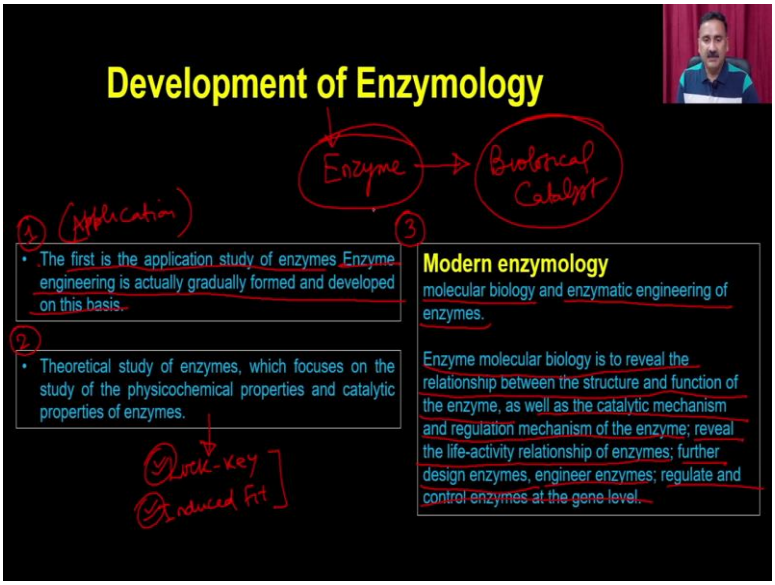
Enzyme Sciences and Technology
Prof. Vishal Trivedi
Department of Biosciences and Bioengineering
Indian Institute of Technology, Guwahati

Module - 01
Introduction
Lecture - 02
Basics of Enzyme

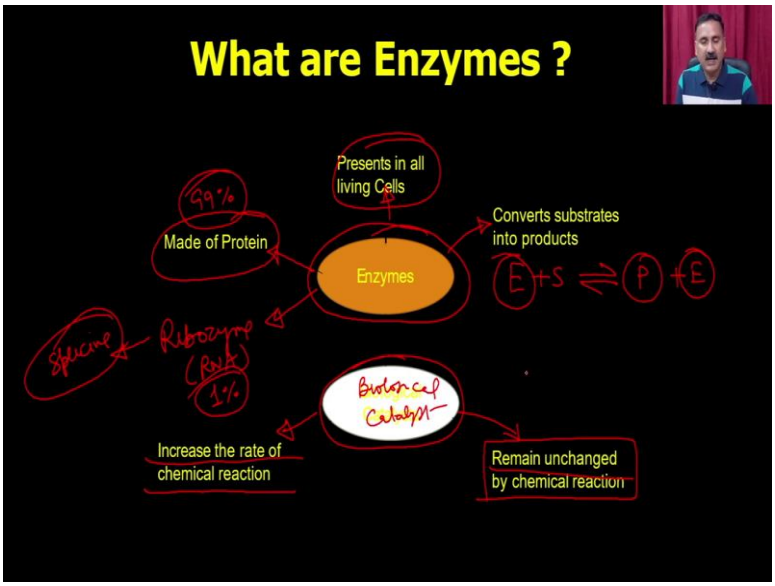
Hello everyone. This is Dr. Vishal Trivedi from Department of Biosciences and Bioengineering IIT Guwahati and the course Enzyme Science and Technology, we are going to discuss about the different properties of the enzyme. So, in this context in today's lecture we are first going to discuss about the Basics of Enzymes and then we also going to discuss about the different properties of the enzymes.

(Refer Slide Time: 01:09)



So, the question comes what is enzyme? Ok, right. Enzyme is also known as the biological catalyst right. So, let us see what are the different properties of the enzyme.

(Refer Slide Time: 01:33)



So, enzymes are the molecules ok which are present in the living cell right which are present in the living cell. They are mostly been made up of the proteins, although there are exceptions that you are actually also have the ribozymes right which is made up of the RNA. And, ribozymes are very specifically not catalyzing all the reactions, they are only doing the catalyzing the splicing reactions.

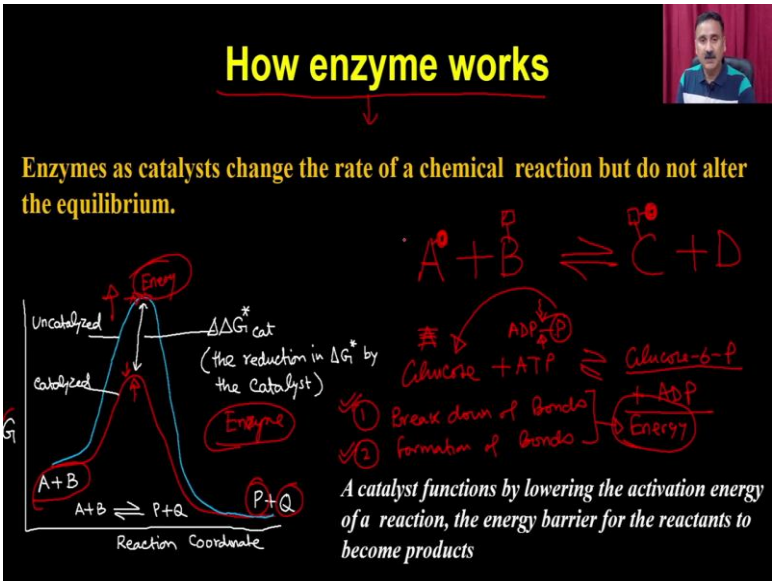
So, if we ignore the ribozymes, it is true that most of the enzymes are made up of the proteins. So, if I say it 99 percent enzymes are made up of the proteins, only maybe 1 percent or less than 1 percent enzymes are made up of the RNA or the ribozymes. And, these ribozymes are only doing the one activity that is they are involved in the splicing.

Then the enzymes are converting the substrate into the product right, you know that the enzymes are going to interact with the substrate and that is how they are actually going to form the product and the enzyme is actually going to be released right. So, enzyme is because it is a biological catalyst so, it is not going to participate into the reaction, it is only going to react chain the reactions.

Biological if they are biological catalyst right so, they are the biological catalyst and what they are doing as a biological catalyst? They are increasing the rate of the chemical reactions ok, but they are going to be remain unchanged after the chemical reactions ok. So, that is what the enzyme is interacting with the substrate, but it is actually going to

change the and they are changing the substrate into the product, but they are actually going to be remained unchanged. Now, let us see how the enzyme works ok.

(Refer Slide Time: 03:44)



So, when we talk about the reactions for example, you have a substrate like you have two reactants like A and B and when they are getting converted, they are forming the C and D ok. Now, what is happening? For example, you have the A which is having a group like this right and the B is having a group like this ok.

So, what will happen is that the A is if A has to convert it into C which actually having a group like this ok. So, exactly what is happening is that this A which is added to this particular group is taken out right. So, this group is going to be removed from here and it is actually going to be connected to this right and that is how the B is going to be get converted into C and A is actually going to be a D actually, because A is no longer having that A group. So, that is how it is actually going to be this.

Let me take the real example right for example, the glucose plus ATP right and it is going to form the glucose 6 phosphate right and it is going to form the ADP right. So, now, if I say so, here the glucose; so, here if I break this it will say ADP and phosphate. So, this is what is this ok.

Now, in this reaction what is happening is that the bond between the ADP and this phosphate is actually going to be broken down and then this molecule is actually going to

be transferred onto the glucose right. And, that is how you are going to have the synthesis of glucose 6 phosphate and the remaining molecule ADP is get converted into ATP. This means, if I have to catalyze a chemical reaction, if I have to catalyze any such reactions I have to have the breakdown of the bonds right that is first thing right.

You are actually going to have the breakdown of the bond between ADP and phosphate right and then we also should have the formation of bonds right. Now, this event and this event, both of these events are associated with the high amount of energy which means they actually require a energy. So, that the molecules are actually going to have lot of energy and that is how they are actually going to have the ability to break the bond and as far as the ability to form the new bonds right.

So, if you plot the amount of energy what is going to be you know developed right. So, if the you see that right how much free energy is associated with these molecules. So, you can actually be able to have the two energy right, you can actually have the A and B when they are and they can be actually present in two different conditions. One is where you have the non-catalyzed reactions.

So, if a non catalyzed reactions what will happen is that all these has to be done without the help of the enzymes right. So, it all these require the more in amount of energy which means A and B has to you have to heat them and such a way that they are actually going to cross this energy barrier right, this is the energy barrier what you have right.

So, this is once you break once you cross allow them to cross this energy barrier, the bond between the phosphate and the ADP is going to be broken down and the bond between the glucose and phosphate is actually going to be formed and that is how they are actually going to form the P and Q. This means if I do not add the catalyst, I have to heat the sample to such a high temperature that they will cross this particular barrier ok.

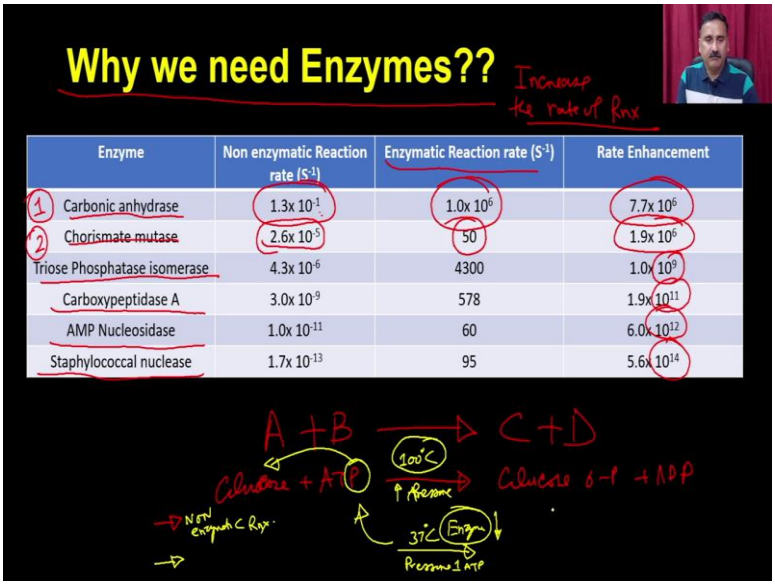
Now, if I add the enzyme ok. So, what will what the enzyme is going to do is it is actually going to go and bind the substrate ok. And, then the molecules which are present like for example, the side chains amino acid side chain and all that, they will actually going to facilitate this process of removal of the breaking of the bond and as well as the formation of the new bonds.

And, because of that you do not need to heat the sample to such a high temperature, the same reaction can be done even at the lower temperature or even at the normal temperature. So, because of that you are these substrates were very easily be able to cross this particular energy barrier and that is how they will actually going to form the more and more products. This is the event what is actually going to be done by the enzyme.

So, what enzymes are doing is they are lowering the activation energy. So, this difference of the energy what you see is actually being called as the activation energy. So, what basically enzymes are doing is they are reducing the activation energy and once they reduce the activation energy the A and B are spontaneously, B getting converted to P and Q ok.

Because, the enzyme itself provide them a suitable environment, enzyme helps the substrate to break the bond and as well as to form the bonds. So, the process for which you require the high temperature, you require the more energy that is also going to be taken care. So, this is the way the enzyme actually works. But the question is why we need more enzymes?

(Refer Slide Time: 10:05)



So, why we need the enzymes? Enzyme is required actually to increase the rate of reaction right, that is the main purpose of the enzyme because the enzyme does not participate into the reactions they only increase the rate of reactions. So, I have taken few

examples for example, I have taken the example of carbonic anhydrase. So, what you see here is the enzyme reaction rates right.

So, if you have a non-enzymatic reaction rates, your reaction rates are very low for example, 10 to power minus 1. But whereas, if you have the enzymes, the enzymes are actually going to have the reaction rate which is 10 to power 6 which means there is a rate enhancement of approximately 10 to power 6. Same is true for even for the other enzymes like chorismate mutase, triose phosphatase isomerase, carboxypeptidase, AMP nucleosidase and the staphylococcal nuclease.

All these what you see here is that in the case in the absence of enzyme or the non-enzymatic reactions, the reaction rates are very very low which means these enzyme these reactions are very hard to perform which means in 10 to the power minus 5 seconds right, it will it there will be you know reactions right. Whereas, in the case of enzymatic reactions you can have the 50 reactions in per second right, these many reactions per second right.

So, you will see it almost there will be enhancement of 10 to power 6, 10 to power 9, 11, 12 and 14. So, there will be always a very high enhancement of rate of reactions with the help of the enzymes. And, why it is important? It is important because of the two reason, if you want to convert A plus B right and form the C plus D for example, I have we have taken an example of the glucose right plus ATP; you know that this is the first enzyme, first reactions of the glycolysis right and plus ATP right.

Now, if I have to perform these reactions under the two conditions. For example, under the non-physiological conditions which means non-enzymatic reactions. Now, if I have to perform this reaction under the non-enzymatic conditions, what we will have to do is I have to heat these reactions at 100 degree Celsius, I have to increase the atmospheric pressure right, I have to increase the pressure.

So, that the conditions are more and more aggressive right, then only the ATP is actually going to give up the phosphate and then only the phosphate is actually going to be transferred onto the glucose. Now, imagine a biological molecule, imagine a biological organisms right; 100 degree Celsius is a very very high temperature right whereas, in a biology you always have a temperature requirement right.

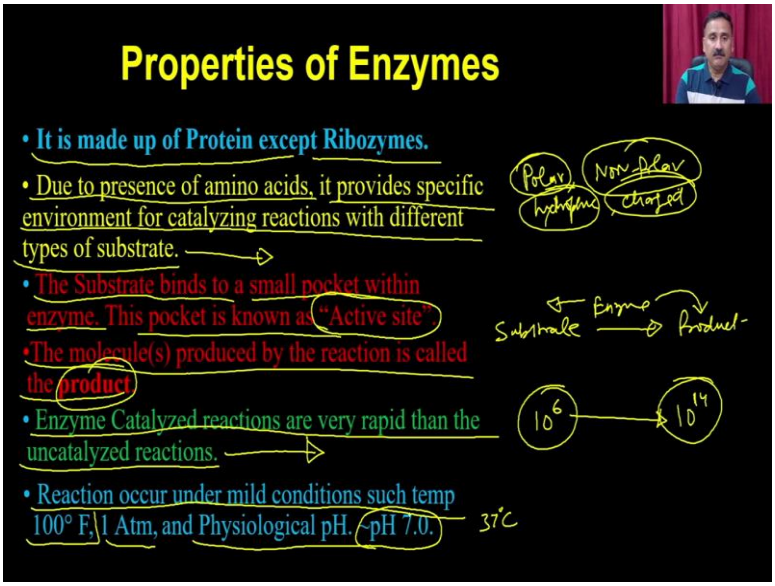
You always have to have the 37 degree Celsius, it cannot go beyond that right because otherwise it is actually going to affect so many parameters. Like you can actually be able to you know change the you know you will actually going to have the fever like conditions right.

So, the temperature is fixed, pressure is also fixed, you cannot work beyond the 1 atmospheric pressure right. So, it cannot go beyond 1 atmospheric pressure. This means you cannot change the conditions if when you are talking about the biological system right for example, the human beings. They cannot work beyond 37, they cannot work more than a 1 temperature right; because otherwise it will cause the damage to the blood vessels and all that right.

This means these parameters cannot be harsh, but you have to catalyze these reactions. So, what is the purpose? You can actually add the enzyme and what the enzyme is going to do, we have already discussed right. It is actually going to lower down the activation energy and because of that you do not have to have the very high temperature. You can actually be able to work with a normal temperature and that is how it is actually going to catalyze its reactions.

Now, let us say take a very briefly we will talk about the different properties of the enzymes.

(Refer Slide Time: 15:00)



So, enzymes are made up of the protein except the ribozymes. They are made up of the amino acids and it provides the specific environment for the catalyzing reaction for the different types of substrates. Because you have the different types of enzymes, you can have the polar amino acids, you can have non-polar amino acid, you can have the charge amino acids like the positively charge amino acid, negatively charge amino acid, you can have the hydrophobic amino acid.

So, you can actually have the different types of amino acids, you can have the polar, non-polar, you can have the hydrophobic, you can have the charge amino acid. So, because of this you are actually going to provide the very very precise and discrete environment and because of that you are actually going to provide the specificity to a particular substrate.

So, enzymes which are for the glucose will not going to bind the fructose, the enzyme which are for the DNA will not bind the RNA. In fact, within the DNA also there are enzyme which actually going to only recognize a particular DNA molecule. So, they are very specific because a combination of these type of amino acids can provide them the specificity.

The enzyme with having a small act area where it actually binds the substrate and this area is called as the active site. So, the substrate binds to a small pocket within the enzyme, this pocket is known as the active site. The molecule produced by the reaction of the enzymatic reaction is called as the product which means the substrate is going to be converted into the product and enzyme this is the enzyme right.

So, enzyme is actually going to interact with the substrate, it is going to do rearrangement, it going to break the bonds, it going to form the bonds and that is how it is actually going to produce the products. Enzyme catalyzed reactions are very rapid than the uncatalyzed reactions right, that we have already taken an example. We have taken an example of carbonic anhydrase, we have taken the chorismate mutase and other kinds of enzymes.

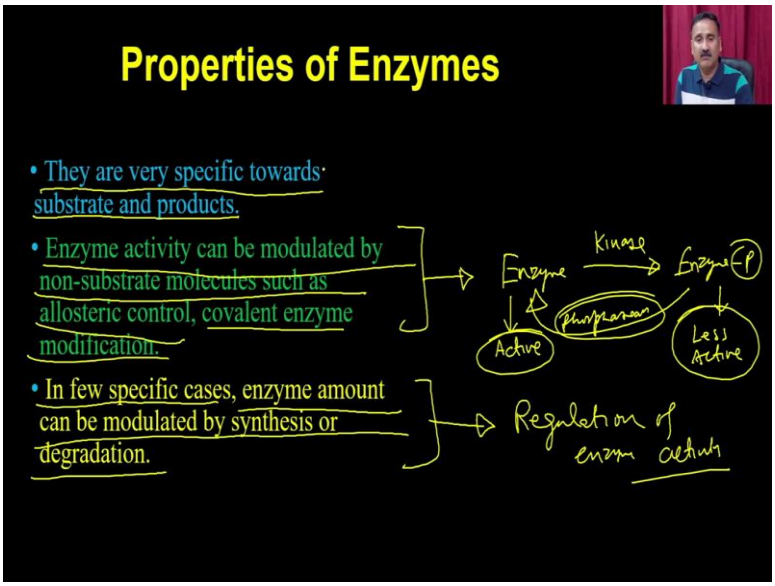
And, we have seen that the reactions are you know up regulating any anywhere from the 10 to power 6 to 10 to power 14 ok. So, reactions are when they you do not have the enzyme, the enzymes and reactions are very slow; when you have the enzyme, the reactions are very fast. Reactions occurs under milder conditions right because the non-

enzymatic reactions are actually require a very harsh condition. For example, 100 degree Fahrenheit, 100 degree you know and so on right.

Whereas, in the case of enzymatic reactions they going to be very mild conditions which means 100 degree Fahrenheit which is actually approximately 37 degree Celsius, you require 1 atmospheric pressure and the physiological pH. Because, many times you might have seen that the in the chemistry lab people are even do like very you know different types of pH and other kinds of things right.

So, even you cannot work under the those condition as well because the physiology does not allow you to go beyond a certain range of the pH right. You can be little bit different from the 7.4, otherwise mostly it is actually going to be remain as 7.4 right. Then, they are very specific, they are very specific towards the substrate and the products right.

(Refer Slide Time: 18:33)



Enzyme activity can be modulated by the non-substrate molecules such as the allosteric controls, covalent modifications and so on. So, this is all we are going to discuss in detail where we are going to talk about the allosteric modulations of the enzyme, we are going to talk about the covalent modification and so on right.

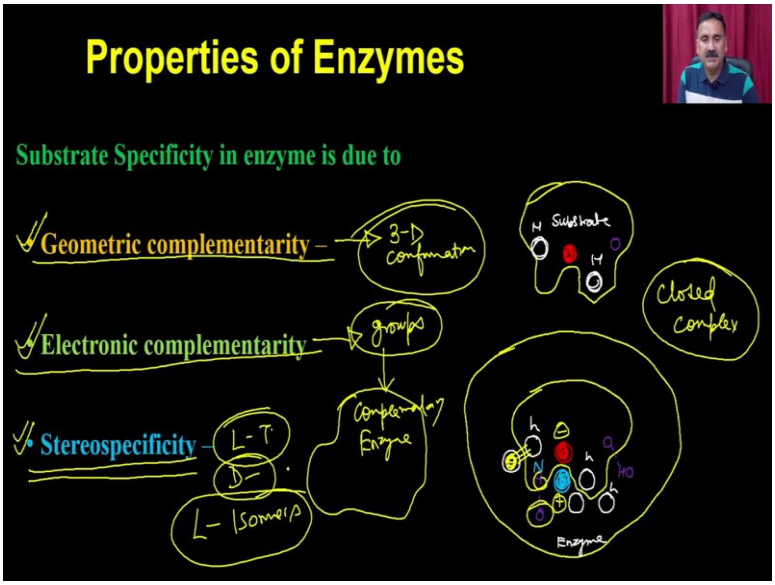
So, in covalent modification is a very different kinds of enzyme, different types of modifications where enzyme is going to be get converted into enzyme phosphate. For example, this is one of the covalent modifications and that enzyme is again going to be

return back. So, you actually going to have one set of kinase, you can actually going to have the one set of phosphatase and the phosphatase job is to convert that into this.

And, in some cases the enzyme is actually going to be active when it is non-phosphorylated and it is actually going to be less active or inactive when it is phosphorylated. But, this kind of active or inactive conditions could vary from enzyme to enzyme. Some enzymes are inactive when they are non-phosphorylated, they are very active when they are phosphorylated so, that depends on the enzyme to enzyme. So, this is one of the classical example of covalent modifications.

Similarly, we can have the allosteric modifications and so on. In some cases, the enzyme amount can be modulated by the synthesis or the degradation. So, that is also we are actually going to discuss when we are talking to talk about the regulation of the enzyme activity. So, now let us talk about how the enzymes are very specific for the substrate. So, enzyme specificity is always been controlled by the three processes.

(Refer Slide Time: 20:29)



One is called as the geometric complementarity, second is called as the electronic complementarity, the third is called as the stereospecificity. So, geometric complementarity means that enzyme is actually going to recognize a particular 3D conformations and they are actually going to active work on that particular theory confirmation.

For example, this is the substrate right so, it has a 3D confirmation like this right and that is actually going to received by the enzyme. If you have another enzyme which is not going to be like this, it is actually going to not going to accept this substrate because then it is actually not going to form a closed complex right. Because, there will be a places where it is not going to be in contact with the enzyme.

So, it has to form a closed complex when the substrate is going to interact with the enzyme. So, this is the enzyme, this is the substrate. Then, we have the electronic complementarity, electronic complementarity means you actually should have the different types of groups what is present onto the substrate and we should have the complementarity groups on the enzyme ok.

So, for example, in this case you can have the hydrophobic groups, you have the charge group, you have the charge group. So, what you see here is when this substrate will come and bind, what it will find is that there is a hydrophobic groups on to the enzyme side it is actually going to form the closed bond right, it is actually going to be show affinity.

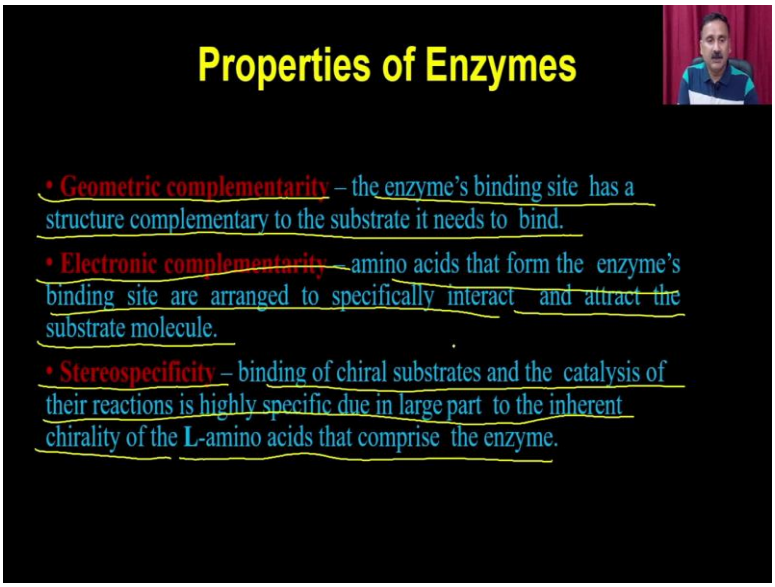
Similarly, there is a hydrogen bonding, there will be hydrogen donor, there is a hydrogen acceptors right. So, that is also present here. Then, you have the you know the negative charge here, you have the positive charge onto the enzyme. So, that is how they are actually going to have the salvage interactions and so on.

So, because this is present at a very precise locations, you are actually going to recognize only this substrate not the other substrate. For example, if you have another substrate where this particular you know positive group is missing right, in that case you have a negative group here, but the positive group is missing. So, this area it is not going to have a very high affinity compared to this substrate.

So, that is how the enzyme can actually be able to discriminate between the one substrate to another substrate. Then, we have the stereospecificity, stereospecificity means the enzyme can also recognize the L type of molecules or the D type of molecule. You know that we have the two different type of stereospecificity, L type isomers or the D types of isomers.

Mostly, the enzymes which are working in the biological system they are always recognizing the L type of isomers right, because the D type of isomers is not very common in the biological system. So, this is the geometrical compatibility.

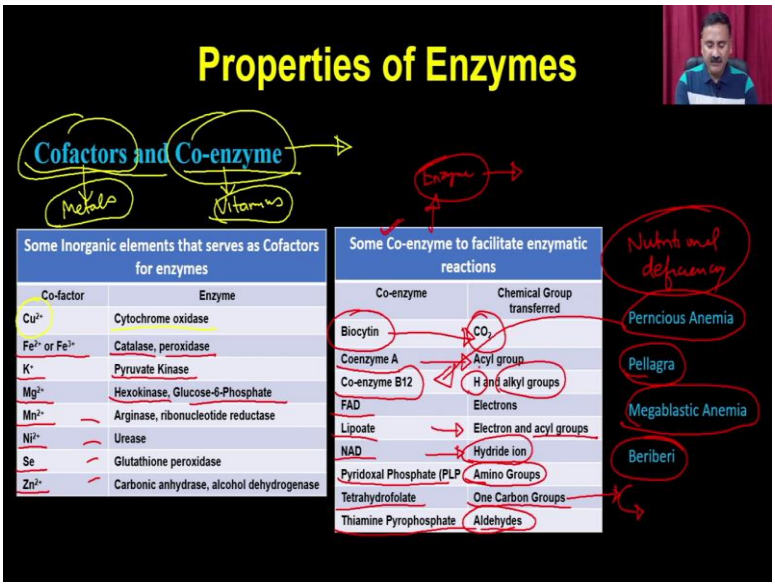
(Refer Slide Time: 23:25)



The enzymes binding site has a structure which is complementarity to the substrate it needs to the binds ok. Then, we have the electronic complementarity. So, amino acid that form the enzymes binding sites are arranged so, specifically interact and attract the substrate molecules. And, then we have the stereospecificity, the binding of the chiral molecule and the catalysis of their reaction is highly specific due to in large part of the inherent chirality of the L amino acid that comprises the enzymes.

Then apart from this, we also requires for example, the enzymes which require the different types of assistance like different types of additional molecules. And, those additional molecules are called as the cofactor as well as the coenzymes.

(Refer Slide Time: 24:12)



So, these are the cofactor and as well as the coenzymes. So, cofactors are mostly the metals right or metal like molecules whereas, the coenzymes are the vitamins. So, here this is the table of the different types of cofactor and as well as the coenzymes which are actually been responsible which are been you know participating into the different types of reactions. Mostly, the cofactors and the coenzymes are participating in facilitating the different types of reactions.

For example, you have a cofactor which is called as the copper, copper 2 plus right and that is present in the cytochrome oxidase. Then, we have the iron so, iron is present in the catalase and as well as the peroxidase. Then, we have the potassium which is present in the pyruvate kinase. Then, we have a magnesium which is present in the hexokinase and glucose 6 phosphate. We have a manganese, nickel, selenium, zinc; all these are present in the different types of enzymes.

Similarly, we have the coenzymes which are also facilitating the different types of chemical reactions. For example, we have the biocytin, the biocytin is working for the decarboxylation reactions. So, it is actually working in terms of transferring the carboxylate groups. Then, we have the coenzyme A, coenzyme A is facilitating the transfer of the acyl group. Then, we have a coenzyme B12 that is working for the transfer of the hydrogen or the alkyl groups between the different molecules.

Then, we have the FAD, lipoate, NAD, pyridoxal phosphate, tetrahydrofolate and thymine pyrophosphates and all these are participating in the one or other reactions. For example, lipoate is participating in the electron and as well as the acyl groups transfers, NAD is working in the hydride ions transfer, pyridoxal phosphate is working in the amino acid group changes.

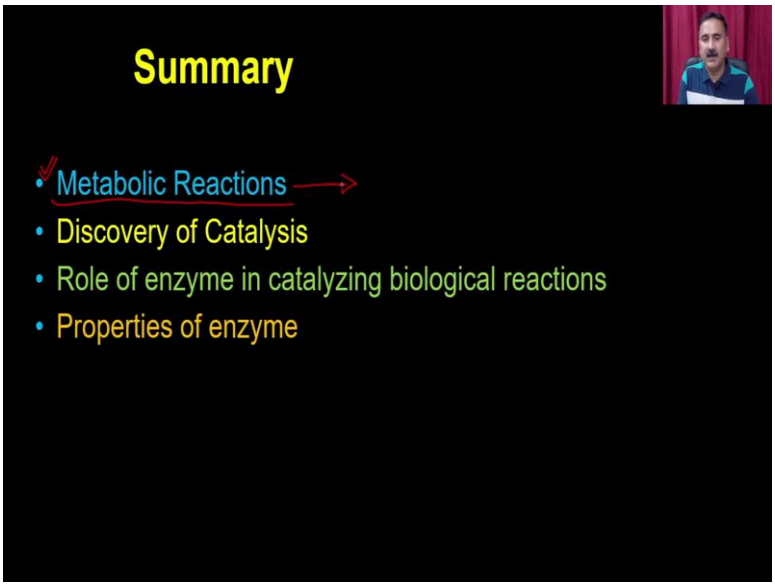
For example, they are actually changing the transferring the amine groups from the one amino acid to another amino acid. Then, we have a tetrahydrofolate which is participating into the one carbon group so, they are actually participating in you know biosynthesis of the nucleic acids and they are working on the salvage pathway.

Then we have the thiamine pyrophosphate, we are working in the transferring of the aldehyde. And, because the these coenzymes are helping the enzymes right they are helping the enzymes, enzyme cannot function the optimally and because of that the deficiency of the coenzymes or the cofactor is leading to the different types of disease.

For example, you can have the pernicious anemia if there will be a deficiency of the vitamin B12. You can have the pellagra, if you have a deficiency of other vitamins. Then, we have the megablastic anemia and as well as the beriberi. On all these diseases what you see is actually because of the nutritional deficiency.

So, you cannot have the some of these molecules in your nutrition and that is how you are actually going to develop some of these diseases. Because, these coenzymes are not present and that is how these enzymes will not be able to function and that is how they will not be able to facilitate these chemical reactions.

(Refer Slide Time: 27:41)



So, what we have learned today? We have learned today about that the enzymes are very important for running the metabolic reactions right. We have discussed about the digestion, we have discussed about the catabolic reactions, we have developed we discussed about the anabolic reactions.

Then, we also discussed about how the people have discovered the process of catalysis, how the scientists have observed that the addition of acid is simply adding the acid into the sucrose into the starch solution is facilitating the breakdown of the starch into the different into the smaller sugars.

And, that is how the Kirchhoff has you know observed that and that is how the other scientist Berzelius has pointed the term catalysis. The process in which the one pro one molecule is getting converted into another molecule with the help of the another molecule, but that molecule is not getting consumed or participate into the reactions.

And then we also discussed about the role of enzyme in the catalyzing the different types of biological reactions and we at the end we also discussed some of the classical properties of the enzymes.

So, with this I would like to conclude my lecture here.

Thank you.