**Biochemistry**
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**Lecture -19**
**Vitamins and Coenzymes - I**

We start our discussion on vitamins and coenzymes. We will have two lectures. And vitamins are something that you studied from your school levels, knowing that what will happen, if you do not have sufficient amount of vitamin A or B and so on and so forth.

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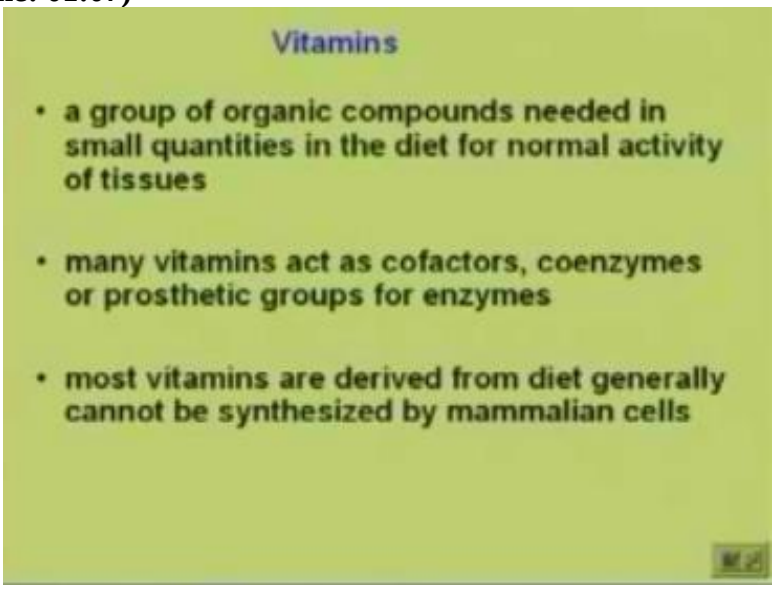


So, the first thing that we know is that, vitamins are basically a group of organic compounds that are needed in small quantities in the diet for normal activities of tissues. And it is something that you must supplement in your diet, because vitamins, the basic requirements of vitamins are basic structures of vitamins, the organic compounds are not synthesized in the body. So they have to be supplemented.

It does not mean that you have to take vitamin tablets, because there are lots of vitamins in the food materials that we consume. In dietary intake itself, you can get the amount of vitamins that you need. Now the reason, why we need these vitamins is because they act as cofactors, coenzymes or prosthetic groups for many enzymatic activities.

And considering that all the activities that go on, all the biochemical reactions that go on in our body are due to the presence of enzymes, are due to the enzymatic reactions, it is

essential that these vitamins are present, so that the cofactors, coenzymes and prosthetic groups, whatever is required for the enzymes are available for the enzymes to act.

And we will see some specific examples, where we have these vitamins acting. This is what I mentioned before that most vitamins are derived from diet and they generally cannot be synthesized by mammalian cells.

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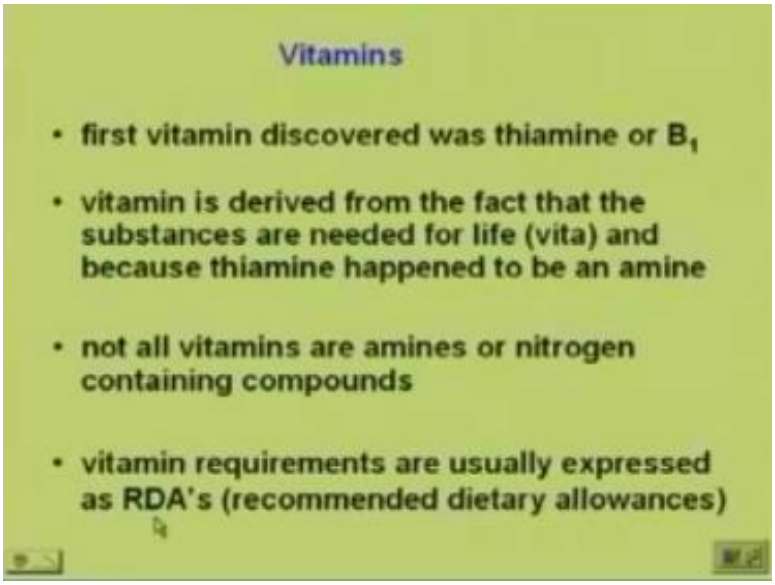


What else do we need to know about vitamins? Well, the first vitamin that was discovered was thiamine or vitamin B1. And the name vitamin itself comes from two words. Vita, which means life and min from the amine because thiamine, which was the first vitamin that was discovered happens to be an amine. But this does not necessarily make all the vitamins having amine groups.

It is just that the first one happened to be an amine and it happened to be essential for life, which is why the name vitamins came about. Now, usually the vitamin requirements are expressed as RDA's that is your recommended dietary allowance or you daily allowance of vitamins, something that you have to consume in your food, for proper action of the enzymatic reaction that are actually going to take place.

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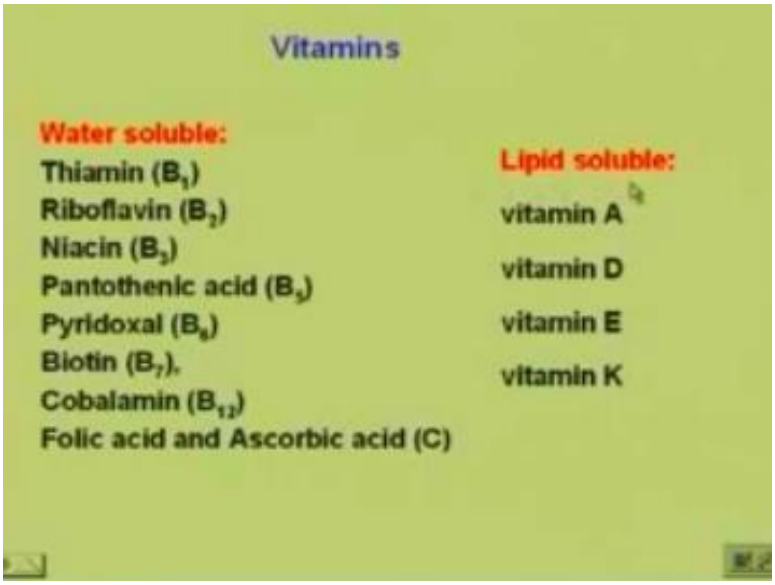


There are two sets of vitamins. They are water soluble vitamins and lipid soluble vitamins. So, when we speak about water soluble vitamins, you will see all the B vitamins that is B complex as it is called, we have B1, B2, B3, B5, B6, B7, B12, vitamin C. All of these are water soluble. So, they happen to be in your blood, flowing in the blood and all. The lipid soluble membranes are vitamin A, D, E and K.

So now, if you are to have a lipid, what does it mean? It means, you have to have some amount of fatty acid, right, so you have to consume some amount of fat as well, so that these vitamins are soluble. So these are the two sets, the water soluble set and the lipid soluble set.

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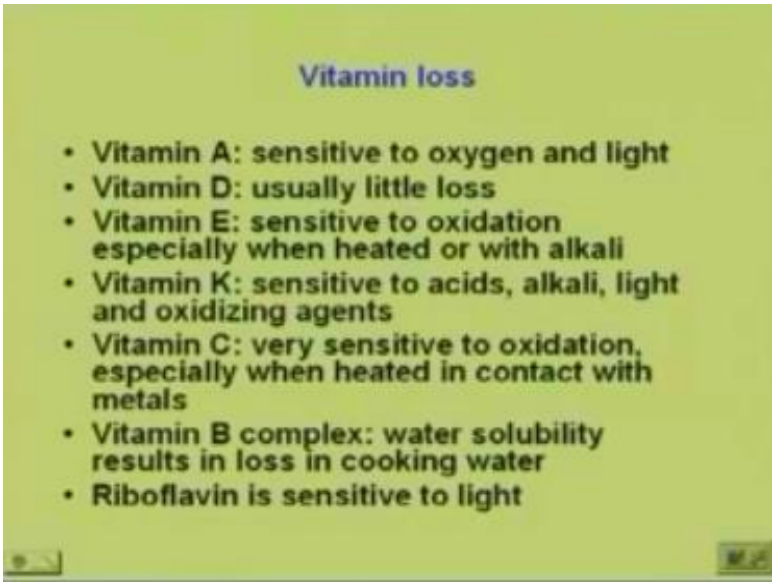


Now, I won't go into the diseases that occur because you don't have sufficient amount of certain vitamin. That is something you studied from school, I guess, class ten, twelve, you

studied all that, what happens if you know, you don't have sufficient amount of vitamin A is night blindness and so on and so forth. But what you need to know is how vitamin loss can actually take place. When it is loss? That is usually due to storage of a vitamin.

When we look at vitamin A, it is sensitive to oxygen and light, ok which means too much of oxygen or too much of light is essentially going to result in a loss of vitamin A. Vitamin D is not usually lost and it is synthesized by the UV radiation that we get from the sun. It is synthesized in the body itself. Vitamin E is sensitive to oxidation, especially when it is heated with an alkali.

Now, the reason, why we are talking about these, because later on when we study the structures of some of these vitamins, we are not going to go into the detail about all the vitamins, but some of the ones that are actually important for some other reactions that we are going to do, when we do the bio energetics part of this course. Vitamin K is sensitive to acids, alkali, light and oxidizing agents.

Vitamin C is extremely sensitive to oxidation, especially when heated in contact with metals. And vitamin B complex is the water solubility, because all of these vitamin B are soluble in water, if you happen to heat or cook basically, you lose some of the vitamins in the food itself. And Riboflavin is sensitive to light. This is another, one of the set of vitamins B. So, the vitamin loss we see here is mostly due to storage.

It is not something that you can keep vitamin for a very long period of time. And if you look at vitamin bottles, they say that extra vitamins have been added, due to the loss that happens on storage. And this loss is due to either they are sensitive to oxygen or sensitive to light and so on and so forth. So, excess vitamins are always added to the vitamin supplement that you find in bottle is because of this loss that occurs.

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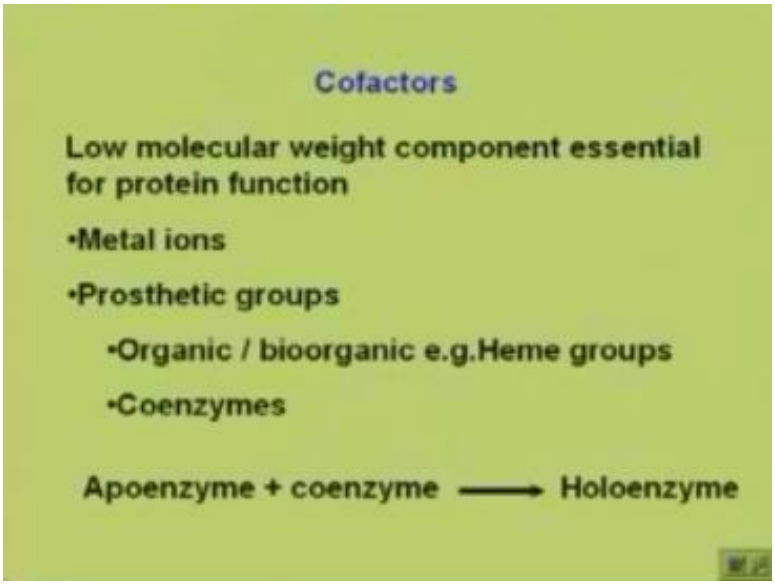


Now, we look at cofactors. We are going to study the vitamins, but since the vitamins and cofactors actually come together, we are going to see how they actually complement one another. So, we have a low molecular weight component that is essential for protein function. Now, when we studied enzymatic reactions previously, enzymatic mechanism of ribonucleus, chymotrypsin or lysozyme, we found that the enzymes themselves are sufficient in bringing about a specific reaction.

But, enzymes that require a cofactor or a coenzyme for the complete action of its enzyme are what is written here, we have an Apoenzyme that along with the coenzyme is going to form the holoenzyme, which is what is going to take part in the reaction. So what we need is, we need the specific cofactor or coenzyme that is going to essentially be large variety of organic compounds or even metal ions.

So, what this means is that these factors are essential for the enzymatic reaction of a certain number of enzymes, which is why they are called cofactors or coenzymes. And these can be organic or bio, like the heme group. The heme group and hemoglobin which we studied is not part of the protein chain. But, obviously you understand, it is essential for the binding of the oxygen. Because, that is where the oxygen binds.

So that is an example of where you would have an essential part of the protein molecule that is not a part of polypeptide chain. It is something extra.

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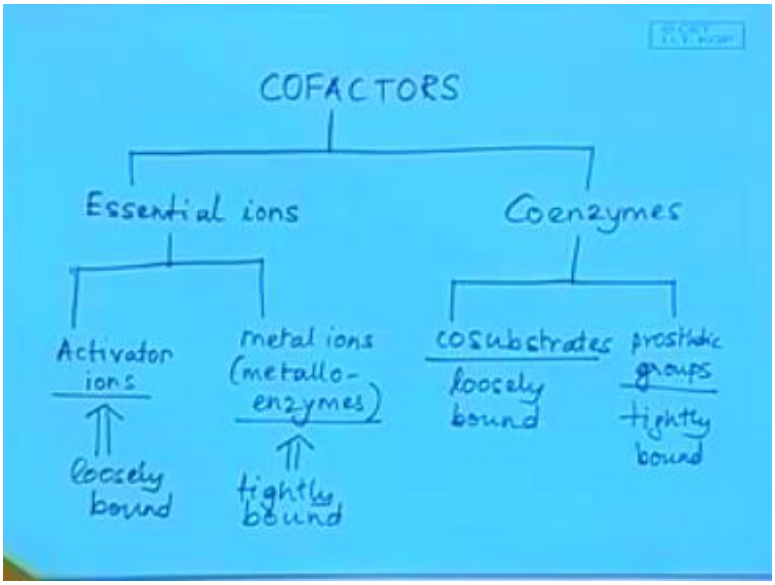


So, if you just look at these cofactors, these cofactors that are actually essential for the overall action of the enzymes. We can probably divide them into two groups. We can have essential ions that are necessary for action. Or we can have coenzymes. Again, we can divide each of these into two groups, which is going to basically cover all the different types of materials that you could have for an enzyme to act, if it cannot act by itself.

For essential ions, you have activator ions or metal ions. Metal ions are those belonging to metalloenzymes. Metalloenzymes are enzymes that have an essential metallic part to it, for their function. These activator enzymes or activator ions rather are loosely bound, whereas the metal ions in metalloenzymes are very tightly bound. In the coenzymes, we have, we could divide them into what are called cosubstrates that are loosely bound and we have prosthetic groups that are tightly bound.

So, we basically have these groups that, all of these together, not necessarily all of them, one of them or none of them in the case of normal enzymes, some of the enzymes that do not, that are not complex enzymes. These enzymes that require such cofactors, would be called complex enzymes. So, the other enzymes that do not require such cofactors are like ribonucleus or lysozyme that can act by themselves depending on the side groups that are present.

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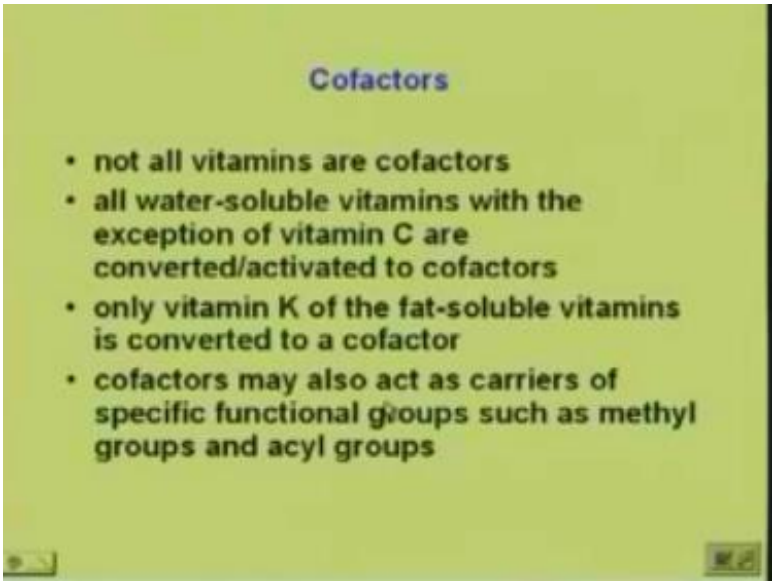


Now, these cofactors are actually formed from the vitamins. Not all vitamins however are cofactors. And all the water soluble vitamins with the exception of vitamin C are converted or activated into cofactors. This is what is extremely important. We are going to see how a certain vitamin is converted to a cofactor and how that cofactor takes part in a reaction, an enzymatic reaction. That is what is important.

And if you obviously, if you do not have the vitamin, it cannot form the cofactor and if cannot form the cofactor, the enzymatic reaction, which requires or the enzyme that requires this cofactor cannot act. Only vitamin K of the fat soluble set is converted to a cofactor. And the cofactors can also act as carriers of specific functional groups such as methyl groups and acyl groups.

Now, this is essential, when we have a synthetic mechanism going on in the body. We have biosynthesis. For the synthesis, we need a set of enzymes. These enzymes are going to result in the transfer of certain chemical groups. For example, if we want to transfer a methyl group, we would have an enzyme that would be called methyltransferase. So, from the name itself, you can say, what the action or what the mechanism, we cannot say the mechanism, but we can understand what the enzyme actually does.

If it is methyltransferase, it is going to be involved in the transfer of a methyl group. But, how does it do it? For that we need specific cofactors, we need to know how methyltransferase actually works, but the cofactor that is going to help in bringing about the methyl group that is going to be transferred. That is where we are going to get it.

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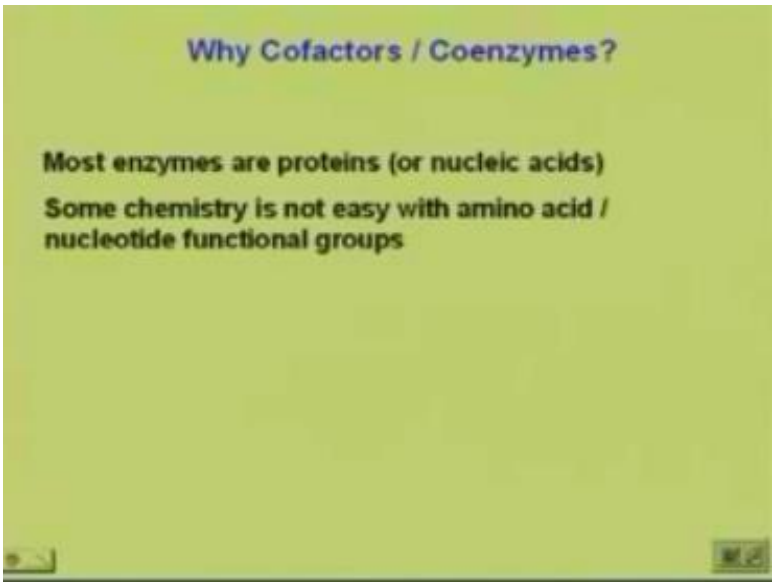


Now, the reason why we need these cofactors or coenzymes is because most of these enzymes are proteins or nucleic acids. And all the chemistry is not possible, just with the amino acids. You understand that because the amino acids are limited in their backbone structure. Why? Because they are limited in the number of R groups that you have. You would have acetic groups, basic groups, but it does not mean that all the reactions that are going on in the body are possible with just those few amino acids. Which is why we require these cofactors and coenzymes for the proper functioning or the proper mechanisms of all these enzymes to act.

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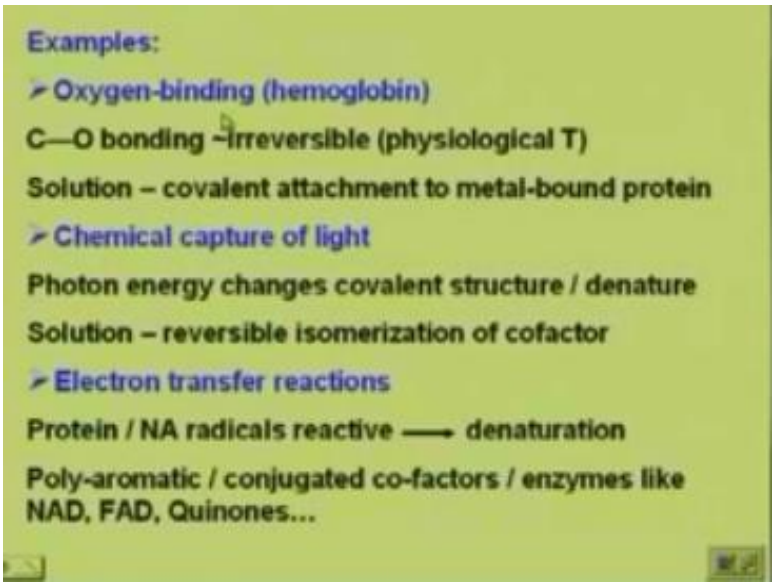


For example, these are the three examples that I have there. One would be the oxygen binding hemoglobin. We understand what do we need here, we need the heme for the oxygen binding

to occur. And it is, only if you have that CO bonding, then only it is going to carry, you know that hemoglobin is a transport protein. It is going to only then be possible for it to carry the oxygen from one level to the other.

So, it is the polypeptide chain that just acts as a cap hold. It acts as just structural frame work to hold the heme group. And, it is the heme that actually doing the function. It is the heme that is taking up the oxygen, it is the heme that is releasing the oxygen, it is the heme that is binding the CO2 and it is the heme that is releasing it. So, if you do not have the heme group, but it is not just sufficient to have heme group floating about in the body.

It has to be in a correct orientation, it has to be held in correct position and this is possible with the polypeptide chain alone. This is how it acts as a cofactor or a coenzyme to the protein hemoglobin. For example, if we are looking at a chemical capture of light, a photon energy changes the covalent structure of a protein. So what is going to happen then? This cofactor will then come to the rescue, to bring it back to where it is supposed to be.

You can have free radical formation. For example, in this electron transfer reaction that we are going to study later on in bio energetics, there are certain protein and nucleic acid radicals that is extremely dangerous to the body. So, they have to be scavenged immediately. But the body has such quality control, it will take place, take action immediately and there are other poly-aromatic, conjugated cofactors and certain enzymes also, that will take care of the free radicals that are found, so that they do not escape and damage the cells.

So, this is why we require the cofactors and these cofactors are basically essential and we require them for specific enzymatic reactions. How are these cofactors formed? These cofactors are formed only if you take the vitamins. So you have the vitamins, the vitamins are transferred or activated into their cofactors and then the cofactors will help in the specific enzymatic reactions.

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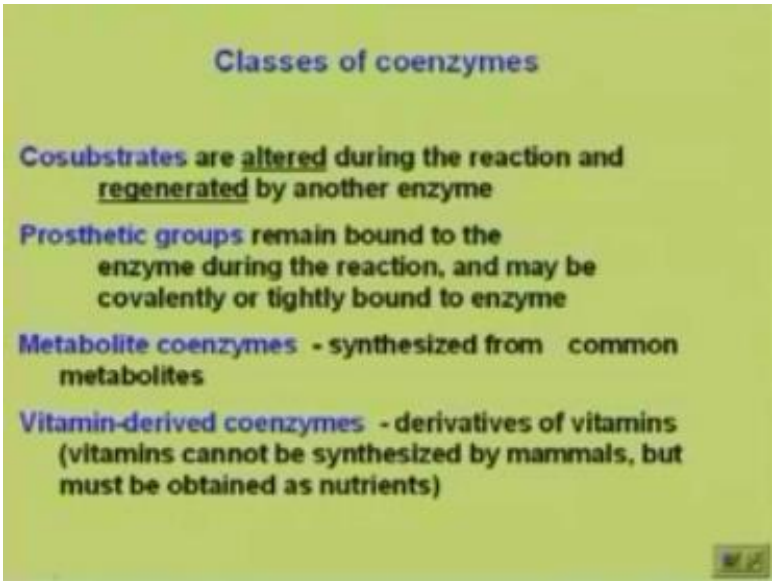


These are the different classes of coenzymes, as I was mentioning, we have cosubstrates that are altered during the reaction, but they are regenerated by another enzyme, so, that they can act again. We have prosthetic groups that remain bound to the enzyme during the reaction, and they may be covalently or extremely tightly bound to the enzyme. We have metabolite enzymes that are actually synthesized from common metabolites.

We have vitamin derived coenzymes, which are the ones that we are going to be interested in right now. What are these? These are derivatives of vitamins and as I mentioned before, they cannot be synthesized by mammals, so they must be obtained as nutrients. So, that they can be converted into cofactors or coenzymes and then used in the specific enzymatic reactions.

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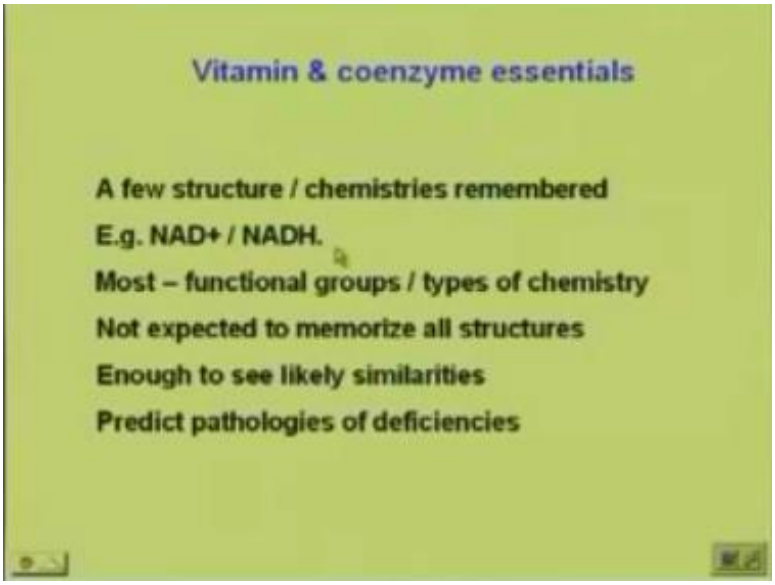

Now, what you need to know from the vitamins and the coenzymes. You need to know of few structures. For example, NAD+, NADH. We will see exactly where these are or what these are. And we have to know basically the functional groups and the types of chemistry that is involved. You are not expected to memorize all the structures at all.

But you should be clever enough to recognize the similarities or basically look at it and understand what sort of moiety it is going to transfer or accept, what is the key part or the key reactive part in the specific cofactor that is going to take part in the reaction. So, this is what you are expected to know.

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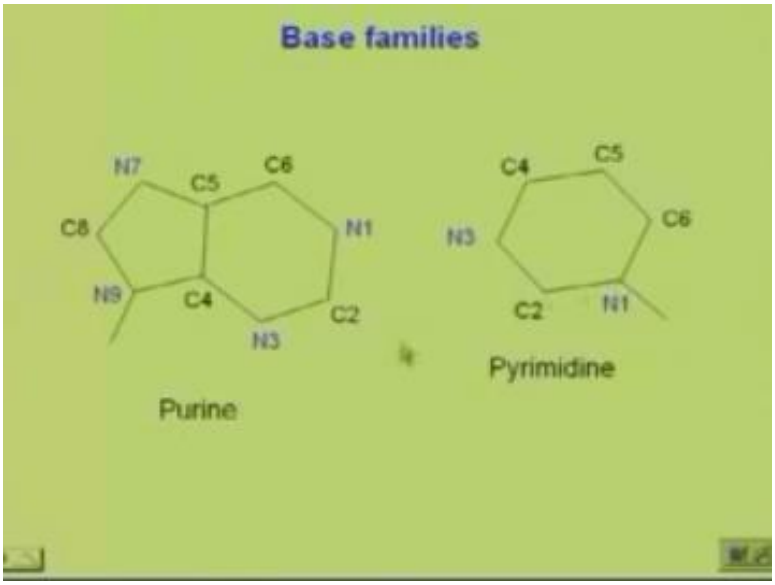


Before, we get into all that, we have to know some certain structures. Now, these structures, I am explaining right now, so I do not have to do it again, when we are going to go to nucleic acids. There are certain base families, this is something you might or might not heard about before. We have purines and pyrimidines. Now, these are essential basis that form the basic structural frame work of DNA and RNA. But they are a part of a lot of vitamins.

And since we are going to look at the structure of vitamins, without this background, it doesn't make sense to go in there. So, this is the numbering that we see in a purine, so this is what would be called as purine nitrogenous base. This is a pyrimidine nitrogenous base. And the extra bond that does not have anything attached to it is what is attached to usually a sugar moiety in some compounds and definitely to a sugar moiety in DNA and RNA. It is just the sugar that is different for DNA and RNA.

So, it is these nitrogenous bases that have this specific numbering scheme, that are essential parts of DNA and RNA. It is this part that is attached to the sugar moiety, so you see what are the types, since it is attached to sugar moiety, it is going to be a glycosidic bond. This is what we studied in the last class. When we are looking at carbohydrate bonding, we have a glycocydic bond, in this case, it is going to be an N glycosidic bond because it is attached to the nitrogen being attached to the sugar moiety.

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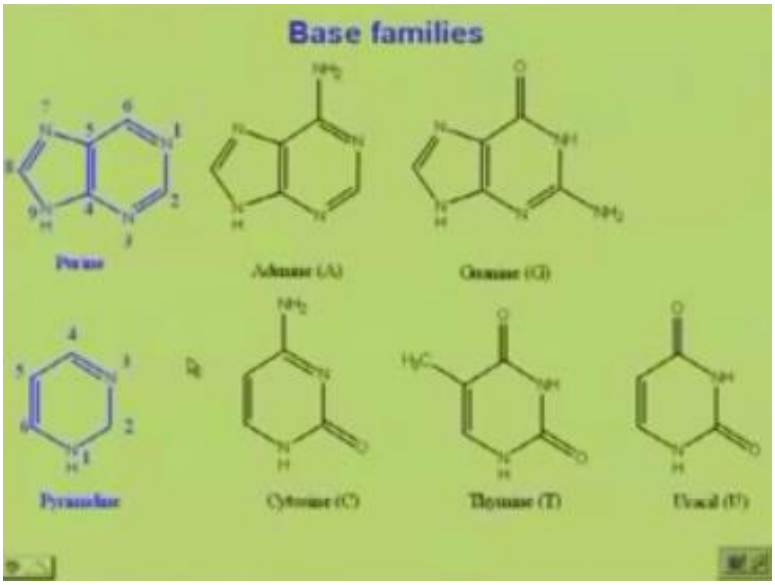


So this is essentially, a purine family, this is the pyrimidine family. In the purine family, the essential basis that we are going to be interested in, are going to be adenine and guanine. And these structure, you are expected to remember. If you know the basic structural framework of what a purine is and what a pyrimidine is, the Purine has adenine and guanine to it. The essential differences are, is you can see an NH2 here, which is a carbonyl group here.

We have an additional NH2 on the carbon 2 for the guanine. In the pyrimidine set, we have these three, the cytosine, the thiamine and the uracil. Because, usually when you write DNA sequences or RNA sequences, just write in the protein sequence, you do not speak about the back bone, you just mention the amino acids one after the other. When you write a protein sequence, the primary structure of a protein sequence, what do we have?

We just write, which amino acid is after which amino acid, because we know that each amino acid is linked to another by a peptide bond. So, once we have this peptide bond, we know exactly how it is linked together. Similarly, when we study the structures of DNA and RNA,

we will see, it is just sufficient to know which bases are connected one after the other. Whether it is AGTC or AGGC or ATTA, you will know exactly what the structure is.

So, these are the base families again, we have two purines here and three pyrimidines here. And these are going to be parts of the structures that are going to form some of the vitamins, definitely the DNA and RNA.

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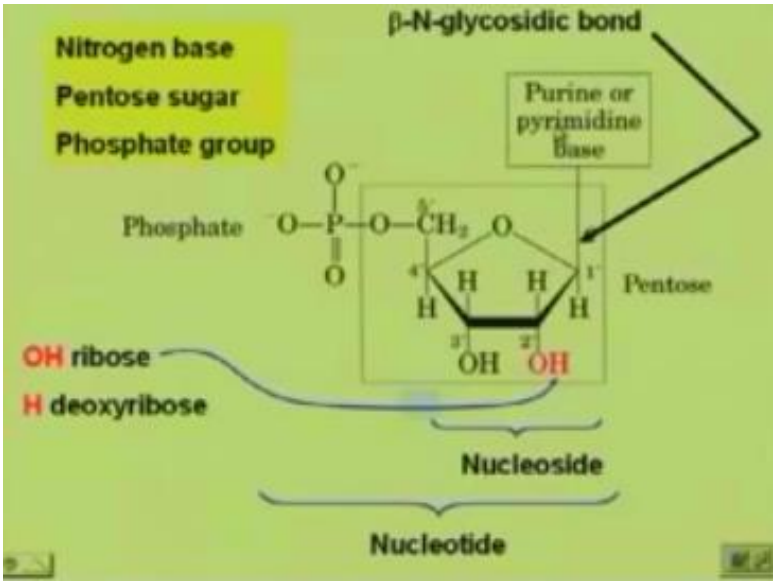


So, what do we have? Usually, when we have this bond is what is attached to the purine or pyrimidine base, ok. Now, where it is attached? This is where it is attached. So this would be the purine based, this would be the pyrimidine based. It is attached here. It is an N glycosidic bond. Not only an N glycosidic bond, it is a beta N glycosidic bond. Why is it a beta? Where is the CH2OH of the sugar? It is cyst to where the basis.

Now you recognize this as being a sugar. It is a pentose sugar. This is the oxygen. This is number one, two, three, four, five. Forget about the phosphate for now. This was CH2OH originally, when it was the pentose sugar. These are marked with the prime because the base is marked with one, two, three, four, five, six, seven, eight, nine, depending on how many there are.

So, the sugar is marked with the prime. So, we have a pentose sugar. Usually, when we have a RNA or DNA, it is the ribose sugar. So, we have one prime, two prime, three prime, four prime and five prime here. This sugar, the two prime OH is what is missing in deoxyribose.

So, when the deoxyribose is mentioned, it is not the three primes that is missing, it is the two prime that is missing.

By default, DNA, the two prime is not mentioned. Deoxyribose means it is missing the oxygen at the two prime position. So, we have the pentose sugar and we have the purine or pyrimidine base attached to it. The base attached to the sugar is called a nucleoside. As soon as the CH2OH is esterified with the phosphate group, it is called a nucleotide. These are essentially different. They are not the same. A nucleotide does not have the phosphate.

So what do we have? We have the purine or pyrimidine base attached to the sugar to form what is called a nucleoside. It is attached by a beta N glycosidic bond. As soon as this is attached to the phosphate, we have what is called a nucleotide. Now, these are the essential units in a DNA or RNA protein nucleotide chain. A nucleic acid chain essentially has these links, one after the other. So, we have a nitrogen base.

These are the essential moieties that you have, a nitrogen base, a pentose sugar and a phosphate group. This is what comprises a nucleotide. So you know that a nucleoside is the sugar and the base. The sugar, the base on phosphate together form the nucleotide.

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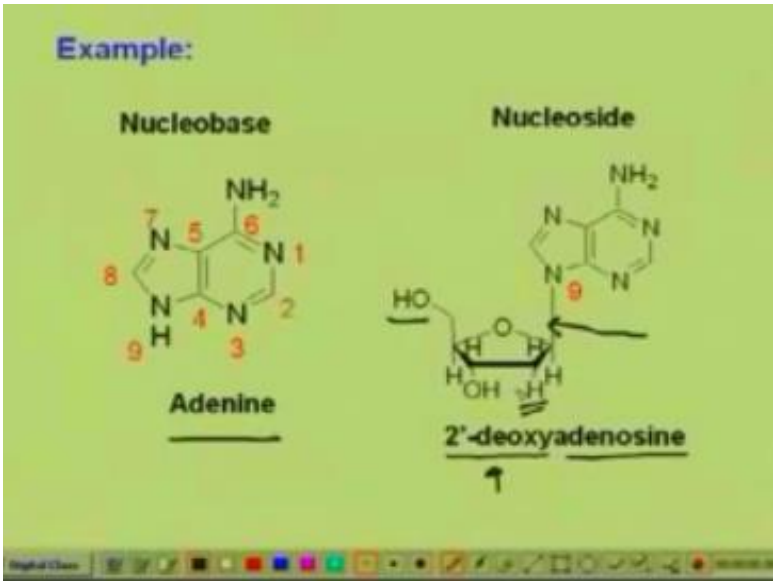


So, let us look at the specific example. This is adenine. This is the base. The nucleobase is called (()) (26:55) what it is called a purine or pyrimidine? It's a purine. A purine that now, is to form the nucleoside, it has to be linked to the sugar moiety. This is missing the OH at this position. So, we have adenine that is a purine. It is linked here. What is it linked to? The

sugar. What is missing here? OH is missing. So, it is two prime deoxy. So this essentially tells you what sugar you have. And you have adenosine, now because it is attached to the sugar moiety. This is still OH. So, we have a purine nucleobase, attached to the sugar forming a nucleoside.

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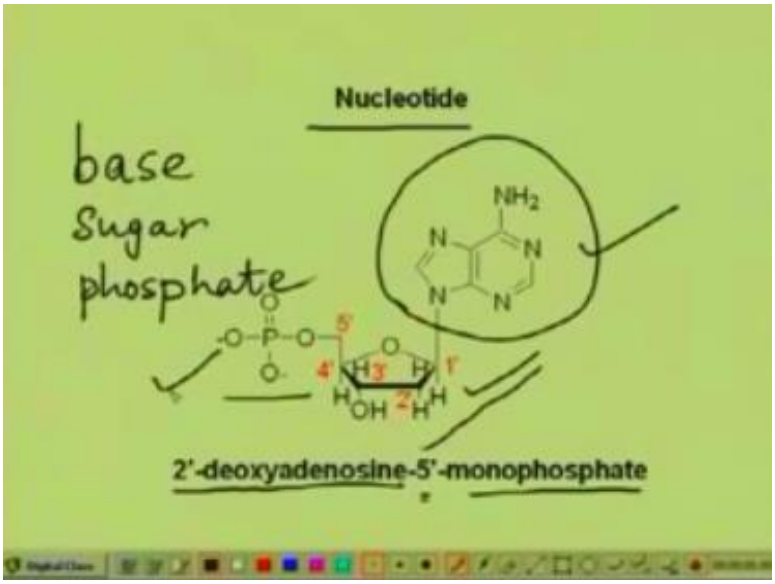


Then now, if we want to form a nucleotide, this is what we are going to have. We have now the base that was attached to the sugar, to form two prime deoxyadenosines. This is the nucleoside. It is now linked to a phosphate, it is linked to the five prime, this is usually not mentioned, because it is always linked to that OH. So five, this is the complete nomenclature, the correct nomenclature that you would write for this nucleotide.

You have the adenine base here, attached to a deoxyribose sugar. Since, adenine is attached to the deoxyribose sugar, it is two prime deoxyadenosines. It is attached to the phosphate at five prime position. So, it is five prime monophosphate. So, this is now a nucleotide. Because what are the essentials units for the nucleotide? You have the base, you have the sugar and you have the phosphate. That is a nucleotide.

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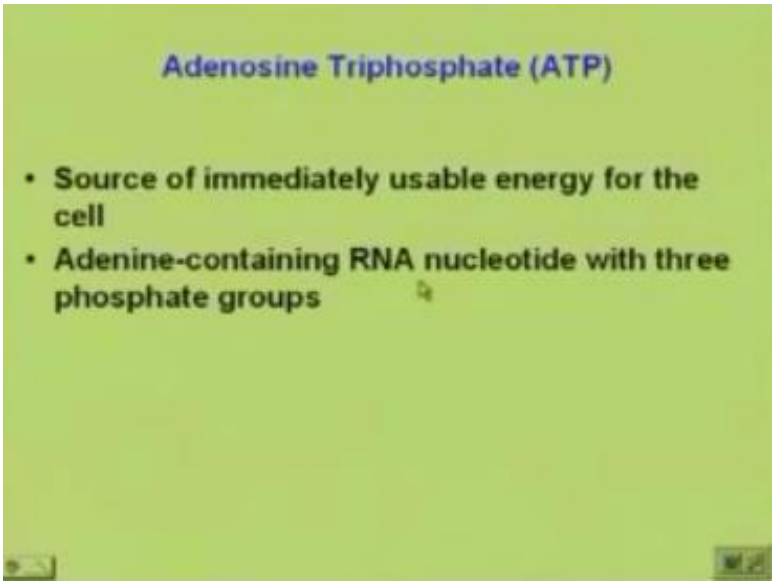


So, now we are going to talk about ATP, adenosine triphosphate. The one that we spoke on previous slide had a single phosphate, AMP, monophosphate. This ATP molecule is extremely important in our body, extremely important, without it you would have no usable energy at all. It is the breaking of the high energy phosphate bonds in ATP that provides us with a lot of the negative free energy that is required to drive other non-spontaneous reactions that have positive delta gene.

This is something that we will understand very clearly, when we do bioenergetics. So, adenosine triphosphate is adenine containing RNA nucleotide. How is it different from the previous one? It has the OH at the two prime position. We will see what it looks like in the next slide.

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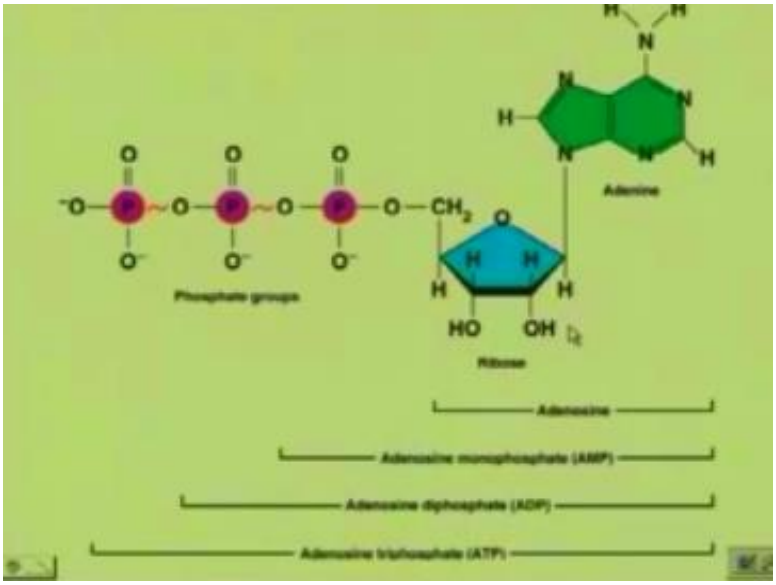

This is what it is. This is the adenine, this is the ribose. I do not have to call it as deoxyribose. Why? Because the OH is very much there. The OH is there at the two prime position. So, this is my ribose sugar. This is my adenine. With one phosphate, it is adenine monophosphate, AMP. When you write the deoxy AMP, you just write a small d and an AMP. So, the previous one was also an AMP. But, we should have written the or we must write a small d.

The one I am talking about is this one. This is also an AMP, but with a small d before it, prefixing the AMP, which signifies that this OH is missing. So, as the AMP is just written like dAMP. This is not nothing much.

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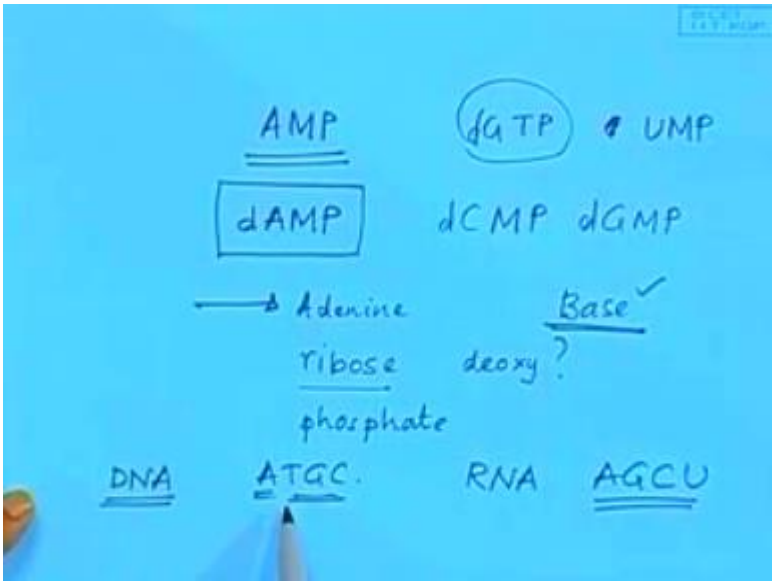


So, instead of the AMP, would be where you would have the ribose. If you have the deoxyribose, it is just this. That is the way it is written. So now, what you need to know, all you need to know to write the structure of AMP or dAMP is what adenine is, what the ribose sugar is, whether it is deoxy and the phosphate. That's all the information, you need to write this.

Now, if I have CMP or GMP, or the d forms of each of these, you know exactly how to write it. Because all you need to know is the structure of adenine obviously, but they are the bases, right. Once you know the structure of the base, you can write what GTP is even, GTP, GMP, UMP, whatever, all the possible combinations. But, what you need to know for that is, you need to know the structure of the bases, which is why I mentioned, you need to know the structure of the purines and the pyrimidines.

And you need to know the structure of ribose sugar. And you need to know whether it is, from this alone you know that this is not deoxy, it is ribose. If I write the d in front of it, only then you have to make it deoxy. Right. So when we write, when I say the structure of DNA and I just write ATGC, if it is DNA, you know all of them are with the small d. Because DNA is deoxy ribonucleic acid. So, all of these do actually or rather do not have the two prime oxygen.

But if I say, write the structure of RNA, for AGCU, you see it actually as U, then you will know that all these have the two prime OH. But, these do not have the two prime OH. So, let us get back to what ATP is. We have our AMP, ADP, ATP. So, we have the adenine base, we have the sugar, we have one phosphate for AMP, two phosphates for ADP and three phosphates for ATP.

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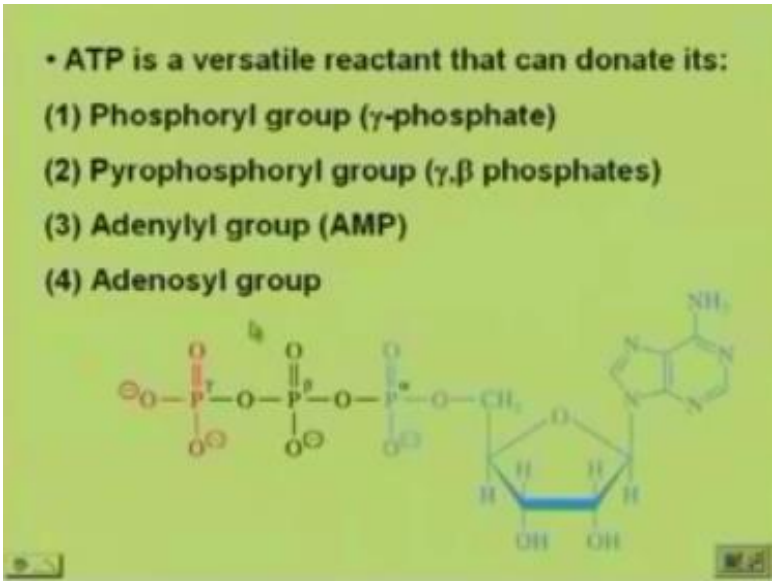


Now, this ATP, as I mentioned is extremely important. Because, it can donate its phosphoryl group that is the gamma phosphate. It can give its pyrophosphoryl group, ok, this, these two P-P. That's what it is called. It can give its adenylyl group, which is this part, the blue part. Or it can give it adenosyl group. So, but the one that we would be most interested later on is ATP forming ADP and PI. So, there is certain cleavage that occurs here that is going to result in ADP and PI. So it is extremely versatile. You will see in the next slide, where I have some of the examples.

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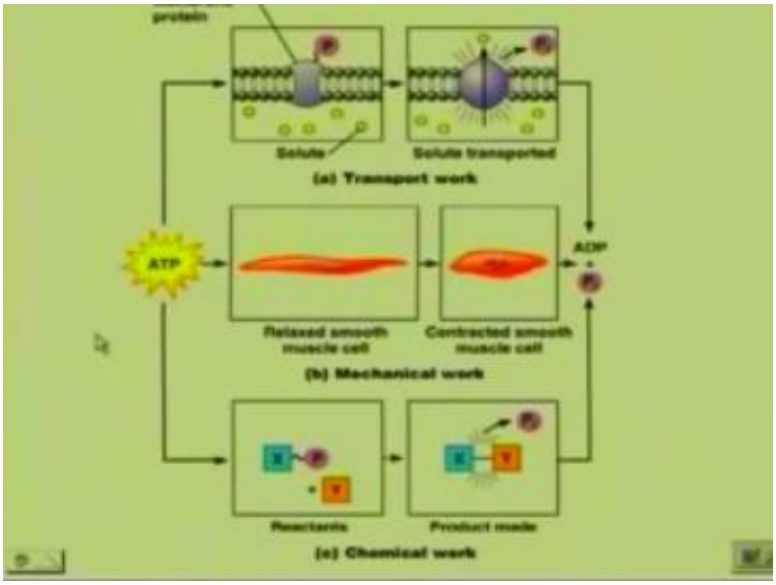


Remember when we did the transfer for the membrane protein, we had a certain reaction up pump. Why do we need a pump there? We were pushing something out of the cell that already has a higher concentration inside. If there is a high concentration on one side, it would not require more of the specific ion, right. But, we learned that it is essential for some ions to go in the cell and some of the ions to come out of the cell. Now for that, we need work.

And this energy is supplied by the ATP. That itself will break the last high energy phosphate bond to form ADP and PI and we have basically transport work, mechanical work and chemical work. All of these require ATP. So, the transport is what we see in membrane transport, in our muscles, the relaxed of, the smoothing of the muscles. This is by the muscle filaments that act in the myosin, these are the muscle filaments.

They also require the ATP, then also specific reactants that if you have the phosphate being removed or added or whatever, it is going to require the ATP.

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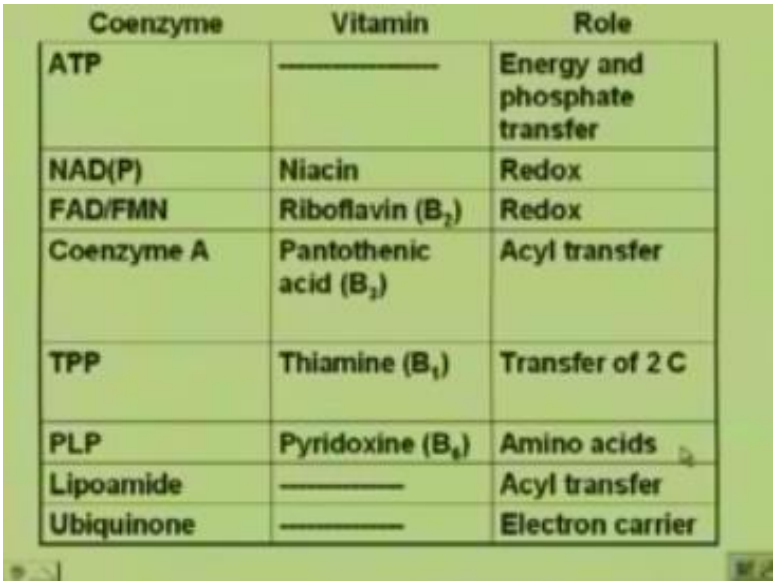

| Coenzyme | Vitamin | Role |
|---|---|---|
| ATP | ---------- | Energy and phosphate transfer |
| NAD(P) | Niacin | Redox |
| FAD/FMN | Riboflavin ($B_2$) | Redox |
| Coenzyme A | Pantothenic acid ($B_3$) | Acyl transfer |
| TPP | Thiamine ($B_1$) | Transfer of 2 C |
| PLP | Pyridoxine ($B_6$) | Amino acids |
| Lipoamide | ---------- | Acyl transfer |
| Ubiquinone | ---------- | Electron carrier |

Now, these are the specific vitamins that we are going to talk about. These are the coenzymes that they form. So, the one that we just looked at ATP is not derived from any vitamin. It has a nitrogenous base to it, it has a sugar to it and it has the phosphate to it. In the nucleotide, it does not have a vitamin that it is derived from. But, it nevertheless put in such a table because it has extremely important role in energy and phosphate transfer.

Because the first step that we will see in glucose breakdown, is the formation of glucose six phosphate. And that phosphate comes from ATP. In NAD(P), which is something we will do in our next class, Niacin, its involvement is in redox reaction. FAD/FMN, Riboflavin, so these are what it is coming from. So this vitamin results in this coenzyme. That is involved in a redox reaction.

We have coenzyme A that is another very important coenzyme rather itself, very important moiety that is required for a specific enzymatic reaction that is involved in the transfer of acyl moiety. What is the acyl moiety? CH3CO. So, the transfer of this acyl moiety, due to coenzyme A is only possible, if you have sufficient amount of pantothenic acid. Thiamine, vitamin B1, it transfers two carbon atoms.

So you recognize, how this is important in the biosynthesis? In the biosynthesis of compounds, where you need two carbons to come into the picture, you will have TPP coming in the picture that is thiamine pyrophosphate. Now, for thiamine pyrophosphate, for TPP to come in the picture, it means you have to have some thiamine to form the TPP. If you do not

have vitamin B1, it cannot form the TPP and the specific enzyme that requires TPP to transfer the two carbons will not be able to act. It is as simple as that.

Pyridoxine, vitamin B6 that is going to form PLP. This PLP is required in aminotransferases. So an enzyme such as an aminotransferase requires PLP to act on it. For its action, you need the PLP to be formed. The PLP is formed only, if you have the pyridoxine and so on and so forth. And these are the other coenzymes. Ubiquinone is very important enzyme because it carries electrons. And it is all involved in large number of free radical reactions, because it is efficient in the carrying of electrons. Some of these, we will see in bioenergetics part.

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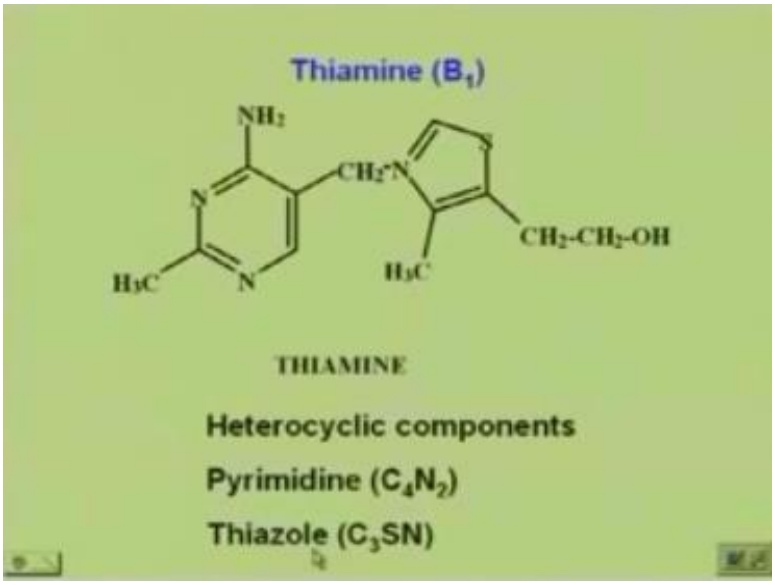


This is thiamine, vitamin B1. It has, you recognize, a pyrimidine base to it. It has a pyrimidine. These are the heterocyclic components it has. It has a pyrimidine and it has thiazole group to it. Now, this thiamine is what you supplement to your diet with. You take thiamine in certain food items. Rice, cereals have thiamine, certain other dietary constitutes would have thiamine. But thiamine itself cannot act. It has to be transferred to the cofactor. It has to be activated.

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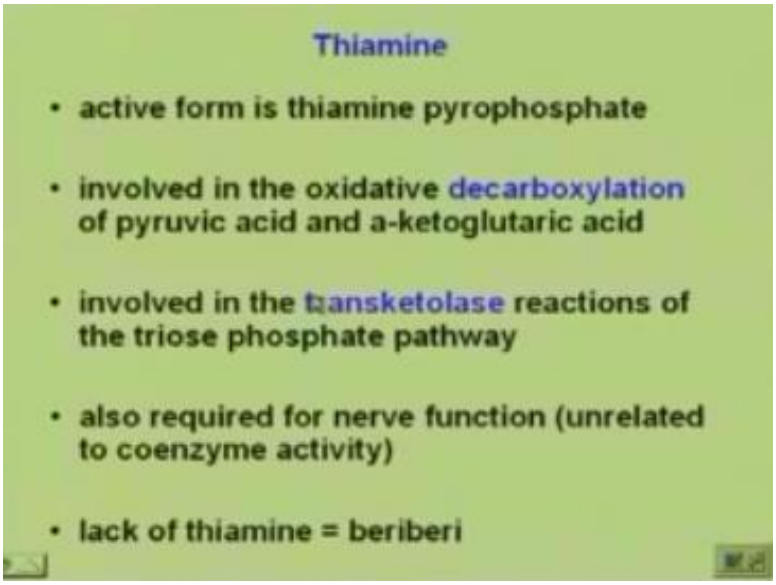


So, the active form is actually thiamine pyrophosphate, TPP. That is what is going to be formed from thiamine that is what going to be formed from your vitamin B1. It is involved in the oxidative decarboxylation of pyruvic acid and this is alpha ketoglutaric acid. We will see what these reactions are in a minute. It is also involved in the transketolase reactions. Now the different pathways that we are talking about here, is what we were going to study, when we do the bioenergetics or hard carbohydrate metabolism takes place because we are going to have break down of glucose.

If you have breakdown of a carbohydrate, the carbon moieties that are broken down from it have to go somewhere because they are in the body, either they have to be synthesized for some other compounds to be formed or they have to be broken down completely for the energy to be obtained. So, for that to occur, you have to have these cofactors that are going to help the enzymes do their action.

This is also required for nerve function, but it's actually independent of its coenzyme activity and the lack of thiamine causes a disease called beriberi.

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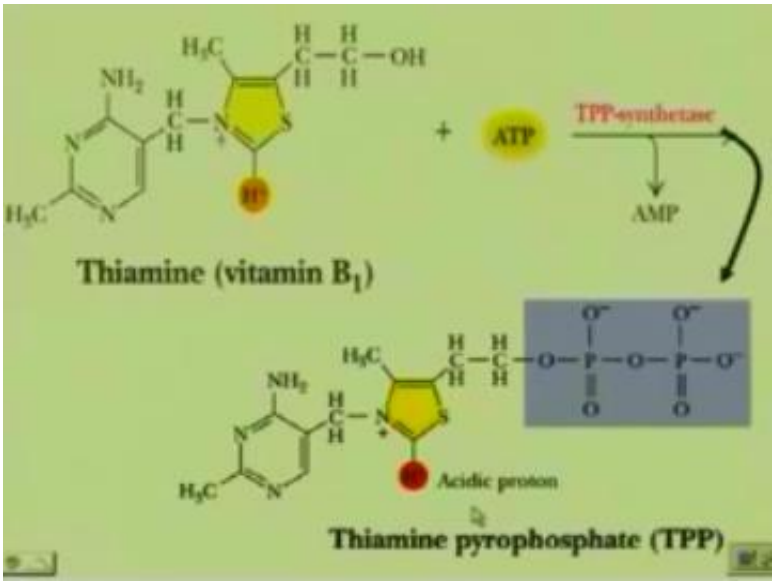


Now, what is happening here? This is thiamine. This is an acidic proton. In the forming of TPP, the enzyme that is required is, see these enzymes should be able to say very easily, even though you might not know the specific name, it is forming TPP, so it has to be TPP synthetase, as simple as that. Because, it has got to make TPP. What else can it do? It has to be a synthetic part that is going to make TPP from thiamine.

So, it is the enzymatic reaction that is taking place, that is going to take thiamine from thiamine pyrophosphate. And you recognize, since you have two phosphates that are added here, it requires ATP, as simple as that. This is what I was mentioning, when I said that you have to understand what the reaction possibly requires. For example, if you know that thiamine has to form thiamine pyrophosphate, it means that it has to add two phosphates to it.

If it has to add two phosphate moieties, where it is going to get it from? ATP. What will ATP form? AMP, because it is losing two of its phosphates. So, thiamine with TPP synthetase and with ATP, is going to form thiamine pyrophosphate and AMP is found. So, this is our TPP. So, now you have the vitamin. The vitamin you took in your diet has been transformed into TPP and this TPP is going to act as a cofactor to a certain enzymatic reaction. What is that reaction?

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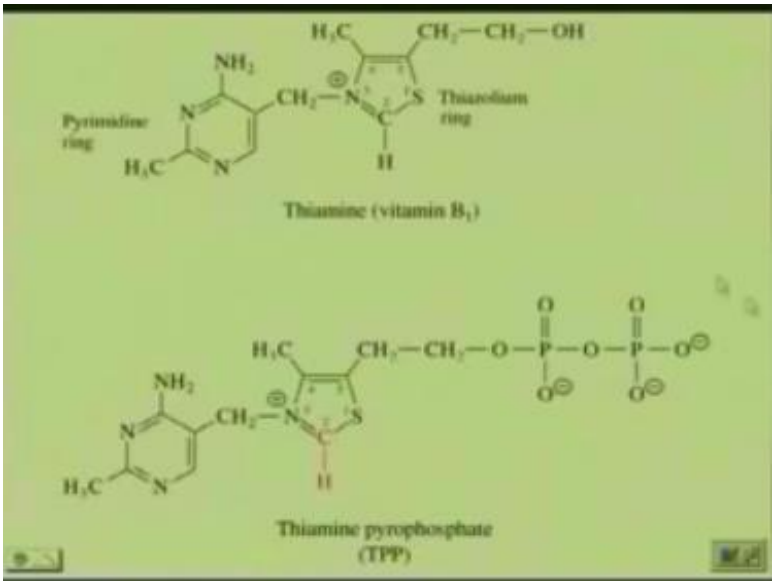


Let us look at the structures once more, which I have here. We have the pyrimidine ring, the thiazolium ring, which is vitamin B1. What we do? A certain enzyme, transformed it to TPP. The enzyme was TPP synthetase and it required ATP because we added two phosphates to it. So, we had thiamine in our diet. We now have TPP, which is now going to act.

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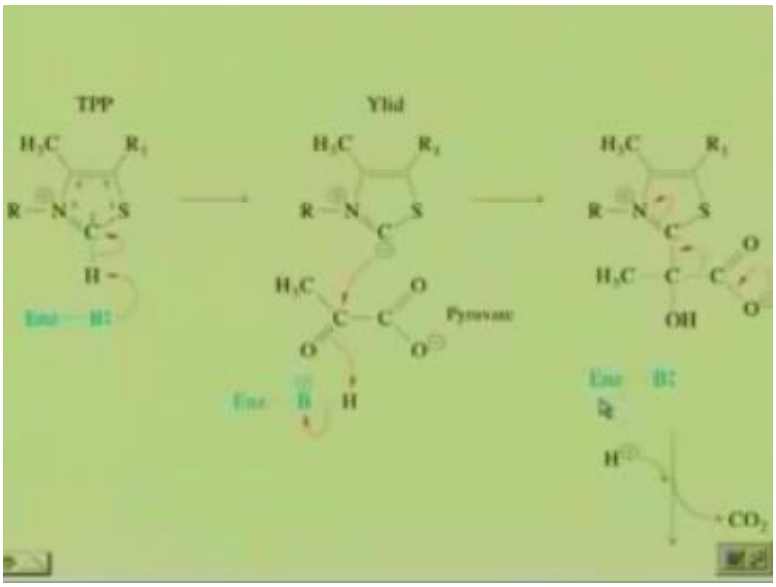


What is it going to do? We have the enzyme here. The enzyme, this is two electrons that are going to take up the acidic hydrogen. So, this is going to be a basic. This is going to be an electron pair, donor. What is it going to do? It is going to take up this acidic hydrogen that was available in the thiasolium moiety of TPP. Now, this is the rest of the molecule, this R and this R. We do not need that part.

It is needed for the reaction, but we do not need that for our understanding of what it is going to do. Now, in the loss of this proton, it forms what is called an ylid. You have studied this in organic reactions, an ylid. Now, this is the pyruvate moiety. What is pyruvate? CH3COCOOH. Right. Now, what happens is, this ylid then now acts on the pyruvate, which then takes up, let us see what is happening here.

We have the ylid negative charge here that acts on the carbonyl moiety of CH3CO group of pyruvate, the acetyl group of pyruvate. In doing so, this H that was taken up by the enzyme from TPP is taken up by pyruvate. What you have to recognize here, is how the whole activity is taking place because of the formation of TPP. If TPP were not found, this would not be possible. Why? Because you would not have the ylid.

If you do not have the ylid formation, you cannot have this hydrogen being taken up by the CH3CO moiety of pyruvate. You also have to recognize that the enzyme that was acting here, has to get back where it started from, like the previous enzymatic mechanisms that we have studied. So, what is happening here is, we have now a direct link with the TPP and the pyruvate moiety.

Because, the negative charge acted on this C, this O- took up the proton from the enzyme. What do we now have? We have the TPP part, so this is the TPP part. And what is this part? It is the pyruvate part. The pyruvate is now going to lose CO2. So, what is the action of TPP in this particular enzymatic reaction? It is decarboxylation. But, that decarboxylation cannot take place, without the enzyme, without TPP.

It has to be both together, that are going to act on the enzyme. Or basically, TPP is acting with the enzyme, in getting the CO2 out of pyruvate. That is what we are looking at. So, essentially what is then going to happen is we are going to lose the carbon dioxide and we are going to get the enzyme identical to what we started with. And, we are also going to look at what happens. This we will do in the next class then.

So basically, what we looking at here is we consider what vitamins actually are required for. Vitamins are required to produce cofactors. They are not synthesized by mammalian cells, so they have to be taken in our diet. They have to be supplemented in the diet. And the reason

we require them is so that they form these cofactors and in forming the cofactors that are essential for certain enzymes to actually have their mechanisms take place.

For example, in the one that we looked here, we found out how the TPP actually works to form the ylid. Without the TPP, the ylid is not formed. Without the ylid, the CO2 cannot be released. So, then the TPP can be divided or formed again, but the enzyme has to get back to where it started from. But, that would not be possible without the TPP. So, we understand that these cofactors are required and they are formed only, if you have the vitamins.

So, the vitamins from the cofactors, the cofactors or coenzymes help the enzymes to act. We will see some other enzymatic reactions and how the cofactors are formed from the vitamins in our next class. Thank you.