**Inorganic Chemistry of Life Principles & Properties**
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**Lecture - 53**
**Highlights of the course - Part I**

Welcome you all to the next class on Inorganic Chemistry of Life Principles and Perspectives. So far, we have tried to more or less complete the syllabus that is assigned to this. So, therefore, what I thought was since, this is a course I better do some kind of a revision. So, this class maybe one or two more classes, I will be revising the whole aspects that I have talked about till now. In this particular course; course highlights; obviously, I cannot speak every aspect that I have talked for the past 40 - 50 classes or so,

Secondly, after completing that that I will start getting into the tutorial parts where I will explain the some questions, I will also address their answers those kind of things. So, with that the course will come to a conclusion ok; look at the I started with this particular slide in the beginning.

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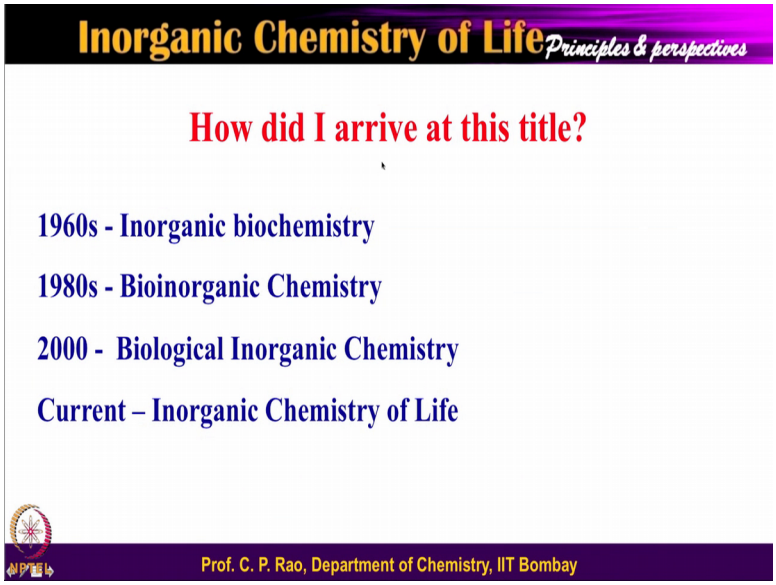


With a title that; how do I arrive at this particular kind of a title as I told you, it is started with in the late early 60s to late 60s, etcetera as inorganic biochemistry where

biochemists have identified some role of inorganic elements here and there therefore, they thought it is it is the inorganic biochemistry.

Then later on the inorganic chemists have come into picture and started looking at this with more details from the perspective of the inorganic chemistry and that has they have named it as a bioinorganic chemistry and so, by that time, the inorganic chemists have taken care of all this, then not only inorganic chemist, the biochemist, the inorganic chemist. A lot of physical chemists organic chemists have jumped into and started trying to make all kinds of studies spectral microscopy studies, comparisons model studies, all these and found that the ions of the inorganic ions have a role across the biological systems. So, therefore, this has taken innominate lecture in the in this particular century as the biological inorganic chemistry.

So, therefore, I thought what I would what I thought was that I take the approach of the biological inorganic chemistry, but add a few aspects to make it inorganic chemistry of life is that where I have taken into consideration the aspects corresponding to the inorganic elements inorganic compounds in the medicine inorganic elements inorganic compounds in diagnosis and therapy etcetera.

So, therefore, I have not only treated with the introduction followed by enzymes, but also I have treated with the these kind of things, as you can clearly see on the next slide very clearly. So, I have already taken you the introduction in the introduction I have covered a lot of aspects elements in biology which of the elements or responsible for biological processes and why all these kinds of things and the their coordination chemistry because their being the inorganic ions.

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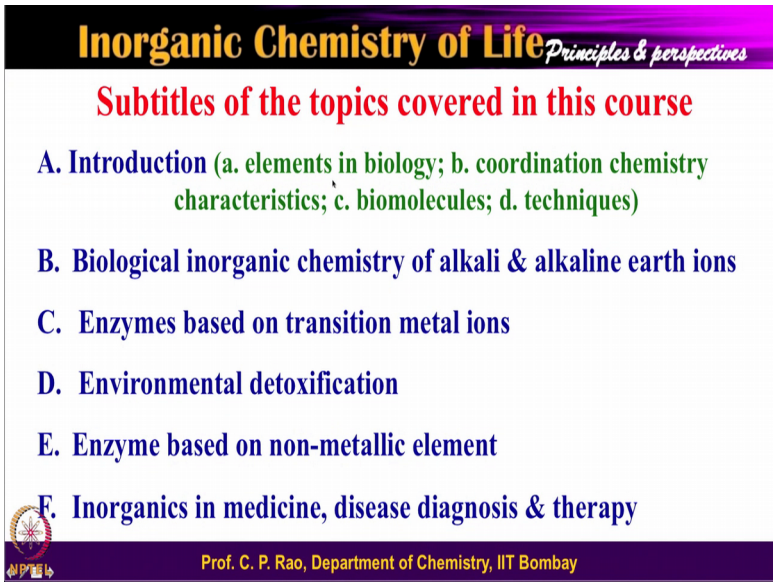


All the coordination chemistry characteristics; how do they support this particular aspect and simple simplistic coordination chemistry features also, I have talked to you, then I have talked to you biomolecules introduced to you by molecules like proteins, nucleic acids, etcetera, all of those and some relevances like protein synthesis and there are host of techniques from spectroscopy from analytical spectroscopy, microscopy life time all these kind of things. So, I have tried to give you a glimpses of all this.

So, therefore, the introduction that I have given you was a was a combination of the elements in the biological systems their coordination chemistry aspects biomolecules and the techniques, then I have explained you few classes on the biological inorganic chemistry alkali and alkaline earth ions anyhow, I am going to give the highlights of all of these again now in this and next one or two classes as I promised and then enzymes, then followed by that about 30; 30 plus classes or so, I have spent on enzymes based on the transition metal ions and then we have looked at some environmental kind of detoxification which is called the mercury mercury detoxification called mercury reductase ok.

That enzyme also we have looked at ok, then we looked at the enzyme based nonmetallic element which is selenium now the selenium in place of the cysteine sulfur instead that a cysteine without sulfur, but in the presence of selenium selenocysteine and that also we

have looked at and then towards the end very recent few hours, I have spent on inorganics in medicine inorganics in the disease diagnosis and therapy ok.

Now, I will go through some highlights of each of these, from now onwards a couple of hours of a or couple of classes that I will be doing that. So, please try to be more attentive for these kind of a highlights which of course, I taught earlier, but this will be in the highlight fashion in the.

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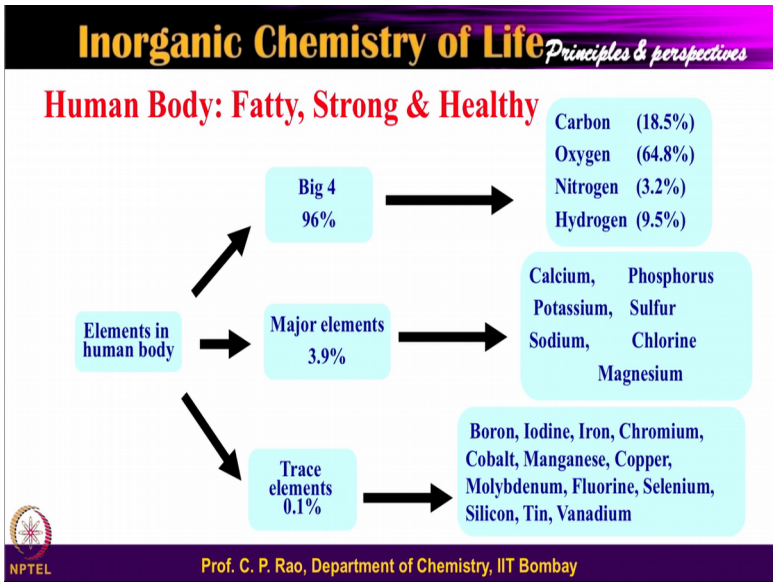


So, beginning the first thing that; I have talked to you is about the looking at the composition of the human body. So, the human body composition if you look at that there are elements which are called the bulk; so, bulk elements are carbon oxygen nitrogen hydrogen, if you take these carbon hydrogen oxygen nitrogen together is the; this is called Big 4 and this will constitute to something like a 96 percent of the weight of a body.

So, this is we refer in the fatty you see in the title fatty; fatty goes to that; then you see some major elements like calcium sodium potassium magnesium all these things and some few other things like phosphorus chloride sulfur which would lead to the anion which will lead to the cations. So, the cationic and anionic these ones and this is around 3.9. So, 96 plus 3.9 is 99.9 and these things other ones which you know you know the bones and all everything is the one which gives the shape to the body that what I am referring to strong.

So, these are the elements which give the strong and what is health. So, it is like a body, then you have a bones and then you have a health. So, the health is given by these trace elements. So, the trace elements itself is 0.1 percent are the total body weight; these are metals, these are non metals also both metals and nonmetals; as you can see about a dozen such elements are there and these elements actually play a great role in biological systems.

So, together I call the body as a fatty when you have carbon hydrogen nex oxygen nitrogen and strong when you have a calcium sodium potassium magnesium phosphorus, etcetera chloride these things and then I would say healthy when you have this trace and ultratrace elements present in the body and that is how we can probably from the point of view of the inorganic chemistry of life, we can classify this into this kind of a aspects effect.

So, all this so many other things I explained, I cannot go into everything, but I will only take to the highlights. So, having talked to you about the bulk elements and the strong the heavy elements and the elements which are present only in the trace and ultra trace elements there with the healthy kind of elements you can see the periodic table.

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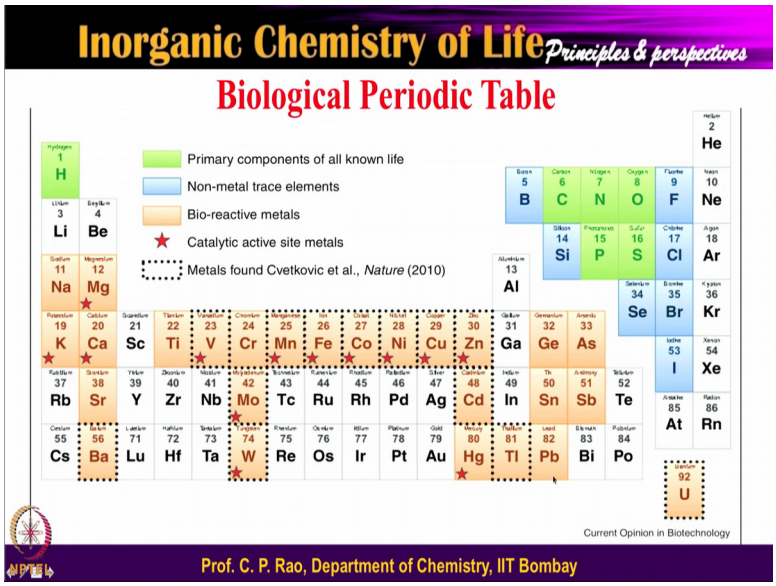


So, the period table where the star ones are all the ones which are the enzymes are also known. So, for example, in the transition metal titanium is known as some kind of a nutrient, but still is not clear whether any enzymes, there is no enzymes vanadium is

chromium also no enzymes, but chromium is a micronutrient, then manganese, iron, cobalt, nickel, copper, zinc, all of them have got enzymes molybdenum also has enzymes tungsten is similar to that of the molybdenum. So, therefore, I will not be treating with this and this is found not in human systems, but in micro organisms where you have the micro organisms which grow at very high temperatures like lava, etcetera. So, therefore, I have given it up.

So, I have also talked to you in this particular course about the mercury mercury detoxification, etcetera and there are some other essential element these are the bulk ones to and the main thing what you can say. So, barring some exception below this is the line where you have the nature has chosen the biological elements above this, but not so much below this barring these ones.

So, this is not clear we try to in the class, I have explained you to compare with their with their abundances in water with their abundance in soil and with their abundance in the human tissue and found; it is not easy to make a direct correlation between the elements that are present in abundant in water abundant in the soil etcetera have not necessarily be a part of the part of the biological system.

On the other hand, there are some elements which are not. So, much present which are present very low concentrations, but they are present in the biological systems and there are some where present in both like ion which is present in both silicon is present in the earth cast, but not present in the human enzyme, but it is as a micro nutrient still no enzyme has been identified that is.

So, like that there are things which are there which are not there. So, therefore, it is nothing to do with exactly the concentration of these elements present in the seawater or the earth crust, rather they are dependent on that.

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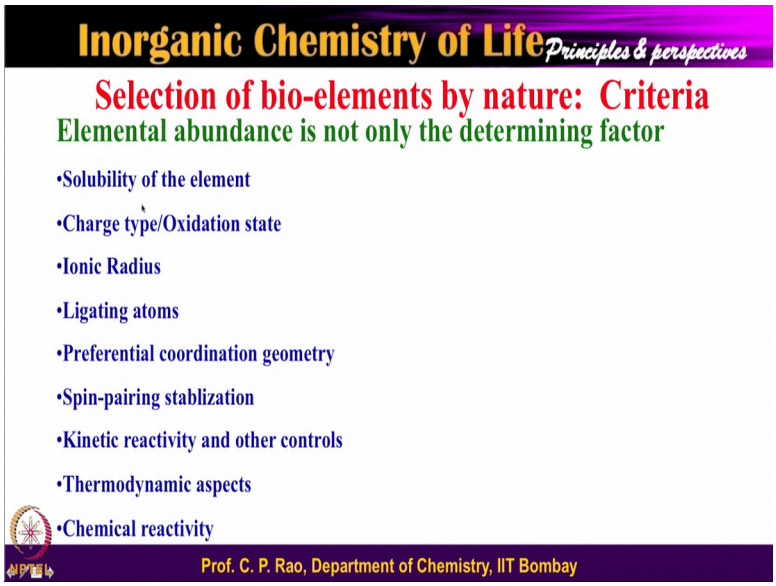


Ion characteristics like a charge size redox potential and if is iron is a cat ion there is coordination chemistry properties like preferential geometry stability thermodynamic parameters, then the lability parameters which involve in the reactivity. So, the sizes oxidation states charges and hs cb characteristics that is hard soft acid based characteristics and the coordination chemistry parameters stability parameters and lability parameters. So, these seem to be more important than the concentration these elements alone in the water or in the or in the earth's crust that.

So, probably the nature has chosen these ones also, I have highlighted to you these essential trace elements are present to certain extent below which is a danger above which is also a danger.

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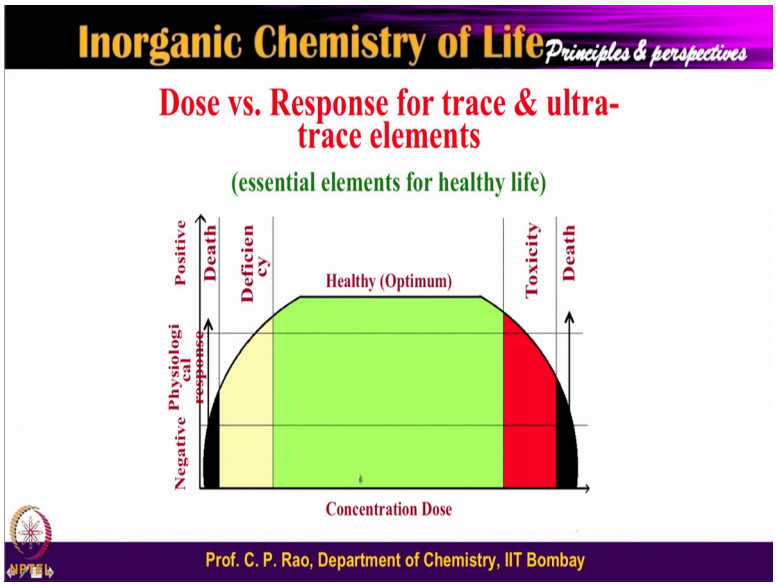


So, below which the concentration is called deficiency above this is called the toxicity. So, the deficiency and toxicity of this so, as we increase the concentration the doses which are these elements these ultra trace and trace and ultra trace on the x axis, their concentration as the dosage and look at the look at the response on the y axis. So, the response slowly increases and response becomes play to and this whole range is good enough beyond that; if you have more concentration the ultra trace and trace then there is a toxicity and toxicity can lead to death also at a very high concentration.

So; that means, under condition that the life has imposed on these elements is the trace and ultra trace elements must be concentrations must be maintained in the body, they cannot be afford to have very low, they cannot be afford to have very high concentrations in both the cases, body will have a problem in terms of its physiology and that is what we have. So, it is very essential.

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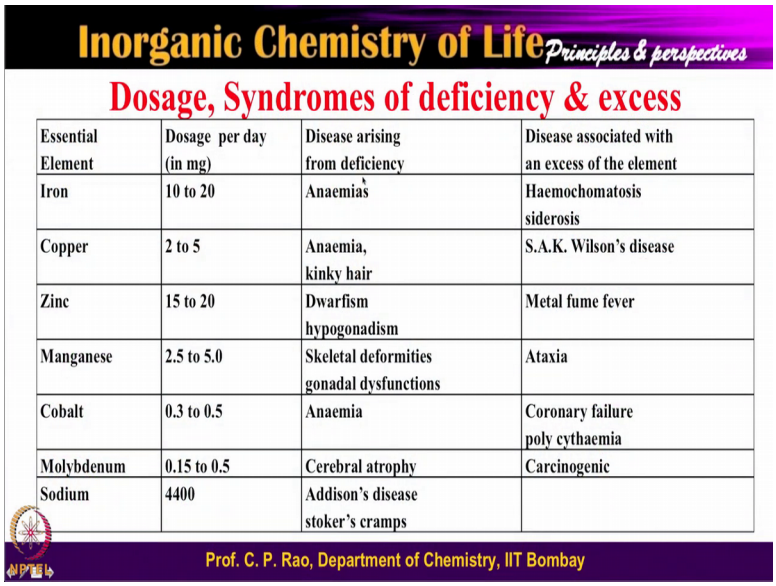


| Essential Element | Dosage per day (in mg) | Disease arising from deficiency | Disease associated with an excess of the element |
|---|---|---|---|
| Iron | 10 to 20 | Anaemias | Haemochomatosis siderosis |
| Copper | 2 to 5 | Anaemia, kinky hair | S.A.K. Wilson's disease |
| Zinc | 15 to 20 | Dwarfism hypogonadism | Metal fume fever |
| Manganese | 2.5 to 5.0 | Skeletal deformities gonadal dysfunctions | Ataxia |
| Cobalt | 0.3 to 0.5 | Anaemia | Coronary failure poly cythaemia |
| Molybdenum | 0.15 to 0.5 | Cerebral atrophy | Carcinogenic |
| Sodium | 4400 | Addison's disease stoker's cramps | |

So, therefore, this slide and maybe 1 or 2 more slides will show you the doses and the deficiency gives some problems and excess will give some problems like copper, iron, these are the things that you will know.

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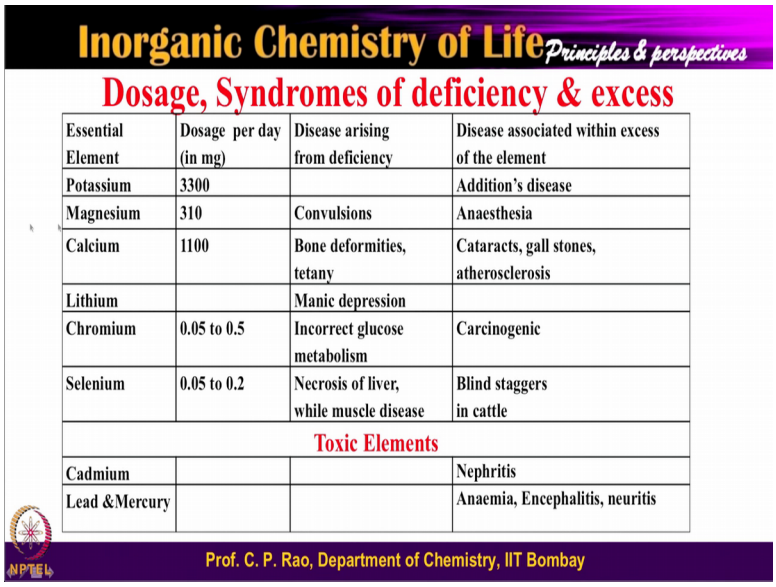


| Essential Element | Dosage per day (in mg) | Disease arising from deficiency | Disease associated within excess of the element |
|---|---|---|---|
| Potassium | 3300 | | Addition's disease |
| Magnesium | 310 | Convulsions | Anaesthesia |
| Calcium | 1100 | Bone deformities, tetany | Cataracts, gall stones, atherosclerosis |
| Lithium | | Manic depression | |
| Chromium | 0.05 to 0.5 | Incorrect glucose metabolism | Carcinogenic |
| Selenium | 0.05 to 0.2 | Necrosis of liver, while muscle disease | Blind staggers in cattle |
| Toxic Elements | | | |
| Cadmium | | | Nephritis |
| Lead &Mercury | | | Anaemia, Encephalitis, neuritis |

So, again some more elements the dosage and deficiency syndrome and excess syndrome ok; So, this is a very essential aspect to be kept in mind. So, because the we cannot take as a much amount as available or something to the biological systems because there

excess will cause a problem there less in the concentration will also cause a problem on this ok.

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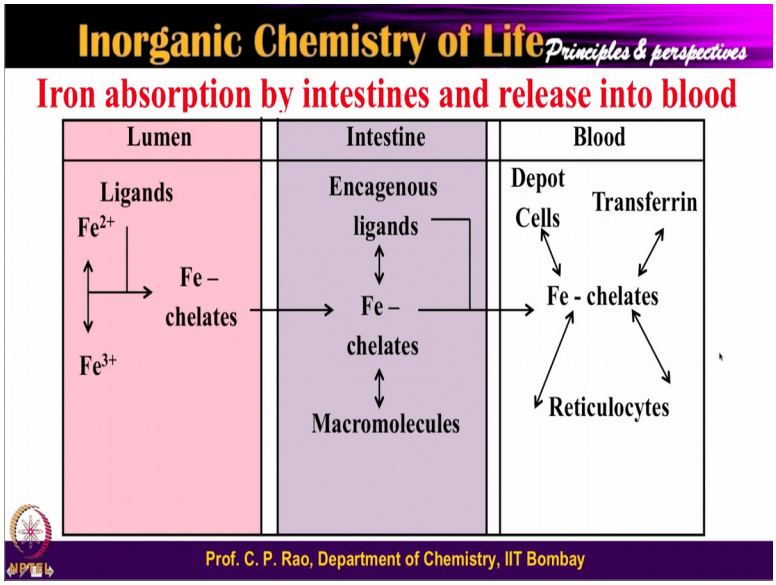


Let us look at another aspect of highlight; that I talked to you earlier is the absorption. So, all these elements we are talking about their presence in the body, but they have to be absorbed.

So, example is taken for iron here iron for exterior into the lumen; lumen has got certain kind of a complex ligands which will exchange and form their chelates and these chelates can pass through the intestine, again, in the intestine, the iron ions can be exchanged, this iron is not neutral iron, these are the ions and they form the chelates if their endogenous ligands and this will be taken into the blood and the blood these chelates are being further transferring their iron to the transferrin reticular side cells, etcetera.

So, this is the kind of a general mechanism and the mechanism very well suited for iron similarly many iron. So, will have their own mechanisms.

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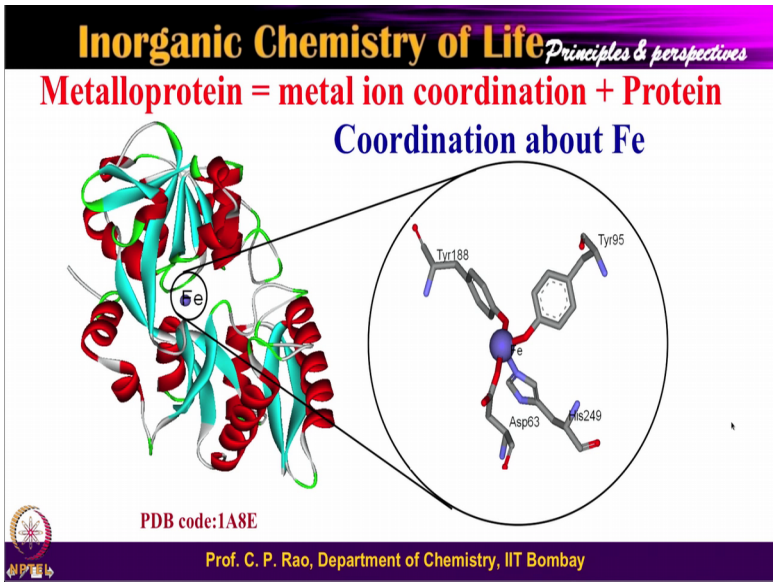


So, with all this, what I have tried to conclude at that stage was that if you take this is as the metalloprotein and the metal ion center, you blow this and see this metal ion center has a tyrosine another tyrosine another histidine another aspartic it and these 4 are bound like a kind of a coordination not like the coordination like the tetrahedral which is basically of course, distorted tetrahedral.

So, therefore, the metal ion maintains a kind of a geometry maintains a coordination sphere of primary and then maintains then surrounding that is the protein. So, therefore, a metalloprotein; nothing wrong in visualizing as metal ion a metal ion coordination plus the protein of this and so, whatever we have realized understood in the general elements in the biological systems is that visualization of the elements or the ions of importance in biology so.

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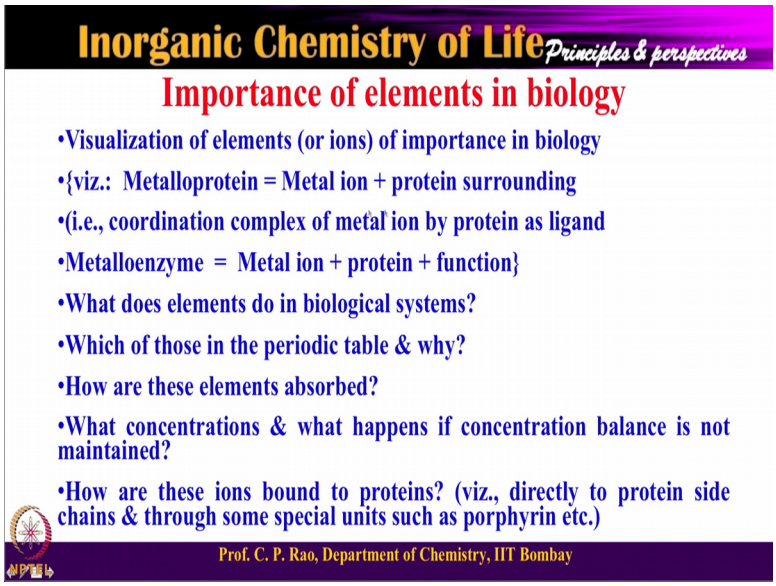


A metalloprotein is a metal ion plus the protein surroundings. So, when the metal ion is present, it is not just a free metal ion; it can be coordination complex of the metal ion by the protein. Now in this context for an inorganic chemist the protein elegant and for a biochemist the inorganic center is just one element.

So, there are the two kinds of things that one would look at the same it is like looking at the half filled glass somebody says the glass has a half filled water and somebody else will say the glass is half empty and this is the kind of a situation that you look at. So, metalloenzyme therefore, is a metal ion plus protein which binds to it plus exhibiting a function. So, metalloprotein to metalloenzymes is the function added.

So, we have taken care of all these things what does elements do in the biological systems and which of those are important in the periodic table, etcetera, I have already explained how are these elements absorb, I have explained what concentrations, what happens if the concentrations are gone up, what happens if the concentrations have gone down ok.

So, how are these bound all these kinds of things, I have I have already addressed just let me tell you, how these are bound once again is a highlight a few of the amino acids are shown here with the carboxylic terminal or phenolic terminal or hydroxy terminal

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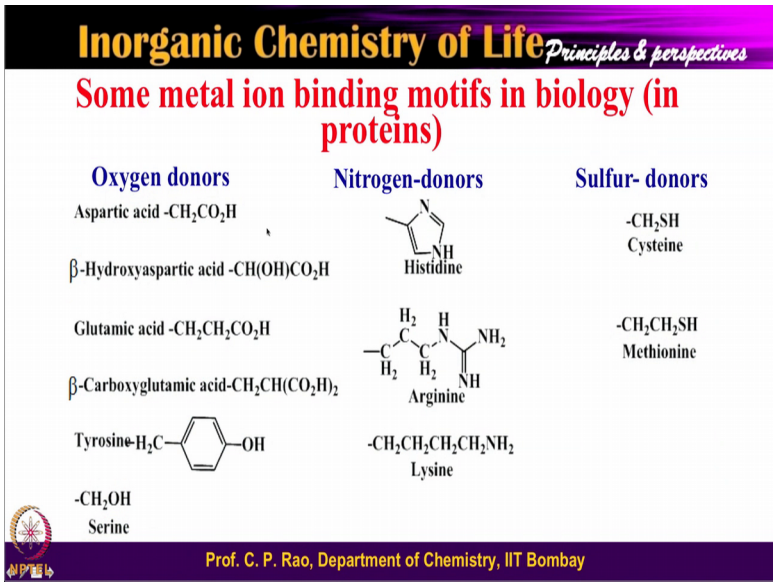


These are the 3 types and here imidazole terminal or arginine terminal or lysine terminal amine or you have here sulfidehydrill function methionine there is an error, it is S CH 3, there is an error here the CH 2, S CH 3; so, methionine.

So, you have the; so, thio type or these ones are these nitrogen ones oxygen kind of things; these are directly coming from the side chains of the proteins.

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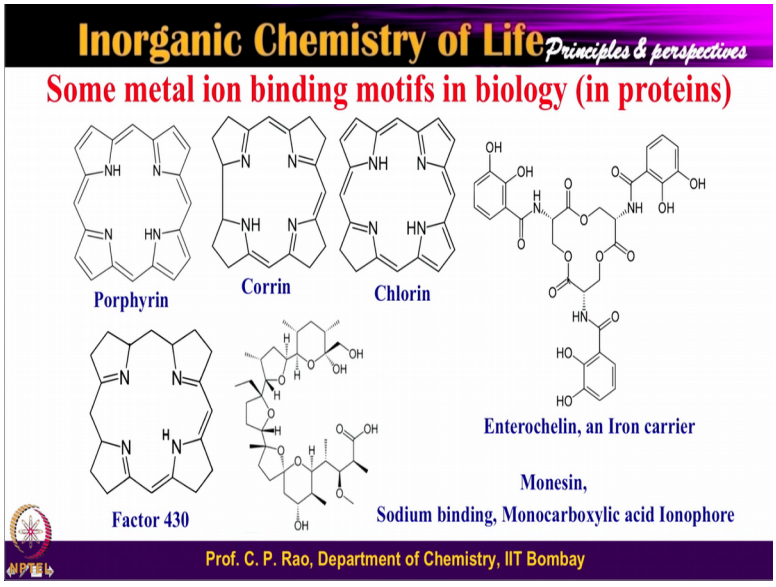


And besides these ones; the side chains are the proteins are very common, there could be carboxylic there could be nitrogen base there could be sulfur ways besides that there are

some special motifs are present in the biological systems like porphyrin shown in the first one corrin, the difference is here this is being a saturated the conjugation is reduced and a chlorine again conjugation is reduced here as well. So, therefore, you have a porphyrin, then corrin chlorine and chlorine etcetera and factor 430; all of these have a ring like structure with 4 nitrogens therefore, 4 nitrogens can be bound to the metal then you can have fifth sixth are can be filled by other systems.

There are a few small molecules also are involved like this one which is for the iron this is antrobactrin in its iron carrier in the lower organisms where this kind of a compound is oozed out into the medium the medium it picks up the iron ions and swallows back. So, therefore, the iron is coming inside, there are some non cyclic ones are also there. So, therefore, these are for some for sodium some for calcium, etcetera, all of these kind. So, these are referred as the ionopher.

So, the binding is one to the side chains all two also to the some special motives and there are some ions are transported by the ionophers as well.

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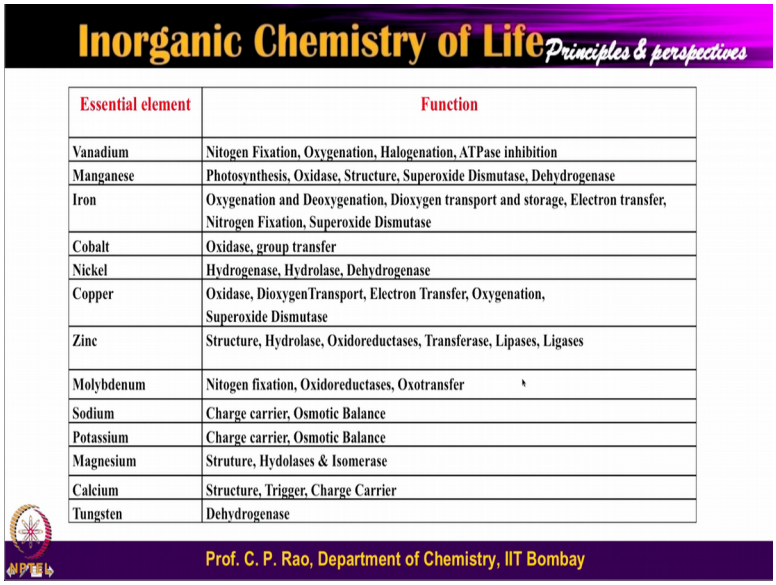


| Essential element | Function |
|---|---|
| Vanadium | Nitogen Fixation, Oxygenation, Halogenation, ATPase inhibition |
| Manganese | Photosynthesis, Oxidase, Structure, Superoxide Dismutase, Dehydrogenase |
| Iron | Oxygenation and Deoxygenation, Dioxygen transport and storage, Electron transfer, Nitrogen Fixation, Superoxide Dismutase |
| Cobalt | Oxidase, group transfer |
| Nickel | Hydrogenase, Hydrolase, Dehydrogenase |
| Copper | Oxidase, DioxygenTransport, Electron Transfer, Oxygenation, Superoxide Dismutase |
| Zinc | Structure, Hydrolase, Oxidoreductases, Transferase, Lipases, Ligases |
| Molybdenum | Nitogen fixation, Oxidoreductases, Oxotransfer |
| Sodium | Charge carrier, Osmotic Balance |
| Potassium | Charge carrier, Osmotic Balance |
| Magnesium | Struture, Hydolases & Isomerase |
| Calcium | Structure, Trigger, Charge Carrier |
| Tungsten | Dehydrogenase |

So, overall what we can say this the these vanadium manganese iron cobalt nickel copper zinc etcetera these are all 4 show variety of functions like oxygenation function, halogenation function, atpase inhibition and nitrogen fixation case of vanadium and manganese several oxidative things, superoxide dismutase dehydrogenase;

dehydrogenase as well as fast photosynthesis. So, important thing is photosynthesis and I have explained all of these under the story of manganese.

Then when you come to the iron you have a oxygenase deoxygenase oxygen transport the iron transport electron transport oxidative reactions reductive reactions oxygenation reduction reductases superoxide dismutase these and ribonucleotide reducyase. So, many things; we have already looked at all these, I will be bringing some highlights anyhow cobalt is its mainly the cobalt in the form of a vitamin B 12 and which does the group transfer reactions in nickel, we have looked at the hydra urease which is a hydrolytic one, then we have looked at hydrogenase, we have looked at the dehydrogenase enzymes too.

Copper again is another enzyme where too many proteins are there too many enzymes are there so, oxidase. So, it will be oxidase dioxygen transport electron transfer. So, oxygenation superoxide dismutase variety of things are there and zinc is also though, it is a non redox metal ion the the kind of reaction that it does is the total spectrum. So, you have a hydrolyzed kind oxido reductase kind transferring its kind lypis kind like eases is all of these kinds of things, then the molybdenum is nitrogen fixation most important then oxido reductase which is basically a part of the oxo transfer is the process in it.

Then you have sodium potassium magnesium calcium which are all charge carriers osmotic balance and magnesium is can do hydrolase structure isomerase calcium when it comes the trigger functions also there and. So, all of these kind of a functions I have tried to highlight this is the thing one part of the introductory aspect that I have covered based on the elements present in biology, then I have quickly explained a little bit on the biological molecules protein synthesis that kind of thing.

So, proteins are governed by primary structure which is the amino acid sequence secondary structure which is alpha helical beta sheet tertiary structure.

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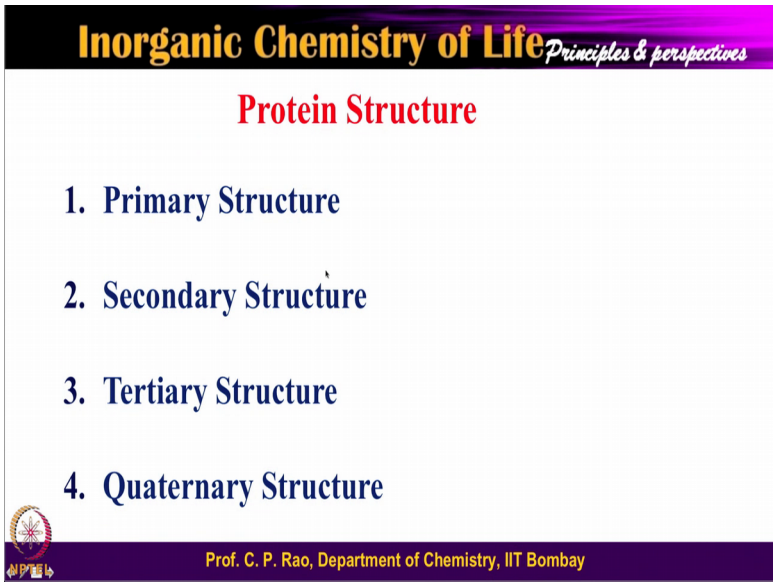


All of these are integrated into the quaternary structure; such tertiary structure systems are joined together to form a quaternary structure ok. So, that is where we have looked at.

So, here you see this is the sequence.

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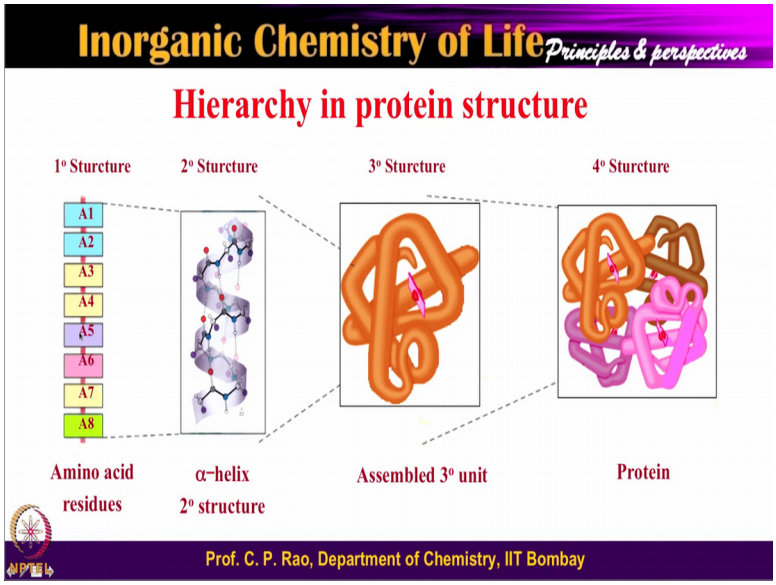


It is the primary structure, this is one of the alpha helix it can be beta sheet also secondary structure. So, all of these are integrated into the polypeptide chain is called

tertiary structure such tertiary structures are present 1, 2, 3, 4, it is called quaternary structure.

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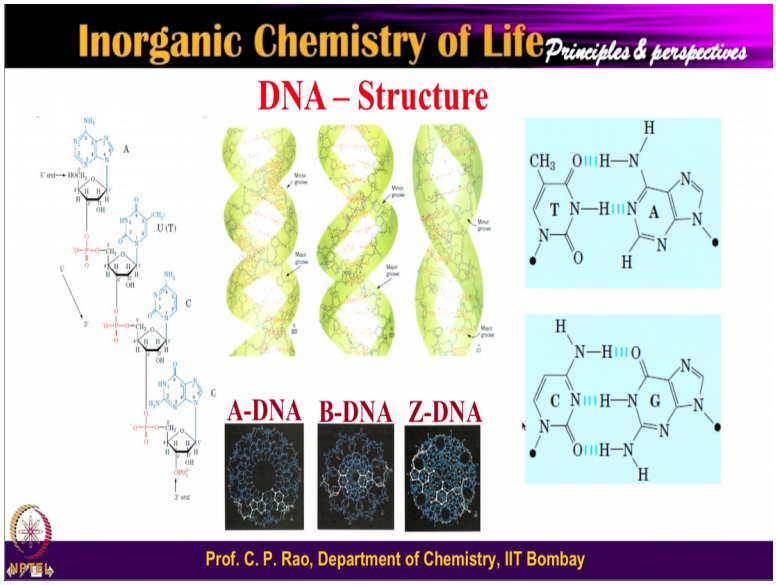


Now, I also looked at the DNA; in DNA is nothing, but phosphate sugar phosphate sugar phosphate sugar and the sugar ok, 3 prime, 5 prime sugar and the phosphate phosphate phosphate and then on this one at the 2 prime, 1 prime, 1 prime sorry of the carbo the like a moiety or ribose moiety, you have your nucleic base. So, therefore, nucleic base comes as a side chain.

Now, in this forms a kind of helical structure you can see that the nucleic bases are coming like this like this, etcetera at these 3 3 different kind of DNAs; A DNA, B DNA, Z DNA, if you look at that; their structure morphology are different, they are stabilized by certain hydrogen bonds called complementarity complementarity of the of the residues in terms of their hydrogen bonds T with Ath and C with G ok.

So, and these are the different kinds of things. So, you have a grooves like major groove and it groove like the minor groove this side. So, major groove this side and minor groove that side. So, these are some of the things where molecules can bind also, they can also disrupt this the nuclear base stacking and enter two that is also possible.

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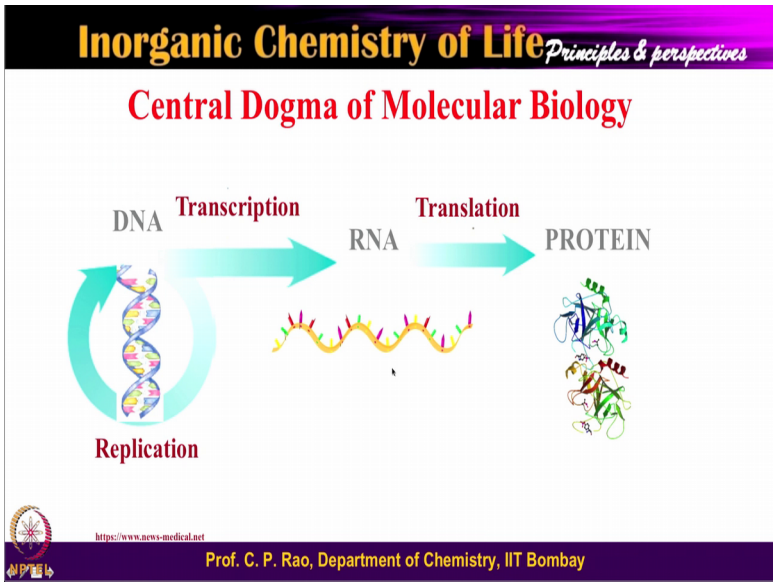


Now some important aspects of the biochemical thing is that the protein synthesis protein synthesis is basically from the DNA; DNA you have replication and then that is being reformed by process the M-RNA, then that is being translated.

So, this transcription then M-RNA translation then gives the protein kind of thing, this is basically referred as a central dogma molecular biology. So, after the protein is synthesized, protein will start maturing, it is called the makes into the secondary tertiary quaternary structures that is called the protein maturation. So, basically protein becomes the structure.

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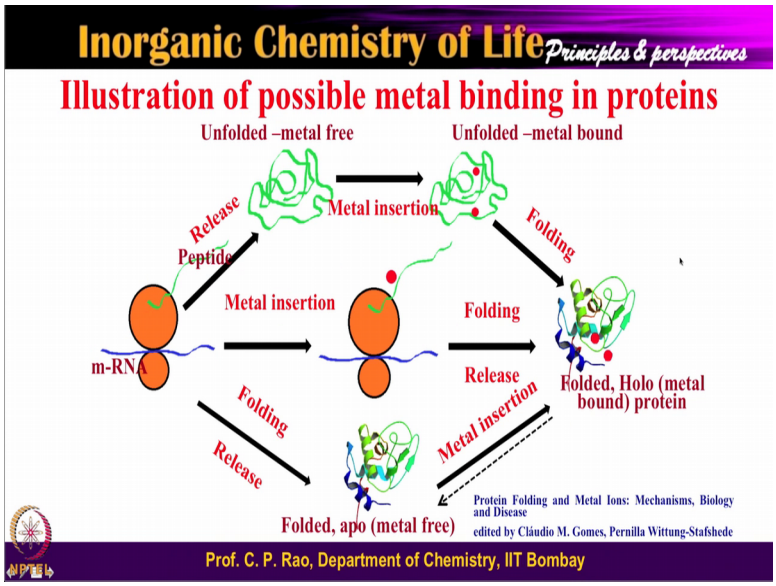


So, when will the metalloprotein form? So, metalloprotein forms after the protein is formed only in various ways. So, you have a protein as a polypeptide. So, polypeptide can make into it is a kind of a folds and these folds can be with without the metal initially and the metal ion can be inserted and the folds get stabilized and the further that stabilization in.

So, folding you can use stabilization, you can get the regular holoprotein. So, this is the holoenzyme or holoprotein. So, this is metal bond this alternate way is this m-RNA and then you have a polypeptide, you can introduce the metal ion prior to the folding and this will make into the folding and it can also form. So, metal insertion can happen before folding or after folding its possible. So, the folding will give this one the metal insertion will give that one.

So, and the reverse of for the release of this, so, these are the kinds of things; that means, when you take the holoprotein and remove the metal ion some of these pos things are possible to go back to the unfolded state etcetera.

So, and when you have a original protein protein plus the metal ion holoprotein, there may or may not be some changes, but there is possibility of some changes in the protein confirmations to and then I explained the because see, we were looking at in the metalloprotein and metalloenzymes metal ion is there. Now, you can replace the metal ion by another one, then you can see whether the function is working or not working.

On the other hand, if you want to see the kind of a primary residues that which are bound to that.

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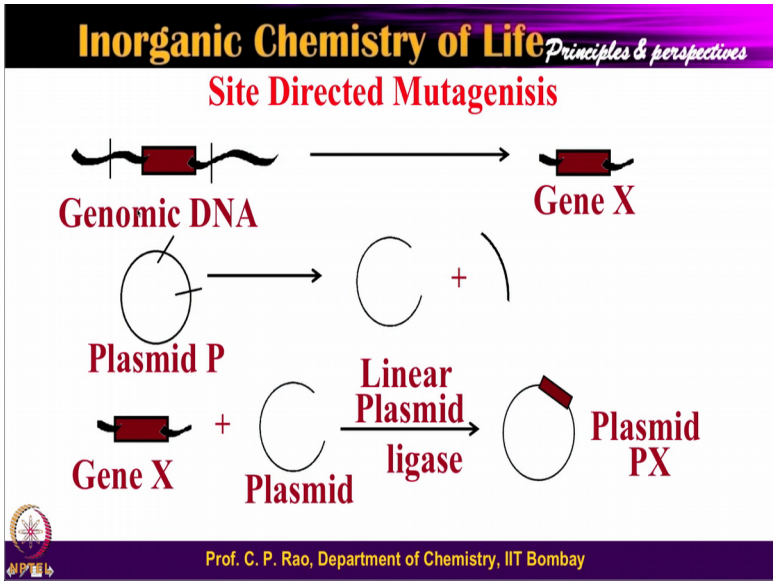


And you want to know whether a particular histidine is definitely essential or not for this metalloenzyme to function what you need to do you need to trim or replace that particular histidine by some other amino acid which is not a histidine and that is what is done by site targeted mutagenesis.

So, you have a genomic DNA, you have a gene and this gene can be put into the plasmid by cutting the plasmid and then put that one. So, you have done in plasmid and this plasmid you express now. So, when you express this know, you get the same protein with whatever the modification you have done you have done single mutation and only on one place you have amino acid being different. So, if for each mutation you have 3 codons are there that is a codon 3 the nucleic nucleotide centers are present and that is what the thing is. So, these are some of the important ones.

So, coming to the understanding of the of the metal center as I said the metal center in the metalloenzymes are very important in terms of their binding their coordination coordination number nature the coordination because the certain of these things are dependent on the HSC B principles and their geometries also.

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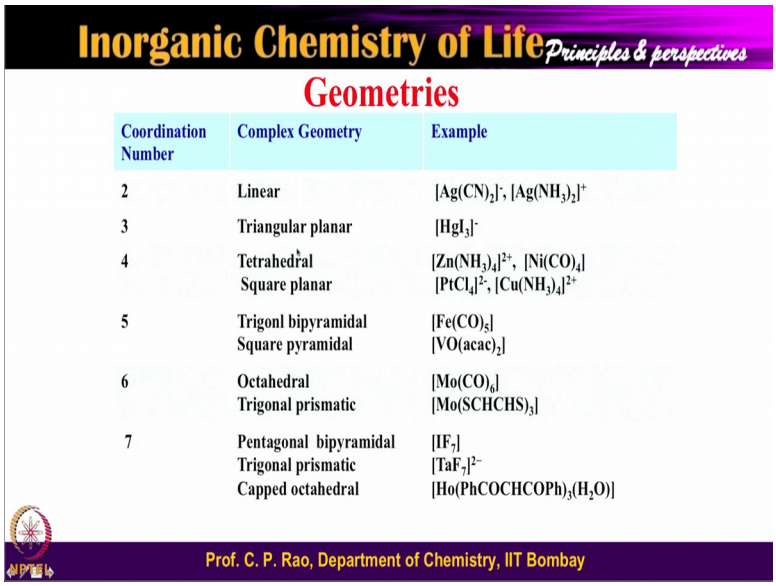


So, geometry can be linear can be triangular can be tetrahedral can be trigonl bipyramidal octahedral prismatic pentagonal bipyramidal trigonal; So, mostly transition metal ions will be showing 4, 6; occasionally 2, but very rarely 7. So, that is why I have given the geometries of 2 here and some examples too.

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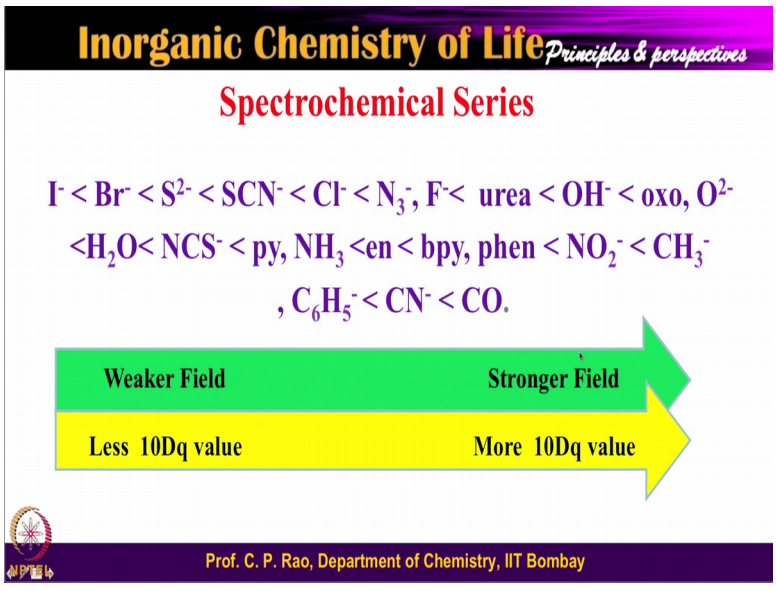


And in the coordination chemistry, we have studied something called crystal field theory which I have not taught in my course, but I presume that you are aware and try to look at. So, these are all the weak ligands which will split the energy levels of the D energy

levels under the under the let us say octahedral field as t 2 g easy, this will split to a less extent as you go towards right further and more and more splitting more and more and extremes splitting come in cyanide and carbon monoxide kind of ligands methyl ligand or phenyl ligand, etcetera zeroth anion; so, carbon.

So, this is this side or the weak field; these are called the strong field and there the difference delta, delta q is small little bit more and large greater. So, how do I explain this? Obviously, so, this is a very important thing many times, people tend to explain this by the electronegativity principle which is definitely not correct you have to in invoke the bonding character and in the bonding it is not the sigma bonding character it is the pi bonding character. So, those which are pi acidic more of it and they are in the higher side ok.

So, therefore, pyacidic character increase from left to the right some of them may be pi bases and then not so much py and py acid kind of thing.

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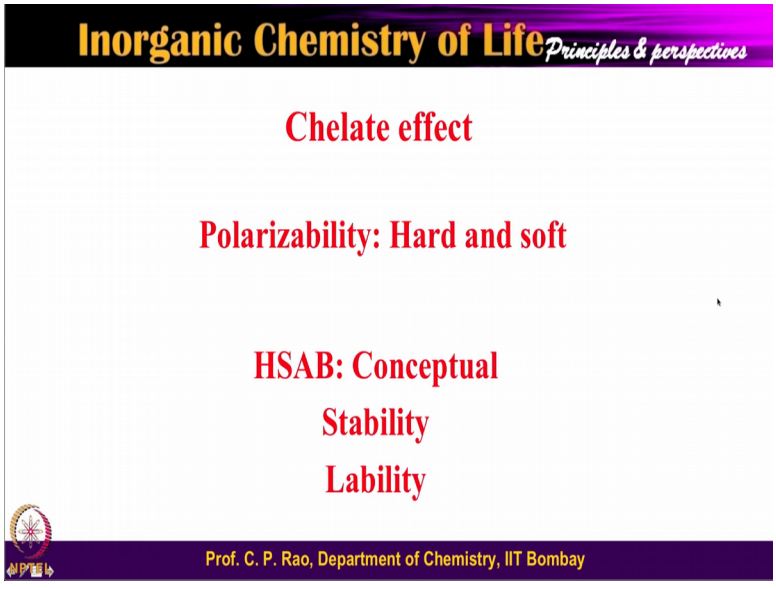


So, that is how we can make that. So, overall the coordination chemistry parameter that is required to understand the role of inorganic elements in the coordination sphere in a metalloprotein and metalloenzymes is there chelate affects their polarizability is hard soft acid base characteristics which you know very well, I am not going to explain again.

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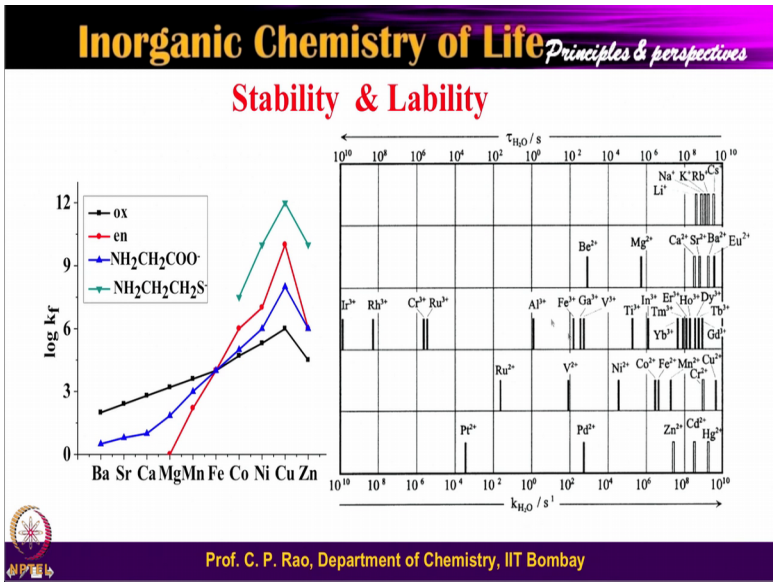


Now, and their stability and their reliability aspects of it and I have tried to explain the stability as you see from the earing million plots, how they are there depending upon the ligands there things will be and keeping the metal ion constant changing the ligands, we are keeping the ligand constant changing the metal you can try to find out the stabilizations of it and these will follow the Hs a be kind of a principle.

On the other hand the metalloenzymes they are not only metal ion is present and then very stable coordination sphere is there they also do some function that is called lability. So, here the lability is given; lability rates are given based on the water exchange reactions.

So, there is not based on the water exchange reactions. So, in the water exchange reactions how fast and outside outside water will get exchanged with the inside water. So, as you see in a given group like lithium sodium potassium as the heaviness increases the the lability increases.

Similarly, beryllium magnesium calcium, etcetera and if you look at these from compared to mono cat ions the di cat ions are less labile tri cat ions are. So, what les labelled tetra cations are so much more less leeway as the heaviness increases the lability increases as the charge increases the lability decreases and of course, in place in case of the transition metal whatever you have to see you have to see not only this, whether they

are low spin or high spin because the low spin and the high spin they do their sizes will vary spin density will vary and that is the reason why we are looking at all of these.

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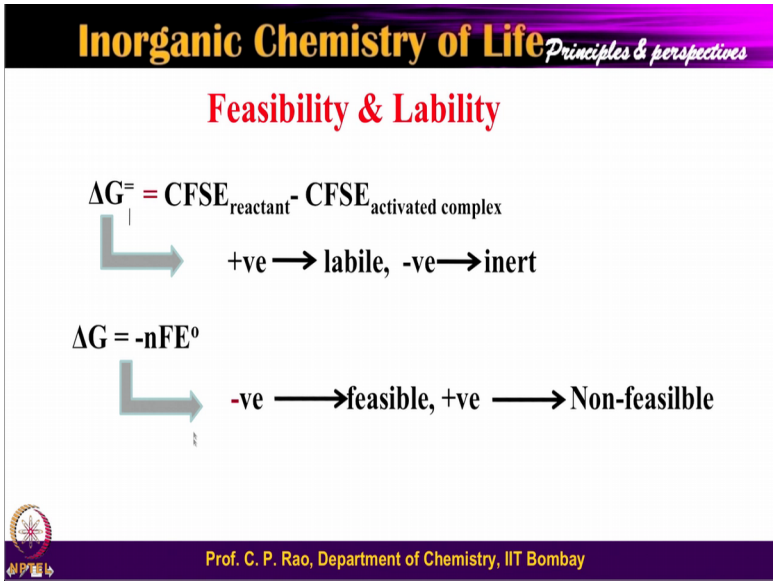


So, in effect; what we are saying is stability means formation of the complex means delta G is minus nFE. So, delta G minus nFE, if the potential is positive minus nFE negative is feasible, if the potential is negative is positive is not feasible.

On the other hand, lability means a change of the reaction means this one delta G dagger double dagger, this is the crystal field stabilization energy of the reactant minus crystal field stabilization energy of the activated complex, if it is positive a syllable F is negative, it is basically inert kind of thing.

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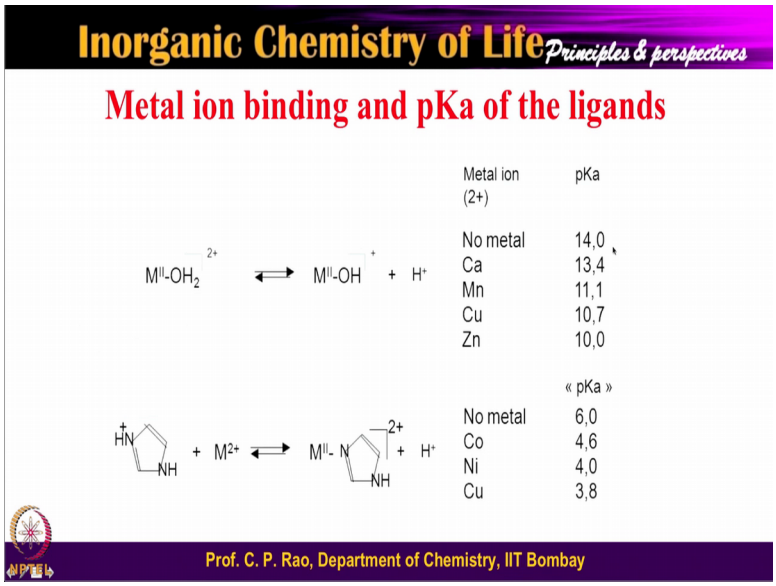


So, therefore, what we have learned the in metalloproteins the metal ions can bind to the side chain upon deprotonation, they can bind to the and form a coordination complex in some cases, they can be bound to the to the special cores like porphyrins, etcetera and these things and how do we understand that it is binding to the side chains polarization of the group, it could be a carboxyl group polarization water group polarization or imidazole group example is shown here when the metal ion is bound to the water and the peak here the water will reduce, it can go from fourteen to ten and going from no metal to the zinc.

Similarly, imidazole on going from the no metal which is six to about four when you go to the nickel or copper; so, therefore, in presence of the metal ions all these can be polarized and they are all bound.

So, with this I have been able to explain you the highlights of the a metal ions their roles biological roles and their the existence as a coordination complex therefore, their existence their think as a stability and since they are functioning they are functioning as a lability of it and I will continue with this revision part in the next class.

Thank you.