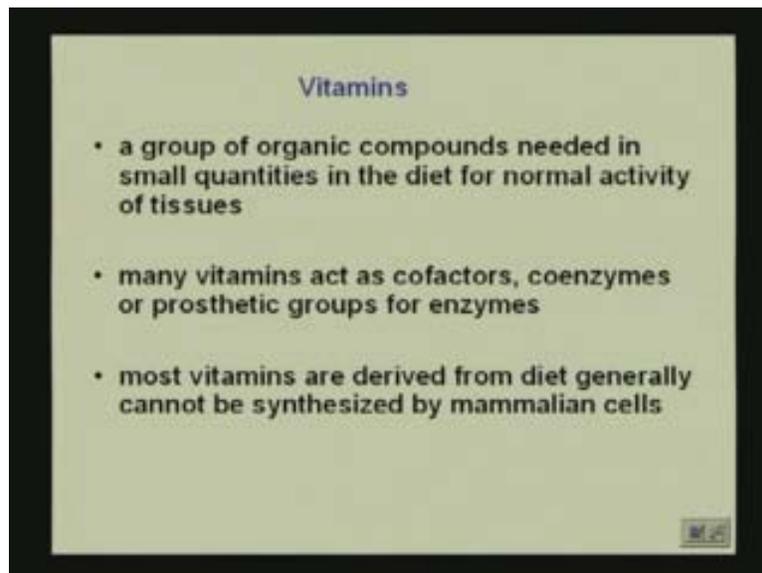


Biochemistry –I
Professor S. Dasgupta
Department of Chemistry
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Lecture - 18
Vitamins and Coenzymes-I

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. 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 activity of tissues and this is something that you must supplement in your diet because the basic structure of vitamins are not synthesized in the body.

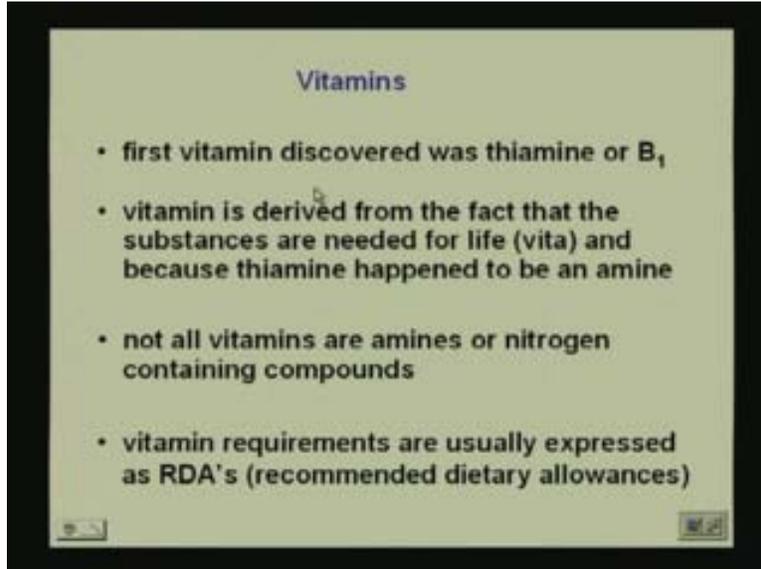
They have to be supplemented; it does not mean you have to take vitamin tablets because there are a lot of vitamins in the food material that we consume. So by dietary intake you can get the amount of vitamins that you need. The reason why we need these vitamins is because they act as cofactors, coenzymes or prosthetic groups for many enzymatic activities. And considering that all the activities and all the biochemical reactions that go on in our body are due to the presence of enzymes or due to the enzymatic reactions, it is essential that these vitamins are present so that the co factors, co enzymes prosthetic groups whatever is required for the enzyme are available for the enzymes to act.

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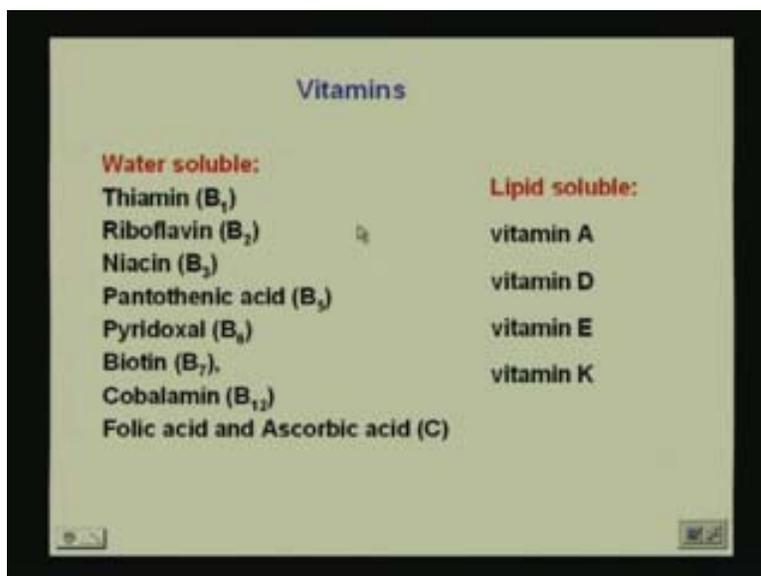
We will see some specific examples where we have these vitamins are acting this is what I mentioned before that most vitamins are derived from diet and they generally cannot be synthesized by mammalian cells.

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The first vitamin that was discovered was thiamine or vitamin B₁. The name vitamins itself comes from two words. Vita means life and min comes from the amine because thiamine, which was the first vitamin that was discovered, happens to be an amine. It does not necessarily make all the vitamins having amine groups. It 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.

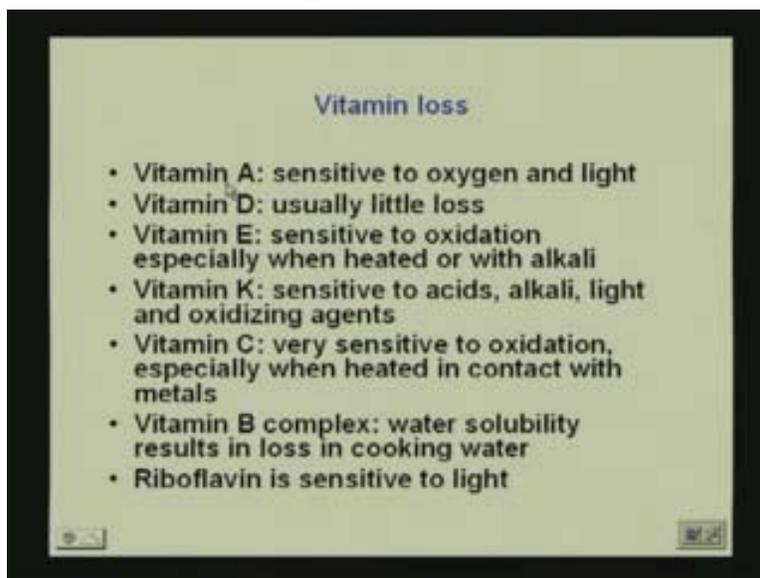
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Usually the vitamins requirements are expressed as RDA's, which are your recommended dietary allowances. So your daily allowance of vitamins is something that we have to consume for proper action of the enzymatic reactions that are actually going to take place.

There are two sets of vitamins. They are water soluble vitamins and lipid soluble vitamins. When we speak about water soluble vitamins, you will see all the B vitamins i.e. the B complex as it is called you have B₁, B₃, B₅, B₆, B₇, B₁₂ and vitamin C; all of these are water soluble. They happen to be in your blood. And all the lipids soluble membranes are the vitamin A, D, E and K. If you have to have lipids, what it means is you have to have some amount of fatty acids, so you have to consume some amount of fat as well so that these vitamins are soluble. These are the two sets the water soluble and the lipid soluble set.

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I won't go into the diseases that occur because you do not have sufficient amount of a certain vitamin. That is something you studied from school as to what happens if you do not have sufficient amount of vitamins A, you get eye blindness and so on so forth. But what we need to know is how vitamin loss can actually take place when it is lost usually due to storage of a vitamin.

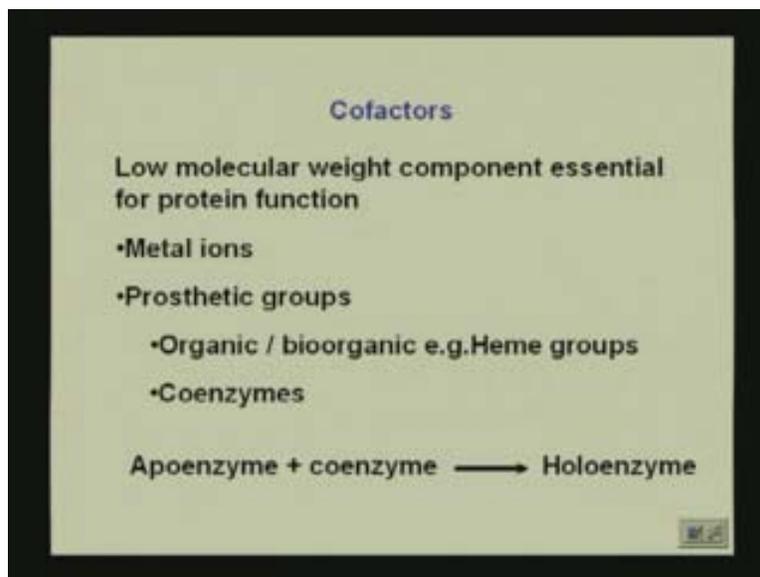
When we look at vitamin A, it is sensitive to oxygen and light which means too much oxygen or too much light is essentially going to result in a loss of vitamin A. Vitamin D is not usually lost and it is synthesized by the UV radiation that we get from the sun. This is synthesized in the body itself.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

So the vitamin loss that we see here is mostly due to storage. It is not something that you can keep a 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 either due to their sensitivity to oxygen or light and so on so forth. Excess vitamins are always added to vitamin supplement that you find in bottles because of this loss that occurs.

We look at cofactors, we are going to study. Since the vitamins and cofactors actually come together, we are going to see how they actually complement one another.

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We have a low molecular weight component that is essential for protein function. When we studied the enzymatic reactions previously, enzymatic mechanism of ribonuclease, chymotrypsin or lysozyme, we found that the enzyme themselves was sufficient in bringing about a specific reaction. But enzymes that are require a cofactor or a coenzyme for the complete action of its enzyme or what is written here we have an apoenzyme that along with coenzyme is going to form the holoenzyme which is what is going to part in the reaction. What we need is this specific cofactor of coenzyme that is going to essentially be a large variety of organic compounds or even metal ions.

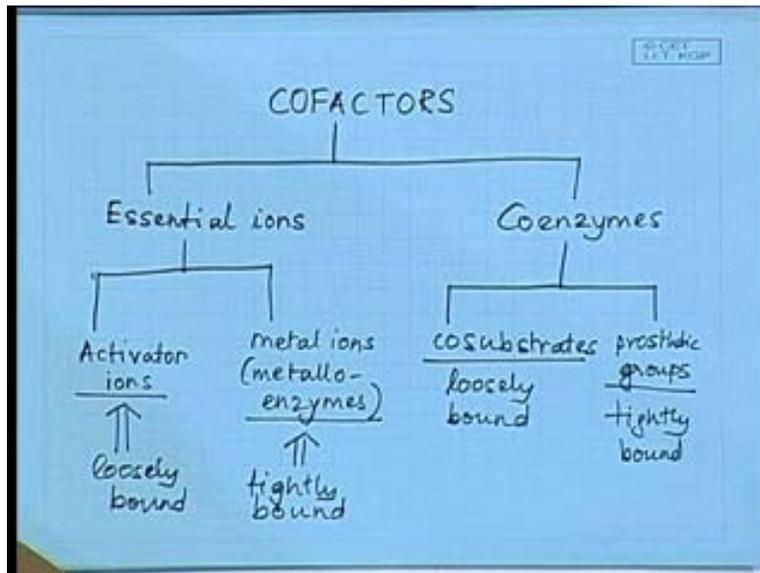
What this means is that these factors are essential for the enzymatic reaction of a certain number of enzymes which is why they are called cofactors or coenzymes. These can be

organic or by like the heme groups the heme groups in hemoglobin which we studied is not part of the protein chain.

Obviously you understand it is essential for the binding of the oxygen because that's where the oxygen binds. That is an example of where you would have essential part of the protein molecule that is not a part of the polypeptide chain, it is something extra. If we just look at these factors these cofactors are actually essential for the overall action of the enzyme. We can probably divide 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.

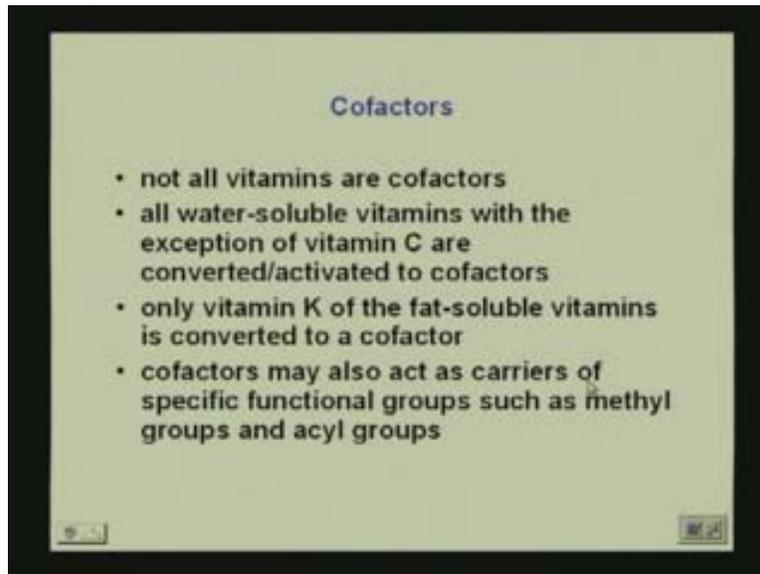
For essential ions you can have activator ions or metal ions. Metal ions are those belonging to metalloenzymes. Metalloenzymes are enzymes that have an essential metallic part to it for their function. These activator enzymes or activator ions rather are loosely bound. Whereas the metalions in metalloenzymes a very tightly bound. In the coenzymes, we could divide them into what a called co-substrates 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 that are not complex enzymes, these enzymes are such required cofactors will be called complex enzymes. The other enzymes do not require such cofactors would be like ribonuclease or lysozyme that can act by themselves, depending on the side groups that are present.

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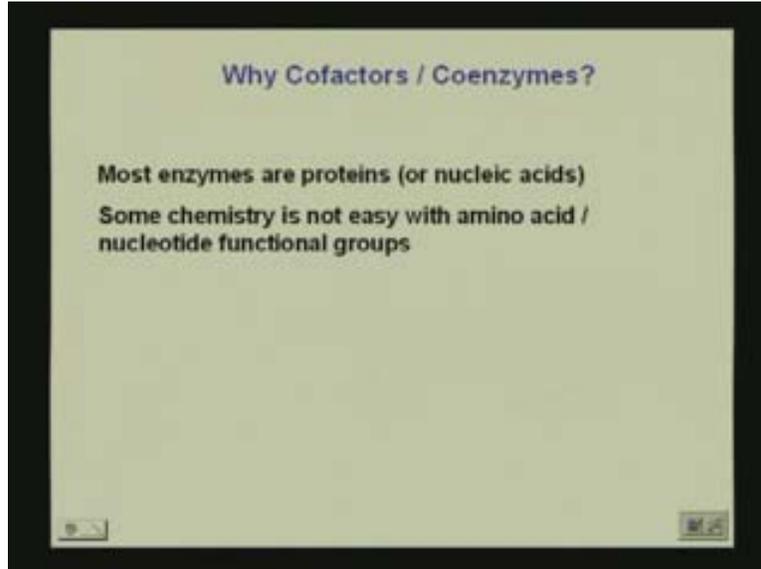
These cofactors are actually formed from the vitamins. not all vitamins however all cofactors and all water soluble vitamins with the exception vitamin C are converted or activated into cofactors and this is what is extremely important. We are going to see how a certain vitamin is converted to a cofactor and how that cofactor takes part in an enzymatic reaction.

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That is what is important and obviously if you do not have vitamin, it cannot form the cofactor and if you cannot form the cofactor, the enzymatic reaction which 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. 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 methyl transferase.

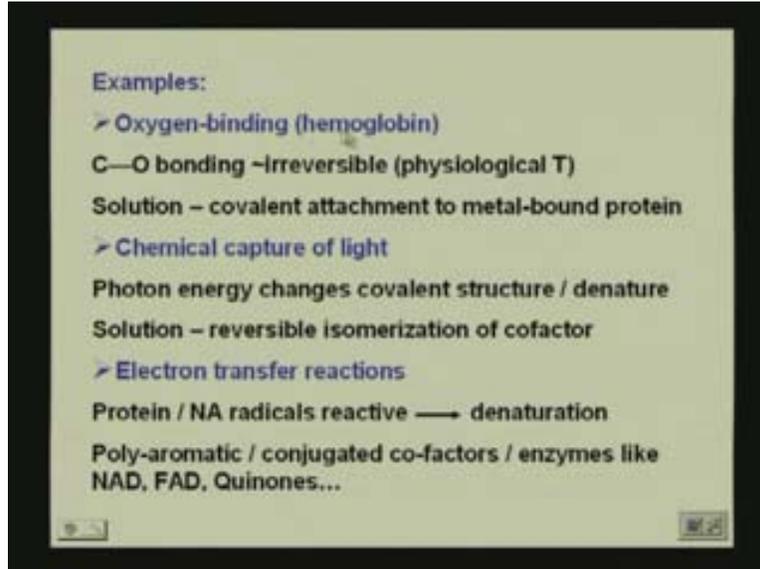
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So from the name itself you can say what the enzymes actually do if it is a methyl transferase. It is going to be involved in the transfer of methyl group but how does it do it. For that we need specific cofactors. We need to know how methyl transferases actually work. But the cofactor is the one that are going to help in bringing about the methyl group that is going to be transferred. That is where we are going to get it.

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 because they are limited in the number of R groups that you have. You would have acidic groups, basic groups but it does not mean that all the reactions that are going on in the body are possible which just those a few amino acids which is why we require these cofactors and coenzymes for the proper functioning or the proper mechanisms for all the enzymes to act.

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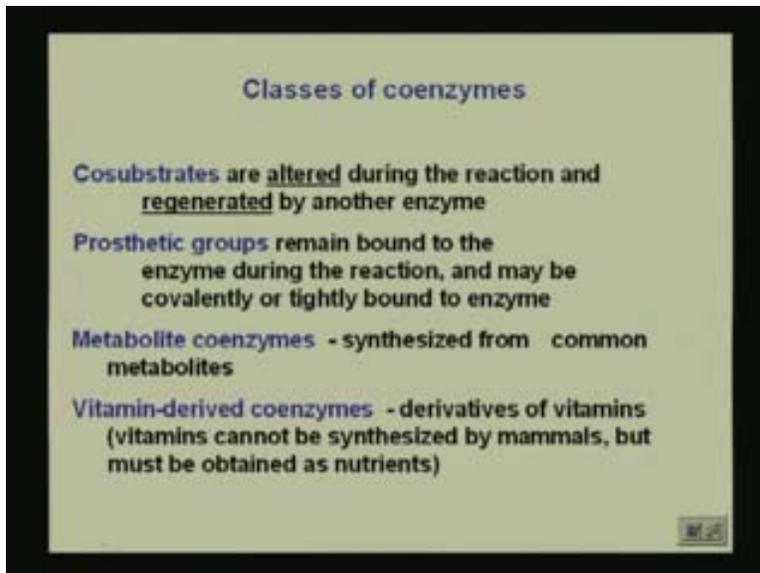
For example; these are three examples that I have here. One would be oxygen binding (hemoglobin). We understand what we need here? We need the heme for the oxygen binding to occur. It is only a few that have C-O bonding only then is it going to carry. You know that hemoglobin is the transport protein. Only then is it possible for it to carry the oxygen from one level to the other. So it is the polypeptide chain that just act as a scaffold.

It acts as just structural frame work to hold the heme group and it is the heme that is actually doing the function. It is heme that is taking up the oxygen, releasing the oxygen, binding the CO₂ and releasing it. If you do not have the heme group but it is not just sufficient to have the heme group floating about in the body. It has to be in a current orientation. It has to be held in the correct position and this is possible with polypeptide chain alone. So this is how it acts as the cofactor or coenzyme to the protein hemoglobin. For example if we looking in the chemical capture of light a photon energy changes the covalent structures of the protein. What is going to happen then, this cofactor will then come to rescue to bring it back to where it is supposed to be. You can have free radical formation.

For example; in these electron transfer reactions that we are going to study later on in bio energetic, there are certain proteins or nucleic acidic radicals that are extremely dangerous to the body. They have to be scavenged immediately but the body has such quality control it will take action immediately and there are other poly-aromatic conjugated co-factors certain enzymes also that will take care of the free radicals that are formed. They do not escape and damage the cells so this is why we require these cofactors. They are basically essential and require them for the specific enzymatic reactions and these cofactors are formed only if you take the vitamins.

You have the vitamins. The vitamins are transferred or activated into the cofactors and then the cofactors will help in the specific enzymatic reactions.

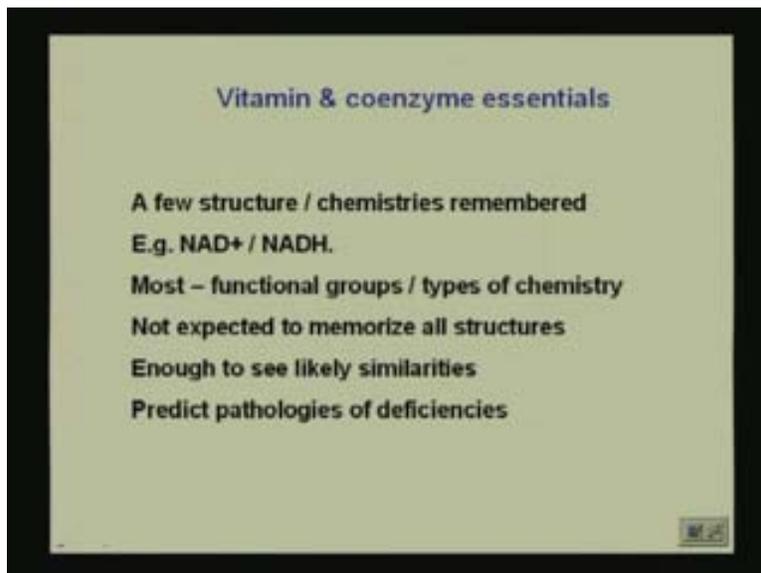
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These are the different classes of coenzymes as I was mentioning we have co-substrates that alter during the reaction but they are regenerated by another enzyme so that they can act again. We have prosthetic group that remain bound to the enzyme during the reaction and may be covalently or extremely tightly bound to the enzyme. We have metabolite enzyme that actually synthesized from common metabolites. We have vitamin-derived coenzymes which is the one that we are going to be interested. These are derivatives of vitamins and as I mention 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.

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

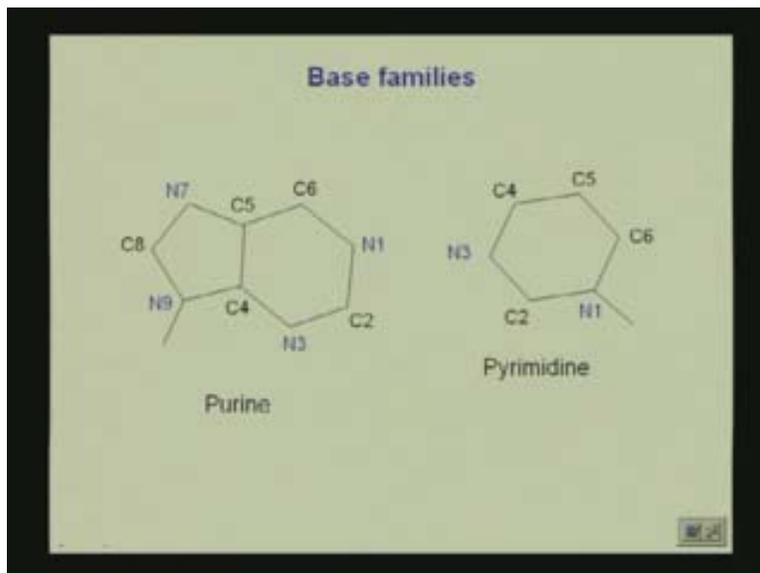
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You should be clever enough to recognize the similarities or basically look at it understands what sort of moiety it is going to transfer or accept, what is the key part or the key reactive part in the specific cofactor that is going to take part in the reaction. This is what you are expected to know. Now before we get into all that we have to know some certain structures. These structures I am explaining right now. I do not have to do it again when we are going to go to nucleic acid.

There are certain base families this is probably something you might or might not heard about before. We have purines and pyrimidines now these are essential bases that form the basic structural frame work of DNA and RNA. They are a part of a lot of vitamins and since we are going to look at the structure vitamins, without this background it does not make sense go in depth. This is the numbering that we see in a purine. This is what would be called purine nitrogen as base. This is the pyrimidine nitrogen as 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 sugar that is different for the DNA and RNA. These nitrogen is bases that have this specific numbering scheme that are essential parts of DNA and RNA.

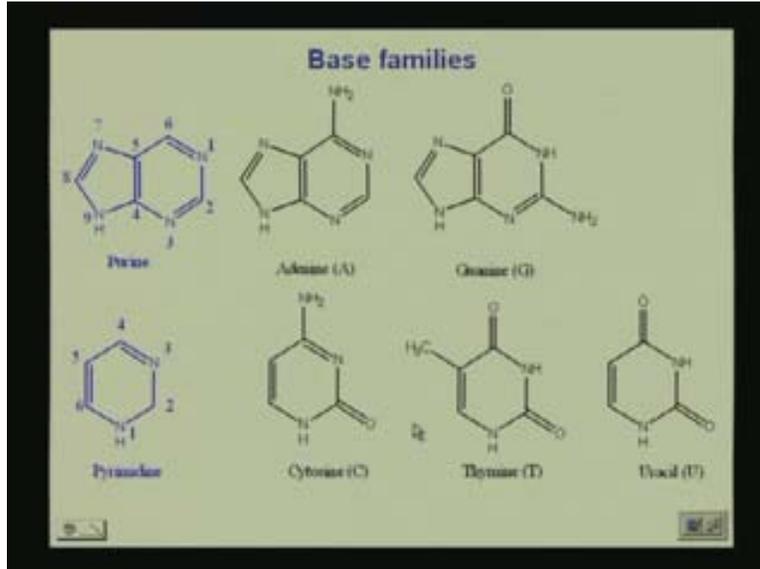
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It is this part that is attached to the sugar moiety so you see what are the since it is attached to a sugar moiety it is going to be a glycosidic bond. This is what we studied in a last class. When we are looking at carbohydrates bonding, we have a glycosidic 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.

This is the essentially a purine family. This is the pyrimidine family (Refer Slide Time: 20:52). In the purine family essential basis that we are going to be interested in are going to be adenine and guanine and these structures you are expected to remember. If you know the basic structural framework of what purine is and what pyrimidine is; the purine has adenine and guanine to it. The essential differences are as you can see NH_2 here which is in carbonyl group here.

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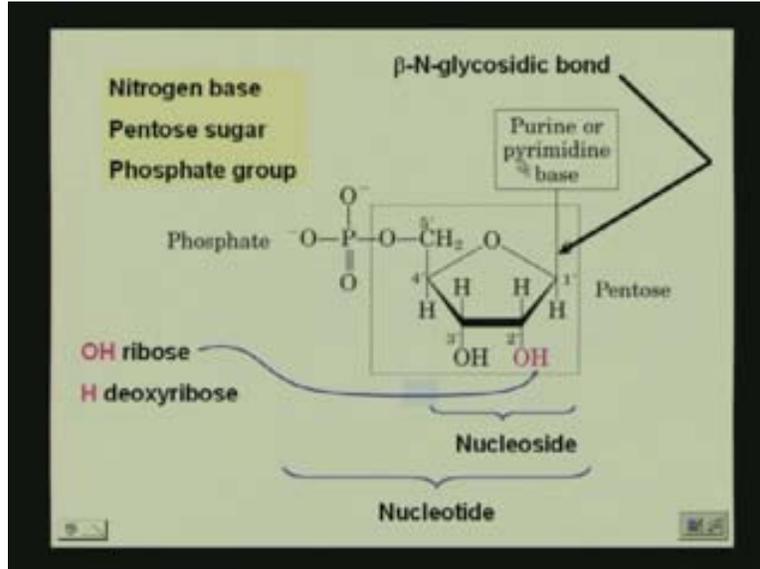


We have an additional NH₂ on the carbon 2 for the guanine. In the pyrimidine set, we have these three the cytosine the thymine and the uracil. Because usually when you write DNA sequences or RNA sequences just like in the protein sequence, you do not speak about the backbone you just mention the amino acid one after the other.

When we write a protein sequence the primary structure of the protein sequence we just write like; which amino acid is after which amino acid because we know that each amino acid is linked to another by a peptide bond. Once we have the 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 basis are connected one after the other. So 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. These are going to be parts of structures that are going to form some of the vitamins, definitely the RNA and DNA.

Usually when we have this bond, what is attached to the purine or the pyrimidine base? Where is it attached? This would be the purine and pyrimidine bases. It is attached here. It is an N-glycosidic bond not only an N-glycosidic bond it is β N-glycosidic bond. Why it is beta? Where is the CH₂OH of the sugar? It is cis to where the base is now. You recognize this is being a sugar. It is a pentose sugar. This is the oxygen number 1, 2, 3, 4 and 5 (Refer Slide Time: 23:57).

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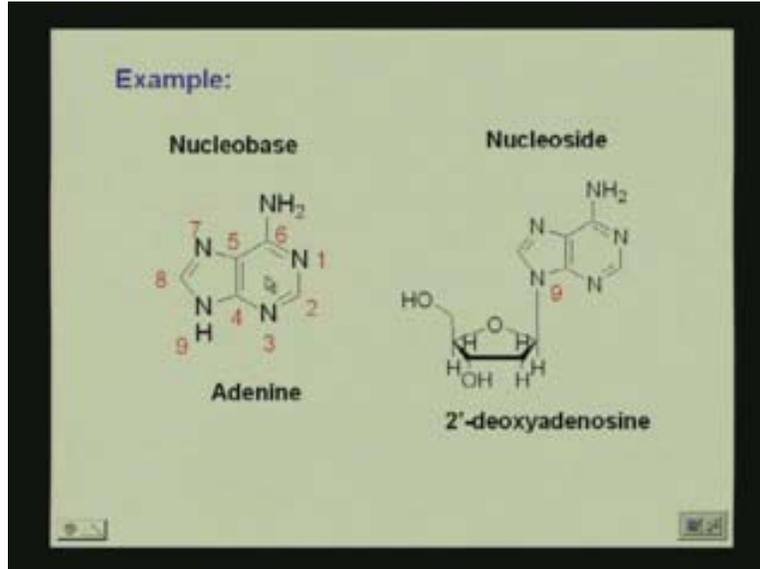


Forget about the phosphate. This was CH_2OH originally when it was the pentose sugar. These are marked with the prime because the base is marked with the 1, 2, 3, 4, 5, 6, 7, 8 and 9 depending on how many there are. The sugar is marked with the prime so we have a pentose sugar usually, when we have RNA or DNA, it is ribose sugar. We have 1', 2', 3', 4' and 5' here. This sugar, the 2' OH is what is missing in deoxyribose. When deoxyribose is mentioned, it is not the three prime that is missing. It is that 2' that is missing by default DNA. The 2' is not mentioned deoxyribose means it is missing the oxygen at the 2'.

We have the pentose sugar and purine or pyrimidine base attached to it. The base attached to the sugar is called a nucleoside. As soon as the CH_2OH is esterified with the phosphate group, it is called a nucleotide. These are essentially different. A nucleotide does not have the phosphate. What do we have we have the purine or the pyrimidine base attached to the sugar to form what is called a nucleoside. it is attached by a beta N glycosidic bond.

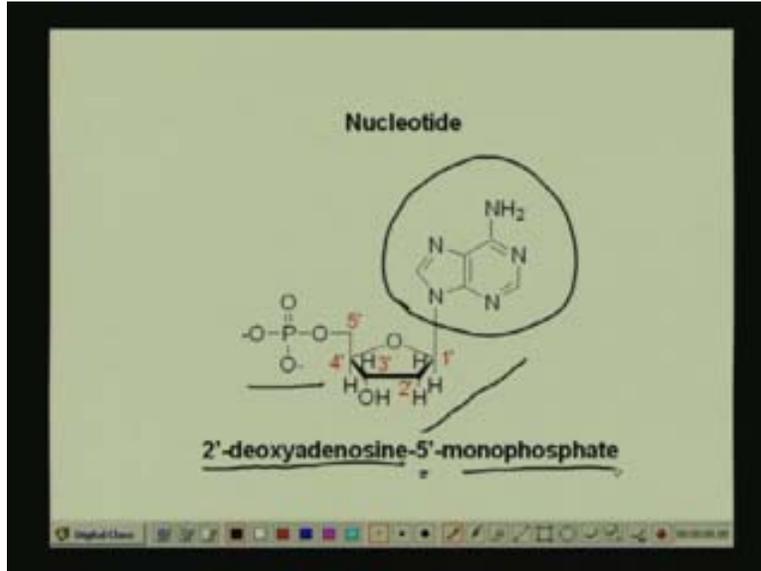
As soon as this is attached to the phosphate, we have what is called a nucleotide. These are the essential units in DNA or RNA nucleotide chain. A nucleic acid chain essentially has these linked one after the other. These are the essential moieties that you have; a nitrogen base, a pentose sugar and a phosphate group. This is what comprises a nucleotide. You know that the nucleoside is the sugar on the base. The sugar the base on phosphate together form the nucleotide. Let us look at the specific example. This is adenine, this is the base then nucleo base is called itself purine or pyrimidine, it is a purine a purine that now if to form the nucleoside it has to be linked to the sugar moiety.

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This is missing the OH at this position so we have adenine, that adenine that is the 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. This essentially tells you what sugar you have. You have the adenosine now because it is attached to the sugar moiety. This is still OH (Refer Slide Time: 28:00). We have a purine, nucleobase attached to the sugar forming a nucleoside. Then now if you 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 2' deoxyadenosine. This is the nucleoside. It is now linked to a phosphate. It is linked to the 5' this is usually not mentioned because it is always linked to that. This is the complete nomenclature, the correct nomenclature that you write for the nucleotide you have the adenine base here attached to deoxyribose sugar. Since adenine is attached to the deoxyribose sugar, it is 2' deoxyadenosine. It is attached with phosphate at the 5' position. So it is 5' monophosphate.

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This is now a nucleotide because you have the essential units for the nucleotide which are the base, the sugar and the phosphate. Now we are going to speak about adenosine triphosphate (ATP). The one that we spoke on the previous slide has a single phosphate i.e. adenosine monophosphate (AMP). This ATP molecule is extremely important in our body. Without it, you would have no useful energy at all. It is the breaking of the high energy phosphate bonds in ATP that provides us with a lot of negative free energy that is required to drive other non-spontaneous reactions that has positive ΔG . This is something that we will understand very clearly when we do bioenergetics. It is adenosine triphosphate that is containing RNA nucleotide. How is it different from the previous one? It has the OH at the 2' position. We will see what it look like in the next slide.

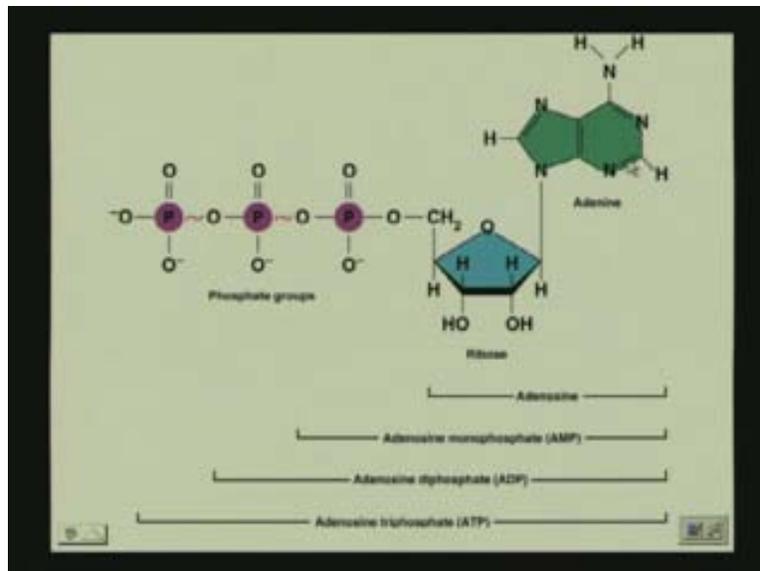
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Adenosine Triphosphate (ATP)

- Source of immediately usable energy for the cell
- Adenine-containing RNA nucleotide with three phosphate groups

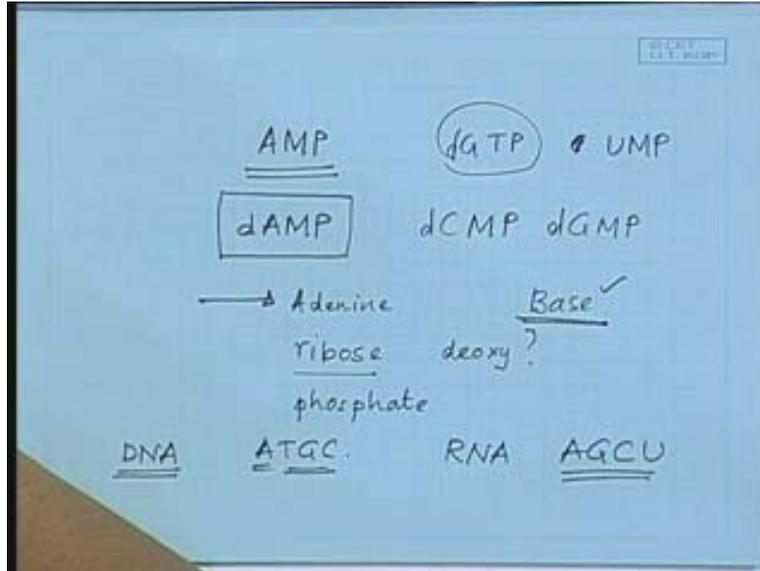
This is what it is this is the adenine this is the ribose. I do not have to call it deoxyribose why because the OH is very much there.

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The OH is there at the 2' position. So this is my ribose sugar and adenine with one phosphate. It is AMP. When you write the deoxy AMP, you just write a dAMP. The previous one was also an AMP but we must write a d. so instead of the AMP would be where you would have ribose, if you have the deoxyribose; it is just this that is the way it is written.

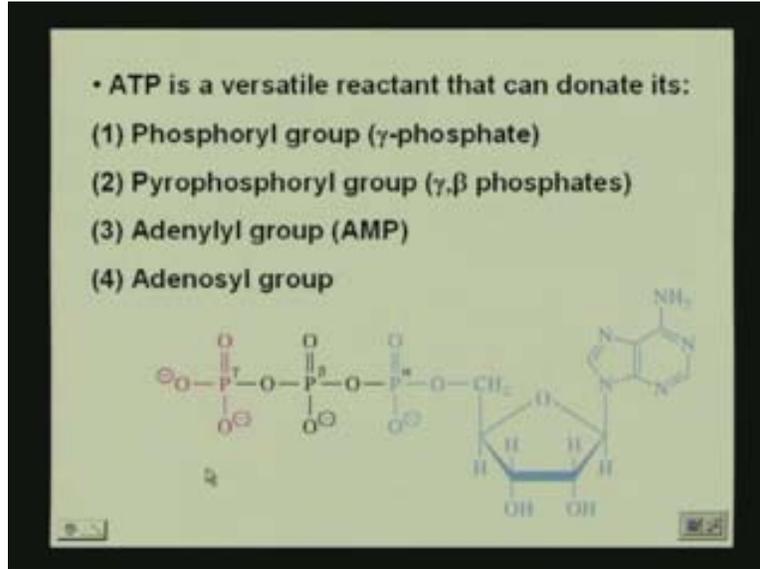
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All you need to know to write the structure of AMP or dAMP, what adenine and the ribose sugar is, whether it is deoxy and the phosphate that is all the information you need to write this. If I have CMP or GMP or the d forms of each of these, you know exactly how to write it? Because all you need to know is the structure of the base. Once you know the structure of the base, you can write what GTP, GMP and UMP and all the possible combinations. But what you need to know is the structure of the bases which is why I am mentioning, you need to know the structure of purine and pyrimidine and the ribose sugar.

If I write the d in front of it only then you have to make it deoxy. So when I say the structure of DNA and I just write ATGC, if it is DNA you know all of them are with d because DNA is deoxyribonucleic acid. So all of these do not have the 2' oxygen. If I say, write the structure of RNA for AGCU, you will see it actually as U then you will know that all these have the 2' OH but these do not have the 2' OH. Let us get back to what ATP is. We have our AMP, ADP and ATP. We have the adenine base, the sugar, and one phosphate for AMP, two phosphates for ADP and three phosphates for ATP.

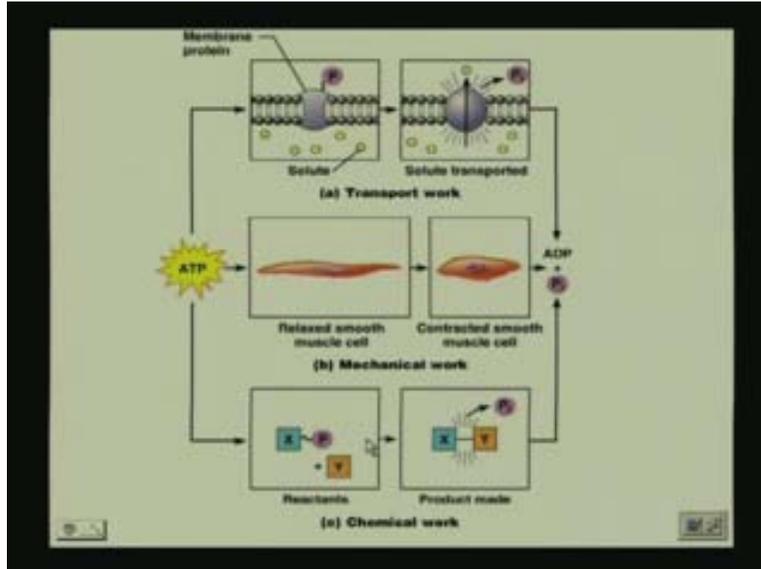
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This ATP as I mentioned extremely important because it can donate its phosphoryl group i.e. the γ phosphate. It can give its pyrophosphoryl group. This is pp. It can give its adenylyl group which is the blue part or it can give its adenosyl group. So but the one that we would be most interested later on is ATP forming ADP and Pi. So there is certain cleavage occurring here that is going to result in ADP and Pi. It is extremely versatile you see in the next slide. I have some of the examples remember when we did the transfer for the membrane protein.

We had a certain reaction, a pump. We need a pump there because you are pushing something out of the cell that already has higher concentration inside. If there is high concentration on one side it would not require more of the specific ion but we learn that it is essential for some ions to go in the cell and some of the ions to come out of the cell. 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. We have basically transport work, mechanical work and chemical work. All of these require ATP. The transport is what we see in the membrane transport, in our muscles the relaxed or the smoothing of the muscles. This is by the filaments. The muscle filament, actin and the myosin also require the ATP. Then also specific reactants that if you have the phosphate being removed or added, it is going to require the ATP.

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These are the specific vitamins that we are going to talk about. These are the coenzymes that they form. The one that just we looked at ATP is not derived from any vitamin. It has a nitrogen base to it, it has the sugar to it and it has a phosphate to it in the nucleotide. It does not have a vitamin that it is derived from. But it nevertheless is usually put in such a table because it is extremely important role in energy and phosphate transfer. The first step that we will see in glucose breakdown is the formation of glucose 6 phosphate and that phosphate comes from ATP.

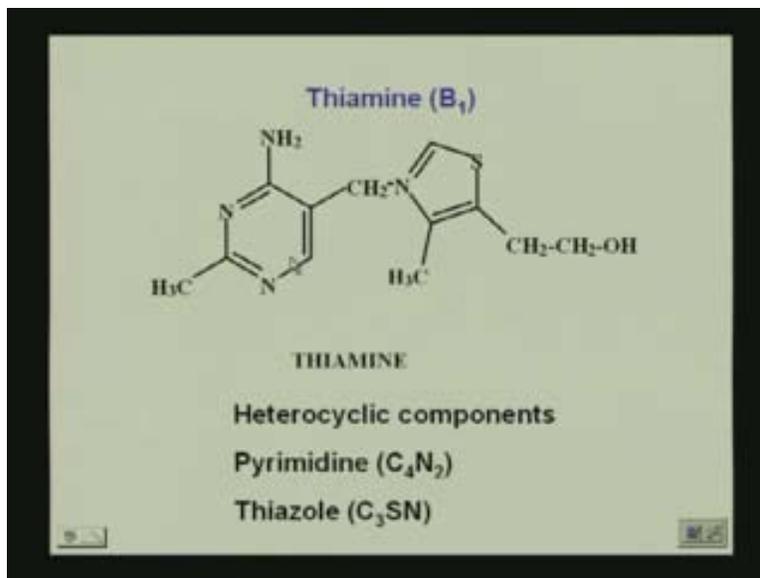
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Coenzyme	Vitamin	Role
ATP	-----	Energy and phosphate transfer
NAD(P)	Niacin	Redox
FAD/FMN	Riboflavin (B ₂)	Redox
Coenzyme A	Pantothenic acid (B ₅)	Acyl transfer
TPP	Thiamine (B ₁)	Transfer of 2 C
PLP	Pyridoxine (B ₆)	Amino acids
Lipoamide	-----	Acyl transfer
Ubiquinone	-----	Electron carrier

NAD(P) which is something we will do in our next class. Niacin is involved in a redox reaction. FAD/FMN Riboflavin so these are what it is coming from. These vitamins result in the coenzyme that is involved in the redox reaction. We have coenzyme A which is another very important coenzyme, rather it's very important moiety that is required for the specific enzymatic reaction that involved in the transfer of the acyl moiety (CH_3CO). The transfer of this acyl moiety due to coenzyme A is only possible if you have sufficient amount of pantothenic acid. Thiamine (vitamin B_1) transfers two carbon atoms. So you recognize how this is important in the biosynthesis. In the biosynthesis of compounds, where you need 2 carbons to come into the picture, you will have TPP coming into the picture; i.e. thiamine pyrophosphate (TPP). For the thiamine pyrophosphate for TPP to come in the picture, it means you have to have some thiamine to form TPP.

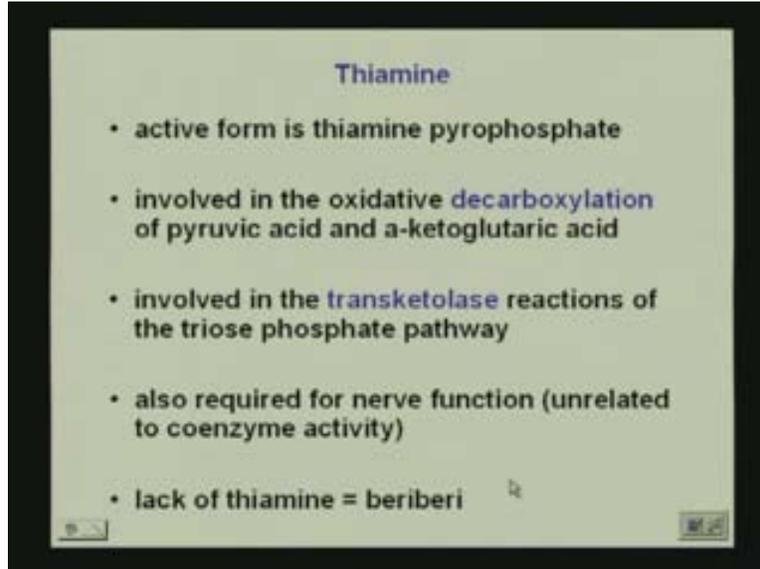
If you do not have vitamin B_1 , it cannot form the TPP. The specific enzyme that requires TPP to transfer of 2 carbons will not be able to act. This is as simple as that. Pyridoxine (vitamin B_6) is going to form PLP. This PLP is required in aminotransferases. So an enzyme such as an aminotransferase 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. These are the other coenzymes. Ubiquinone is the very important enzyme because it carries electrons. It is involved in large number of the free radical reactions because it is efficient in the carrying of electrons. Some of these we will see in the bio energetic part. This is thiamine vitamin B_1 it has a pyrimidine base to it.

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These are the heterocyclic components. It has the pyrimidine and thiazole group to it. This thiamine is what you supplement your diet with. You take thiamine in certain food items. Rice, cereals have thiamine. Certain other dietary constituent have thiamine but thiamine itself cannot act, it has to be transferred to a cofactor. It has to be activated so the active form is actually thiamine pyrophosphate TPP, which is what is going to be formed from thiamine that is what is going to be formed from your vitamin B_1 .

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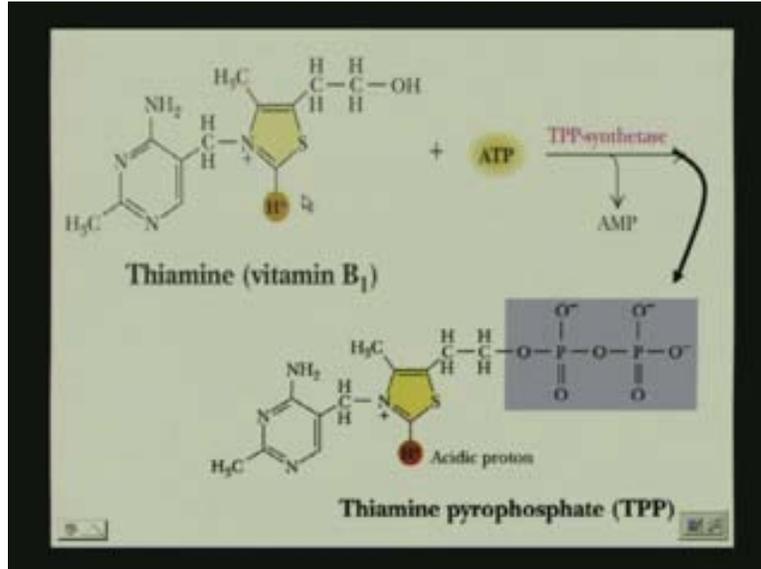


It is involved in the oxidative decarboxylation of pyruvic acid. This is α ketoglutaric acid. We will see what these reactions are in the minutes. It is also involved in the transketolase reactions. The different pathways that we are talking about here are, what we are going to study when we do the bioenergetic or carbohydrate metabolism takes place, because we are going to have breakdown of the glucose.

If you have breakdown of carbohydrates, the carbon moieties that are broken down from it has to go some where 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 is actually independent of its coenzyme activity and lack of thiamine causes a disease called beriberi.

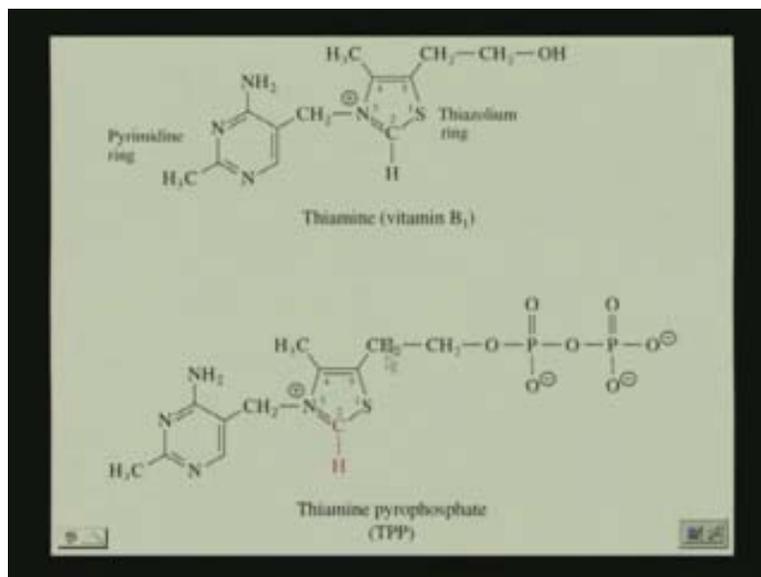
This is thiamine, an acidic proton in the forming of TPP. The enzymes that is required is these enzymes should be able to say very easily even though you might not know the specific name it is forming TPP so it has to be TPP synthetase, it got to make TPP. What else can it do? It has to be a synthetic part that is going to make TPP from thiamine. It is an 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 that requires ATP.

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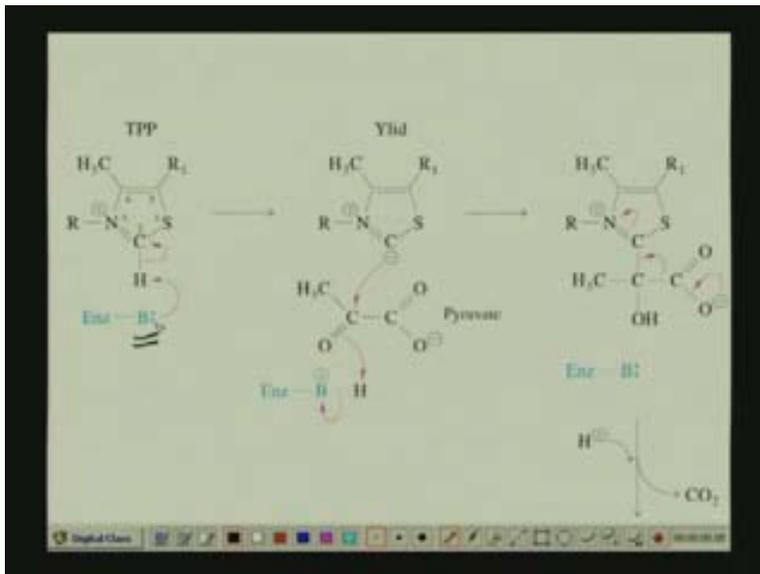
This is what I was mentioning when I said that you have to understand what the reaction possibly requires. For example; if you know that thiamine has to form thiamine pyrophosphate it means that it has to add two phosphates to it. If it has to add two phosphate moieties it is going to get it from ATP. ATP forms AMP because it's losing two of its phosphates. So thiamine TPP synthetase and with ATP is going to form thiamine pyrophosphate and AMP. So this is TPP. You have the vitamin that you took in. This vitamin that you have taken in your diet has been transformed into TPP and this TPP is going to act as a cofactor to a certain enzymatic reaction.

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Let us look at the structures once more which I have here. We have the pyrimidine ring, the thiasolium ring which is vitamin B₁, a certain enzyme transformed into TPP. The enzyme was TPP synthetic and it requires ATP because we added two phosphates to it. We had thiamine in our diet; we now have TPP which is now going to act. We have the enzyme here. These are two electrons that are going to take up the acidic hydrogen.

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This is going to be a basic an electron pair donor. It is going to take up this acidic hydrogen that was available in the thiasolium moiety of TPP. This is the rest of the molecule this R and this (Refer Slide Time: 36:44) R. We do not need that part it needed for the reaction. We do not need for understanding. What it is going to do? Now in the loss of this proton, it forms what is called an ylide. You studied in the organic reactions. Now this is the pyruvate moiety (Refer Slide Time: 47:18).

What is pyruvate? CH_3COCOOH . this ylide then now acts on the pyruvate which then takes up. we have the ylides negative charged here that acts on the carbonyl moiety of CH_3CO group of pyruvate, the acetyl group of pyruvate. By 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 formed, this would not be possible. Why? Because you would not have the ylide. If you do not have the ylide formation you cannot have this hydrogen being taken up by the CH_3CO 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 mechanism that we studied. 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 proton from the enzyme. We have the TPP path and the pyruvate path. The pyruvate is now going to lose CO_2 . What is the action of TPP in this particular enzymatic reaction? It is decarboxylation. But the

decarboxylation cannot take place without the enzyme TPP. It has to be both together that are going to act on the enzyme. Basically TPP is acting with the enzyme in getting the CO_2 out of pyruvate. That is what we are looking. So essentially what is then going to happen is we are going to lose the CO_2 and we are going to get the enzyme identical to what we started. So basically what we looking at here is, we consider what vitamins actually are required. They 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 can form these cofactors and in forming the cofactors that are essential for certain enzymes to actually have their mechanism take place. For example; in the one that we looked here, we found out how the TPP actually works to form the ylide. Without the TPP the ylide is not formed and without the ylide the CO_2 cannot be released. Then the TPP can be degraded or formed again but the enzyme has to get back to where it started from. But that would not be possible without the TPP. We understand that these cofactors are required and they are formed only if you have the vitamins. So the vitamins form the cofactors and cofactors form coenzymes help the enzyme to act.