be very strong to very strong, they can do hydrolysis, they can do redox, they can do oxidative reductive kind of things and then all kinds of functions are possible and when you take the vanadium when you take the manganese iron, cobalt, nickel each of these metal ion, I will be taking the corresponding enzymes and explaining you later.

So, therefore, I am given you very brief and how these metal ions activate the proteins and convert the proteins into metalloenzymes, metal activated enzymes and simple classification of the enzyme activities. So, these things will go in the next 10 plus hour or so, explaining based using each one of the metal ion, each one of these metal ions which are involved.

Thank you very much.