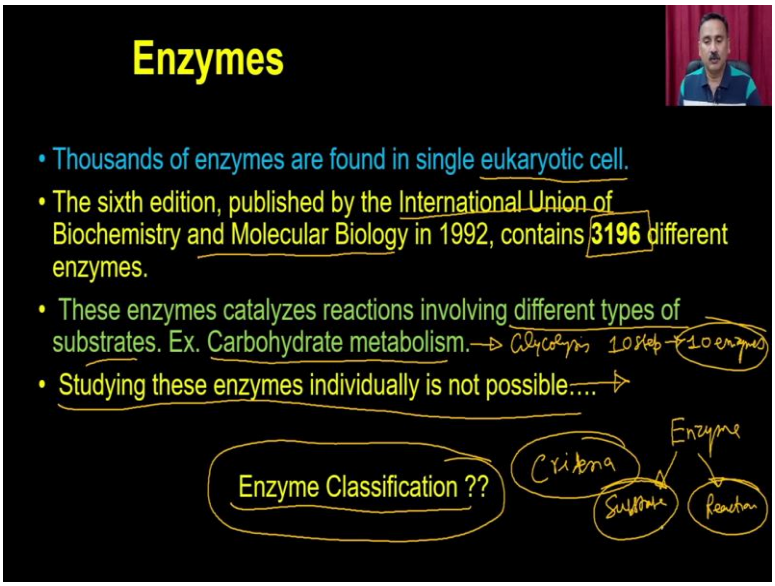
Enzyme Sciences and Technology
Prof. Vishal Trivedi
Department of Biosciences and Bioengineering
Indian Institute of Technology, Guwahati

Module - 01
Introduction
Lecture - 03
Enzyme Classification (Part-I)

Hello everyone this is Doctor Vishal Trivedi from department of Biosciences and Bioengineering IIT Guwahati. And in the course Enzyme Science and Technology we are discussing about the different properties of the enzymes. And in this context if you recall in the previous lecture we have discussed about the general properties of the enzymes and what are the historical aspects of the development of these field of enzymology and so on.

So, in the current lecture we are going to discuss more about the enzyme classification and how the enzymes are being classified. So, the first question comes why there is a need to classify the enzymes?

(Refer Slide Time: 01:25)



So, what you see here is that we have the thousands of enzymes, which are being found and in a single eukaryotic cell, right. Whether it is eukaryotic cell or the prokaryotic cell you will have the thousands of enzymes and these enzymes are present in the cytosol

they are present in the different organelles some of the enzymes are even part of the plasma membranes and so on.

So, as per the sixth edition published by the International Union of the Biochemistry and Molecular Biology there are 3196 different enzymes what are present. And I am sure by now the number could be even in some more also. So, these enzymes actually catalyze the reactions involving the different types of substrate. For example, we you might see that we have the different types of enzymes which are present in the carbohydrate metabolisms, right.

For example, in the glycolysis or itself we have the 10 steps. And in these 10 steps we have the 10 different enzymes which are participating into the catalyzing the different reactions. And they are you know working with the different types of substrates. And because the enzyme number is very high because they can be able to utilize the different types of substrates it is very very difficult actually to study these enzyme individually.

And that is why it is studying these enzyme individually is not possible which means if you can actually group them together it is easy to study them. Because then you can actually be able to study one of the representative from the group and that itself is going to help you to understand the general mechanism like.

So, for that purpose it is very important that we should do the enzyme classifications, but the when people thought of enzyme classifications the number was very high, they were utilizing the different types of substrates. The question comes how you can be able to classify the enzymes?

So, if you recall or if you might have heard about the classifications of the even the you know different types of organisms for example, if you talk about the classifications of the bacteria, classification of viruses, classifications of animals, plants, fungi or any other groups they are being classified by taking some criteria. So, you actually require some you know criteria on which you can be able to do the enzyme classifications.

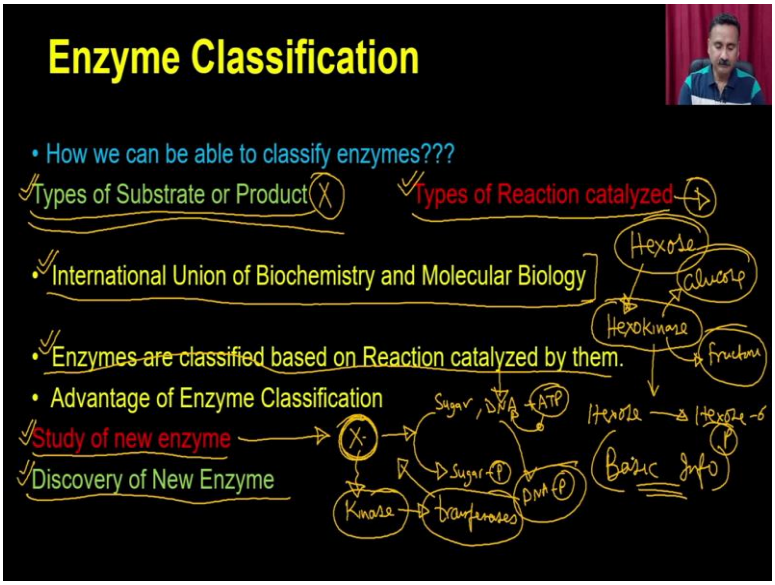
In the case of the organisms, it is easy because you can be able to look at the some of the properties and phenotypes and all those kind of thing and that is how you can be able to adopt that as a criteria and you can be able to classify them but in the case of enzyme. Also, the first question comes how you are actually going to do a enzyme classification

and what would be the criteria and on which you can be able to do the enzyme classifications.

So, for enzyme the as like you know for the animals or the plants, they can be classified based on whether they are you know having the four legs, whether they are having the feathers, whether they are actually having the bones and so on. Similarly, for the enzymes it has only the two properties one is enzyme, what substrate it is utilizing and the other is what kind of reaction it is catalyzing, ok.

So, enzymes are only doing this, right. So, that is why the enzyme classification cannot be based on any other para type, but also on the basis of only the substrate and the reactions.

(Refer Slide Time: 05:23)



So, that is what it is written, right. So, if you want to classify the enzyme can be only classified either by the type of substrate or product what it is utilizing or type of the reaction it is actually catalyzing. Now, if you see the type of substrate or the product, you will see that the same enzyme is actually utilizing the different types of substrate under the different conditions. For example, you have the hexose, right. So, this is a hexose sugar right, and this can be accepted by the enzyme which is called as hexokinase, right.

So, hexokinase is also that you know accepting the glucose hexokinase is also accepting the fructose. So, how you can be able to classify these kind of enzymes, which are

actually taking up the different types of substrates? That is why the classifying the enzyme based on the substrate is not possible because the substrate can be changed for a single enzyme.

Let us now we have taken an example of the hexokinase which actually accept the different types of six membered sugars, right. Whether it is glucose, fructose, mannose or any other sugar hexose is hexokinase is actually going to accept these sugars and it will catalyze the reaction. So, that is why the idea of classifying the enzyme based on the substrate or the product was turned down.

And then the people have started you know classifying the enzyme whether it can be classified based on the reaction it catalyzes, right. So, as for the reaction is catalyzed hexokinase whether it will take the same example of hexokinase. Hexokinase will actually perform the same kind of reactions whether it is accepting the glucose as a substrate or the fructose as a substrate.

So, what it is going to do is it is actually going to convert the hexose into the hexose 6 phosphate, right. So, it is actually going to phosphorylate sugar whether it is the glucose or whether it is fructose or whether it is mannose. So, that is something which is more and more stable right, in terms of the reaction it is catalyzing.

And that will not change even under the different conditions whether the substrate specificity can be modulated for an enzyme, but the reaction it catalyzes cannot be changed until you change the other parameters. So, that is how that could be the one of the criteria what on based on which the people have started you know classifying the enzymes.

And the union which actually performing these tasks is called as the international union of biochemistry and molecular biology, which is actually a union of the different biochemist and scientists who are working in this particular field. So, they have actually have a set of guidelines on based on which they are taking up the different types of reaction what enzyme is catalyzing and that is how it is actually classifying the different types of enzymes.

So, enzymes are classified based on the reaction catalyzes by them, ok. And what is the advantage, why they are we are doing the enzyme classifications? Because it helps to

study the new enzyme and it helps to study the discovery of the new enzyme as well. So, you can imagine that if I have identified the new enzyme. For example, if I have identified an enzyme X, right.

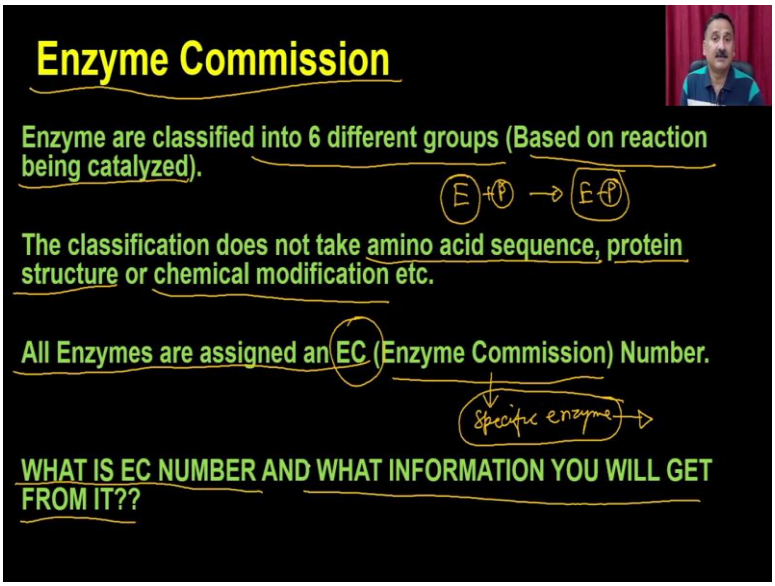
And if this X is actually doing this it is actually taking up a sugar and it is converting that into the sugar phosphate or it will taking up the DNA and it is converting the DNA into the DNA phosphates. So, what will say is that this enzyme, which is X actually which is just identified the new is having the similar kind of properties what the enzyme kinase group is actually having or in general what we will say is it is actually the having the properties which is similar to the enzyme belonging to the group transferases.

And that is how we can actually be able to say what are the different properties of the kinetic properties or enzymatic properties associated with this particular enzyme. So, if it is a transferase, it requires the two substrates. So, that it can be able to transfer the group from one substrate to another substrate for example, in this case it is actually going to use the ATP and that is how it is actually going to transfer the phosphate from the ATP to the DNA. And that is how it is actually going to form the DNA phosphate.

So, a lot of information's you can be able to gather about this new enzyme what you have discovered or what you have identified in a organisms or in a particular cell without doing much experiment. Because once you know that it is a transferase, this it is a kinase then you can be able to do like similarity search you can do sort of experiments and without doing much experiment you can be able to get the basic information about this particular enzyme.

Because how you going to deal this enzyme that all information you are going to get. The specialized information or the specialized feature of that particular enzyme for that you might have to do the research and that you actually going to get in due course of time. But the basic information will help you to understand and to perform the experiments.

(Refer Slide Time: 11:16)



So, as I said you know there is a there is a commission which actually there is a committee which actually classifies the enzyme. So, they have actually formed the enzyme commission. And the enzyme commission has actually classified the enzyme into the 6 different groups based on the reaction what they have been catalyzed, ok.

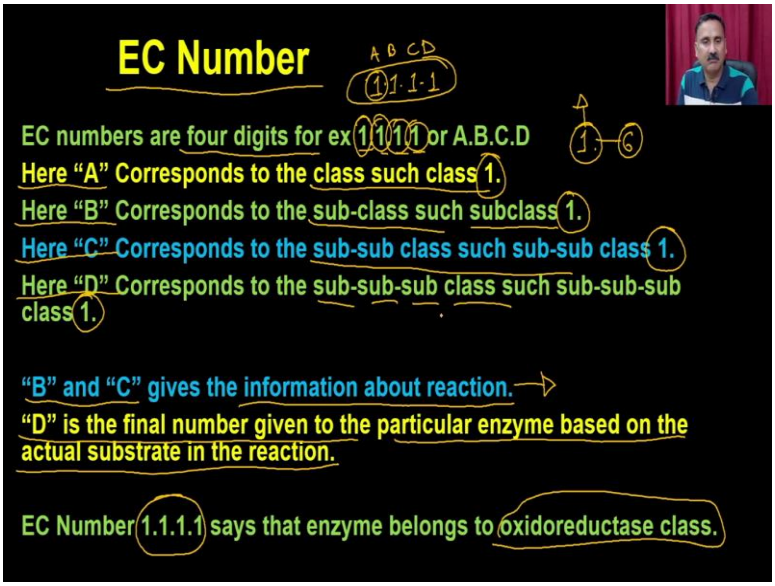
This reaction this reaction mechanism what is catalyzed is not taking into the consideration the amino acid of the sequence like the protein, the enzyme what you are the it is not taking up into the consideration the protein structures or the chemical modifications, ok.

For example, if you have the enzyme E and if it is getting phosphorylated it is actually going to form the enzyme phosphorylated enzyme. So, that kind of chemical modifications are not going to taken up into the consideration. Only the native enzyme is actually going to be taken up into the consideration. And all the enzymes are actually going to be classified based on the reaction what is being catalyzes.

And all the enzymes are assigned a number which is called as the (Refer Time: 12:26) enzyme commission number. And these enzyme commission number are specific for that particular enzyme what you are going to talk about, right. So, that enzyme when you say ok this enzyme is having this enzyme commission number you are actually going to say that this is the whole classifications.

So, what is the what is the enzyme commission number and what information you will get from a enzyme commission number, right. So, enzyme commission number is a very very powerful tool or powerful term which actually going to give you a lot of information about that particular enzyme.

(Refer Slide Time: 13:07)



So, what is enzyme commission number? So, enzyme commission number is a number which actually contains the four digits for example, 1 dot 1 1 dot 1 and 1 dot 1, ok. So, 1 1 1 and 1 and it is actually going to be written like this 1 dot 1 dot 1 dot and all these four 1 are corresponding to a particular information or it is actually going to give you the classification of that particular enzyme.

So, if I say that A A A all these are A B C D, what is a and what is B what is C and what is D is corresponding. So, here the A which means the first digit is corresponding to the class which actually this particular enzyme is belonging. As I said you know what the enzyme commission has classified the enzyme into the 6 classes. So, all these are from 1 to 6. So, depending on what number you see in the in place of A you are going to say that the enzyme is belonging to the enzyme class A, right or enzyme class 1.

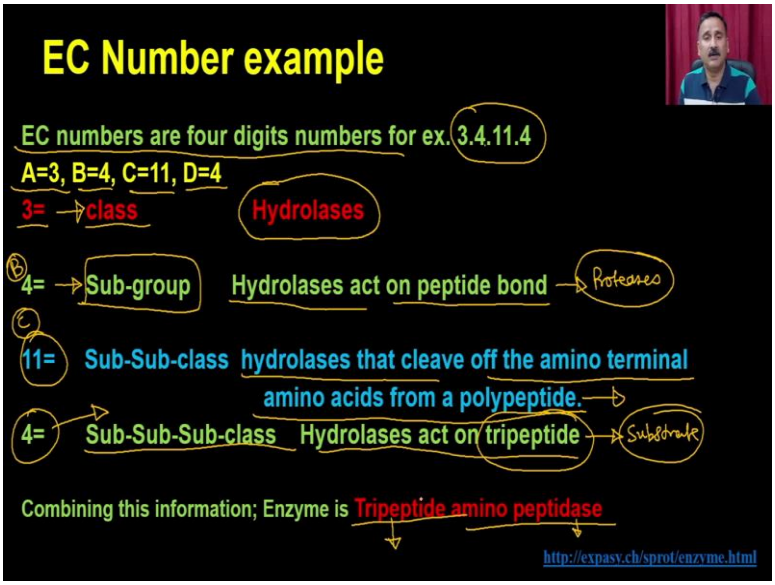
Then the number B is corresponding to the subclass under the class actually subclass. So, it will subclass 1 then the C is corresponding to the sub sub subclass and that also is 1 in this case and then the D is actually going to say sub sub and sub class and that also is actually the 1.

So, if you combine these 4 and you will say that it this is an enzyme which belongs to the oxidoreductase class actually because the first one is the oxidoreductase class. What information the B and C is actually going to give you? The B and C is actually going to give you the information about the reaction what this enzyme is going to catalyze which means the type of reactions.

Whereas D is the final number and that is the number what is being given particularly based on the actual substrate what is involved in this particular reaction. So, you see from the enzyme commission number you will get the full informations, you will get the information about which class it is belonging, what kind of reaction it did catalyzing and what substrate, ok.

And that is why this enzyme commission number is a very very powerful tool to utilize and that is how it is actually going to help the scientist to give get the full information about that particular enzymes. Now, I will take an example of the some of the enzyme commission number and understand how what it means actually.

(Refer Slide Time: 15:54)



So, a EC number are four digit number as we said, ok. So, for example, this is the EC number 3 dot 4 dot 11 dot 4. So, what it says is, A is equal to 3 B is equal to 4 C is equal is 11 and D is equal to 4. So, when it says A is equal to 3, 3 means it is actually corresponding to the class of that particular enzyme.

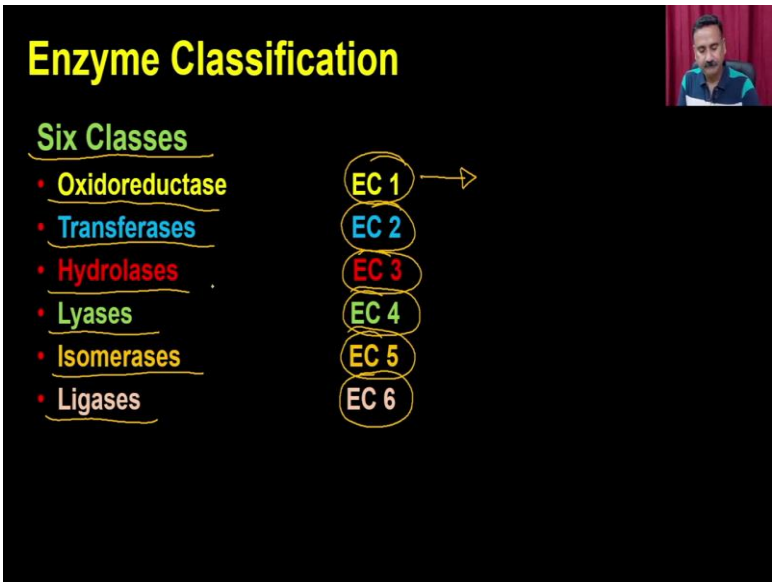
So, class of the 3 if the in front of the class it is saying 3, then the enzyme is belonging to a group which is called as the hydrolysis. If the B is 4 which means it is actually denoting the subgroup, right. So, the subgroup 4 within the 3 is belonging to the hydrolysis that act on the peptide bond which means all these are actually the proteases which are actually going to work on the peptide bonds.

Then if you have the C which is 11 which means the sub subclass is 11 then these are the enzyme like the hydrolases that cleaves off the amino terminal amino acid from a polypeptide, ok. So, that is a kind of the information. So, what you see here is. So, from the B and from the C we are getting the information about the type of reaction it is catalyzing. First is it is a hydrolysis which is act on the peptide bond, the second is that it is actually going to work from the amino terminal side of the enzyme.

And then the number 4 is going to give you the information about the sub sub sub class and that is the hydrolases which act on to the tripeptide. So, this actually gives you the information about the substrates, ok. Now if you combine all these information's. So, what it says is that it is a tripeptide it is a enzyme which works from the amino terminal side and it is actually a protease. So, if you combine all these you will say that the enzyme is tripeptide amino peptidase.

So, peptidase means the enzyme which actually is going to work on the peptide bond, amino means it is actually going to choose the enzyme for choose the substrate from the amino side and the tripeptide means it is actually going to work on a tripeptide it is not going to work on the bigger proteins it is only going to work on the tripeptides.

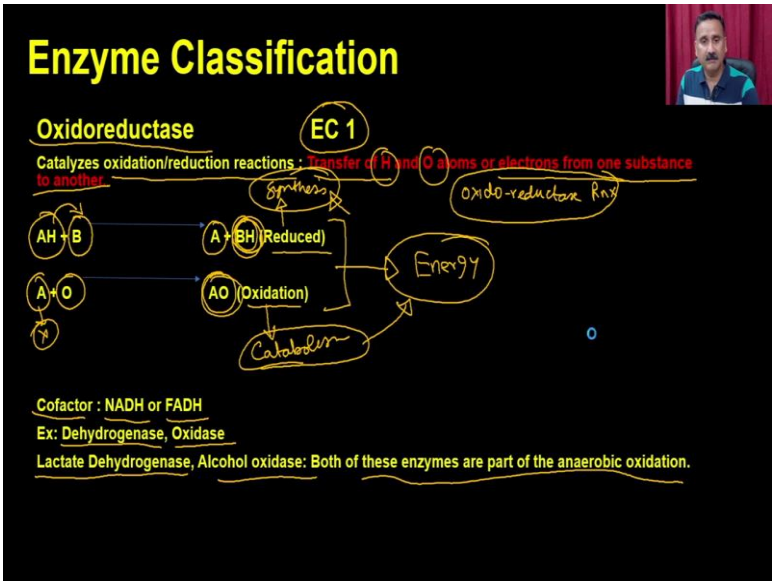
(Refer Slide Time: 18:30)



So, based on the enzyme commissions there are six classes. One is called as the oxidoreductase. So, that is the EC number 1, then we have the transferases that is the EC number 2. Then we have the hydrolases that is the EC number 3, then we have the lyases these are the EC number 4, then we have the isomerases and these are the EC number 5 and then we have the ligases and these are the EC number 6.

So, let us take the EC a enzymes from the each group and we will see how the these groups are further being classified under the enzyme commissions.

(Refer Slide Time: 19:15)



So, first is oxidoreductase. As I said it is the EC 1. And what are the enzymes are going what reaction it is going to catalyze? It is actually going to catalyze oxidoreduction reduction which means it is actually going to have the transfer of proton hydrogen or the oxygen or it is actually going to have the withdrawal of the electron from the one substrate to another substrate. So, it is actually going to catalyze the oxidoreductase reactions.

Which means it is actually going to catalyze the withdrawal or the addition of the electron from the one substance to another substance. Which means if you have a substrate AH if you add the another substrate B this enzyme is actually going to withdraw the pro proton or the hydrogen from this and it is actually going to transfer that onto the B. And that is how it is actually going to form the A plus BH. So, this is a reduction reactions.

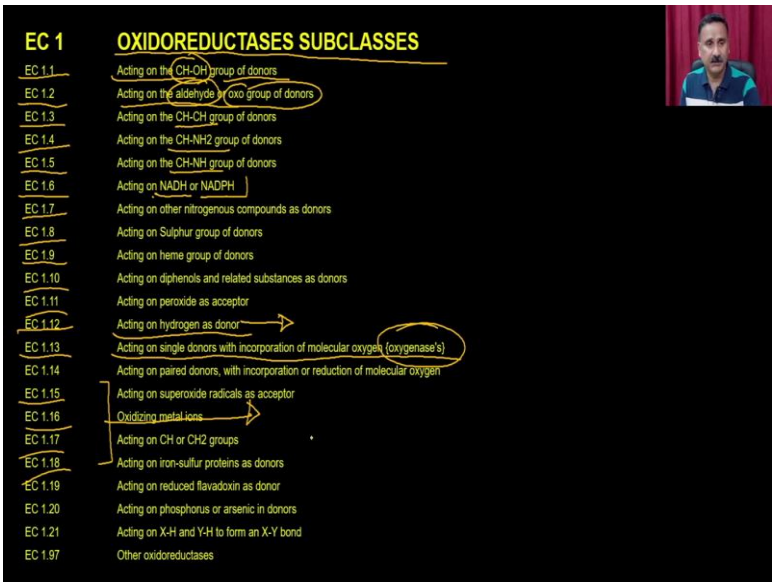
Similarly, you can have the A and it is actually going to add the oxygen and that is how it is actually going to form the AO and that is how it is actually going to this is a oxidation reaction. So, basically these enzymes are going to catalyze the oxidation and reduction reaction. And that is why these enzymes are very important in terms of running the metabolism and acquiring the energy for the system.

Because when they are actually going to do the oxidation, they are actually going to be a part of the catabolism and when they are going to do the reduction, they are actually going to be a part of the synthesis phase. Because reduction means you have synthesized a new molecule, oxidation means you have destroyed this actually, ok.

Because there is no A available now it is the AO what is available. So, that is, but in the synthesis part you are actually going to have the energy which is going to be taken up whereas, in the catabolic reactions the energy is going to be produced. Since the electrons are involved in these enzymes of reactions you are always going to use the different types of co factors.

For example, in this case NADH or FADH can be a co-factor. Examples are the dehydrogenases and oxidases for example, the lactate dehydrogenase alcohol oxidase both of these enzymes are part of the anaerobic oxidations. Now the oxidoreductase class is further being divided based on the subclass and sub subclass.

(Refer Slide Time: 21:59)

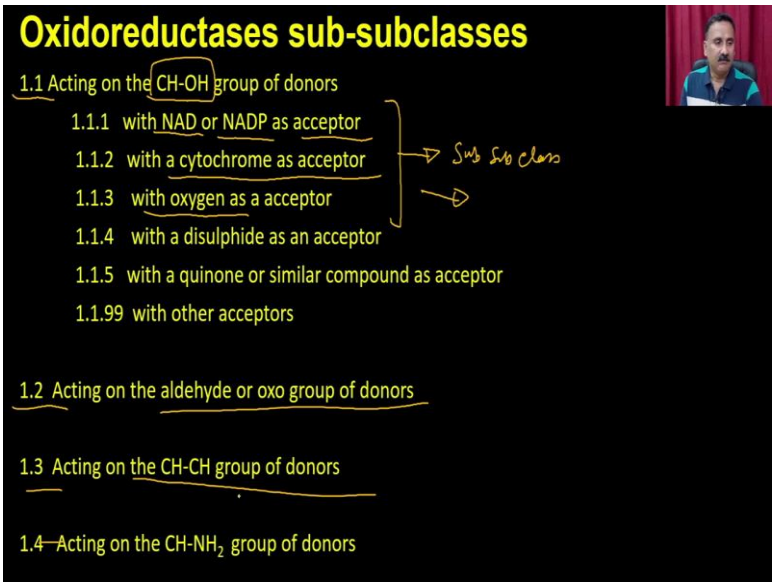


So, what are the subclasses are present within the oxidoreductase class? So, you have the EC 1.1 which is these are the enzyme which are acting onto the CH and OH group of the donors. Then we have the EC 1 2 these are the enzyme which are actually or acting on the aldehyde or the oxo group of the donor, then we have EC 1 3, 1 4, 1 5, 1 6 all these are actually going to act on the different types of C substrate like CH substrate, CH NH 2 substrate CH NH 2 sometime they are also acting on the NADH or NADPH.

Then we have the EC 1.7, 1.8, 9, 10, 11 and all these are actually the different types of enzymes which are part of these particular mechanisms and they are actually acting on the different types of substrates. Then we have the EC 12 which is actually acting on the hydrogen as a donor, right. And then EC 13 which is acting on the single donor with a incorporation of molecular oxygen.

So, these are the oxygenases then we have the EC 15, 16, 17, 18 all these are actually the different classes or different subclasses which is within the oxidoreductase and they are actually working on the different types of substrates or catalyzing the different types of reactions.

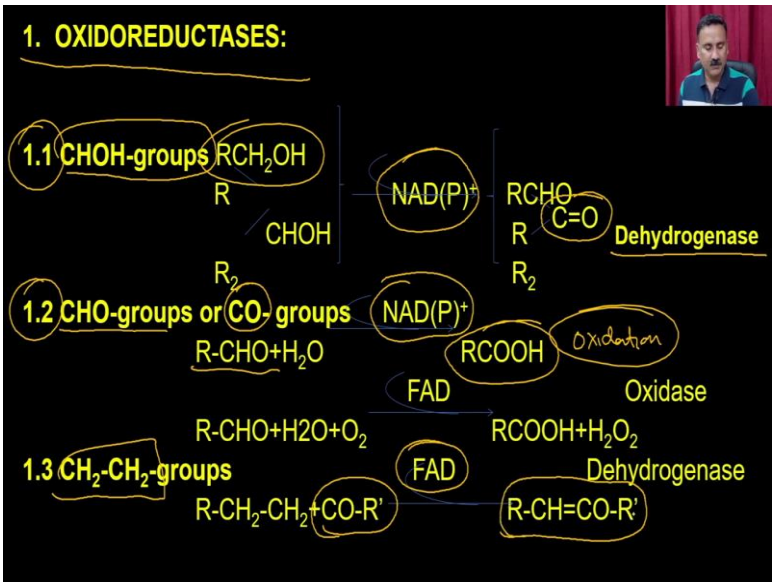
(Refer Slide Time: 23:25)



Then we have the sub subclass within oxidoreductase. So, you what you see here is the 1.1 which is actually the enzyme which are going to work on the CH OH group can still be further having the subgroups like the enzyme which are using the NAD or NADP as a acceptor or you can have the enzyme which are actually having the cytochrome as a acceptor or you can have a enzyme which is having the oxygen as an acceptor.

So, based on the acceptor it is can be further classified into the sub subclass and that actually is going to give you the this EC numbers, ok. Then we have the 1.2 which is aldehyde enzymes which are acting on the aldehydes or 1.3, 1.4 these are the enzyme which are working on are acting on the alkenes or the amines.

(Refer Slide Time: 24:19)

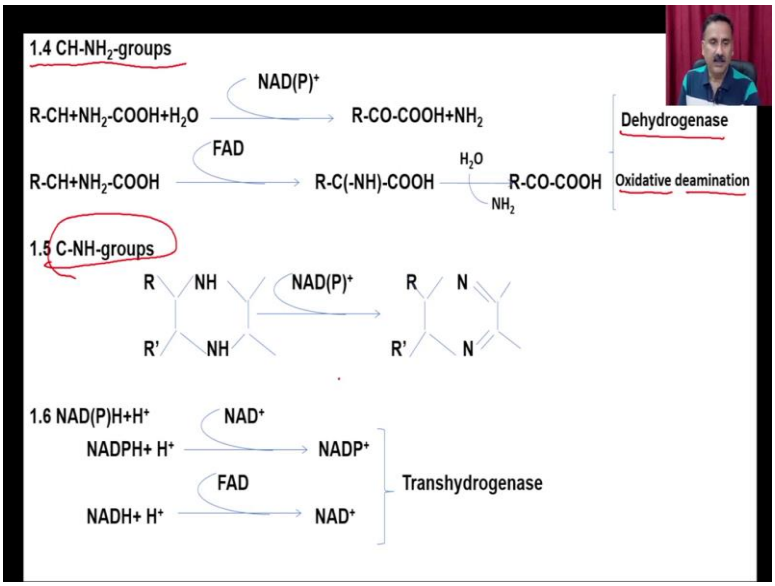


Then we have the and these are the reactions what the oxidoreductase is going to catalyze. So, in for example, in the case of 1.1 it is actually going to work on to the alcohol groups like the RCH 2 OH and with the help of the acceptors it is actually going to convert them into the aldehyde.

And that is how it is actually going to have the dehydrogenases. Then we have the 1.2 the enzyme, which are actually going to act onto the aldehydes or the carbonyl groups and they are actually going to convert the aldehyde into the acid and that is how it is actually a oxidation reaction, right. Because you are converting the aldehyde into the acid and it is actually going to use the NAD plus as a co factor.

Then we have the enzyme which is going to work on to the alkenes. So, in this case you can have the CH 2 CH 2 and with the this another group and it is going to use the FAD as the molecule and that is how it is actually going to bring the in the double bond into this, ok and.

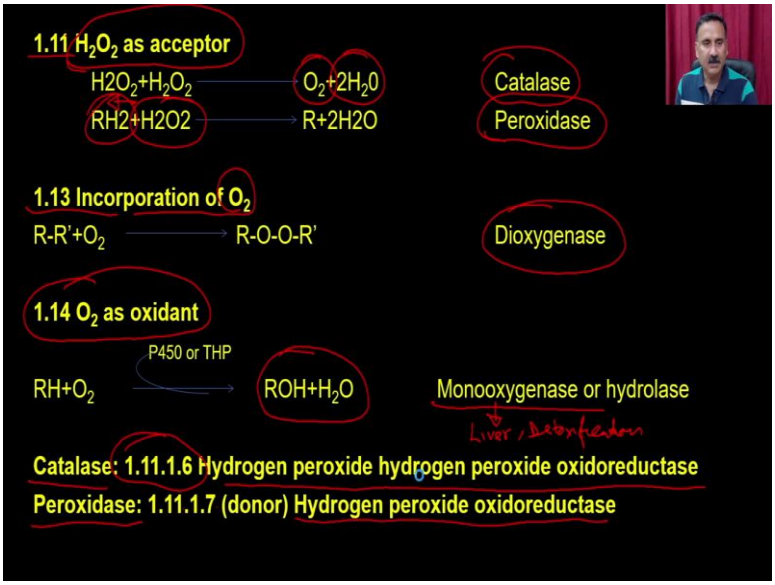
(Refer Slide Time: 25:32)



Then we have the another molecules like the another molecules like CH NH 2 group. So, 1.4 is belonging CH 2 NH 2 group and that also is going to catalyze the dehydrogenation reactions or oxidative deamination. So, these and these small these kind of reactions are very common in terms of the some of the detoxification reactions what we are going to discuss in detail also.

Then we have this C NH group the enzymes which are going to work on C NH 2 group C NH groups and that is going to be catalyzed this kind of reactions.

(Refer Slide Time: 26:15)

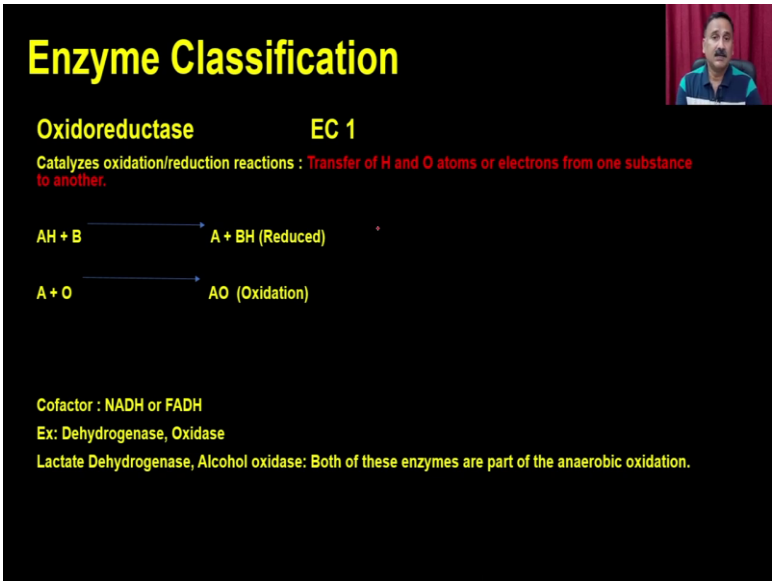


And then we have the 1.11 which is the where the H 2 O 2 is going to be used as an acceptor. So, you see that the H 2 O 2 is going to produce the water molecule and the oxygen and that is the classical reaction going to be catalyzed by the catalysis or sometime the H 2 O 2 is going to be used for the oxidation of the substrate and that is actually going to be a classical reaction what is being catalyzed by the peroxidases.

Then we have the 1.13 and that is the incorporation of the oxygen, right. So, that is called as the dioxygenases. And then we have the 1.14 which is where the oxygen can be used as an oxidant and in that case these are actually going to oxidize the molecule and it is going to be a part of the monooxygenase system. These monooxygenase systems are present within the liver for the detoxification reactions for catalyzing the detoxification reactions for catalyze the detoxification reactions, ok.

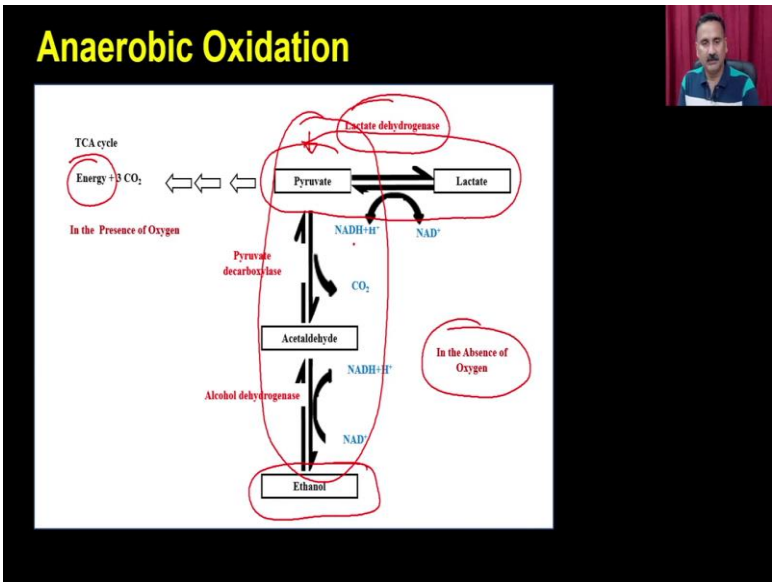
So, catalysts you will see that this is the EC number for catalysts which is 1 1 1 11.1.6, right. Because 1 11.1.6 and that is how it is a hydrogen peroxide oxidoreductase. Similarly, we can have the peroxidase which is 1.11.1.7 and there you have the hydrogen peroxide oxidoreductase.

(Refer Slide Time: 27:42)



Now, when we talk about the oxidoreductase, oxidoreductase play a very crucial role in terms of the many of the metabolic reactions and many of the metabolic processes so, one of the metabolic processes where the oxidoreductase play very crucial role is the anaerobic oxidations.

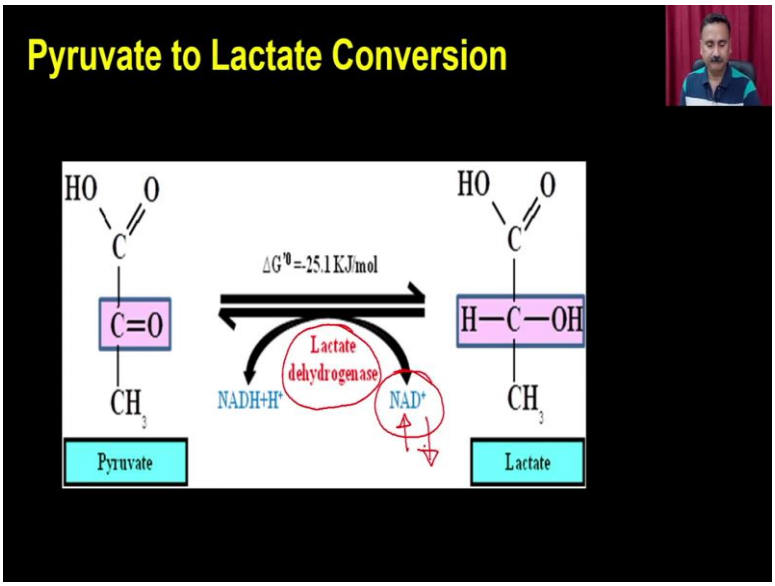
(Refer Slide Time: 28:00)



So, within the anaerobic oxidation what you have is you are under the oxygen in the presence of oxygen the pyruvate what is being synthesized by the glycolysis is actually going to be entered into the TCA cycle and that is how it is actually going to produce the energy.

Whereas in the absence of oxygen the pyruvate is going to be converted into either into the lactate or it is going to be converted into the ethanol. And this is the pathway where you will see that lot of the oxidoreductase enzymes are involved like for example, whether the pyruvate is getting converted into lactate that is being catalyzed by the lactate dehydrogenase and you are going to use NADH as a co factor.

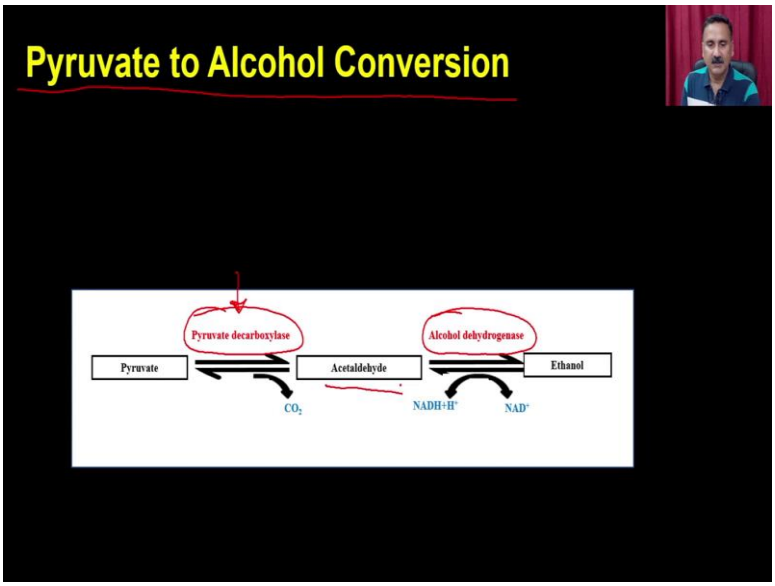
(Refer Slide Time: 28:45)



So, that is a reaction what is going to be catalyzed by one of the classical oxidoreductase enzyme that is the lactate dehydrogenase which is converting the pyruvate into the lactate. And this is a survival pathway because you cannot have the continuous supply of NAD plus if there is a if there is a no oxygen, right.

If there is a no availability of oxygen that there will be a shortage of NAD plus and that is how this is a kind of a rescue mechanism through which the NAD is going to be generated and that is actually going to be part available for catalyzing the different types of reactions.

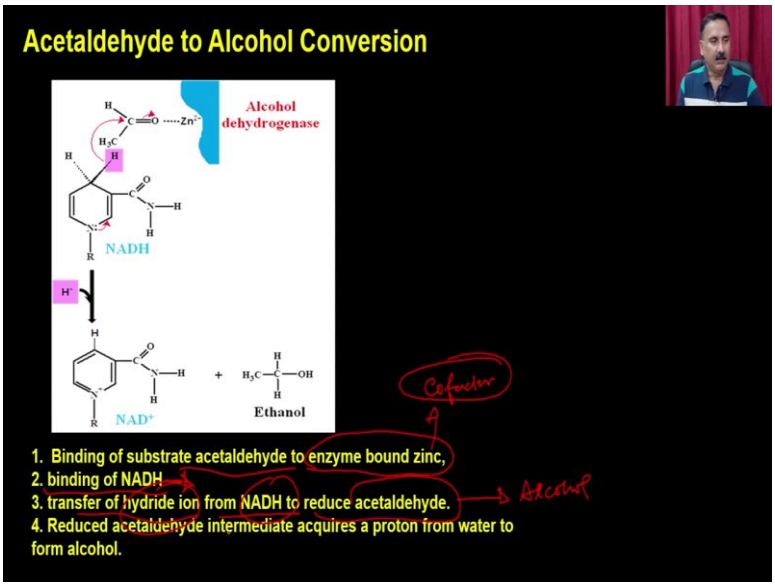
(Refer Slide Time: 29:26)



So, when we talk about the anaerobic oxidations, the pyruvate is going to be converted into alcohol in two step process. The first step, which is going to be catalyzed by the pyruvate decarboxylase and the second step, which is actually going to be catalyzed by the alcohol dehydrogenations.

So, this step we are not going to discuss because this is a step what is being catalyzed by the lyases, right. So, that is we are not going to catalyze reaction what we are not going to discuss at this moment because that also we are going to discuss when we are going to take up the lyase group what we are going to discuss is this, right. So, acetaldehyde is getting converted into the ethanol.

(Refer Slide Time: 30:08)



And this reaction is involving the co factors like the NAD plus and NADH and that is how what you see here is this is the mechanism through which the alcohol dehydrogenase is converting the acetaldehyde into the alcohol. So, what will happen is that the substrate acetaldehyde is actually going to form the enzyme bound zinc.

So, what you see here is that the zinc is actually also a very very important co factor what is involved into the reaction mechanism, then it is actually going to bind the NADH and then there will be a transfer of the hydride ion from the NADH to reduce acetaldehyde and once you reduce the acetaldehyde it is actually going to form the alcohol.

So, with this I would like to conclude my lecture here in our subsequent lecture we are going to discuss more aspects related to enzymes.

Thank you.