Organic Chemistry In Biology And Drug Development
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Lecture - 35
Chemistry of Cofactors/Coenzymes

Welcome to this course on Organic Chemistry and Biology and Drug Development. In the last session, we have discussed the various techniques by which a DNA can be amplified. One is called polymerase chain reaction, other was the rDNA technology. So, far; that means, we have covered; we started with the building blocks of living systems amino acids, carbohydrates and I have shown some structure of lipids.

And then we went to for the study of amino acid chemistry and then the proteins, their structure determination, their detection, their separation and the proteins which act as bio catalysts; that is the enzyme chemistry. And this was followed by the we went to different types of enzyme inhibitors, then we went to the nucleic acids and we covered the DNA, RNA, central dogma of biology .

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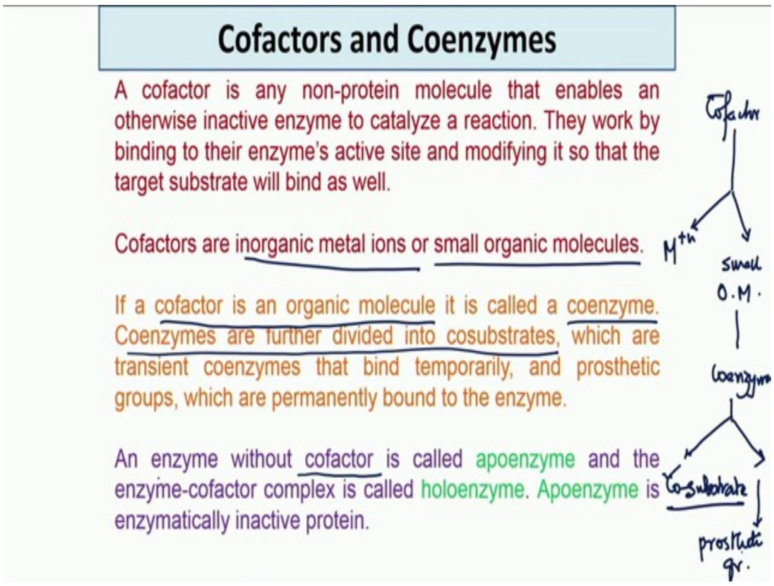

Today, we will again go back to the enzyme chemistry because I told you that in many of the enzymatic reactions; there is a need of a small organic molecule or a metal ion for the enzyme to enzyme to show its catalytic activity.

Now, the; a general term is used for that which is called a cofactor. So, cofactor basically is any non protein molecule that enables an otherwise inactive enzyme to catalyze a reaction. They work by binding to the enzymes active site and modify it so that the target substrate will bind as well ok.

So that means, in many enzymatic reactions what we find that apart from the enzyme which is catalytically inactive when it is alone; There are some small molecules or metal ions which are required for the enzyme to show its activity. The mechanism for that is generally that the cofactor goes and binds to the active site of the enzyme or sometimes the cofactor is already bound to the active site. And then as you add the substrate; the substrate is converted to the product.

Now, cofactor can be an inorganic metal ions or metal ions or small organic molecules. So, when it is a small organic molecule, it is called a coenzyme. Coenzymes can be divided into two categories; in one the coenzyme can be added from outside. So at the time of reaction, it goes and binds temporarily to the enzyme active site and then is released. And then so that it can catalyze other enzyme the molecules of the same enzyme so that the turnover number gets increased. In some enzymatic reactions, this coenzyme is permanently bound to the enzyme ok.

Now, when an enzyme is without the cofactor; that means, when the cofactor; now remember again what I just said that cofactor is the very general term. So, it could be metal ions or it could be small organic molecules. Now, if it is small organic molecules then it is called a coenzyme. So, remember coenzymes are also cofactors and these coenzyme can be divided into two; one is where it goes and binds temporarily as the reaction proceeds and then is released again. So, in that case it is also called a co substrate you can because the substrate also very similar; it does the reaction by going into the active site and then changes into the product.

Here the coenzymes are transiently bound and then the reaction is catalyzed and in many occasions; the coenzymes are released. But in another form which has to be again converted to the coenzyme form; so in that case it is called a co substrate.

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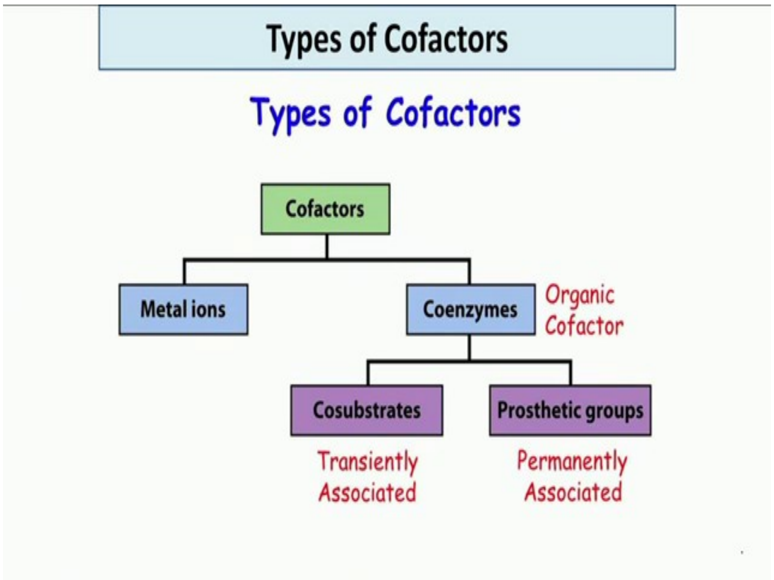

Where cofactor is bound to the enzyme that is called prosthetic groups. So, one is co substrate another is prosthetic group . Now we know that there are cofactors which are essential for the enzyme to show its activity. Cofactors can be two types metal ions or coenzymes; coenzymes are basically when the cofactor is organic. And then if it is transiently associated, then that is called co substrates and if it is permanently bound to the enzyme that is called a prosthetic group.

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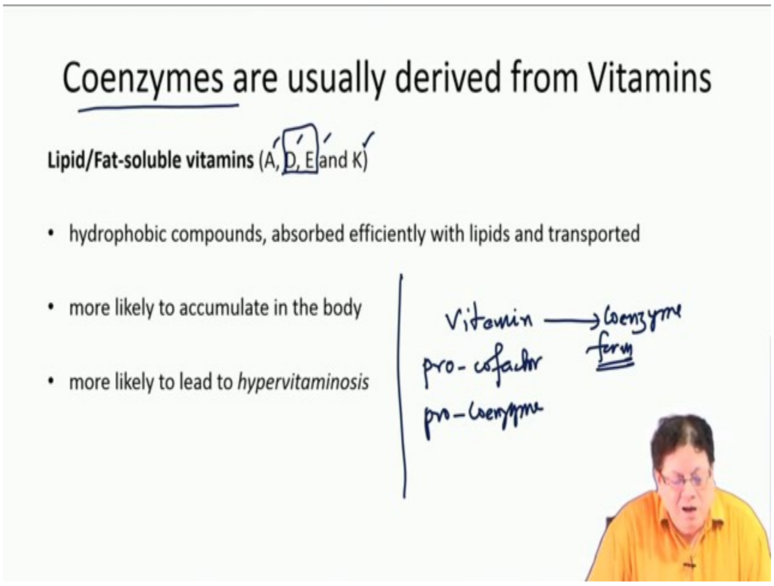

Now, one thing is very clear that some of the cofactors are metal ions. So, we will discuss the metal ion catalyzed enzymatic reactions separately. But when the cofactor is organic; they are called coenzymes and they are usually derived from the vitamins.

So, vitamins are small molecules which are organic molecules, which are essential for the body's growth and many of these metabolic activities. So, these metabolic activities or growth is related to some transformation which is catalyzed by the enzymes. Many of these vitamins are actually they are precursor to the coenzyme. So, it is said that coenzymes are usually derived from vitamins. The vitamin itself is not the cofactor or the coenzyme. So, vitamin is a pro cofactor or you can call it pro-coenzyme .

So, this has to be converted by some chemical transformation to the actual coenzyme form. Vitamins are pro-coenzymes and in the body system, they are converted into another form which is the active form. That means, real coenzyme takes part in the reaction that helped the enzyme to catalyze the reaction.

Now, these vitamins you know they are classified into two groups one is lipid or fat soluble vitamins *i.e* it is soluble in organic solvents. So, lipids are basically the bio materials which are soluble in the organic solvent. So, lipid soluble vitamins these are A, D, E and K. But many of these like A and K are, they are actually precursor to the coenzymes. Vitamin D and E have a different function. Although they do not actually help in any enzymatic catalysis, they have some different function.

That means all vitamins are not precursors to coenzymes. The lipid soluble vitamins, A and K are the coenzymes. D is actually a hormone and E is basically an antioxidant which removes the reactive oxygen species in the living system. We will talk about this reactive oxygen species later on because that is a very important one which is basically the causative agent for breakdown of cells. And then that causes aging and diseases like cancer or other degenerative diseases. So, A and K are precursors to coenzymes.

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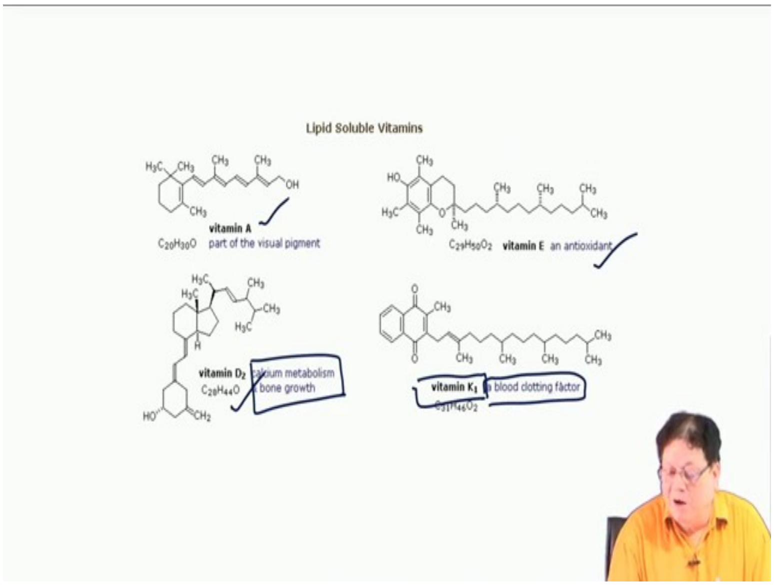

These are the structures of lipid soluble vitamins. Vitamin E is an antioxidant. Vitamin D_2 is required for calcium metabolism and bone growth. So, this is not a cofactor Vitamin K_1 participates in blood clotting.

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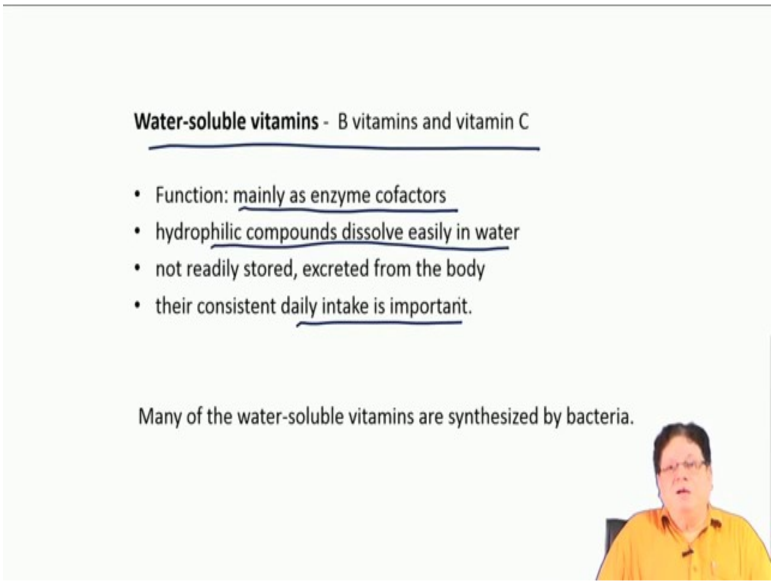

On the other hand, there are water soluble vitamins like Vitamin B and C. Their function is mainly as enzyme cofactors. Their hydrophilic part easily dissolves in water. They are not stored in the body, not readily stored excreted from the body. And they are consistent daily intake is very important because they take part in the many of these enzymatic reactions which are involved in metabolism.

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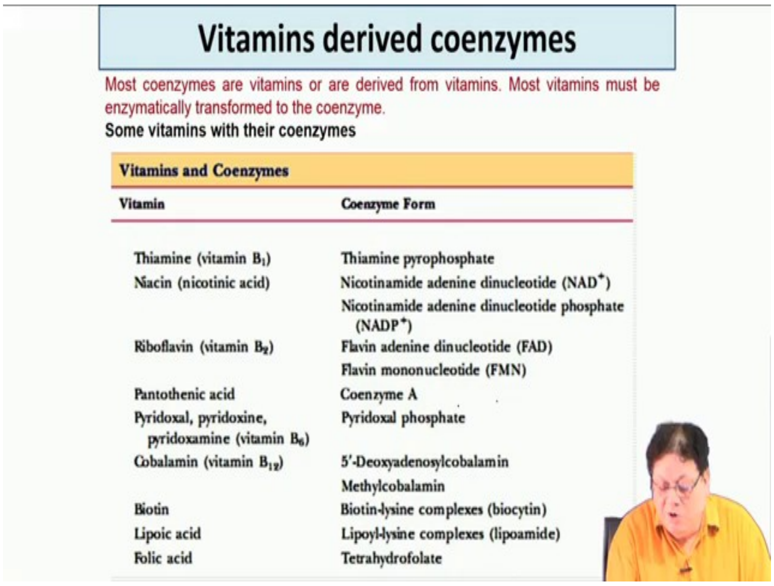

I have shown that vitamins. So, this is the some of the B vitamins. The names are given here. We will go into the structure later on. So, there is thiamine B_1 these are you have read in the school level; there as thiamine, niacin, riboflavin, pantothenic acid, pyridoxal, cobalamin, biotin lipoic acid and folic acid.

We will try to cover their chemistry or the biochemistry. And as I said this is the vitamin of coenzyme form; like thiamine has to be converted into thiamine pyrophosphate, niacin has to be converted into NAD^+ or $NADP^+$. And then, so all these are basically converted into the actual active coenzyme.

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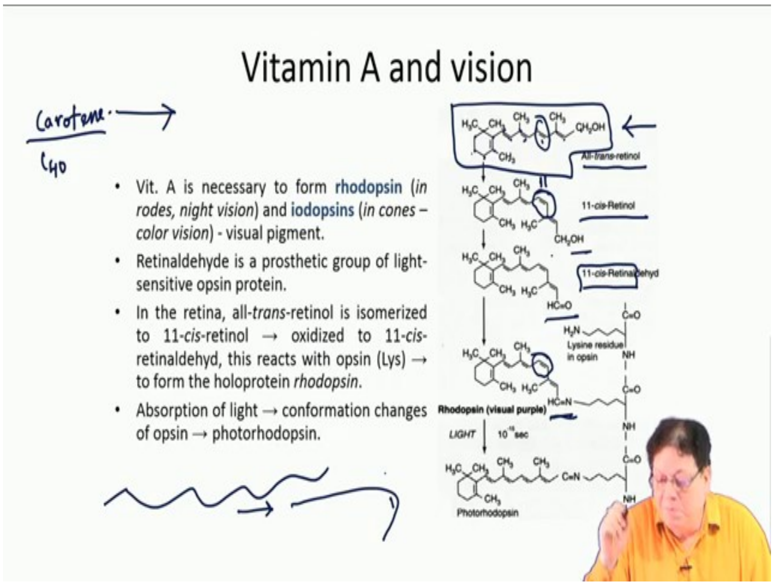

Now, we will start with vitamin A that is one of the coenzyme. Now, we know that vitamin A is a necessary for our vision. Now, the chemistry of vision is very interesting. Here we start with a molecule which is called retinol this is the structure of retinol. Look at the structure; if you count the number of carbon atoms 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 and 20. So, these 20; that means, it is a diterpene and it is derived from the biosynthesis of terpene.

Now, actually what happens? This retinol we get from carotene that we know that if we take lot of carrots, then it protects our vision. So, carotene what is the orange yellow pigment that is present in the carrots. So, carotene actually is a formula which has got the double the number of carbon atoms it is a C_{40} unit. So, from C_{40} unit it goes to the this retinol. You see that double bond stereochemistry and that is all trans; so this is called all trans retinol. Now, this is converted by an enzyme called retinol isomerase. So the double bond here you see that is now present in the cis form. It is a cis form and if you do the numbering, this is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11. So, that position is called 11 that is why this is called 11-cis-retinol.

This 11-cis-retinol is oxidized by the retinal dehydrogenase. Dehydrogenase is a redox enzyme. So, that oxidizes the CH_2OH into CHO; so that becomes 11-cis-retinal ok. You do not have to write aldehyde; you can write just 11-cis-retinal. Here *al* means aldehyde.

And then there is a protein called opsin that has a lysine residue and this lysine then reacts and forms the this imine. Lysine, has a side chain of amine and that reacts with the aldehyde and forms the imine. Then this is what is called the rhodopsin and then as light falls on it, then there is this change in configuration of the double bond that 11-cis double bond goes into the 11-all trans form.

So, there is a change in the geometry of the molecule. Because cisoid means you have to have a system which looks like this because this is a cis double bond and transoid means now you can have a flat straight chain. So, there is a change in the overall geometry of the molecule and that creates a signal. It is called the optical signal and that is then processed in the brain and that is and then we see that whatever is in front of us.

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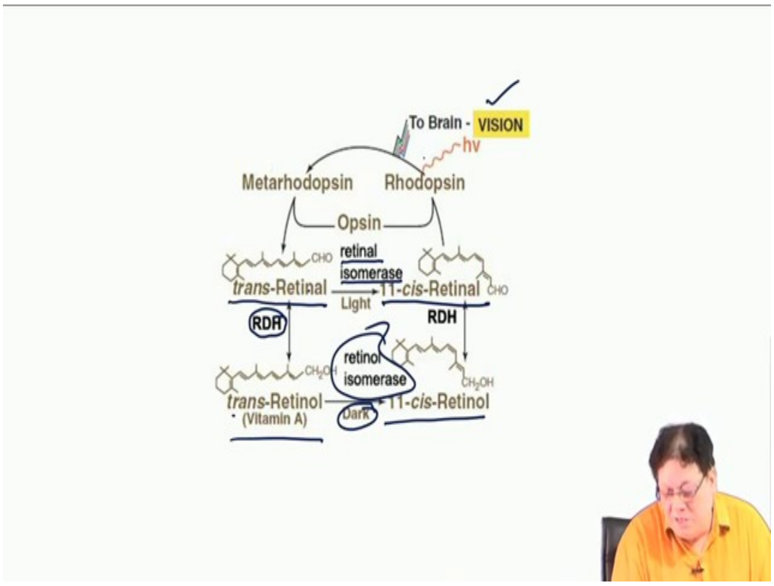

So, this is what is we are telling see; first it is that trans-retinal. I told about the retinol isomer is; similarly you have retinal isomerase. Retinal isomerase is aldehyde that all trans aldehyde goes to 11-cis-retinal. Then it reacts with the opsin protein and forms the rhodopsin. Rhodopsin in presence of light creates a conformational change and that creates a signal and ultimately that signal is processed in the brain and that gives us the vision.

Now, to see the next set of images what we have to do? Now the rhodopsin is photo excited and then it is transferred to the trans-retinal. Trans retinal does not react with the opsin protein, so it is released now and then via retinal isomerase you can go into 11 cis; cis one again back to cis and then cis goes to the rhodopsin forms the rhodopsin .

You can also have the retinol that there is retinol dehydrogenase that can reduce it to the alcohol because I told you retinol dehydrogenase is a redox enzyme. So, depending on the substrate either it will oxidize or it will reduce. So, it goes to the trans-retinol and then that goes to the retinol isomerase works in the dark, ok. When there is no light it can; it is an enzyme that is that converts it to 11 cis retinol and then it again; basically this is a reversible process.

So, when you need the aldehyde it goes to the aldehyde whatever aldehyde is there that forms the rhodopsin. So, basically what happens? That as first trans-retinal is converted into the 11 cis; 11 cis reacts with the rhodopsin forms the rhodopsin and then as light is incident upon this rhodopsin molecule, then you have this change of configuration from cis to trans and that creates a change of conformational geometry and then creates a signal processed in the brain .

And then what happens to this? Because here it is it becomes the trans so that has to be converted again back to the aldehyde; either by retinal isomerase or it can be reduced through retinol trans retinal and then 11 *cis* retinol and then goes to the aldehyde; so, that is the simplified biochemistry of a vision.

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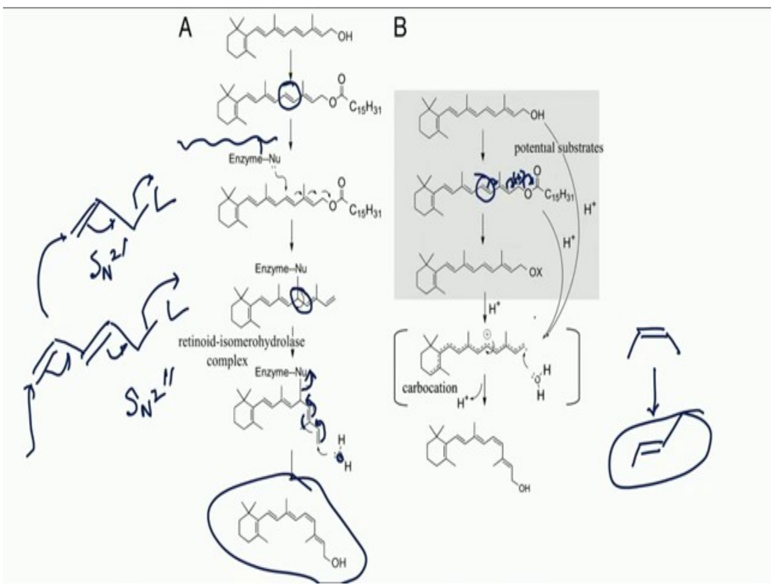

Now, if you want to know that a how these in light I think we know that when we shine light because the cis configuration is the is the relatively unstable one as compared to the trans. So, when light is incident on this double bond. So, cis can go to the trans, because trans is the thermodynamically more stable one.

So, that goes where the excited state where there is no there; there basically there is single bond at the; and then it rotates and then releases the releases the light and then it goes to the trans form. So, that is the mechanism of the light is incident on rhodopsin . Rhodopsin contains the cis configuration.

But the question is how this trans one by retinol you can ask that retinol isomerase converts the trans to the cis, but which double bond? It is only the 11th double bond which is least substituted one in this case because all are tri substituted or tetra substituted. So, this is the one which nature has picked to do the right summarization reversible isomerization.

So, mechanism is that this OH of retinol is converted to an fatty acid ester. Retinol isomerase is the nucleophile that adds to the double bond. Because this double bond can do what is called S_N^{2}” type of reaction. What is S_N^{2}’? That if you have a leaving group here and if the nucleophile attacks here that is called S_N^{2}’. ok, but in this case it is another double bond here. So, it is you can say that this is S_N^{2}”. So, that goes here that comes here L leaves.

You have a double bond here and enzyme nucleophile has added to the double bond. But what it has created? It has created a single bond here. Single bonds are free to rotate. So, at that time it rotates, forms the cis configuration and then in the cis configuration water comes and attacks this. The bond migration goes like this. So, this goes here, that goes there; this leaves. So, that creates the cisoid geometry; this is one mechanism. Another mechanism is via just without attacking by the nucleophile, you can release it because this gives an allyl this is a very stable carbocation.

So, now you can draw many of the resonating structures. So, one of the resonating structures where there will be no double bond here because that will be stabilized like this. And at that time it rotates and goes to the cis and then water comes and attacks the terminal carbon. So, these are the basically the two mechanisms that have been proposed for this retinol to trans-retinol to 11-cis-retinol.

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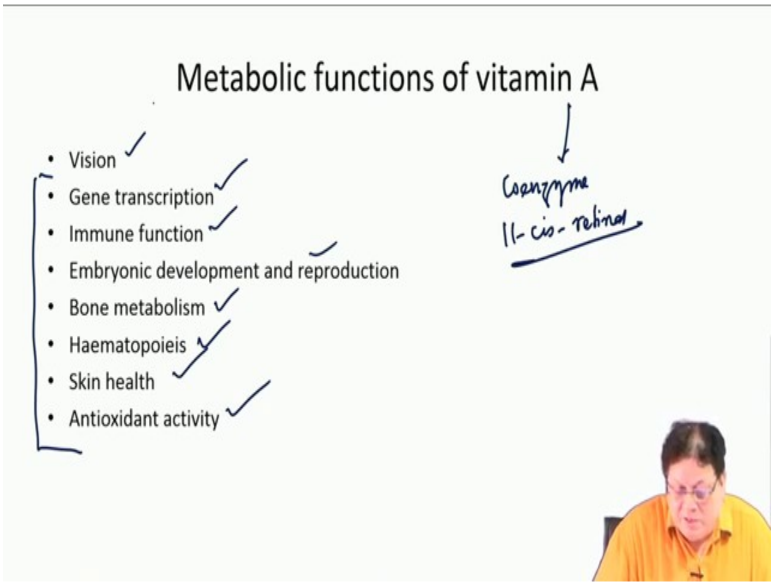

So, it takes care of the vitamin A biochemistry. Apart from vision, vitamin A plays a part in gene transcription, immune function, embryonic development reproduction, bone metabolism, hematopoiesis; that means, red blood cell formation, skin health and antioxidant activity. Because it has got lot of double bonds, so it can consume radicals very quickly; radicals can add to it oxygen radicals.

So, vitamin A is not only important for vision biochemistry where it acts as a coenzyme, but it is also important in other many other functions. So, a deficiency causes many types of problems.

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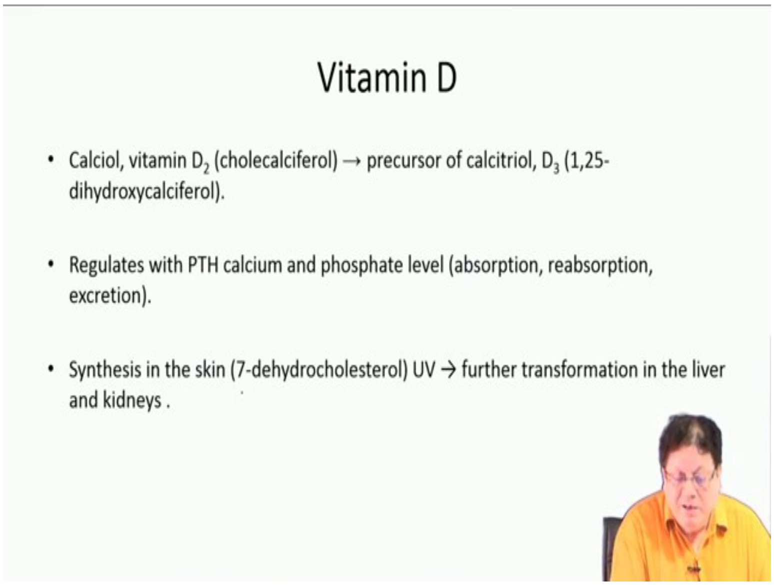

Vitamin D is not a coenzyme. It is basically a hormone. Now what is a hormone? Hormone is usually secreted by glands and then it is transmitted through the blood stream to different places in the body. And then it has got its receptors. Receptors are basically proteins and then it binds to the receptor. Then it creates a signal that starts a cascade of reactions; so that is what is hormone.

Now, is it is not a coenzyme not a precursor of coenzyme.

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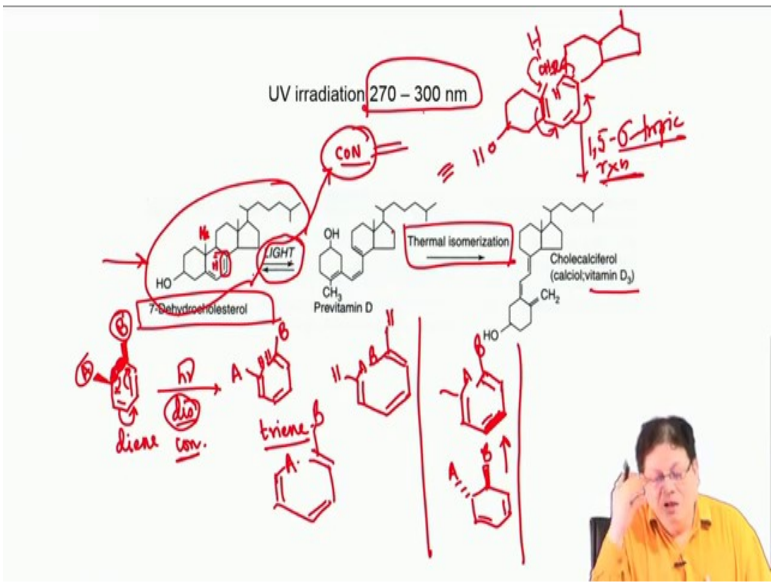

The vitamin D_3 is obtained from a well known substrate 7-Dehydrocholesterol which is having an extra double bond 7-Dehydro; that means, 1, 2, 3, 4, 5, 6, 7. The extra double bond is there. So, that is called 7-Dehydro cholesterol, otherwise cholesterol is a saturated framework here.

Now, these 7-Dehydro cholesterol in presence of light it has got a 6 membered ring with a diene framework. And I think those who have studied organic chemistry and pericyclic reaction, they know very well that these type of molecules when we shine light then it can open up. The process is called an electrocyclic ring opening reaction.

You have a double bond here. So, from a cyclic diene you get an acyclic triene. Now, if you have substituents here, suppose I have these substituents A and B. Now, there are two modes of electro cyclization or this ring opening and the two modes are one is dis rotatory and the other is what is called con rotatory.

Now, dis rotatory means the orbitals here which forms the sigma bond, they move in opposite direction. If it goes in the clockwise direction, that goes in the anti clockwise direction. So, if this is clockwise that is anti clockwise. So, if in dis rotary motion what happens, that this A and B which are earlier cis to each other; they go in the they appear in the same direction; that means, see the double bond geometry is like this; this is a sp^2 carbon. So, the question is where will be the A and B? The A and B will be either both are outside and this is a hydrogen suppose or they could be inside or they could be here B and there A and there hydrogen.

So, A or B either inwards; both go inwards or they could be outward. So, both are possible for both the isomers for dis rotatory motion. For con rotatory motion because they move in the same direction; so both are clockwise, then what happens? This A and B; one goes inward and the other goes outward. So, basically you can get one of the product I will show that if this is B, then A will be this.

Now, the; if you have A and B trans to each other then what will happen? That if it is dis rotatory, now if it is trans relation then it will be just the opposite. For dis rotatory; one will go inside another will go outside; if you have the trans relationship. So, I should write that; so what I am talking about is this, this is the double bond. So, if you have say B here and A is alpha so, then that goes to the double bond here triene, but now the A and B are trans to each other. So, in dis rotatory they maintain their trans relationship;

that means, if one goes inward, the other goes the outward and if A is outward then B will be inward. So, that is for dis rotatory and for con rotatory A and B will be either both outward or both inward.

Now, what happens here? Why nature has picked up light. If it is light and it is a 6 electron ring opening process. So, there are rules for that and the rule says that 6 electron in presence of light will undergo a con rotatory motion and four electrons on the other hand, we will go for just a second 6 electron heat is dis rotatory 6 electron, light is con rotatory.

So, if it is con rotatory, then this hydrogen is alpha and this methyl is beta this methyl is beta. So, what will happen now? As it is con rotator, so actually this is written in little bit different way. So, maybe I will write that in the proper perspective; that means, the product which is obtained here; that is this double bond here and a double bond here and a double bond there. And then you have this ring; you have a 5 membered ring that is you have a ring here also and that is the OH and you have a methyl.

Now, because the methyl hydrogen are trans to each other to start with so, with light it will go in con rotatory fashion, so they will now come both inwards, ok. They will not be outwards because it will be very difficult if you have outward, it will be very difficult to make these to complete these rings ok. So, that is why this process has to be con rotatory in order to have a ring opening reaction and that is why it is; it is done only in light, ok.

And then there is a thermal isomerization which is called a 1, 5-hydrogen shift sigmatropic reaction; this is called sigmatropic reaction. Sigmatropic reaction one of the hydrogen here of the methyl that goes to the 5 position that goes here. So, basically what you will end up is a double bond at the ring junction and a double bond here and a double bond CH_2. This is how double bond CH_2 means the methyl that is the double bond CH_2; so, 1, 5 sigmatropic and the before a light induced con rotatory motion. So, this is the first derivative of the first vitamin D which is biosynthesized inside the body.

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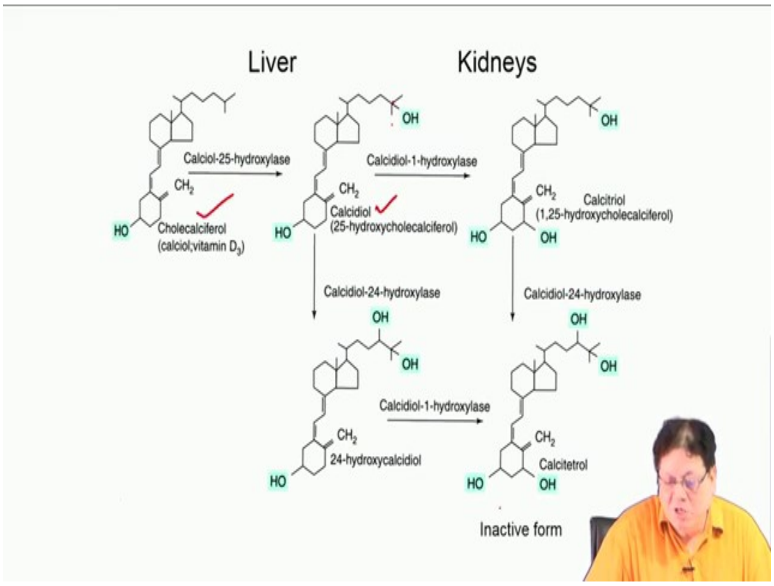

And then there are hydroxylation that can take place. Hydroxylation at this point that gives it to gives you what is called this is called cholecalciferol, the initial vitamin that is D_3. Then; then calcidiol that is this is the 25th carbon; so 25-hydroxycholecalciferol and then another form is calcitriol and that is another further hydroxylation. So, this is trial, this is diol and this is monoal these are the three forms of vitamin D_3.

What is their function? Their function is basically to maintain the proper calcium ion which supports the development of the bone. When babies are born usually the vitamin D are put on outside with a message of this oil which contains this cholesterol. So, as you shine light; they have to put into the light to in order to have this con rotatory ring opening and then thermal isomerization.

So, the first one is photochemical and it has to be photochemical because then you what you need is a con rotatory motion; you do not need any dis rotatory motion. So, that is the, and this UV it requires the radiation of 270 to 300 nanometers. So, you require exposure to sunlight, but not in the visible light. It happens in the long wavelength UV light, but that is there always along with the sunlight .

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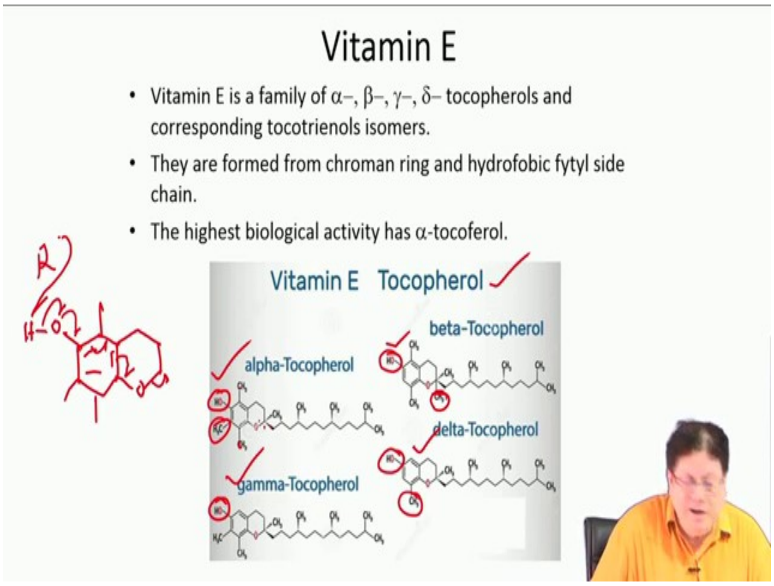

Vitamin E is family of α-, β-, γ-, δ-tocopherol. Vitamin E is fat soluble vitamin and it has long fatty chain. It contains a OH group at gamma beta/delta/alpha/gamma position.

alpha-Tocopherol has three methyls, gamma and beta-Tocopherol have two methyls, delta-Tocopherol has got only one methyl. So, tocopherol is the name given to the vitamin E and this is an antioxidant. Antioxidant means it can neutralize radicals.

α-, β-, γ-, δ-tocopherol contain phenolic OH functional group. When a radical produced in the body; this will immediately donate the hydrogen and then it becomes O dot. This is highly stable because this O dot can go into resonance and form via resonance.

So, there is a radical which is tertiary radical that is generated. So, it is basically a reversible connection between this radical here at the carbon or the oxygen radical. So, basically it neutralizes any radical that is produced in the body and these radicals are basically outcome of reactive oxygen species.

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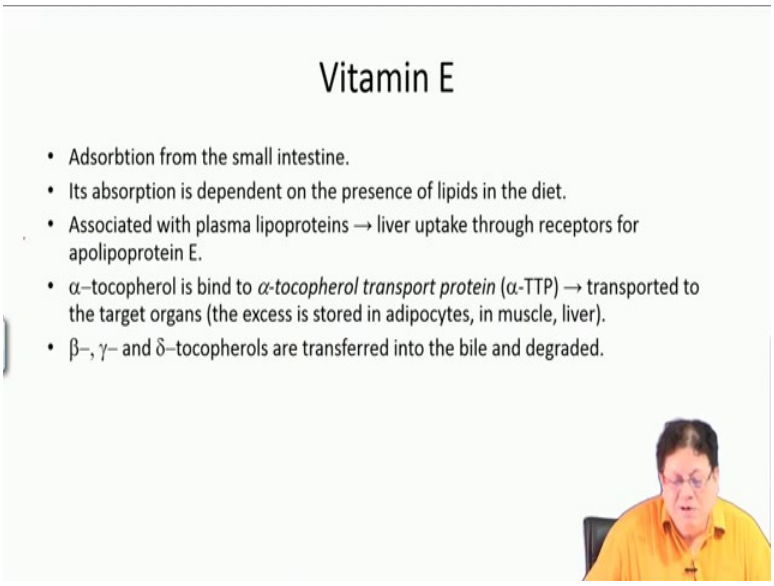

So, vitamin E is very important because these radicals are associated with degenerative diseases or mutation which can cause cancer diseases like cancer and then also other like gout, aging all these things are there.

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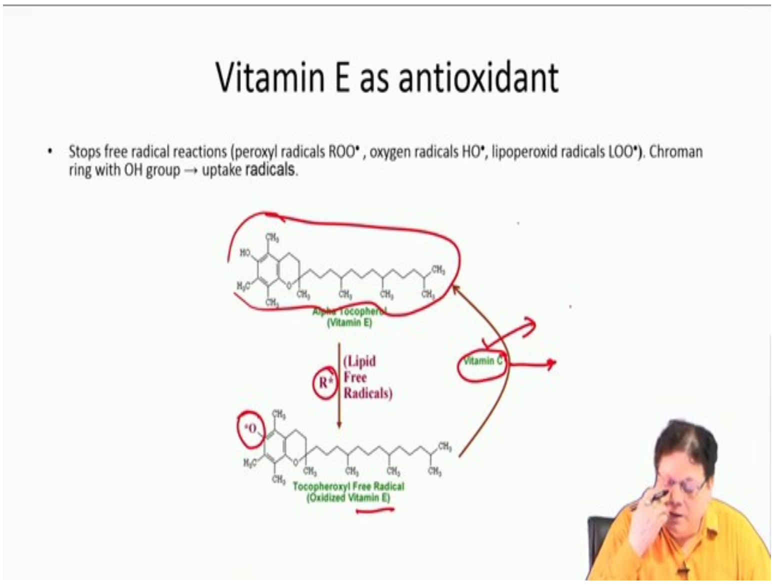

Vitamin E as say I already told you that vitamin E forms this radical here, it abstracts any R·. It donates the hydrogen forms this radical. This radical is very stable because it can go into this tertiary radical, but it has to be brought back to the again the original form. It cannot be in the radical form.

Now, the vitamin C is to bring the the vitamin E free radical into the tocopherol. Vitamin C is also an antioxidant. So, this itself is oxidized and then it is reduced. We will discuss vitamin C chemistry later.

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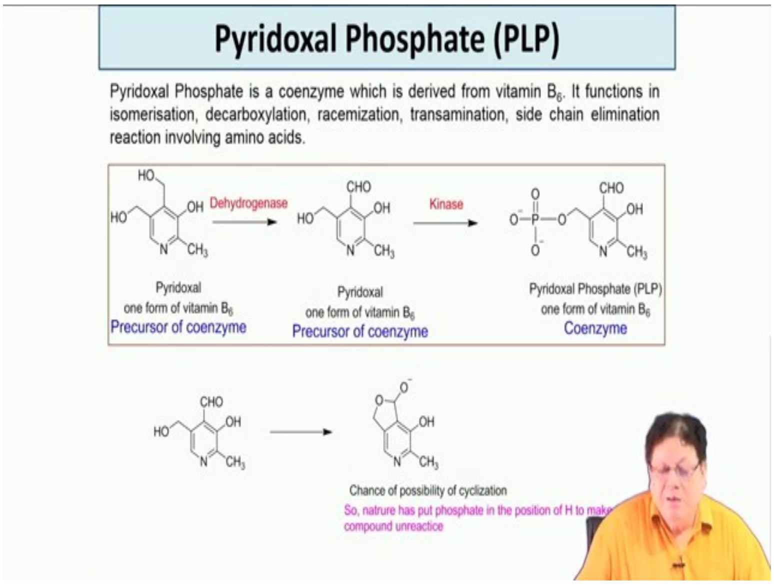

So, that is basically the chemistry of biochemistry of the lipid soluble vitamin. We have not discussed vitamin K yet, that will come little later in the next session.

Thank you.