Introduction to Biomolecules
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Lecture – 7
Synthesis of Polypeptides And Enzymes (Part 1/5)

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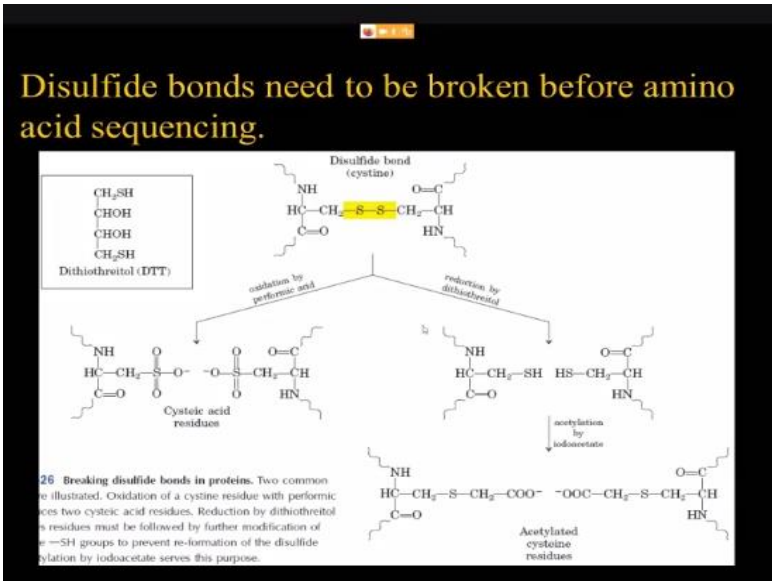

So yesterday we were discussing about advanced degradation for amino acid sequencing of proteins. So, we will continue that. So today we are primarily going to look at how to synthesize polypeptides okay. But before going into that one small detail that we need to know with respect to the amino acid sequencing that is if you remember I talked about two cysteine residues.

If they are juxtaposed in the three-dimensional structure such that this disulfide bond cystine can form then that stabilizes the protein structure. So, when you are going to do sequencing this covalent bond needs to be broken. So then only you are going to have the undisturbed chain where that PTC adduct based cleavage and sequencing can continue. And how do you do this? So, this is done by using the reducing agent like dithiothreitol.

And that converts this disulfide bond into two sulfhydryl groups, but then they can get oxidized to disulfide bond again and to prevent that that needs to be derivatized and that is done using iodoacetic acid in this particular case and this acetalized cystines are not going to form the covalent bond. So, this is one way. Another way is that it is oxidized using

performic acid to have this sulfate groups on the cysteine so called cystic acid and they again are not going to form covalent bonds. So, this is the way by which this disulfide bond is broken.

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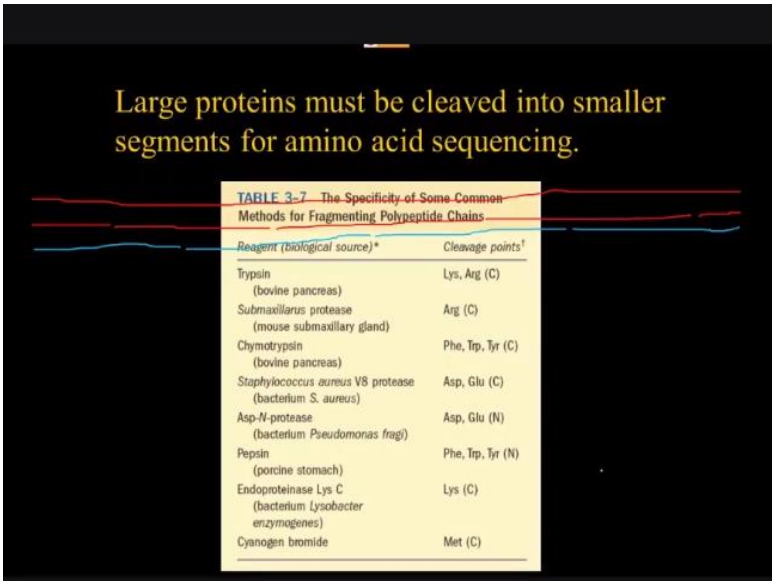

And one other really important point is that you are not going to take, average protein is roughly about 300 amino acids. So, you are not going to be able to take 300 amino acid polypeptide and keep cleaving one amino acid at a time from the N-terminus up to the 300th amino acid because each step in this reaction is not going to be going to completion. So, therefore incomplete products will accumulate.

And eventually as the cycle number increases the quantity of the incomplete products, the background noise, the detection will overwhelm the actual signal. So, you would not be able to sequence up beyond a certain number of cycles. So, to address this what is normally done is that the polypeptide chain is cleaved or broken into smaller oligopeptides and that is usually done using proteases, a few examples are listed here and like this is not protein.

So, this is a cyanogen bromide, this also helps in cleaning. So, each one of these proteases are specific to the cleavage point like this trypsin which is a common enzyme produced by the pancreas cleaves on the C-terminal side of lysine or arginine and so on the other ones okay. So using these proteases you break the polypeptide chain into the smaller peptides and these peptides are separated using high performance liquid chromatography.

This is a modified version of chromatography techniques that we learnt and the separated peptides are then individually sequenced using the Edman degradation. So now for example if I have a polypeptide, let us try using a blackboard here. Let us say this is a long chain okay. So now what is it? That is the margin, so I am going to go this side. So, let us say this is a long polypeptide chain and you use the trypsin and it cleaved into let us say these many peptides.

Now the way i have drawn here you know this is 1, this is 2, this is 3 and 4 and 5, but when it is cleaved in solution and when they are a mixture and you separate these peptides how do you know this one is after this and this one is after this? So, this sequence you will not know, so although individually each oligopeptides amino acid sequence you will be able to determine, but you will not be able to get them aligned and get this full polypeptide sequence in order.

To address this what people do is they digest with two different enzymes in two different separate reactions. So, you get overlapping peptides. For example if this is how the trypsin is going to cut, then I use let us say chymotrypsin and then I get overlapping peptides. For example, I may get another one like this. Now we can be more advanced with this. Let us try out all this, pink color, let us choose blue okay.

So now with chymotrypsin I might get like this. So now you see these two will have overlap up to this then after that this portion of this peptide will overlap with this. And based on this portion being present in this and this portion right away following that in this particular peptide, you know that this comes after this and so on.

So, by generating these overlapping peptides by using two different proteases, then you get a mixture of overlapping peptides and sequencing all of them eventually leads to getting the entire sequence of the polypeptide, entire polypeptide okay.

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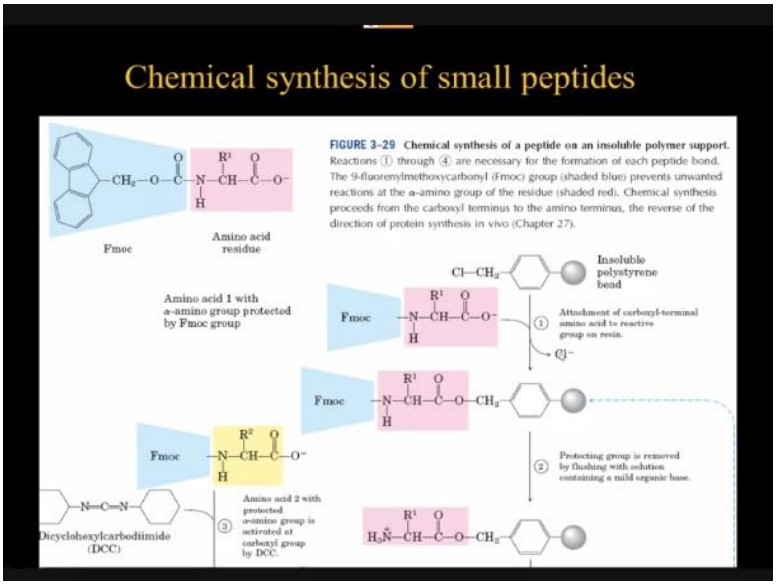

So now let us go and look at how the opposite is done that is how do we join the amino acids chemically to make a polypeptide. So, this method as I kind of mentioned in couple of lectures ago is not for synthesizing a 300 amino acid protein okay. So in that situation, what we actually do is we use the our DNA and clone it in a plasmid put in bacterium and use the biological system.

Like the bacterial translation machinery to make the protein synthesis machinery like using the ribosome you synthesize the protein in bacterium. So chemically the synthesizing is usually for shorter peptides maybe it is less than 20 amino acids is what is a routine, but still it is pretty expensive. So only when it is absolutely essential so most of the time what the common application of this peptide synthesis is the shorter peptides are used as epitopes to raise antibodies for specific parts of a protein okay.

And those epitope specific antibodies making is one very common application where these oligopeptides are normally synthesized. So how do we do this synthesis? So again, this is a solid phase synthesis. What I mean by solid phase is the starting reaction. Like for example if this is the first molecule that is going to be part of the reaction, it is covalently attached to a resin like the chromatography resin we are talking about.

Usually in these things these resins are flat, disc like, paper like material put at the bottom of a plastic tube like the microfuge tube that you may have seen and the required reactants are added and incubated at different temperature or if mixing is required steering all of that is done with the tube. And after every step the molecules added, the solvents or whatever they

are all flushed out. So, the reaction happens in solid phase, so attached to a solid matrix so that is what we call as solid phase.

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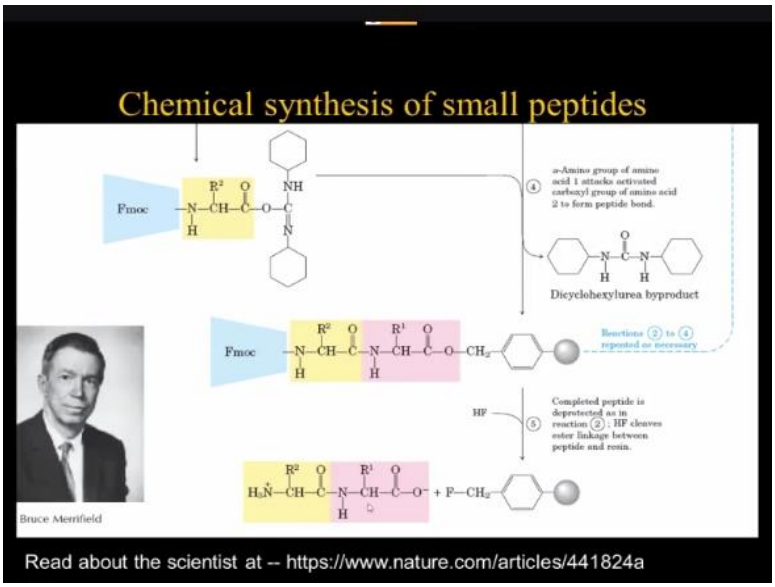

So, this was pioneered by a scientist by name Bruce Merryfield okay. So, he again had a really fantastic achievement. So, it will be really inspiring for you if you go to this website and read about the Bruce Merrifield and invention and optimization of this solid phase synthesis of peptides. Okay now let us look at the chemistry of how this is done. So, in all these reactions the main point is the functional groups that are not supposed to react.

They are all protected okay by modification like methylation and so on. And then the functional group that must react is activated by attaching to a group where that bond is more labile and easy for reaction with the next one. So these are the two main concepts we need to remember in this synthetic process. So here in this particular case this amino group is protected by adding this Fmoc group okay.

So, if you want to know the Fmoc expansion right it is here, you can read this. So this Fmoc group is attached to the amino group and this bond is not going to get readily broken under the conditions that we are going to do for the synthesis. And by this way, this amino group is not available as a functional group to react, so only the carboxyl group is available. Now this carboxyl group is first attached to this matrix.

This intermediate molecule where this chlorine carbon bond very readily reacts with the carboxyl group to form the first link okay. So this is the reactive group, this chlorinated

methyl group is the reactive group and that reacts with the carboxyl group to form this bond. And once this is done to add the next amino acid you need to deprotect, you need to take this Fmoc out and make this amino group available and that is done by using a mild organic phase.

So essentially imagine this is the filter paper and this liquid is added and after the required time of incubation then you add an organic base solution and wash it and that cleaves this and this goes away and then you add the next one and so on that is how it goes. So now the amino group is regenerated. So next we need to do two things. One the next incoming amino acid its carboxyl group needs to be energized or activated.

And that is done by combining with this dicyclohexylcarbodiimide or DCC or the product is there in the next slide. And similarly, you need to yeah so that leads to the formation of this okay. So this DCC reacts with this carboxyl group and this is a reactive group and this is remaining protected with the Fmoc. And this then is combined with this amino group here and after the reaction it becomes the dicyclohexylurea and you have this carboxyl group attached to the amino group here okay.

This is the first amino acid and this is second. Now using hydrofluoric acid um finally like after this is repeated like so now the third cycle will be again you need to deprotect this using mold organic base as we saw in step 2, then like step 3 the next amino acid needs to be activated with the DCC and then you will combine the two they will react and the chain will grow.

And once the required polypeptide chain is done, then you use hydrofluoric acid to cleave this from the matrix and the peptide comes out okay. So, this is the Merrifield's solid phase based synthesis. So here the main point is the Fmoc protected amino acids that is the main ingredient for success of this synthesis okay.

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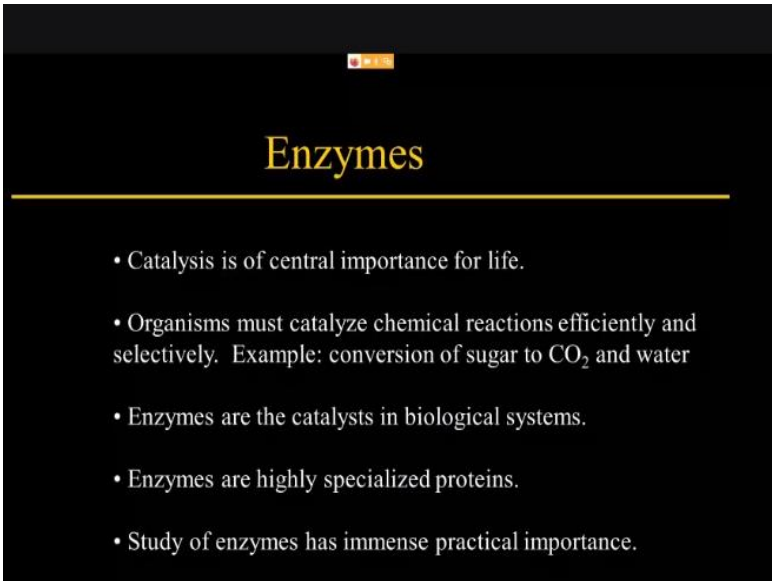

So, with this our general discussion of proteins is complete. So now we are going to look at a specific group of proteins which are very important for all the biochemical reactions be it DNA replication or DNA repair or getting energy from the food you ate and so on. Like synthesizing the bio membrane. synthesizing polysaccharides, extracellular matrix like everything is a chemical reaction.

It is not simple one chemical reaction, it is a series of chemical reactions and each one of those individual steps of those series are catalyzed by enzymes okay. So, at the end of this lecture, we will have a brief discussion on why all these reactions require a catalyst and why they do not happen spontaneously, what is the advantage of it? So, we will see that at the end. So first let us focus on what are enzymes.

So, first catalysis is centrally important because without a catalyst the reaction rate is so low that the life as you know cannot exist okay. With the lifetime and with the specific duration for each events of life none of that will be possible without a catalyst because the rates of uncatalyzed biochemical reactions are extremely small, it is beyond the time scale in which life normally operates on this planet.

And these reactions have to happen very efficiently and they have to be very selectively. So, the substrate specificity is a substrate being the reactant in these reactions. So, the catalyst has to be very specific for those substrates. So, the selectivity is very important and we have already seen one good example where you know you can eat starch and obtain glucose from it but not by eating cellulose, although both are made up of polymer of glucose.

One has an alpha linkage and another one has beta linkage and we do not have the enzyme that cleaves the alpha linkage does not cleave the beta linkage okay. So, they are very specific. Here I have given a big global example like conversion of sugar ultimately through a large series of steps to carbon dioxide and water. The simplest products that you find in the environment from which sugars can be assembled.

So, the enzymes that catalyze this conversion this series they are not going to do any other such conversions, enzymes are very specific. If even enzyme is going to phosphorylate glucose at the sixth position it is not going to do that for the sixth position of fructose or galactose okay, very closely related other monosaccharides. Enzymes are the catalysts, so this already we have stated multiple times and they are highly specialized proteins okay.

So that is an important thing. So, these are globular proteins meaning these are folded not as fibers. So, there are proteins that are long fibers, a good example is collagen present in our extracellular matrix which is the material present among the cells okay, in between cells, not within the cell but outside cells but among the cells. It is like the cement. If you think cells as bricks and the cement that you use to hold to stick the bricks that sort of thing is what is extracellular matrix.

So that is where you find collagen that is a long fiber and similarly the main component in your hair is a protein called keratin that is again a long fiber like molecule. But enzymes are not that kind, enzymes are folded, the polypeptide chain is folded into a globular shape okay. It is spherical and they have as very specific structures in those globular shape which are very specific for binding the substrate and making sure the bond cleavage or the condensation of making new bond can really happen.

So, the enzyme active site where all these reactions happen have specific structures to enable the reaction to happen. So that is the sense in which the enzymes are highly specialized proteins. And study of enzymes is not simply to understand how life happens that is the primary goal of biology, but primary goal of studying enzymes in biology like how the various events of life happen but outside that there is a lot of applications for enzymes.

The immobilized enzymes are used for commercial scale manufacturing of many compounds

of commercial importance. So, therefore a good knowledge of what are enzymes, how they catalyze reactions, how to work with enzymes is really important regardless of which area of biology you are going to pursue either for a basic science curiosity driven research or an industrial application.

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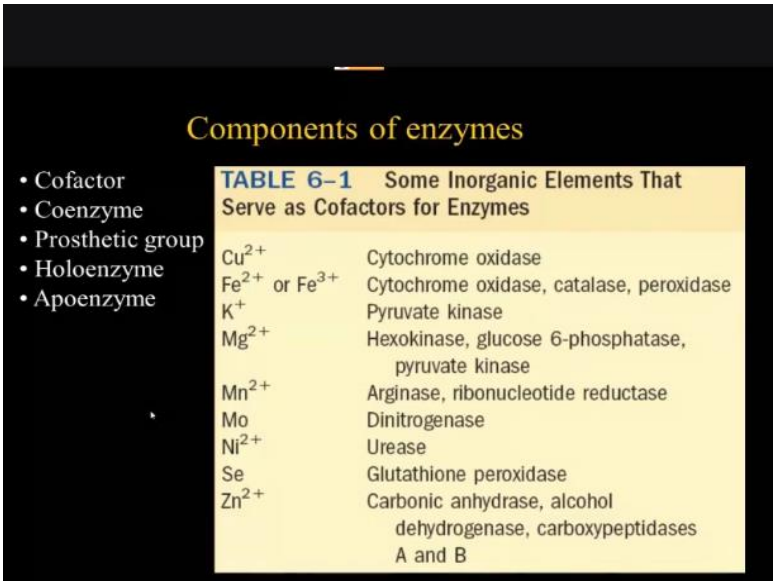

So, a few important components non-peptide components we need to familiarize ourselves in the context of enzymes. So, most enzymes are not catalyzing the reaction simply using that polypeptide chain alone. So embedded in this polypeptide chain are other uh molecules okay, so they are called cofactors. And when the cofactor is usually a metal ion like the ones listed here with respect to in which enzyme you find them.

So, these are called cofactors and sometimes such non amino acid moiety present in enzymes can be small organic molecules and they are called coenzymes. Almost all the vitamins, B complex vitamins that we are require as nutrients in our food these vitamins function as coenzymes. We will see examples when we consider individual biochemical pathways, at that time I will point out which vitamin is functioning as a coenzyme for which enzyme and what is the reaction catalyzed.

So that will help you to appreciate the importance of vitamins in biochemistry. And when these cofactors, coenzymes, etc., are very tightly bound to the active site okay, more tightly than the other ones they are called prosthetic groups okay. So prosthetic groups are not different from these. When a coenzyme or cofactor is tightly bound to the enzyme, they are called the prosthetic groups.

The polypeptide chain under the given cofactor both together when you have complete enzyme that can have the enzymatic activity you call that a holoenzyme. And if it is merely the polypeptide chain without the required non polypeptide moiety then you call that apoenzyme. Apoenzyme is just the polypeptide chain, all of when this apoenzyme is bound with the given coenzyme then it is called a holoenzyme okay.

So these are some of the common terms used in enzymology. So, it is a good idea to remember them and clearly know the distinction among them in terms of their definitions.

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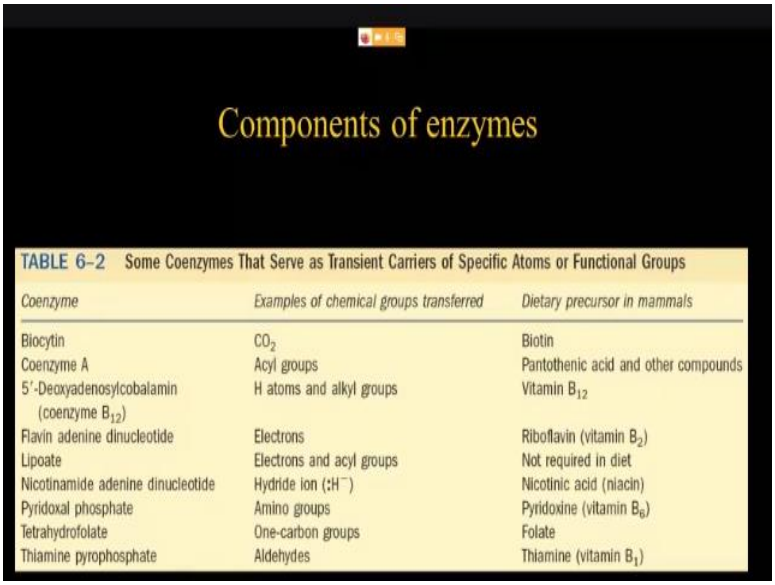

Okay here is a list of coenzymes. So this will become a lot clearer when e actually consider the individual reactions, but this table gives you a list. So biocytin, carbon dioxide transferred, biotin is the vitamin that functions as the coenzyme here. Pantothenic acid another B complex vitamin that is a present as coenzyme A, this pantothenic acid is part of coenzyme A and that is involved in acyl group transfers okay.

So, this is important in glycolysis itself the process by which glucose is converted into pyruvate and so on. So here you have a list of coenzymes and what is the group transfer in which they are involved. So, they are primarily involved in group transfers. Group transfer meaning one group is being transferred from one molecule to another molecule. For example biotin carries carbon dioxide from one molecule temporarily on it in a covalent form and to another molecule.

So, biotin will be in the enzyme active site and helps in transferring this carbon dioxide group or one carbon group they often call from this to one substrate to the other one. And similarly, coenzyme A is involved in acyl group transfer okay and these are involved in electron transfers. They are present in the electron transport chain where the pyruvate is ultimately oxidized to carbon dioxide and water and then you get the maximum amount of energy okay.

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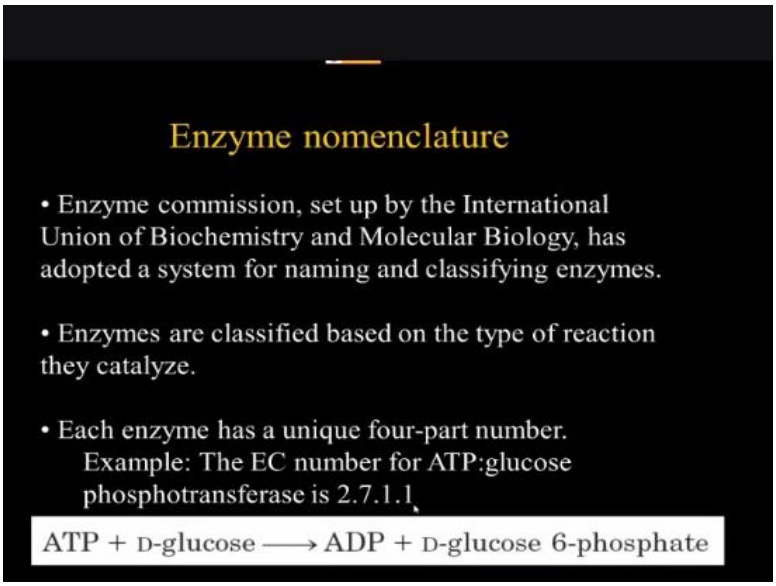

So, another aspect of enzyme we need to know is that there are large number of enzymes, so how to classify them, catalogue them and have unique identity for each one of them? So, you might have discovered one enzyme and with respect to its activity on one particular substrate and a certain condition and based on that you would have named it. You may not even have considered the substrate.

Maybe you would have considered the name of the organism where you isolated and you gave a name or based on that organism. Someone else working continents away discovered the same enzyme from some other source and gave a different name and if this process goes on how anyone is going to know that these two scientists are talking about the same polypeptide.

So to avoid such confusions and to make sure we clearly understand the enzymes based on the reaction they catalyze, they have been classified and given a hierarchical numbering system okay, a four part number. So, this is the larger group than this which is larger than this and so on. So, if you have a primary group of 1, 2, 3, 4, 5, 6 groups and within the 6 groups you have a sub group like this 7 is 2.7.

In under 2 this is seventh sub group and in that you have sub-sub group and then sub-sub-sub group which is basically the fourth digit is not a group, it specifically identifies a given enzyme. So, the main focus is type of the reaction that they catalyze. So that is the basis used by this International Union of Biochemistry and Molecular Biology that has a subcommittee called enzyme commission and this enzyme commission is the one that gives the name.

So, if you discover a new enzyme you need to contact enzyme commission, describe them what is the substrate, what is the product and what kind of reaction s catalyzed and then they will give you this number and when you publish that this number will be essential to include in the manuscript to submit to the journal. So here is one example where the enzyme commission's number is 2.7.1.1. What this is?

It is ATP glucose phosphorus transferase. What it means is from ATP it transfers the phospho group to glucose to making glucose 6-phosphate okay. ATP plus glucose gives you ADP like the phosphate is removed, adenosine triphosphate, now it is adenosine diphosphate and the other phosphate removed from this is attached to glucose okay. So now let us look at how it got these numbers?

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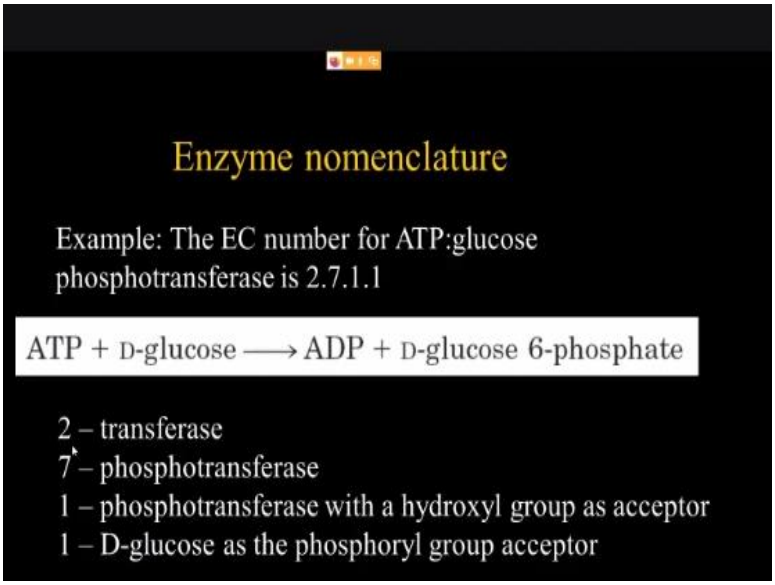

So, number 2 is transferase okay. I told you there are 6 major groups, in that the second group is all transferases are group 2. Since this is a transfer reaction so it is a transferase, so it is number 2. Now what kind of a group is transferred among the transferases? The seventh subgroup of transferases are all transferring phospho group and this is transferring a phospho

group, therefore it is 2.7. Now where is this phosphate getting transferred?

So, for that to answer that you have subgroups within 7 and this is number 1 because it transfers the phosphor group with a hydroxyl group as acceptor. In the glucose you are familiar that you have hydroxyl groups, the alcohol groups and to one of them the phosphate group is transferred. So, it is going to be ester linkage. This phosphate is an acid and there an alcohol, so acid alcohol reaction is ester.

And to a hydroxyl group acceptor when phosphor group is transferred that is a sub group 1 under subgroup 7. So therefore, this is 2.7.1. And then which hydroxyl group is the acceptor here? So, it is the D glucose is the acceptor and that is how it gets 2.7.1.1. So, when you come to the fourth digit it specifically defines that enzyme. No longer it is a group okay, this is a specific enzyme.

So, this is the only enzyme that transfers a phospho group to a hydroxyl group of glucose okay. So, this is how you have this hierarchical four-part number system for naming the enzymes.

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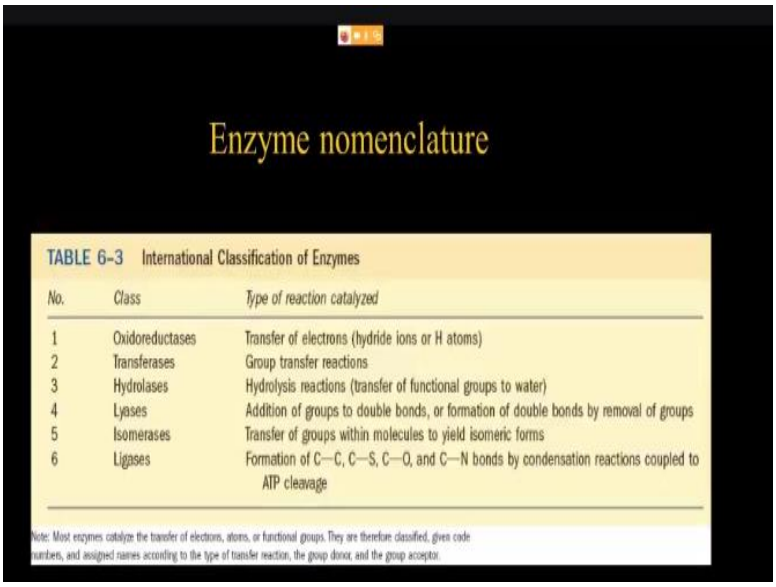

And we are not going to learn all the enzymes and memorize them, we will only focus on the first hierarchy the largest one what are the six groups that I said. Sub-sub group and all is for biochemistry majoring students. So here we would not worry about those. So, these are the 6 groups. The first one oxidoreductase transfer of electrons either as hydride ions or proton. So those are the oxidoreductases, either they oxidize or reduce okay, so electron transfer.

And transfer of other groups is transferases okay like the one that we saw in the previous example transfer of phosphate group. Hydrolases they break bonds where the originally bond is made by condensation through dehydration. So, this is familiar to us that is how polysaccharides are made, polypeptides are made, polynucleotides are made. They are all condensation reactions releasing water molecule okay.

Dehydration dependent condensation reactions. So, to break them you add water so that you call as hydrolysis and those enzymes are hydrolases. And other group breaking like addition of groups to double bonds or formation of double bond by removing groups those are lyases because they lyase and therefore their lyases. You need to distinguish this from hydrolases because here it is water molecule is involved.

And the addition of water is required or removal of water is required and in that sense these are distinguished. So, this is like for example breakdown of starch into glucose is actually a lysis but it is a hydrolysis okay water involving and they are large number deserving their own major subgroup and that is why you have separate group hydrolases from lyases. And rearranging groups.

Remember stereoisomers cannot be converted one to the other without breaking and rejoining the bonds because either a double bond or an arrangement sequential arrangement around a chiral carbon generates these isomers and one cannot become the other by bond rotation because that's not possible under those conditions and to convert one to the other you have to break and rejoin. So, we learned this under stereochemistry.

And enzymes that do this like converting glucose 6-phosphate to fructose 6-phosphate that requires enzymes called isomerase. So, they transfer groups within the molecule to yield other isomeric forms. Then you have ligases which joint, they make these bonds carbon-carbon, carbon-sulfur, carbon-oxygen, carbon-nitrogen like peptide bond and these bond formations are catalyzed by ligases.

So, these are a major like under each one of them you have very large number of enzymes that distinct from the other five and that is why you have these 6 major groups okay. So, within this we have subgroups that we saw in the example of ATP glucose

phosphotransferase, alright.

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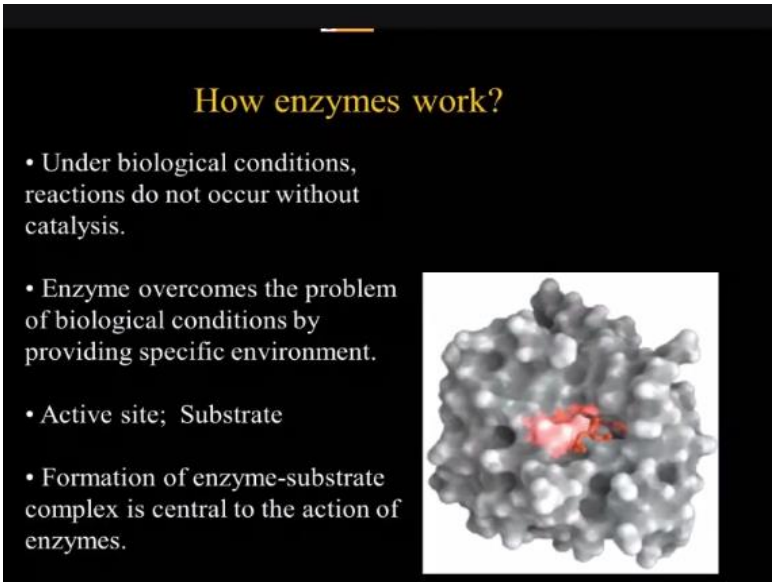

So, these are all like setting the stage, tidying up the workspace to work. Now what is actually the work? So, we are going to look at how do enzymes function. They are like magic and they really make impossible things possible. So how do they do that? So, under biological conditions reactions do not occur without catalysis, this I told you at the beginning. Enzymes overcome this problem of by providing specific environment.

So that is the main thing we are going to learn about enzymes and the specific environment is actually made possible by the overall three diamond structure as shown in this space filling model on the right where the substrate molecule is shown colored and you need a site a workspace where the substrate selectively fades and nothing else fades and then there should be other groups that enable the reaction to happen.

So that is the specific environment it provides and in that one important concept like a term that we need to know is the active site. So active site is the site of the enzyme molecule where the reaction actually happens. And then the reactant I told you earlier we call substrate okay, so remember this term called a substrate. In enzyme catalyzed reactions the reactants we call them substrates, it can be singular or plural you made.

Like for example this previous enzyme it has two substrates ATP plus glucose gives ADP plus glucose 6-phosphate. Some may be like one like starch binds and gets cleaved into glucose and starch chain minus one glucose. So, these two terms you need to know. Then

formation of enzyme-substrate complex, so that it's a recent concept. The idea that substrate and the enzyme actually form a complex okay. So, we need to first remember that.

So, the substrate binds to the enzyme, binding meaning multiple interactions between the groups on the substrate and the groups on the enzyme. It need not be always functional group, you do not need to be thinking carboxyl group or hydroxyl group or alcohol group and amino group, sulfuryl group and so on. Quite often it is hydrogen bonds okay, the hydrogen bonded to oxygen or other electronegative atoms coming nearer another one where hydrogen bonding is possible.

It could be hydrophobic interaction. So you have the phenylalanine, tryptophan, those kind of aromatic rings or you have leucine, isoleucine kind of hydrophobic aliphatic chains and they coming next to each other may lead to hydrophobic interactions. So, it is a variety of non-covalent interactions and it is not only covalent interactions, occasionally there may even be covalent bond formation.

So, these variety of interactions that happen, multiple interactions okay that happened between the substrate and enzyme groups is central to the action of enzymes okay so that is the main point. So, this is really crux of the enzyme substrate binding and conversion of substrate to product. This is central to the enzyme-based catalysis, right.

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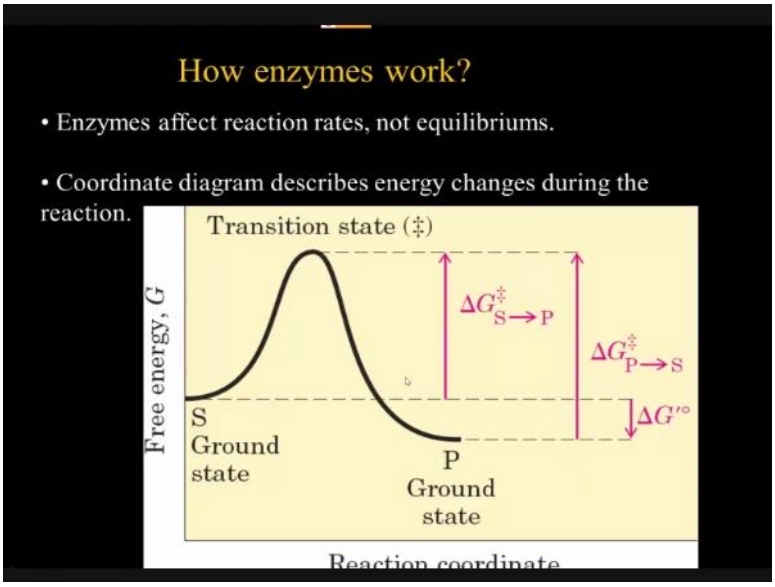

So, the other important point remember about enzymes is like any other catalyst, they affect the reaction rates not the equilibrium okay. So, the concentration of C D upon A B for a

certain K e like the equilibrium constant will be still the same that is not going to change. Only when C D reaches a certain concentration, the forward and reverse reactions will be equal and you will get the K e equilibrium value that is not going to be affected.

But how quickly you will reach the equilibrium that is where enzymes influence okay. They affect reaction rates not equilibrium. So that is indicated here the reaction coordinate so where the x-axis essentially you define the energy state, ground state for the substrate and the product. So that plotted against the free energy is what this diagram is reaction coordinate. So, compared to the entropy of substrate so this is higher.

So, the free energy available is lower okay. So only under that condition a reaction can happen. So, the net change has to be negative, then only reaction is going to happen. But then it is not going to spontaneously go from S to P, instead for that change to happen it has to cross this transition state which is at a much higher energy level and it has to cross this barrier, we often call this as the activation energy barrier.

The energy required to reach here that is the activation energy barrier and then once it is taken there it can come down to the ground state. So, you consider the example of water present in an overhead tank. So that water is not going to spontaneously flow down to the ground level because at ground level the entropy will be higher, it is not going to flow straight down. It has to climb up the wall of the tank to come down and that climbing up the wall that is the analogy for this activation energy.

Now you kind of understand from this analogy itself that why those activation energy barriers are required. Like for example without the tank walls you cannot store water overhead, it is not going to come to the ground level. So, these activation energy barriers are crucial to make sure the right reactions happen at the right time and not all the time. Otherwise, the complex molecules breakdown into simpler entities and life is not going to happen okay.

So that is why the activation energies and something to lower the activation energy those kinds of things are required. So, this sort of a diagram depicts this activation energy idea and how the enzymes are going to affect that and so on. So, this tells you the difference in the ground state of the substrate to the transition state it has to reach and that is what we call as the activation energy and from there then it is going to come down.

And here you are going to see overall net negative free energy change and that is why the reaction is possible. So, this is another factor we need to remember and this is the actual difference between the substrate and product.

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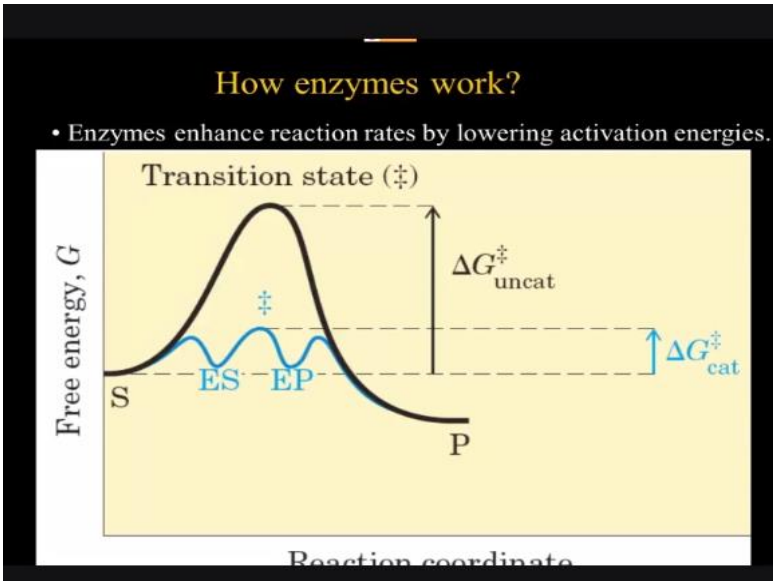

So, in this what the enzymes do is they reduce this activation energy. So, in an energy sense the answer to this question how enzymes work they reduce the activation energy. Normally there is not enough to answer if someone asks you how enzymes work. You can say they reduce the activation energy, but then you need to also answer how do they reduce the activation energy.

So that we will go next, but right now here we let us focus on this reaction coordinate diagram. What you find is that this peak is reduced but it is not reduced into one small peak, you see 2 or 3 or multiple peaks indicating that the substrate conversion to the product may involve a series of intermediate reactions okay and each one having its own activation energy, but the individual ones are lot less than the overall energy required to get to this transition state.

For example, the substrate to bind and make enzyme substrate complex it has a certain activation energy and once that is done then from this state to go here like substrate bound to enzyme getting converted into product bound to enzyme has a certain activation energy requirement but that is not that much. And once you get here, then you again need another hump to really release the product out.

So, each one individually can readily happen compared to this single conversion of substrate, uncatalyzed conversion of substrate product where the energy required to get to transition state is enormous okay. So, this is the delta G for uncatalyzed reaction, this is the one for catalyzed reaction. So that is the rate limiting step because that is the peak. So, this rate limiting step is coming up in the next slide.

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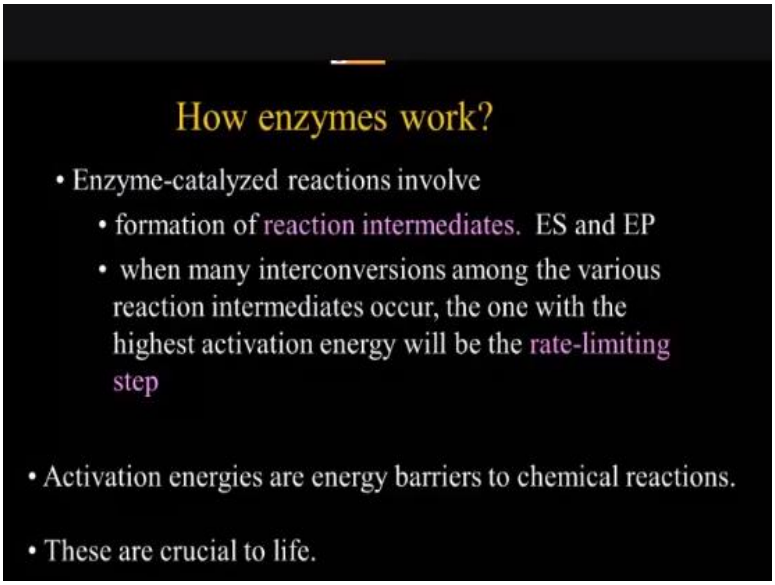

So okay. So, enzyme-catalyzed reactions involve formation of reaction intermediates. We just saw enzyme substrate as an intermediate because here the molecule the moieties of substrate are interacting with the enzyme moieties and as a result these are constrained in a certain way. So, this is not equal to free substrate. So, in that sense it is an intermediate and as a result of those constraints certain bond breaking joining have been made possible.

So, it moves from ES to EP and these two are reaction intermediates. When many interconversions like this among various reaction intermediates occur, the one with the highest activation energy will be limiting the overall rate of the reaction S to P overall conversion the rate at which that can happen will depend on the one that requires the maximum energy.

For example, in this case the ES to EP this conversion is the rate limiting because that requires the highest energy compared to the previous two. So, this is a three-step reaction, three intermediate steps with two intermediates being formed. In that this ES to EP is the one that requires the highest and therefore we call this as the rate limiting step okay. So, this rate

limiting step is in the context of series of intermediates that happen in an enzyme catalyzed reaction.

So, we have this rate limiting idea in another context where you have a series of biochemical reactions where A gets converted to B gets converted to C gets converted to D and so on. So there the formation of D depends on which one of the previous reactions is limiting, which happens the slowest that is going to limit the rate of the formation of D from A in that particular example of A to B to C to D.

So, suppose B to C conversion is the slowest then the rate at which A becomes B depends on the B becoming C and therefore B to C we call as rate limiting step that is in a biochemical pathway context. So that is distinct from this, but idea is the same okay. So here it is a series of intermediate steps in a single overall reaction. So, activation energies are energy barriers for chemical reactions because they act as the overhead tank wall.

So, they are the barrier that holds the water on top of your building okay. Without that the water will freely fall down to the ground and you will not be able to have water coming in your bathroom or tap okay. So, such energy barriers in biology are essential to make sure the right reactions happen at the right time and large complex molecular assemblies or large molecules are actually stable. They do not really breakdown into products.

For example, glucose does not readily breakdown into carbon dioxide and water because glucose breaking down into carbon dioxide water is like water freely falling from 10th floor of a building to the ground. It will very readily happen okay, but if it happens then you will have no glucose okay. So, you will have nothing. So, you will not have lipid bilayer, you will not have polynucleotide in your nucleus. None of that is possible.

So, as a result these activation energy barriers are very crucial to life and when these barriers exist but at the same time for the right reactions to happen. For example, you need to oxidize the carbohydrates you ate in the morning breakfast to produce energy you need certain reactions to happen, you cannot really prevent all reactions from happening and they are selectively made possible by using the enzymes.

So that is how enzymes have evolved during the 3.5 billion years of evolution to selectively

make certain reactions possible under certain conditions okay. So, with this I will stop and we will continue on the enzymes in the next class as well.