Inorganic Chemistry of Life Principles & Properties

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Lecture - 08
General introduction of metalloproteins

Good afternoon, welcome you all for this next lecture on Inorganic Chemistry of Life Principles and Perspectives. In the previous class, I have talked to you about the structures of the proteins or 4 different structures the proteins. Then I talked to you about the nucleic acid; nucleo nucleotide, nucleo nucleoside and nucleic acid and nucleic acid structures like a b z and also talk to you about the base pairing the Watson kind of base pairing which indeed stabilizes the double helical structure and then also have focused on how a protein is being synthesized by the biological system and which through a phenomena called the central dogma of molecular biology that is DNA to mRNA to protein.

And then I also talked to you about the post translational modification after the protein base being synthesized. The protein structure tertiary structure quadrant structures taking place then there are certain unwanted units which are present in that are removed and there are some post translational modification which are even covalent in nature something like acetylation, phosphorylation, o methylation, hydroxylation many of these kinds of things. Also talked to you about the how this is C terminal, N terminal connectivity is being a main all these aspects are being talked in the previous class.

But we have not looked at how a metalloprotein is synthesized. So, let us look at the how the metalloprotein is synthesized.

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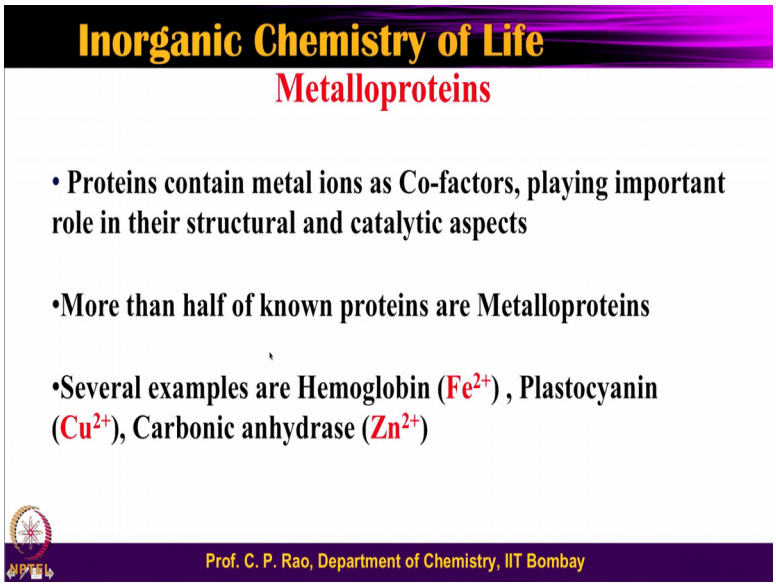

See for making the metalloprotein; it is not that metal ion is coming along with the protein synthesis; it is the protein is first synthesized and then metal ion is being introduced into the protein ok. So, there are various ways these ones; the proteins which contain a variety of metal ion as Co-factors. We have seen the iron we have seen, copper we have seen, nickel we have seen, manganese so many different kinds of a Co-factors, you can call it as metal base Co-factors. The all the all these play important role in either the structure of the protein stabilizing or the catalysts of the protein.

So, they are involved as a structural and catalytic ingredients or the of the protein as a metal centre. And I have already explained to you earlier how this metal centre is; metal centre is connected to the side chains of the protein to form a coordination complex. So that means, in the metalloprotein is formed straight away the coordination complex is not formed; metal protein is initially synthesized, then followed by introducing the metal ion into this ok. So, there are several metalloproteins, metalloenzymes in our body. In fact, almost one-third of bit more than you know one-third of the proteins that are there in the human body or all metalloproteins.

So, that mean there is a huge amount of reactivity functionality responsibility taken by the metalloproteins and metalloenzymes. Generally there is a there is feel for us to ignore the inorganic pots in the biological systems, but this particular course will bring to you how important is inorganic pot this. So, so there are variety of examples hemoglobin,

plastocyanin, carbonic, anhydrase these are just for a few and many examples we have already seen earlier and many more will complete later on.

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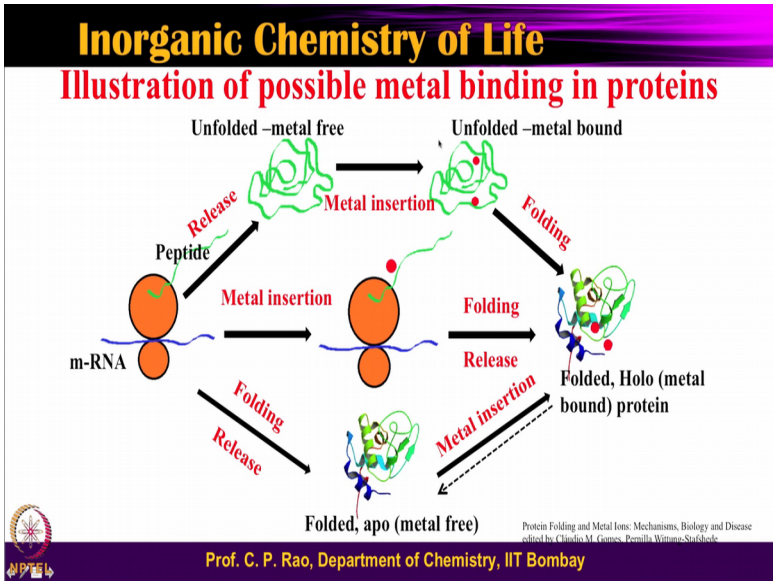

Now, let us look at how such a metal ion is entering into the protein to make the protein into a metalloprotein or to make the protein into a metalloenzyme; so, metalloprotein and metalloenzyme. So, these are something where you can say you have your ribosomal m-RNA and which gives the peptide synthesis which we have seen just a while ago in the previous lecture ok.

So, and this gives the unfolded and the metal free; one of the possibilities is that the metal can be inserted. So, there are 3 ways path shown; path going via top, path going straight, path going through this. There are 3 different ways by which we can think of the introducing the metal ion into this particular proteins. So, the protein synthesis metal ion insertion and then goes into the unfolded, but metal bound then goes into the total folded structure.

So, the structure which I have shown like this are called unfolded, the structures which are shown like this are called the folded. So, this protein is same as this protein, but structure is not the same. So, in other words the primary structure this and the primary structure this is same, but the tertiary structure this is and tertiary structure, the secondary structure this is and secondary structure this; they are not the same.

So, that is one way you can make the metalloprotein. So, the folded Holo; Holo means metal ion present. Holo means metal ion present, so folded Holo protein or metal bound protein. Let us look at the other one the protein and while protein is synthesis in the vicinity, the metal ion is brought and the metal ion can get into the folding process we induce the folding process and the folding will take place.

So, that can also be released and then the folding and release folded apo-protein and then you make their put the metal ion into this or you can take the Holo protein; metal ion removal that is shown by this dotted line. So, if you show by this dotted line you will get the apo-protein and from this side the protein folding will still give the protein, but not in the metallated form. So, this is called apo form apo form plus metal insertion gives the final form.

So, this is the final form of metalloprotein and this is where the metal the protein is synthesized. So, between protein is synthesized and in the metalloprotein is formed, you have these 3 parts which are shown ok. So, now what we need to remember now is that the metal ion is added after the protein is synthesized or during the protein is synthesized which will induce the metal folding or complete folding would have also occurred then followed by introducing the metal ion. So, there are 3 different possible ways; there are 3 different possible ways by which the metalloprotein can be synthesized from the simple protein.

Then other things that one needs to remember at the stages; the protein having the metal ion in it is preferred as a Holo protein. The Holo protein minus the metal ion is referred as the apo-protein; so, here after in future we will be referring to proteins in the form of Holo protein, protein in the form of apo-protein; apo-protein means the metal ion is removed. In fact, one can do that even by using some chelating agents you can remove the two. So, why is it removed? Removed because to do certain kind of a reactions.

Suppose you want to reintroduce a new metal ion or introduce another type of metal ion into this. So, various way various things can be done in order to find whether that particular metal ion is really important or not important. So, so this gives overall feel as how and what a different ways by which the metal ion can be introduced to make the metalloprotein of course, if it shows a function it is called metalloenzyme in that.

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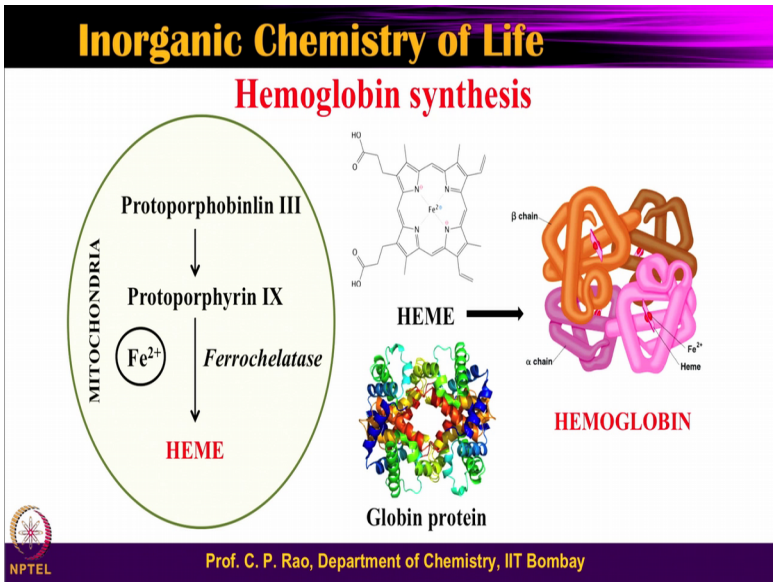

Let us look at one example here; in this particular example it is a bit different kind of an example the example is shown for the heme; so, for the Hemoglobin synthesis. So, the in the hemoglobin synthesis there are many previous steps which we are not showing that; take the Protoporphobinlin III this is one of the stage and this is converted into Protoporphyrin IX and this is the form in which it is present in the hemoglobin.

So, the protoporphyrin IX is the one which is present in the form of the heme the porphyrin in that we see and this is coming from the pre precursor of this and this is still not having any metal ion in this. So now this into this you introduce the metal ion. So, how do you introduce the metal ion? In this particular case, there is something called Ferrochelatase. So, so the ferrochelatase is because the ion and then bringing to make the chelate formation of this and that will be introduced into the protoporphyrin IX; once the ion is introduced the protoporphyrin IX plus iron is called heme.

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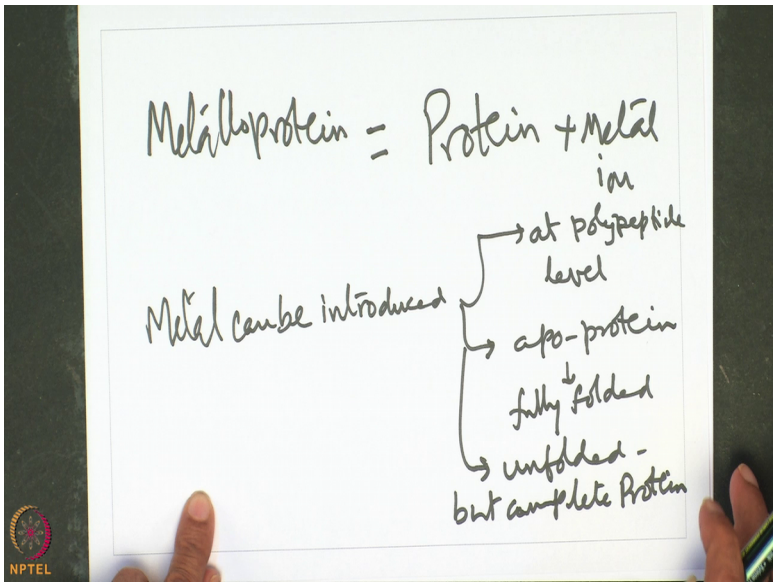

So, we have for the metalloprotein is equal to protein plus metal ion. So, the metal ion can be added at polypeptide level or it can be added in a apo-protein; that means, it is fully folded or it can be unfolded, but complete protein. So, there are different types the different stages where it can be introduced at simple polypeptide level or it can be at the apo-protein; that means, already fully folded and regular proteins is formed into which it can go or other possibility is you have a complete protein, but not folded enough to have the structure into this as well it is possible enzyme ok.

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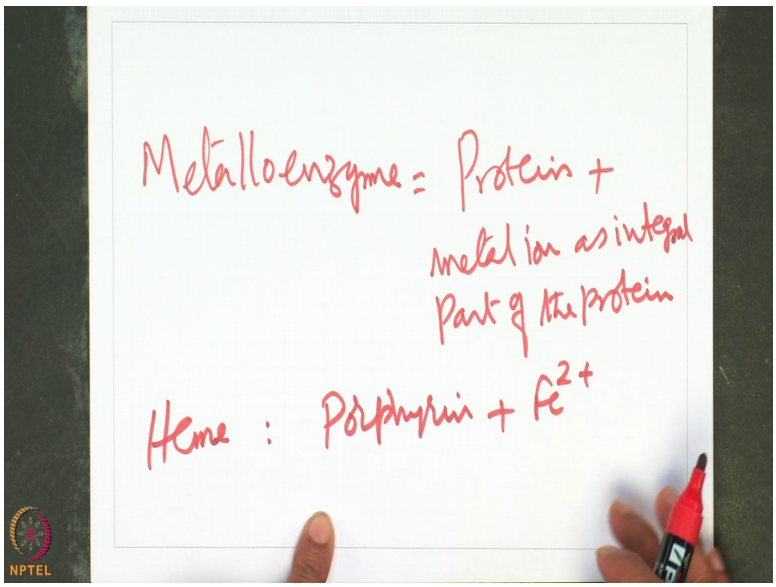

You have protein plus metal ion as integral part of the protein; so, these are some other things that we have looked at. In case of heme proteins; so in case of heme proteins, so heme is porphyrin plus iron ok. So, there are different kinds of porphyrins and the different kind of porphyrins are present in different kind of heme proteins. So, with that we will see whenever the future discussions come on that particular thing.

So, having seen this the protoporphyrin III to proto protoporphyrin IX to hemeby using their ferrochelatase ok and as you can see finally, you will form the entire protein which is a functional part of the protein; so where the protein in the structured form and the heme and the ion present in this.

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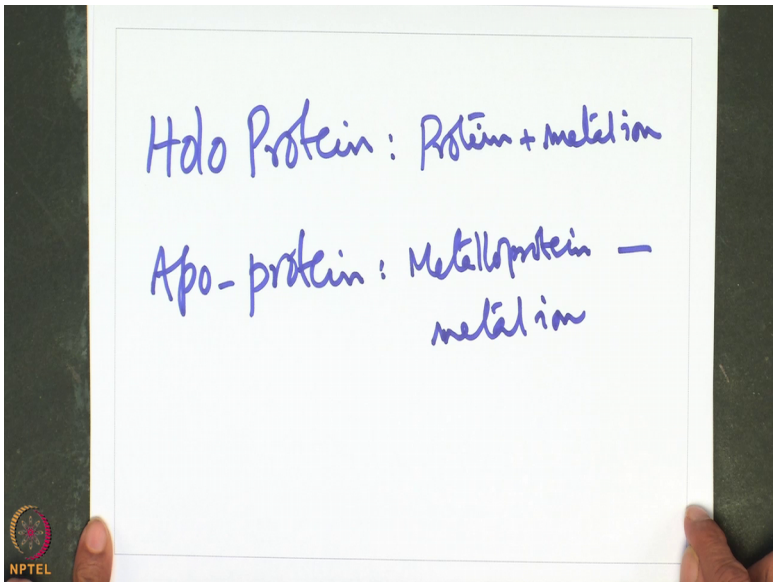

So, you can see in different ways for the same protein, in this context that we need to remember or 2 aspects. One is the holo protein, other is the apo-protein; holo protein is protein plus the metal ion, apo-protein is metalloprotein minus metal ion.

I also mentioned to you, it is a possible by using some kind of a chelaters; you can selectively remove the metal ion and then to make the apo-protein. The other way, you can take the apo-protein at the metal ion you can get the metalloprotein too ok. Now let us look at the other aspect of it.

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You have a protein, you have a metal ion; so binding through their side chains etcetera. So, therefore, this is a primary coordination sphere ok, so; that means, some amino acid 5, then amino acid their 20, then amino acid 40, then amino acid may be 70, may be amino acid 110 whatever. So, different levels of the amino acids are bound to this and forms a coordination complex.

And now as I said earlier the properties of this metal ion n plus is very much different from the metal iron in water or the metal ion in any of the in vitro kind of a systems; because the protein applies its own conformational restrictions, its own the hydrophobic hydrophilic regions influence and thereby it influences the total properties including redox properties of the metal centers of these ones ok.

Now, you want to claim this AA 5 is really bound; how do we know that the AA 5 is the one of the directly bound amino acid in this? AA 5 must be there in the primary structure this is no doubt, but how do you say that the AA 5 is bound or AA 40 is bound to metal centred? The simplest way is you change this AA 5 from one whatever be there amino acid there to a different kind and then look at whether this protein is stable, whether the protein is functional.

So, if the answers is yes the protein is stable, protein is functional, functioning the same way as that; that means, certainly AA 5 is not bound. On the other hand, if you answer were to be that the AA 5 this when you remove this into a different amino acid because

the different amino acid do not have the same kind of a side chain; therefore, will not be binding the same and the rate reaction catalysis will not be the same because you are change the binding partner, which is not binding also in fact.

So, in such case the functional change; if both the stability and the function of the protein changes so; obviously, we can say that particular amino acid is absolutely essential. So, how do we do that kind of a change? So, to do that kind of a change; changing one amino acid to other is referred as the Mutation, it is called mutation.

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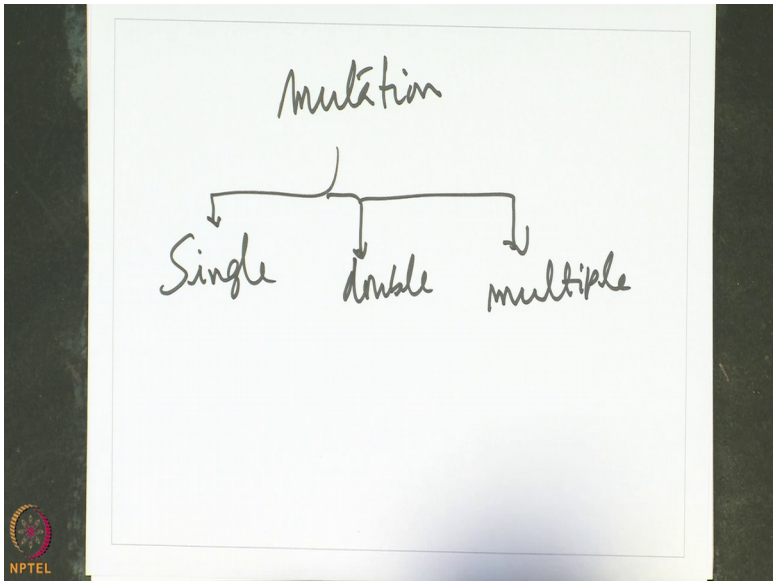

In fact, in the previous example in the previous example I can change this by changing the corresponding codon, I can change this by corresponding codon; codon is a triplet pair triplet from the corresponding nucleotides; I can change this one, I can change this one or I can change more than one. So, therefore, if I change one at a time it is called single mutation, if I change more than one it is double or multiple mutations.

So, mutations could be single, double or multiple. So, I can change only a codon's for 1 amino acid, I can change codon's for 2 of them, I can change the codon's for several of them. So, so this mutation; so by doing this I will get a protein with a change in the amino acid in that particular position. So, let us look at the next slide in this conduction. So, you can do ah; so making specific changes in DNA sequence by the mutation.

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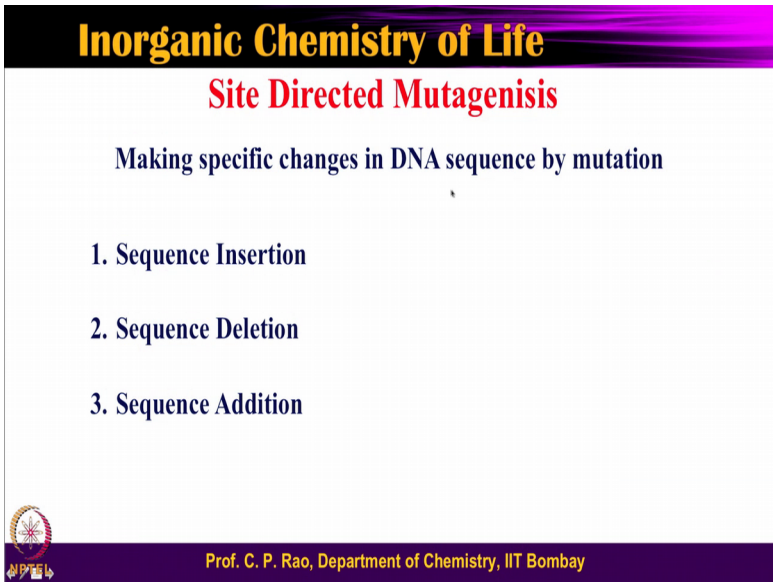

So, you would like to change that should reflect in the protein synthesis. This is this goes through different processes called sequence insertion, sequence deletion, sequence addition; I am not going to go into the details of all this because these kind of things, you will study if you have interest in molecule biology course. But all that I would say is of these are the kinds of operations that need to be done with the DNA; so, those operations.

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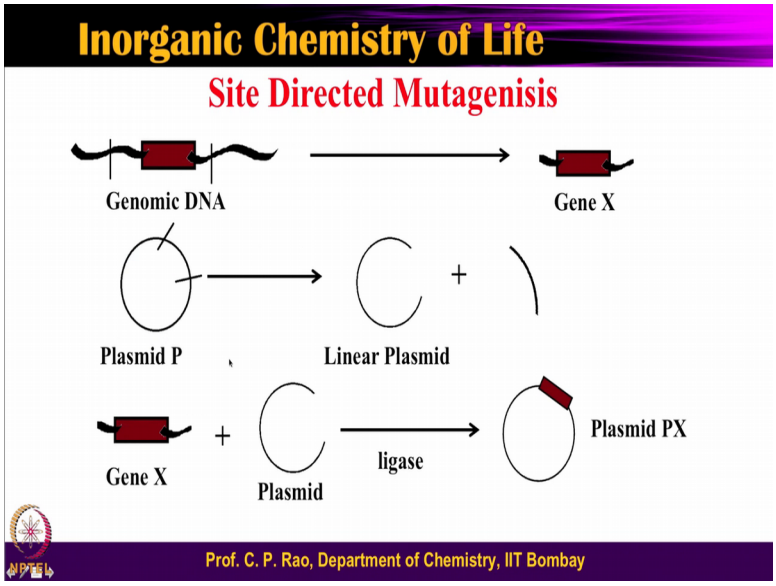

So, you just see suppose you are looking at the single mutation; one mutation and it is at a particular position it is called Site Targeted Mutagenesis at a particular site, you target at a particular site.

So, this is your genome or DNA part of it and you are interested in this particular code codon or region. So, that is what is referred as the Gene X whatever be thin is that and you select this one and this is ready now, then you take a Plasmid and you cut the plasmid here this line and this line and you got a open plasmid. So, from a circular plasmid you got a non circular plasmid or a Linear Plasmid and this pieces out in the position of the piece, you put this guy.

So, this gene what you got; so, this gene should have a information what you wanted to synthesize the triplet code should be there. So, you want to synthesize a particular amino acid, you want to replace a particular amino acid in the previous case; then you cut that pod and put this new piece+; so now it is integrated into this. Now, so you have the plasmid you have integrated of the g; integrated the whole gene with the modification and that is now is put into the some line box; for example, E-coli.

So, you can introduce this into E-coli and express the express the system then you will get a express the protein the protein that comes out will have the modification in this particular thing.

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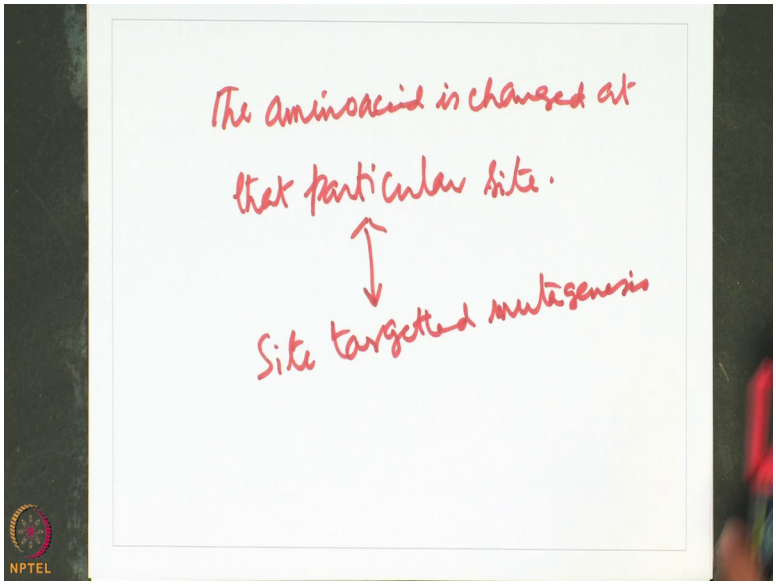

So, in site targeted mutagenesis case you will have so one with this process you will get at that particular site the amino acid is changed at that particular site and this is referred as site targetted is called site targetted mutagenesis. So, once you synthesize this is can be synthesized by using the E-coli cells you put this plasmid into the E coil.

So, this is whole operations are called genetic engineering operations; what I have mentioned is very little portion of this at the tip of the iceberg, not anything details. Somebody who is interested in this more details, they should approach biochemistry molecular biology course more precisely is speaking ok.

So, you have a genome; you select the genome where you have this particular modification is there and then put this the gene into integrate into a plasmid which is already cut and this plasmid this plasmid which is integrated with this gene X is introduced into the live cells and then allow the expression to take place and that will give the protein synthesis aspects as well ok.

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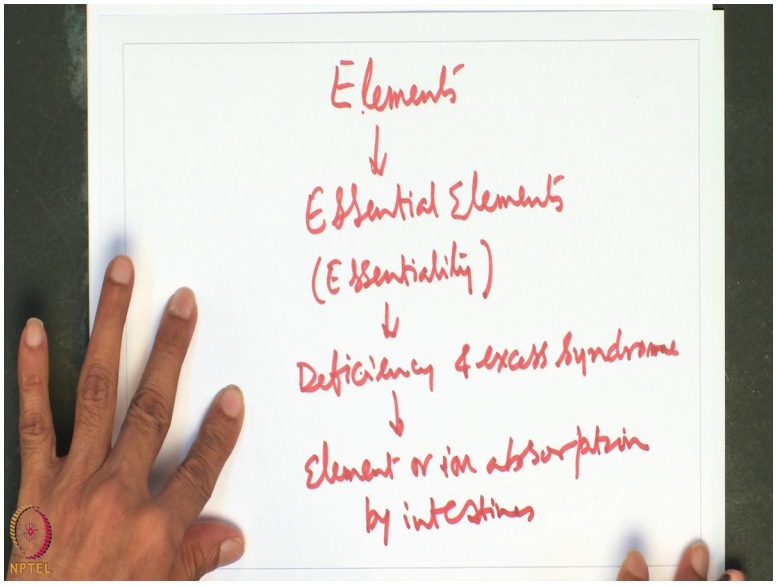

So, thus in this entire process of what I have been doing in the last several hours or lectures is that elements of whichever of essential elements ok.

And of course, we have looked at the essentiality the essentiality is looked at and we have worked out all the criteria. So, essentiality and deficiency and excess syndrome, then we have looked at the element or ion absorption by intestines.

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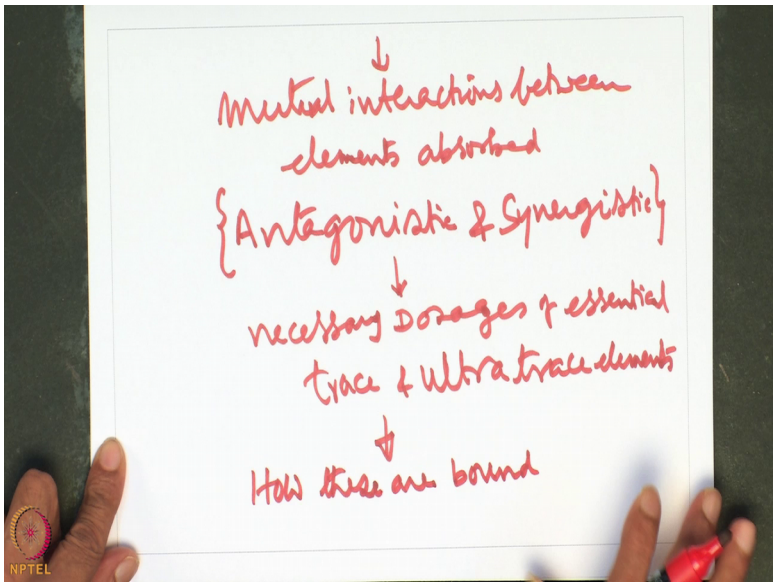

We looked at mutual interactions between the elements absorbed ok. This is Antagonistic and Synergistic; then we have look at the necessary dosages of essential trace and ultra trace and ultra trace elements. Then we started looking at how these are bound and prior to that, we have also looked at why these elements and not the other why these elements are so specific?

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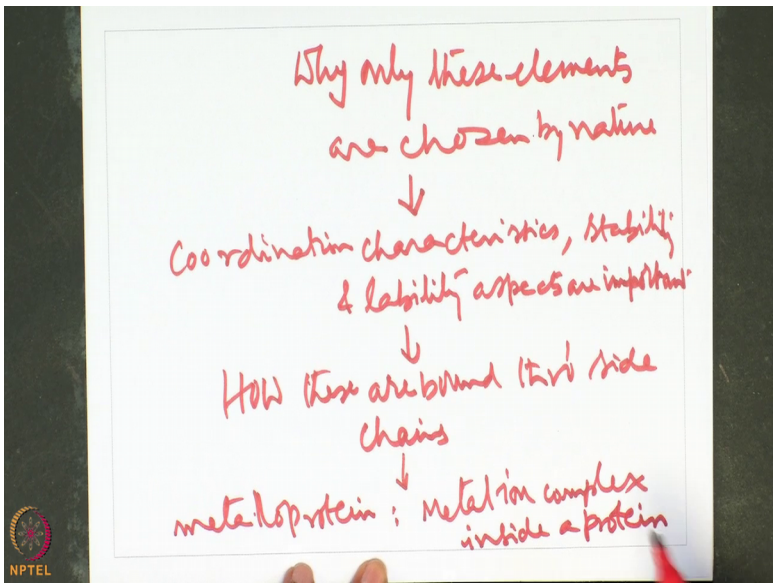

Why only these elements are chosen by nature and there we have looked at variety of coordination, characteristics, stabilities and lability aspects are important and I am still to explain this as stability, lability; which I will be doing in the next lecture in any case.

So, and coordination characteristics stability, then we talked about how these are bound through the side chains, how these are bound through side chains and then we brought the concept of the metalloprotein as; metalloprotein as metalion complex inside a protein, metalion complex inside a protein and then we tried to say that the simple metal ion in water versus metal ion in protein.

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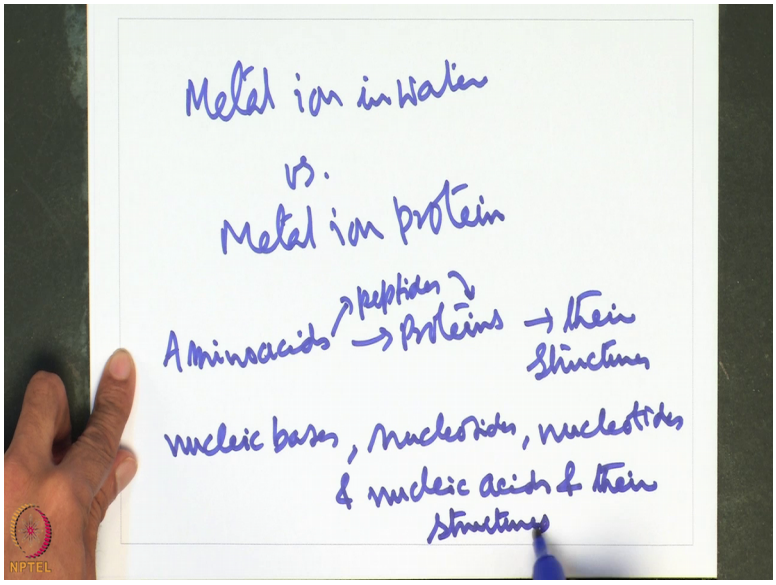

These are absolutely different; the properties of the metal ions are very well modified by the protein as compared to the metal ion and that is why different proteins would bring different kinds of re activities to the metal ions therefore, we have a huge variety of metalloproteins and metalloenzymes functioning in this. Then I have taken to explain you these amino acids proteins and peptides; amino acids proteins in between peptides and proteins and their structures. And we have also looked at nucleic bases, nucleosides, nucleotides and nucleic acids and their structures.

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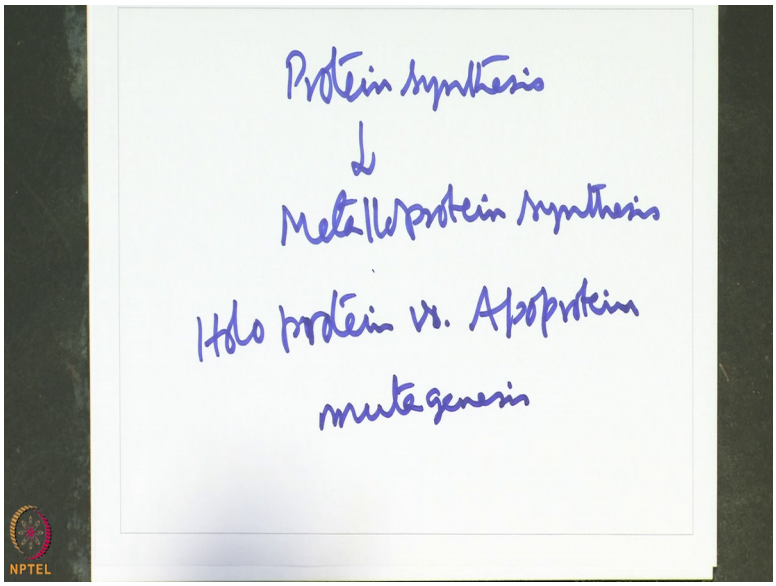

So, we have looked at all of these then we looked at the protein synthesis and then we looked at the metalloprotein synthesis, where the metal ion is introduced into the protein that is being synthesized and then brings the property and different various ways and also now we talked about the holo protein versus apoprotein then we have talked about the mutagenesis.

So, these are all various aspects that encompasses to understand the basic conceptual ways of this role of inorganic elements in biological systems in the form of inorganic chemistry of life and in the next class I will talk to you about the stability, lability, reactivity one of these aspects the metal ions when they are there. So, far we have only looked at the biological side, then we have look at the inorganic chemistry aspects of side.

Thank you very much.