

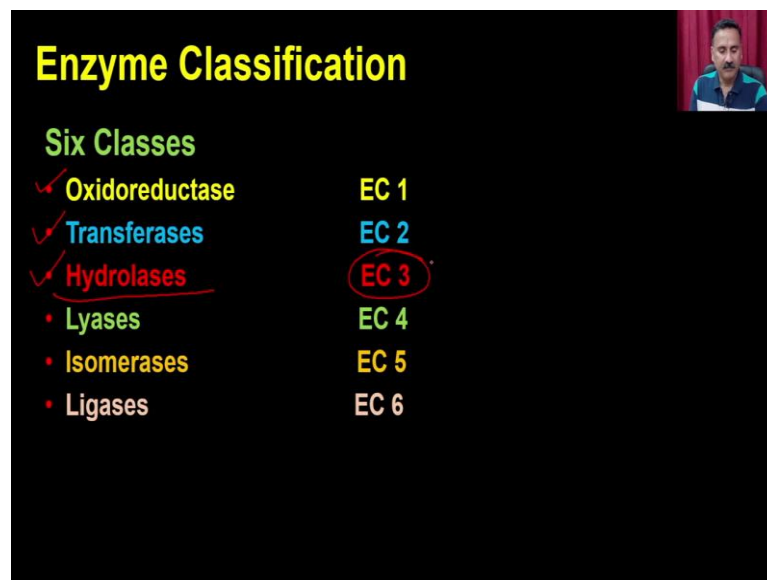
Enzyme Sciences and Technology
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Module - 01
Introduction
Lecture - 04
Enzyme Classification (Part-II)

Hello everyone. This is Dr. 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 this 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.

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Enzyme Classification

Six Classes

✓ Oxidoreductase	EC 1
✓ Transferases	EC 2
✓ <u>Hydrolases</u>	<u>EC 3</u>
• Lyases	EC 4
• Isomerases	EC 5
• Ligases	EC 6

Now, let us move on to the next class and the next class is called as the transferases and transferases are belonging to a group which is called as the EC 2.

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Enzyme Classification

Transferases EC 2

Catalyzes transfer reactions: Transfer of a functional group from one substrate to another. The group transferred may be Methyl, Acyl, Amino or Phospho.

$AB + C \xrightarrow{E} A + BC$

Cofactor: Mg^{2+} , Mn^{2+}

Co-enzyme: Co-enzyme, h

Ex. Kinases, Transferases:

They transfer group, modify the protein/ DNA/ Lipids and carbohydrates. Many of these modifications modulate the activity of enzyme.

Glucose + ADP-P $\xrightarrow{\text{Hexokinase}}$ Glucose-6-P + ADP

Nucleotide on the growing chain

As the name suggests transferases means it is actually going to transfer the group from one substrate molecule to another substrate molecule. So, it actually catalyzes the transfer reactions, which means transfer of a functional group from the one substrate to another substrate.

And, the group what is going to be transferred either would be a methyl group, acyl group, amino groups or the phosphorus group, phospho groups. There are many more groups, but it actually catalyzes, these are the simple examples what I have given. It could actually catalyzes even the carboxyl group, it can also catalyzes the other kind of groups as well. So, what reaction it catalyzes?

If you suppose you have a substrate AB, it reacts with C then you have an enzyme and that enzyme is actually going to you know transfer the group. So, B is a group right on the A and that actually is going to be transferred on to the BC. I can give you an example like for example, the glucose which and then ATP and then you have the a ATP. So, ATP I can just write like this right.

And, then it is actually going to form the glucose, 6 phosphates and the enzyme is hexokinase right and it is actually going to form the ADP. So, what is happening is that this is actually getting transferred onto the glucose and that is how it is actually going to form the glucose phosphate. So, the cofactor, cofactors are magnesium, manganese, in some cases you can also have the coenzyme.

So, coenzyme A for example, this is the very classical coenzyme A which actually participate into the acyl transferases. Examples are kinases, transferases some of the RNA polymerases. So, RNA polymerase is also a transferases because it is transferring the nucleotide from the growing chain right.

So, it is actually transferring the nucleotide from the on the growing RNA chain ok. So, that is how it is actually also belonging to the same group of the transferases. So, they transfer the group, mostly the protein on to the protein, DNA, lipids and the carbohydrate. Many of these modifications modulate the activity of the enzyme.

So, transferases are a very very big group and within these transferases we have the different types of enzymes which actually transfers the group on to the protein, DNA lipids or the carbohydrates and that is how by doing so they are actually changing the properties of these molecules. For example, you know that when the enzymes are getting phosphorylated, it is getting changes it is catalytic activity.

Some in some cases the phosphorylation is making it more active or in some cases the phosphorylation is actually you know making it less active. And, that is how you will see some of the cleavage kind of classification classical examples when you talk about the pyruvate, dehydrogenase or when we talk about the PFK 1 and all those kinds of enzymes.

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EC 2 **Transferase's subclasses**

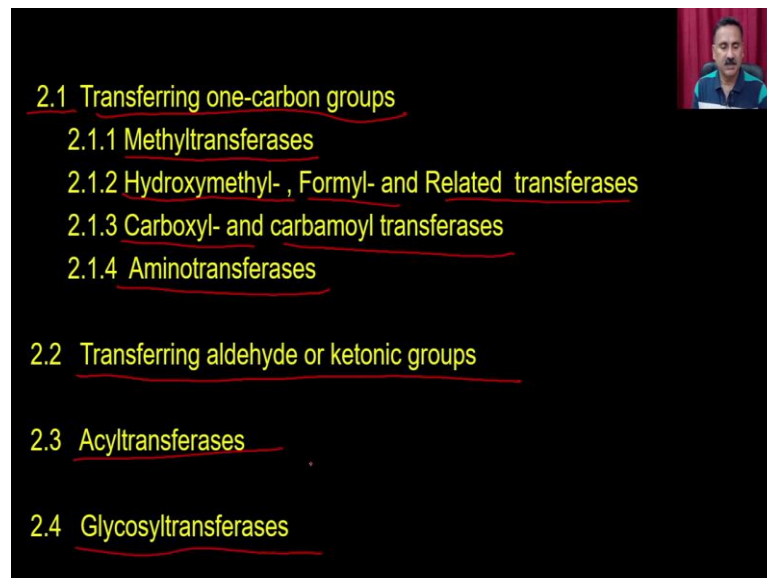
EC 2.1	Transferring one carbon groups
EC 2.2	Transferring aldehyde or ketonic groups
EC 2.3	Acyltransferases → Acyl
EC 2.4	Glycosyltransferases → Glucose
EC 2.5	Transferring alkyl or aryl groups, other than methyl groups (C-C)
EC 2.6	Transferring nitrogenous groups
EC 2.7	Transferring phosphorus- containing groups Kinase
EC 2.8	Transferring Sulphur - containing groups → SH
EC 2.9	Transferring selenium - containing groups

Now, the transferases are further being classified into the subclasses. So, you can have the EC 2.1. So, these are the enzyme which are transferring the one carbon group. Then, we can have the EC 2.2, these are the enzyme which are transferasing the aldehyde or the ketonic group. Then, we have the EC 2.3, these are the acyl transferases. Then we have EC 2.4, these are the glycosyl transferases which means they are actually going to transfer the glucose molecules. This one is actually going to transfer the acyl group.

Then we have the EC 2.5, these are the transferring the alkyl or the aryl groups which means they are actually going to transfer the carbon-carbon other than the methyl groups ok. Then we have EC 2.6, these are the enzyme which are transferring the nitrogenous group. Then we have EC 2.7, these are the groups which are transferring the phosphorus containing groups.

So, this means the kinase is belonging to this particular class that is the EC 2.7. Then we have EC 2.8, these are the enzyme which are transferring the sulphur containing groups. So, sulphur containing group which means the thioester or thiol these kind of groups.

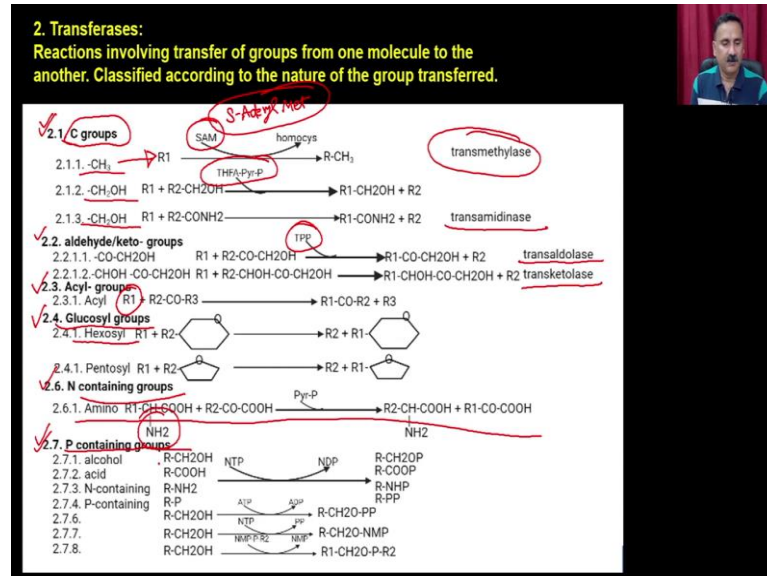
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And, then we have EC 2.9, these are the transferring the selenium containing groups and within these groups you can have also the subgroups. Like for example, 2.1 these are the enzyme which are transferring the one carbon group. So, within that you have the methyl transferases, hydroxy methyl transferases, formyl and related transferases, carboxyl and carbamoyl transferases and the amino transferases.

Similarly, 2.2 group also can have the they are transferring the aldehyde or ketone group and 2.3 acyl groups and the glycosyl transferases.

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The reaction what they are going to catalyze is also being like the 2.1 for example, is working on the C group right. So, it can be methyl transferases or hydroxy methyl transferases and so on and that is how they are actually going to catalyze these kind of reactions.

So, in those cases where you have methyl, it is actually going to use the S adenosyl methionine; S adenosyl methionine as the cofactor and that is how you are actually going to have the methyl transferases. Similarly, you can have the hydroxy methyl transferases where sometime they use the hydroxyl phosphate or the tetrahydrofolate.

And, then we have the hydroxyl methyl transferase and this case we have the transamidinase. Then, the 2.2 is aldehyde or the keto group and these are the reaction what it is actually going to catalyze where they are also using the different types of the cofactors like the. So, these are the transaldolases or the transketolases.

Then we have the 2.3 which is acyl groups and these acyl groups are transferring the acyl group and that is how they are called as the acyl group. Then, we have 2.4 which is the glycosyl groups. So, these are the hexosyl groups and all that. Then we have the 2.6

which is the N containing group. So, they will actually going to catalyze this reactions and that is how they are actually going to transfer the N containing groups.

And, so N they will transfer this N containing group on to the new molecule. And, then we have the 2.7 containing which is P containing groups and that is how it is actually going to transfer the phosphorus from this particular thing.

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Enzyme Classification

Transferases EC 2

Catalyzes transfer reactions : Transfer of a functional group from one substrate to another. The group transferred may be Methyl, Acyl, Amino or Phospho.

Mechanism (Kinase)

The diagram illustrates the mechanism of a kinase reaction. It shows ATP (Adenosine Triphosphate) with its three phosphate groups (labeled P) and a substrate (S) with a hydroxyl group (H-O). The reaction is catalyzed by a kinase, resulting in a phosphorylated product (S-P) and ADP (Adenosine Diphosphate). The diagram uses red circles and arrows to show the transfer of a phosphate group from ATP to the substrate.

Now, if we talk about the mechanism so, mechanism is very very complicated. So, we will take a just example of the kinase for example, ok. So, if I take a kinase so, so you know that the kinase, how the mechanism is actually going, how the kinase is actually going to work.

So, if you talk about the substrate for example, right so, this is the substrate for the kinase. So, which is actually a substrate ok. So, this is the ATP molecule what the kinase is actually going to use for its reactions ok and this is the 6, 5th , 6 carbon ok. So, this is the ATP molecule what it is actually going to use and it is actually going to transfer the groups on to the alcohol group ok.

So, for example, this is actually a protein. So, if it is a protein so, this is not a phosphorus, this is the protein actually and so, it has lot of amino acids. So, terminal amino acid is someplace is serine. So, serine has the hydroxyl group and this lone pair of electron what you see is actually going to act on to the gamma phosphate.

So, in this case what you see here is ATP has 3 phosphate right. So, this is the 1st phosphate, this is the 2nd phosphate, this is the 3rd phosphate and this is called as alpha phosphate, this is called beta phosphate and this is called as the gamma phosphate. So, what it is going to do is these lone pair of electron what is present on to the oxygen is actually going to act on to this bond.

And that is how there will be a internal rearrangement and then this phosphate is actually going to be transferred onto this and that is how it is actually going to form the this ok and that is how it is actually going to form the phosphorylated product; whether this product whether this is a protein or whether it is a carbohydrate or whether it is a DNA.

So, it is actually modifying the all the biomolecules whether it is a carbohydrate whether it is a protein or whether it is a lipid or whether it is a DNA. So, let us take a example of some of these biomolecules.

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The slide is titled "Enzyme Classification" in yellow text. Below the title, it lists "Transferases EC 2" in yellow. A red text box defines the function: "Catalyzes transfer reactions : Transfer of a functional group from one substrate to another. The group transferred may be Methyl, Acyl, Amino or Phospho." Below this, a red diagram titled "Carbohydrate Modifications" shows a reaction: "Glucose" with a circled "OH" group on the left, an arrow labeled "Hexokinase" in the middle, and "Glucose-P" with a circled "P" on the right. Below the arrow, "ATP" is written above "ADP". A red arrow points from "Carbohydrate Modifications" to a box labeled "Glycolysis". Another red arrow points down from "Glucose-P" to "Glucose 1,6, bisphosphate". A small video inset of a man is in the top right corner.

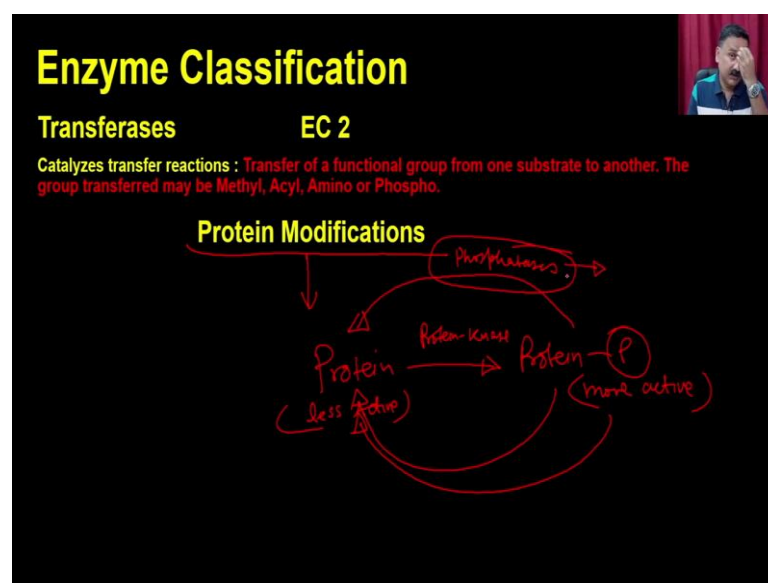
So, in the case of carbohydrate modifications, carbohydrate modifications are very very much found in many carbohydrate metabolisms. So, for example, in the glycolysis right. So, you know that the glycolysis is starting with the first reaction by when the glucose is getting converted into the glucose 6 phosphate.

And, it is a reaction which actually commits this glucose into the glycolysis. Now, from here there will be another round of another round of the kinase reaction and that is how it

is actually going to form the glucose 1, 6 bis phosphate right and that is how you have the two kinase reactions. One which is actually going to be catalyzed by the hexo kinase and the another one which is going to be catalyzed by the another kinase.

And, both the places you are actually going to have the similar kind of mechanism where we have just now we have discussed right, all the glucose also has the OH right and this OH has a lone pair of electron. And, that is actually going to act on to the gamma phosphate of the ATP and that is how it is actually going to you know form the bonds.

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Then, we have the protein modifications, protein modifications can be that where a protein can be converted into a phosphorylated proteins right. So, this is actually being catalyzed by the protein kinase. So, you can have the different types of protein kinases whether it is a serine kinase or threonine kinase or tyrosine kinase and that actually is going to convert into the this protein into the protein phosphate.

And, we have very many classical examples like the AKT, we can have the many enzymes which are actually being converted into this way. And, in some cases this protein which is native protein is less active ok. And, some when it is getting phosphorylated, it is becoming more active right.

But, in some cases this k this whole thing is getting reversed ok which means the phosphorylated protein is less active and the less native protein is more active. Now,

once the phosphorylated protein is formed, it can be converted back into the non-phosphorylated protein and the enzyme is phosphatases ok. So, phosphatases are also going to catalyze this, but phosphatase does not belong to the transferase class.

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Enzyme Classification

Transferases EC 2

Catalyzes transfer reactions : Transfer of a functional group from one substrate to another. The group transferred may be Methyl, Acyl, Amino or Phospho.

DNA Modifications → Methyl transferase

The diagram illustrates the action of EcoRI and EcoRI Methylase on DNA. On the left, EcoRI cleaves unmethylated DNA at its recognition sequence (GAATTC), resulting in fragments with sticky ends (AATTG overhangs). This process leads to DNA degradation. On the right, EcoRI Methylase methylates the DNA at the same recognition sequence, adding a methyl group (CH₃) to the adenine base. This methylated DNA is not cleaved by EcoRI, preventing DNA degradation. Handwritten notes in red ink include 'Methylated - self' and 'non-methylated - non self', with a circled 'Self-Non-self' in the center.

Then, we have the DNA modifications and the DNA modification is actually going to be catalyzed. So, in this case for example, we have talking about the methyl transferases right. So, if you have the methylated DNA that has a very much high significance in terms of asking the restriction enzymes to recognize the self versus non-self.

So, that is actually going to work in terms of giving the ability to these restriction enzyme to recognize which DNA is self and which DNA is non-self. And, you know that the methylated DNA is actually going to be considered as self DNA whereas, the non-methylated DNA is going to be called as non-self DNA which means this non-methylated DNA is going to be degraded by the restriction enzyme systems.

Now, let us move on to the third enzyme. So, we have discussed about the oxidoreductase, we discussed about the transferases and now we are going to talk about the hydrolases.

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Enzyme Classification

Hydrolases EC 3

Catalyzes degradation reactions : Formation of two products from the substrate by hydrolysis using water.

$AB + H_2O \xrightarrow{\text{Enzyme}} AOH + BH$

Ex. Lipases, Amylase, peptidase, phosphatase.

Lipid, Peptide, Phosphorylated product

So, hydrolase is belonging to the EC class 3. Hydrolase is catalyzing the degradation reaction which means the formation of the two product from the substrate by the hydrolyses with the help of the some water. So, water is very important. If water is not involved, it is not the hydrolyses.

So, AB which is a substrate when it reacts with water in the presence of the enzyme, it is actually going to form the AOH plus BH and that is how it is actually going to form the two products and that is how it is actually going to be degraded. Classical examples are lipases, amylases, peptidases and the phosphatases. Lipases the enzyme which is actually going to work on the lipids.

Then, we have the peptidases, the enzyme which are actually going to work on the peptide bonds and the phosphatases which are actually going to work on the phosphorylated products. Now, these hydrolyses are further being classified into the subclass ok.

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EC3 Hydrolases subclasses

- EC 3.1 Acting on ester bonds
- EC 3.2 Glycosylases
- EC 3.3 Acting on ether bonds
- EC 3.4 Acting on peptide bonds (peptidases)
- EC 3.5 Acting on carbon-nitrogen bonds, other than peptide bonds
- EC 3.6 Acting on acid anhydrides
- EC 3.7 Acting on carbon-carbon bonds
- EC 3.8 Acting on halide bonds
- EC 3.9 Acting on phosphorus-nitrogen bonds
- EC 3.10 Acting on sulfur-nitrogen bonds
- EC 3.11 Acting on carbon-phosphorus bonds
- EC 3.12 Acting on sulfur-sulfur bonds
- EC 3.13 Acting on carbon-sulfur bonds

So, for example, the EC 3.1 which is acting on the ester bonds, EC 3.2 which is actually working on these are the glycosylases so, they will work on the glycosidic bonds. Then, you see 3.3 is acting on the ether bonds. So, depending on which bond they are breaking, they are further being classified into the different subgroups.


So, EC 3.4, it is acting on the peptide bond so, these are the peptidases. Then, we see 3.5 which is acting on the carbon and nitrogen bond which means it is actually you know breaking this bond ok. Then, we have the EC 3.6 so, these are the enzyme which are acting on the acid anhydrides. Then, we have the EC 3.7; these are the enzyme which are acting on the carbon-carbon bond which means if they are going to break this bond.

Then we have the EC 3.8, these are the enzyme which are acting onto the halide bonds and then we have the EC 3.9, these are the enzyme which are acting on to the phosphorus and nitrogen bond which means they are actually going to act onto this bond. And, then we have the EC 3.10, these are the sulfur nitrogen bonds. Then we have EC 3.11 which are carbon phosphorus and so on ok and these are being further divided into the sub subgroups right.

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EC3 Hydrolases subclasses

- ✓ EC 3.1 Acting on ester bonds
 - 3.1.1 Carboxylic Ester Hydrolases
 - 3.1.2 Thiol-ester Hydrolases
 - 3.1.3 Phosphoric Monoester Hydrolases
 - 3.1.4 Phosphoric Di-ester Hydrolases
 - 3.1.5 Tri-phosphoric Monoester Hydrolases
- ✓ 3.2 Glycosylases
- 3.3 Acting on ether bonds
- 3.4 Acting on peptide bonds (peptidases)




For example, the EC 3.1 which is actually an enzyme which is acting on the ester bonds are further divided into the subgroups. So, 3.1.1 is the enzyme which is going to work on the carboxylic ester hydrolyses ok. Then we have the thiol ester hydrolases. Then we have the phosphoric monoester hydrolases, phosphoric diester hydrolases and tri phosphoric monoester hydrolases. Similarly, the enzyme belonging to the glycosylases 3.2, 3.3 can be further sub divided into the subclasses.

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3. Hydrolases:

Reactions involving cleavage of a molecule concomitantly with uptake of water.
Classified according to the nature of the substrate.

- ✓ **3.1. Carboxylic acid esterases**
 - 3.1.1. esterases: $R_1COOR_2 + H_2O \rightarrow R_1COOH + HOR_2$
 - 3.1.2. thioesterases: $R_1COSR_2 + H_2O \rightarrow R_1COOH + HSR_2$
 - 3.1.3. phosphomonoesterases: $RO-P \xrightarrow{H_2O} ROH + HO-P$
 - 3.1.4. phosphodiesterases: $R_1-O-P(=O)(OH)-O-R_2 \xrightarrow{H_2O} R_1-OH + HO-P(=O)(OH)-R_2$
 - 3.1.4. phosphodiesterases: $R_1-O-S(=O)(OH)-O-R_2 \xrightarrow{H_2O} R-OH + H_2SO_4$
- 3.2. Glycosides**
 - 3.2.1. glycosidases: $\text{Glycoside} + H_2O \rightarrow \text{Sugars} + ROH$
 - 3.2.1. glycosidases: $\text{Glycoside} + H_2O \rightarrow \text{Sugars} + NH_2$
- 3.4. Proteins and peptides**
 - 3.4.1. aminopeptidases: $R-CH(NH_2)COOH + H_2O \rightarrow R-CHO + NH_2 + COOH$
 - 3.4.2. carboxypeptidases: $R-CH(NH_2)COOH + H_2O \rightarrow R-CH(NH_2)COOH + H_2O$
 - 3.4.3. dipeptidases: $R-CH(NH_2)COOH + H_2O \rightarrow R-CHO + NH_2 + COOH$
 - 3.4.4. proteinases: $R-CONH_2 + H_2O \rightarrow RCOOH + RNH_2$
- 3.5. C-N bonds in non-peptides**
 - $R-CONH_2 + H_2O \rightarrow RCOOH + RNH_2$



The reaction what it is actually going to catalyze for example, the 3.1, 3.1 is the esterases. So, esterases are actually going to catalyze the degradation of the esters right. So, you can have the ester like R_1COOR_2 and that is actually going to degrade by the you know by the carboxylic acid esterases to form the acid and as well as the alcohol. Similarly, all these glycosidases these are actually going to form the degradation of the sugar groups. So, they are actually going to degrade this.

Similarly, we can have the protein and peptides 3.4. So, amino peptidases, carboxypeptidases, di peptidases, proteinase peptidases. So, depending on this they actually are going to act on the different types of substrates and then we also going to have C-N bond in the non-peptide bond and that is also going to work. So, how these enzymes are working? How they are actually acting on the different substrate and cleaving the bonds?

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Enzyme Classification

Hydrolases EC 3

Catalyzes degradation reactions : Formation of two products from the substrate by hydrolysis using water.

Mechanism.

Step 1: E-136 and D-140 present near the zinc binding site to form the S2 and S3 sites. The negatively charged sites form a salt-bridge with an arginine present in an incoming peptide to facilitate strong binding. The F-53 lies at the S1 site and is believed to interact with the aromatic residues found in the substrate.

Step 2: The residue E-49 which lies within hydrogen bonding distance to a water molecule coordinated to zinc is predicted to polarize this water molecule, thereby aligning it for nucleophilic attack on the bond of the substrate.

Step 3: Residues Y-157 and H-231 which will stabilize the oxyanion of a tetrahedral intermediate.

Step 4: Solvent molecules or similar charged amino acids T-79 and R-125 could be involved in hydrogen bonding to stabilize oxyanion of a tetrahedral intermediate.

Metalloprotease

Enzyme-Substrate Complex

Tetrahedral Intermediate

Enzyme-Carboxylate Complex

So, hydrolases are actually catalyzing this because they have the a classical groups of the amino acids present within the active site. And, these amino acids are withdrawing the electrons and they are actually stabilizing the products and that is how they are actually going to cleave. So, for example, I have taken an example of this, this is an enzyme which this is a metalloenzyme right.

So, this is a metalloprotease and which is present in the (Refer Time: 19:51) and it actually catalyzes these reactions. So, in these reactions the substrate like the residues

like the E 136 and one D 140 is actually being present close to the reaction center and that is how they are actually going to stabilize the incoming peptides.

And, then the phenylalanine 53 which is lies at the S1 site and which is actually going to interact with the aromatic residues which are present in the peptide bonds or which are present in the peptide substrates. And, then the E 49 which is within the hydrogen bonding distance of the water molecule; so, it is actually going to stabilize the water molecule because you know that these are the hydrolyses.

So, they are actually going to use the water molecule. So, once the peptide is entering, it is actually going to form this complex and then there will be a nucleophilic attack on to the peptide carbonyl bond. And, that is how it is actually going to form the tetrahedral intermediate and that tetrahedral intermediate is actually going to be stabilized by the different types of the amino acid residues, water present within the active site of this particular metalloprotease.

And, that is how it is actually going to ultimately cleave off the peptide bond and it is actually going to release the product and that is how it is actually going to form the enzyme carboxylate complex. And, from here the enzyme carboxylate complex, the carboxylate and the water molecule is going to be released. And, that is how it is actually going to acquire the native conformation and then native conformation is again going to be ready for taking up the new substrate molecule.

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Enzyme Classification

Hydrolases EC 3

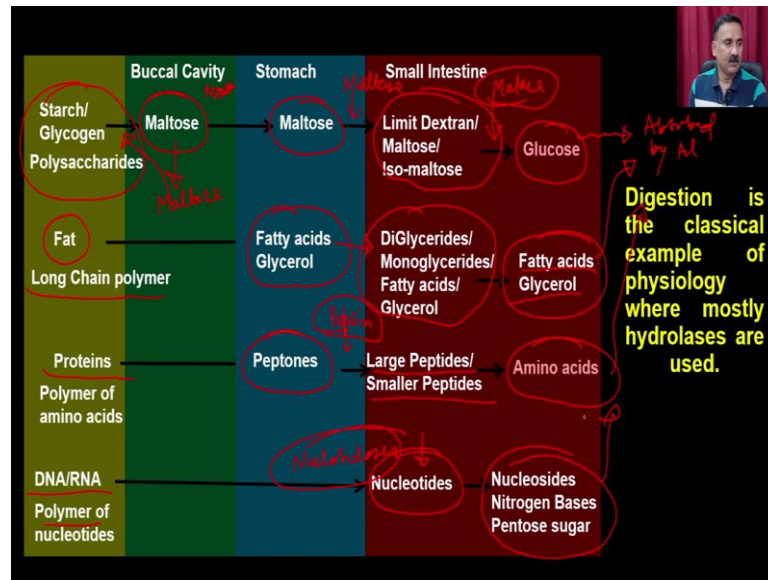
Catalyzes degradation reactions : Formation of two products from the substrate by hydrolysis using water.

$$AB + H_2O \longrightarrow AOH + BH$$

EX: Enzymes present in Digestion System. → Hydrolases

Now, the classical examples where these enzymes are actually having the very very high application is the digestive system ok. So, digestive system is the classical example where the lot of hydrolyses are actually being involved into the catalyst into the digestion process.

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This is what you see here is that if you have a carbohydrate, it will enter into the buccal cavity. So, carbohydrate is the maltose ok. So, once the carbohydrate is present into the buccal cavity which means it is present in our mouth, it is actually going to be get converted into the. So, this polymeric sugar is actually going to be get converted into the maltose and the enzyme, the hydrolytic enzyme what is actually going to catalyze this reaction is called as maltase.

So, once the maltose is entered, it enters into the stomach and from the stomach it is actually going to be get converted into these products. And, there are some enzymes like the maltase and it is actually going to convert the maltose into the limit dextran maltose and iso maltose. And, from here it is actually again going to be act on to the maltase and that is how it is actually going to be converted into the glucose molecule. And, that is how the glucose is actually going to be absorbed by the elementary canal.

Similarly, if a if it is a fat so, fat is a long chain polymer. So, there will be no enzyme available within the mouth which is actually going to you know digest the fat and then within the stomach it is actually going to be digested to the fatty acids glycerol. And,

then it is actually going to be converted into the different types of hydrolytic enzyme into the diglycerides, mono glycerides and so on. And, ultimately it is actually going to be converted into the fatty acid.

And, once it get converted into fatty acid and glycerol, it is also going to be available for the absorption by the for by the small intestine. Then, the for the proteins, protein is actually going to be get converted into the peptones and then the peptone is getting converted into the large peptide. So, at this stage within the stomach it can be act by the by the some of the classical hydrolytic enzyme like the pepsin and all that and ultimately it is going to form the amino acids and that also is available for digestion.

Similarly, we can also have the DNA and RNA and that also is going to be converted into nucleotide within the small intestine and the enzyme is nucleotidase right. So, that is also a the hydrolytic enzymes and that is how it is actually going to form the monomeric molecules and that is how they will be actually going to be absorbed.

Now so far what we have discussed? We have discussed about the oxidoreductase, we discussed about the transferases, we discussed about the hydrolases and now let us talk about the lyases, which is belonging to the EC class 4.

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Enzyme Classification

Lyases **EC 4** *In the absence of water*

Catalyzes non-hydrolytic degradation of chemical bond reaction: Non-hydrolytic addition or removal of group from substrate. C-C, C-N, C-O or C-S bonds may be cleaved.

$R-CO-COOH \xrightarrow[\text{decarboxylation}]{\text{Lyases}} R-COH + CO_2$

Ex. Decarboxylation: Pyruvate Decarboxylase.

So, lyases are actually the enzyme which are belonging to EC class 4. And, what reaction they catalyzes? The reaction they catalyzes the non-hydrolytic degradation of the

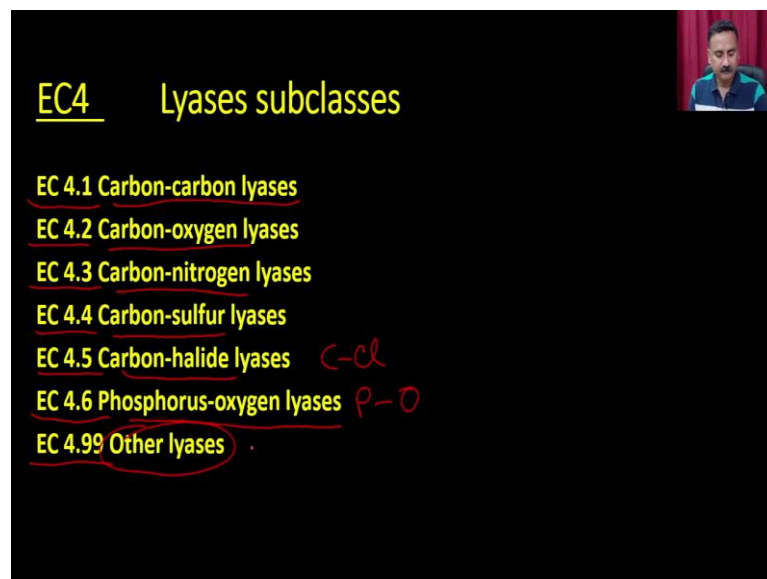
chemical bond which means they are actually going to do the same function what the hydrolyses are doing, but in the absence of water right.

So, lyases are also causing the degradation of the chemical bond which means they are actually going to break the bond, but they will not utilize the water, they will utilize some other molecule. So, the non-hydrolytic addition or the removal of the group from the substrate that is the reaction what is going to be catalyzed by the lyases.

For example, the C-C bond, C-N bond, C-O bond or C-S bond. So, when it going to break this bond, it is actually going to utilize except water some other molecules. For example, R-CO-COOH so, this is the substrate when it get the lyases right; it is actually going to get converted into the carbon dioxide is going to be removed right and it is actually going to form the R-COH.

So, this is actually a decarboxylation reactions right which is actually going to remove the carbon dioxide. Classical example is decarboxylation reaction which is catalyzed by the pyruvate decarboxylase. Now, the lyases can be further divided into subclasses.

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The slide displays a list of enzyme subclasses under the heading 'EC4 Lyases subclasses'. The list includes:

- EC 4.1 Carbon-carbon lyases
- EC 4.2 Carbon-oxygen lyases
- EC 4.3 Carbon-nitrogen lyases
- EC 4.4 Carbon-sulfur lyases
- EC 4.5 Carbon-halide lyases C-Cl
- EC 4.6 Phosphorus-oxygen lyases P-O
- EC 4.99 Other lyases

A small video inset in the top right corner shows a man speaking.

So, these subclasses are 4.1. The lyases which are working on to the carbon-carbon bond. EC 4.2 the carbon oxygen bond. EC 4.3 carbon nitrogen bonds. EC 4.4 carbon sulfur bonds. EC 4.5 carbon halide right so, carbon and chloride. Then, the EC 4.6 the

phosphorus oxygen bond which means this bond and the EC 4.99 which is the other lyases belonging to the other classes.

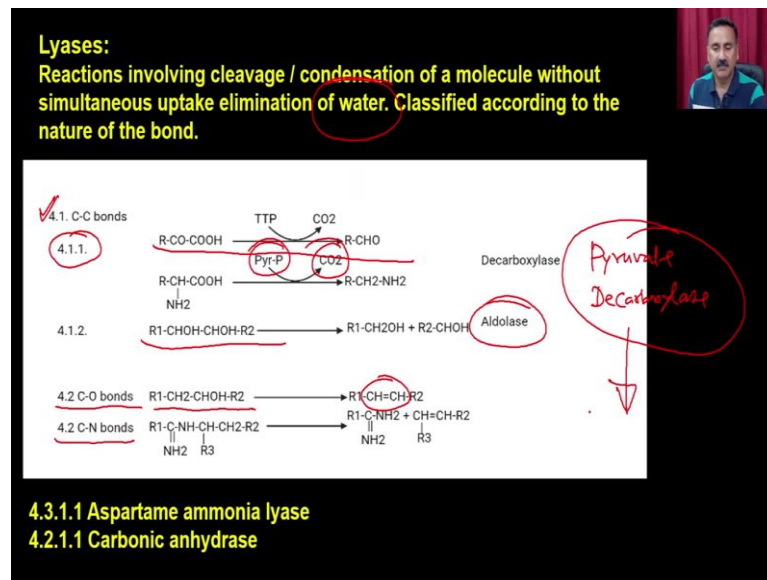
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4. Lyases sub-subclasses

- 4.1 Carbon-carbon lyases
 - 4.1.1 Carboxy-lyases
 - 4.1.2 Aldehyde-lyases
 - 4.1.3 Oxo-acid-lyases
 - 4.1.99 Other Carbon-carbon lyases
- 4.2 Carbon-oxygen lyases →
- 4.3 Carbon-nitrogen lyases →
- 4.4 Carbon-sulfur lyases →
- 4.5 Carbon-halide lyases →

Then, these groups are further sub classified into the sub subclasses. So, for example, the 4.1 the carbon-carbon lyases could be the 4.1.1 is the carboxy lyases. 4.1.2 is aldehyde lyases. 4.1.3 is oxoacid lyases. And, 4.1.99 are the other carbon-carbon lyases. So, this is just a classical way of the classifying the subgroups into the sub subgroups. We have subgroups for this one also, we have subgroups for this one also and we have subgroups for all these groups also.

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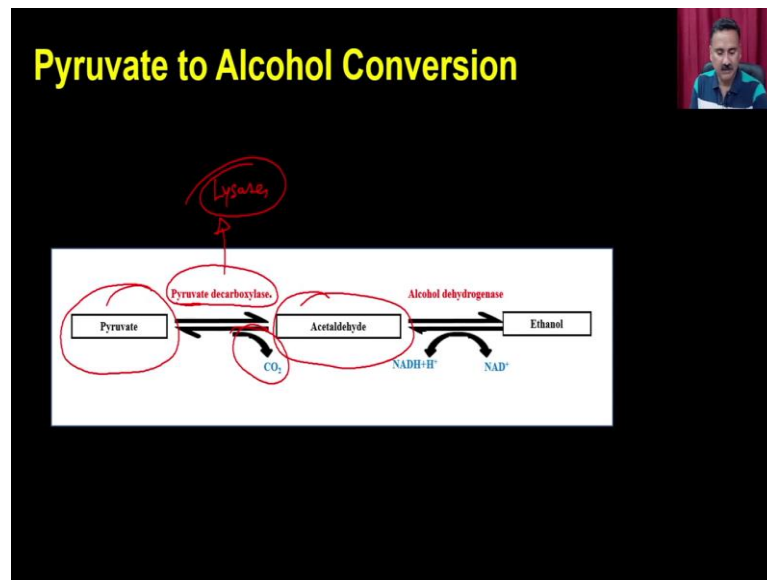


What reaction they catalyzes? So, reaction involving the cleavage or the condensation of the molecule without the help of the water is the part of the lyases which means for example, the 4.1. So, 4.1.1 is actually going to catalyze this reaction, 4.1.1 is actually going to. So, for example, they will utilize the pyridoxal phosphate and that is how they will be catalyzing the decarboxylation reactions.

Similarly, we can have 4.1.2 where it is actually going to catalyze the degradation with the help of the enzyme is aldolases. Then, we have the carbon oxygen bond. So, this is the carbon oxygen bond and it is actually going to form the carbon double bond. So, there will be a loss of carbon oxygen bond here. Then, we have the C-N bond so, it is actually going to act on this and that is how it is actually going to form this.

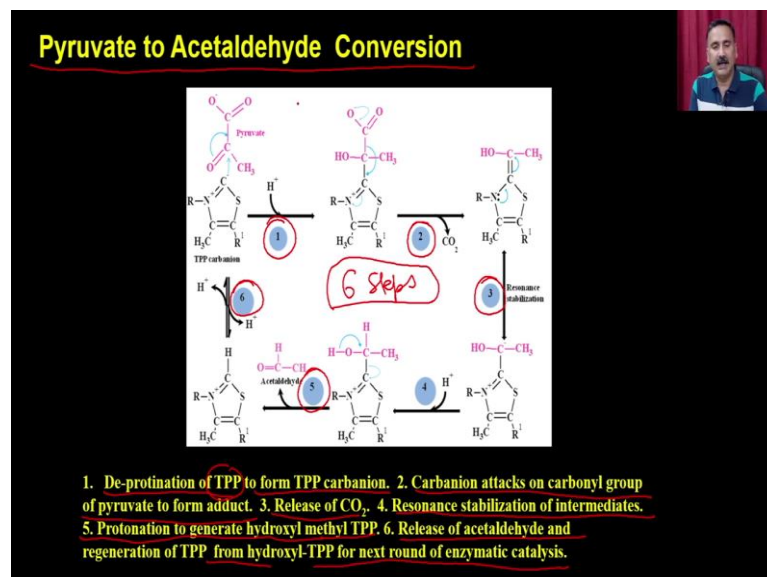
So, classical example in this case is pyruvate decarboxylase ok which is actually be a part of the anaerobic oxidation. And, that is how the pyruvate decarboxylase is actually going to convert the pyruvate into the acetaldehyde and that is how it is actually going to participate into the alcohol production.

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So, pyruvate is getting converted into the acetaldehyde and in this process one molecule of carbon dioxide is going to be removed from the pyruvate and the reaction is going to be catalyzed by the pyruvate decarboxylase which is a lyase. So, let us take see how the pyruvate decarboxylase is catalyzing these reactions.

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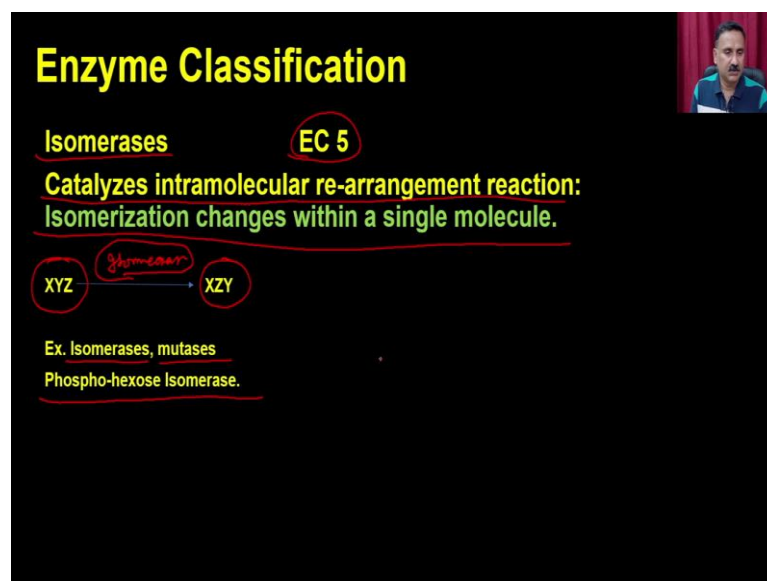
So, pyruvate decarboxylase mediated conversion of pyruvate to acetaldehyde is catalyzed by the pyruvate decarboxylase, it is a 6 step reaction. So, in the step 1 you are going to have the deprotonation of the TPP to form the TPP carbanion ion ok. So, this is

the TPP carbanion ion what is going to be formed in the step 1. Then there is the step 2, you are going to have the carbanion attack on to the carbonyl group of the pyruvate to form the adduct. Then, in the step 3, there will be a release of carbon dioxide.

So, there will be a decarboxylation reactions ok and in the step 4 there will be a resonance stabilization of the intermediate. And, in the step 5 there will be a protonation of the hydroxyl methyl TPP and that is how in the step 6 there will be a release of the acetaldehyde and the regeneration of TPP from the hydroxyl TPP for the next round of the enzymatic catalysis.

So, these are the 6 step what it is going to be catalyzed by the pyruvate decarboxylase to convert the pyruvate into the acetaldehyde and that acetaldehyde is actually going to be taken up by the alcohol de dehydrogenases to convert the acetaldehyde into the alcohol. Now, we have already discussed about the lyases, now we will talk about another class which is called as the isomerases. It belongs to the EC 5.

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Enzyme Classification

Isomerases **EC 5**

Catalyzes intramolecular re-arrangement reaction:
Isomerization changes within a single molecule.

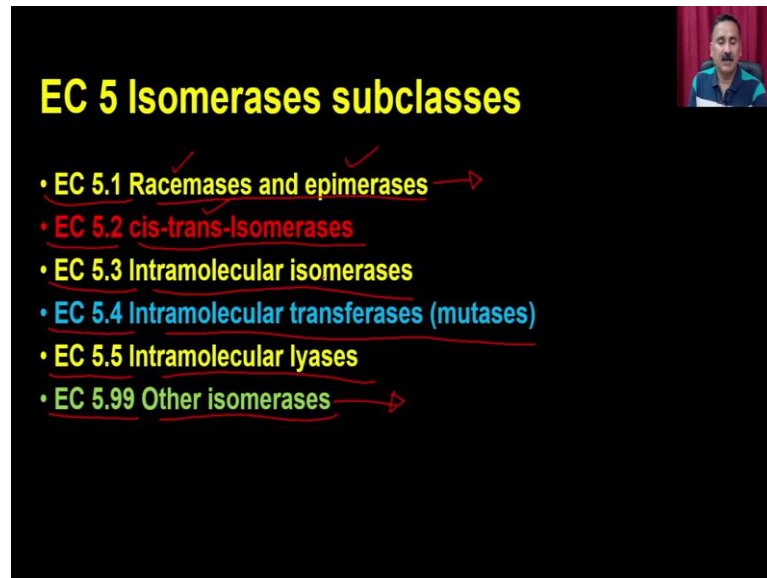
XYZ → XZY
Isomerase

Ex. Isomerases, mutases
Phospho-hexose Isomerase.

So, EC isomerases are the enzyme which belongs to the class EC 5, it catalyzes the reaction of intramolecular rearrangement reaction which means the isomerization changes within the single molecule which means if there is a substrate called XYZ, it is going to be get converted into X Z Y. So, that is going to be catalyzes by the isomerases.

So, in this the groups it is not changing the substrate, it is not degrading the substrate, it is just changing the position of the group within the molecule and that is how it is actually going to cause the generation of the different types of isomers. Examples are isomerases and the mutases. For example, phosphohexose isomerase ok. So, phosphohexose isomerase is actually going to catalyze the isomerizations.

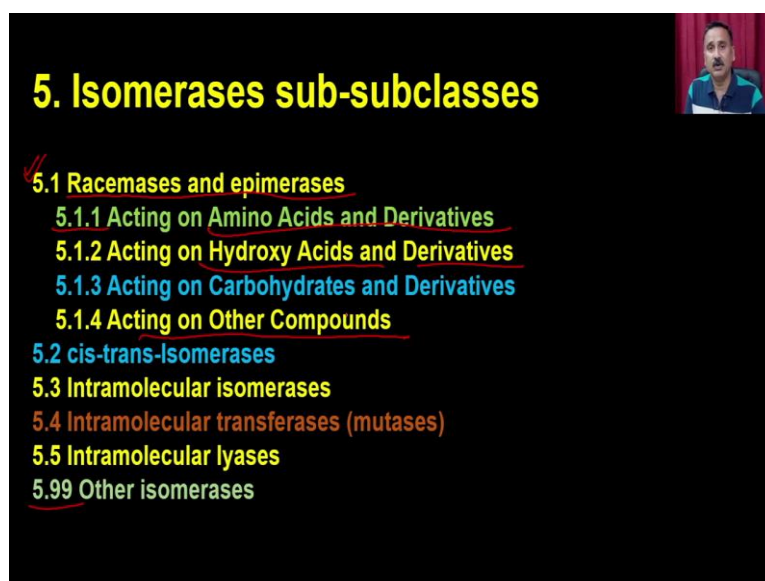
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Isomerization can be further sub classified for example, the EC 5.1 which is the racemases and epimerases, EC 5.2 which is the cis trans isomerases. EC 5.3 intramolecular isomerases, EC 5.4 the intramolecular transferases, EC 5.5 the intramolecular lyases and EC 5.99 that are the other isomerases.

So, what you see here is that all these subgroups are actually catalyzing the isomerization, different types of isomerization whether it is a racemizations, epimerization, cis trans isomerizations and so on. And, all these sub groups are sub classes are further classified into sub subclass.

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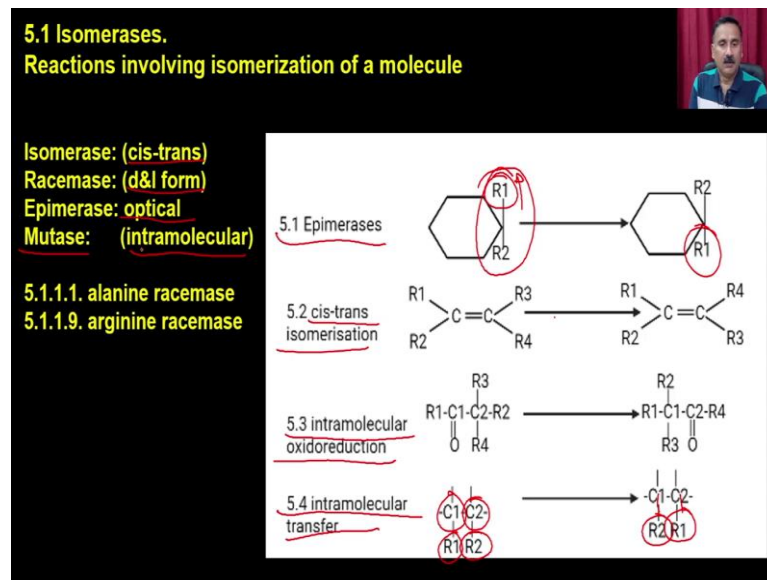
5. Isomerases sub-subclasses

- 5.1 Racemases and epimerases
 - 5.1.1 Acting on Amino Acids and Derivatives
 - 5.1.2 Acting on Hydroxy Acids and Derivatives
 - 5.1.3 Acting on Carbohydrates and Derivatives
 - 5.1.4 Acting on Other Compounds
- 5.2 cis-trans-Isomerases
- 5.3 Intramolecular isomerases
- 5.4 Intramolecular transferases (mutases)
- 5.5 Intramolecular lyases
- 5.99 Other isomerases

So, for example, the 5.1 it is actually going to be catalyzed into racemases and epimerases right. So, within this also you can have the 5.1.1 that is acting on to the amino acids and derivatives, 5.1.2 is acting on to the hydroxy acids and derivatives, 5.1.3 is acting on to the carbohydrate and its derivative and 5.1.4 is acting on to the other compounds.

Similarly 5.2, 5.3, 5.4, 5.5 and 5.99 can be further classified into the subgroups depending on the other type of reaction or bond what it is actually going to act to form the isomers.

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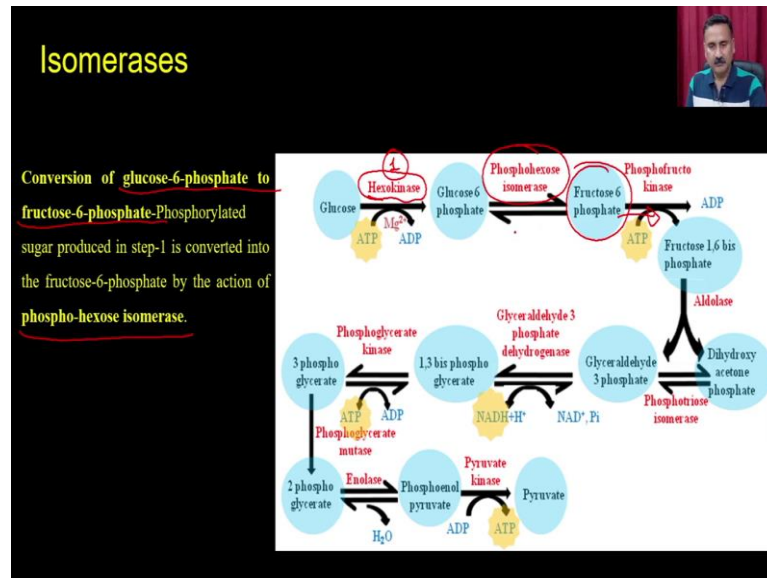
These are the reactions, but it is actually going to catalyze for example, the epimerases. Epimerases are actually going to you know act on to the particular group and that is how you will see that it is actually changing the position of the R 1 from the above to the surface to the lower to the surface.

So, it is actually causing the epimerizations. Then you can also form the cis trans isomerizations; so, it actually can change the position from this side to this side and that is how it is actually going to form the cis trans isomerizations. Then, it also can cause the intramolecular oxidoreduction so, that is how it is actually going to form the isomers.

And, then it also can cause the intramolecular transfers and that is how it is actually going to for example, in this case you see the R 2 is connected to C 2 whereas, R 1 is connected to C 1 and this enzyme has catalyzes the reaction and that is how the R 2 is now connected to C 1 and R 1 is connected to C 2.

So, that is how it is actually going to be catalyze different types of reactions like the cis trans isomerization, d and l racemizations or the epimerizations like the optical rearrangement or some time the intramolecular rearrangement that enzyme is called as the mutases.

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So, isomerization I you might have seen the reactions where the glucose 6 phosphate is getting converted into fructose 6 phosphate and the enzyme is called as the phospho hexose isomerase. So, this is the enzyme what it is going to cause right. So, in the step 1 you are actually going to use the hexokinase to convert the glucose to the glucose 6 phosphate.

And, then this glucose 6 phosphate is acting (Refer Time: 35:18) into the phospho iso isomerase and it is actually going to form the fructose 6 phosphate and that is how the fructose 6 phosphate will further enter into the glycolysis. So, we have discussed about the oxidoreductase, transferases, hydrolases, lyases and isomerases and now we will talk about the ligases.

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Enzyme Classification

Ligases **EC 6** **Ligases**

Catalyzes Joining reaction: Join together two molecules by synthesis of new C-O, C-S, C-N or C-C bonds with simultaneous breakdown of ATP. **Energy**

$$X + Y + \text{ATP} \rightarrow XY + \text{ADP} + \text{P}_i$$

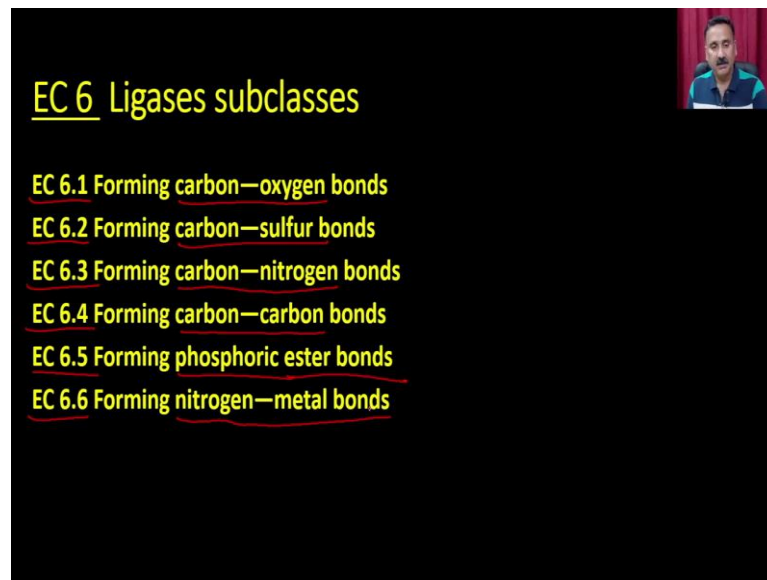
Ex. Synthetases

So, ligases as the name suggests so, ligases are belonging to the EC class 6 and they are catalyzing the joining reactions. So, they join together to two molecule by the synthesis of the new molecule which means C either the formation of C-O bond, C-S bond, C-N bond or C-C bond. So, ligases are opposite to the lyases right.

So, these are these enzymes are breaking down the molecules, these enzymes are synthesizing the new molecule. And, when they are synthesizing the new molecule in many cases they are also breaking down the ATP which means they are actually taking up the energy from the ATP to break the bonds.

So, for example, in this case X plus Y plus ATP and then it is actually going to catalyze the synthesis of X Y and the ATP is going to be broken down into ADP plus Pi. So, when it is broking down the ATP, it is actually releasing some energy. So, ATP is releasing some energy and that is how that released energy is utilized to activate the bonds onto the X and Y and that is how the X and Y are forming the bond together. Examples are synthetases.

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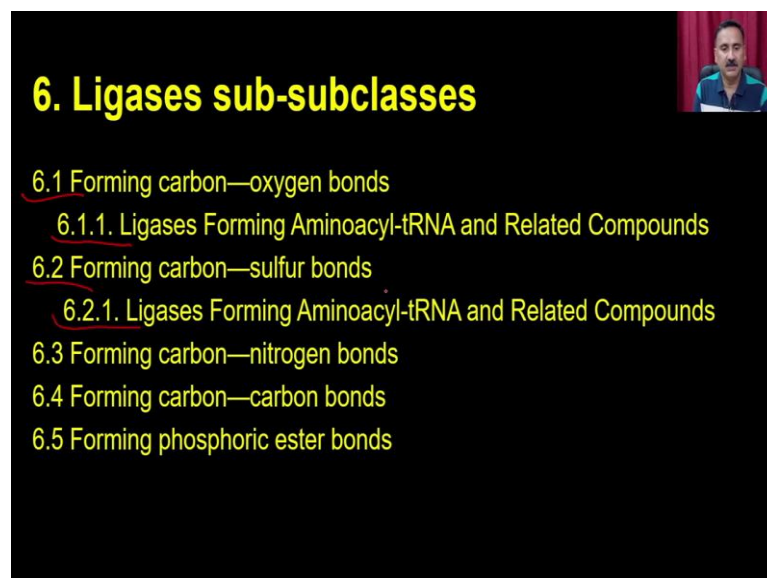
EC 6 Ligases subclasses

- EC 6.1 Forming carbon—oxygen bonds
- EC 6.2 Forming carbon—sulfur bonds
- EC 6.3 Forming carbon—nitrogen bonds
- EC 6.4 Forming carbon—carbon bonds
- EC 6.5 Forming phosphoric ester bonds
- EC 6.6 Forming nitrogen—metal bonds

Ligases are further being classified based on the different types of bonds what is actually going to catalyze. So, for example, EC 6.1 it is forming the bond between carbon and oxygen, EC 6.2 it is forming a bond between carbon and sulfur, EC 6.3 it is forming a bond between carbon and nitrogen.

EC 6.4 it is forming a bond between carbon and carbon, EC 6.5 forming a bond between phosphoric ester. And, then EC 6.6 it is forming a bond between nitrogen and metal and these groups are further being classified into sub subclasses.

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6. Ligases sub-subclasses

- 6.1 Forming carbon—oxygen bonds
 - 6.1.1. Ligases Forming Aminoacyl-tRNA and Related Compounds
- 6.2 Forming carbon—sulfur bonds
 - 6.2.1. Ligases Forming Aminoacyl-tRNA and Related Compounds
- 6.3 Forming carbon—nitrogen bonds
- 6.4 Forming carbon—carbon bonds
- 6.5 Forming phosphoric ester bonds

And, these are the subclasses like for example, 6.1 is being classified into 6.1.1, 6.2 is classified into this subclass and so on.

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6. Ligases.
Reactions involving establishment of a binding between two molecules with simultaneous hydrolysis of an acid anhydride bond. Classified according to the nature of the bond.

6.1 C-O bonds: $\text{RCOOH (amino acid)} + \text{ATP} \rightarrow \text{RCO-AMP} + \text{PP}_i \xrightarrow{\text{tRNA}} \text{RCO-tRNA} + \text{AMP}$ (Enzyme: Synthetase)

6.2 C-S bonds: $\text{RCOOH (fatty acid)} + \text{ATP} \rightarrow \text{RCO-AMP} + \text{PP}_i \xrightarrow{\text{HSCoA}} \text{RCO-tRNA} + \text{AMP}$ (Enzyme: Thiokinase)

6.3 C-N bonds: $\text{RCOOH} + \text{NH}_2\text{R(H)} + \text{ATP} \rightarrow \text{RCONH(R)} + \text{ADP} + \text{P}_i$

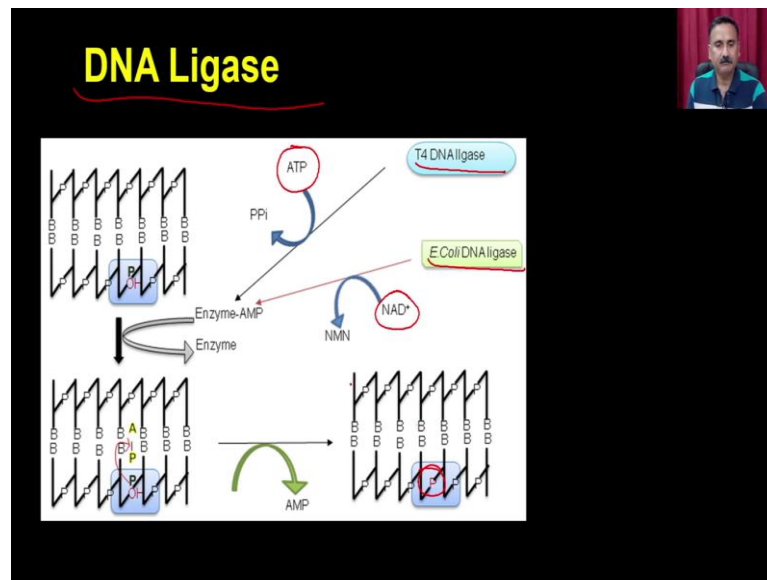
6.4 C-C bonds: $\text{RCO-SCoA} + \text{CO}_2\text{-biotin-enzyme} \rightarrow \text{HCOOC-R-CO-SCoA}$

6.1.1.1. tyrosine tRNA ligase
6.2.1.1. acetate CoA ligase

And, these are the reaction what it is actually going to catalyze. So, for example, 6.1 it is forming the bond between carbon and oxygen. So, for example, this is the acid right amino acid and it is actually going to form this and ultimately it is going to form this. So, that is the synthetases. Then, we can also have the thio kinase. So, thiokinase is forming this bond between the carbon and sulfur right.

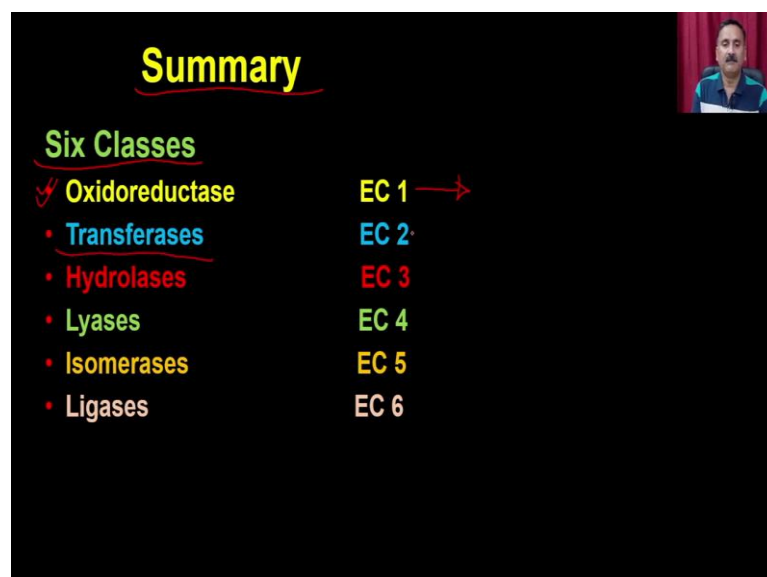
And, then we can also have the C-N bond. So, C-N bond is actually going to synthesize this and you will see in all these reactions ATP is actually working as a source of energy, because you require a energy if you want to synthesize the new compounds. You will get the energy when there will be a breakdown.

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Classical example is DNA ligases. So, DNA ligases could be the NAD dependent DNA ligases or the ATP dependent DNA ligases. Classical example is T4 DNA ligase or the E coli DNA ligases and the what they are going to do is they are actually going to form ester linkage between the two strands of the DNA, two fragments of the DNA and that is how they are actually going to form the phosphodiester linkage. And, that is how they are actually going to link the two DNA molecules.

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So, this is what we have discussed so far. What we have discussed? We have discussed about the classification of the enzyme, we discussed how why it is important for classifying the enzyme and what is the advantage of classifying the enzyme. And, by going through this whole lecture, you might have understood and appreciate that by classifying the these groups into the different classes, we have made the study of an enzyme more and more sophisticated and systematic.

And, because of that you can be able to you know you can be able to recognize or identify the new enzymes, you can be able to you know if you have identified an enzyme, it can be help in terms of characterizing that enzyme as well. So, in summary we have what we have discussed today? We have discussed about the classification of the enzyme into 6 classes.

We discussed about the oxidoreductase, the enzyme which are actually going to catalyze the oxidation reaction reactions when we talk about transferases is. So, transferase is the one of the biggest group what is present in the enzyme classes and they are actually transferring the phosphate groups from the to the different biomolecules whether it is the carbohydrates, DNA, proteins and the lipids. And, that is how they are actually you know changing the biological activity of these all these molecules.

Then we have the hydrolases. So, hydrolases are catalyzing the breakdowns of the different types of substrates and they are always using the water as one of the substrate to catalyze that. The major chunk or the major place where the hydrolyses are actively participating into the reaction is the digestive system where most of the hydrolyses are degrading the polymeric food into the monomeric substances and these monomeric substances are being absorbed by the elementary canal.

Then we have the lyases. So, lyases are also doing the same reaction as the hydrolyses, but they are not using the water as one of the substrate; instead of water they are utilizing the other substrate to catalyze the breakdown of the bonds. And, then we have the isomerases. Isomerases are catalyzing the isomerization of the molecules. So, they can be catalyze the racemizations, epimerizations, cis trans isomers and so on.

And, then at the end we also discussed about the ligases, the molecule which are actually going to join the two molecules and that is how they are actually been part of the

synthesis of the new molecule. 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.