**Organic Chemistry In Biology And Drug Development**
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**Lecture - 39**
**Chemistry of Cofactors/Coenzymes (Contd.)**

Welcome to this session on the course Organic Chemistry in Biology and Drug Development. The last few lectures contain biological pathways combined with the discovery of drugs.

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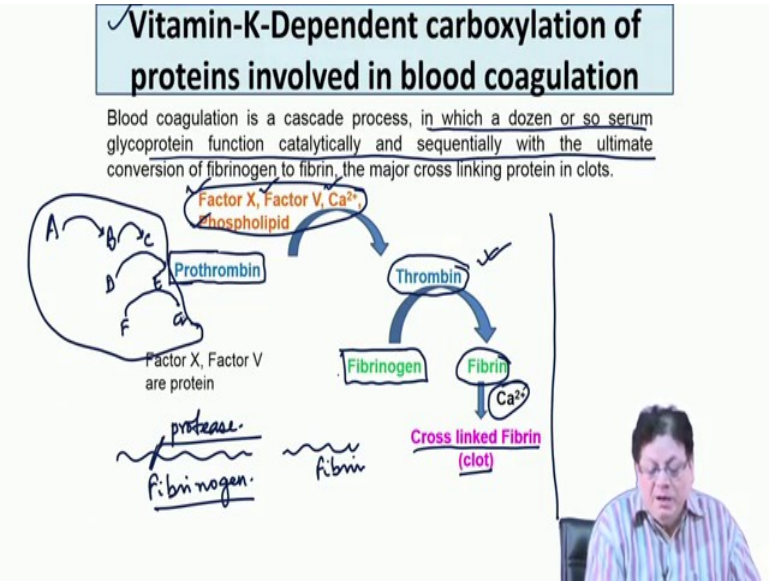


We already learned some medicinal aspects while studying the chemistry in biological system, for example folic acid biosynthesis inhibitions. Now next coenzyme that we are going to discuss is called vitamin K. Now this vitamin K actually involves in blood coagulation. Blood coagulation means whenever there is a cut this the blood would not come outside after few seconds or minutes. Now this K actually stands for a coagulation what it say in German language this starts with the K. There is some blackish thin called clot that covers the cut and this clot process is dependent on the vitamin K. If you have a deficiency vitamin K then blood clotting will be a very slow. So, lot of blood will be lost. In addition to vitamin K, also you need a lot of calcium ions because the clot is made up of a protein which is called fibrin. This fibrin protein in presence of calcium forms cross linking proteins and that makes it is very insoluble mass which is known as the clot.

This vitamin K is helping to form the clot. Now blood clotting process is actually involves a large number of cascade reactions. Cascade reaction means say suppose A goes to B and then B goes to B goes to C and then maybe C catalyzes the reaction of D to E. So, like that E catalyzes the reaction of F to G. So, these are called cascade reactions. So, there are a large number of cascade reactions during blood coagulation. So, serum glycoprotein functions catalytically and ultimately fibrin is formed.

That is the last step in blood coagulation and fibrin in presence of calcium forms the cross linked fibrin which is called the clot. Now what is the starting material for fibrin. It is what is called fibrinogen. Fibrinogen is a larger protein as compared to fibrin. So, at the time of this cut ultimate reaction is that fibrinogen has to be converted to fibrin. So, it is a very large protein. So, you have to take out some portion from the fibrinogen to make the fibrin.

So, basically what happens if this is fibrinogen then you cut here and you get the fibrin? This is true for many of these proteins or enzymes that are present in our body like when you take food that food will be digested by chymotrypsin trypsin and pepsin. Chymotrypsin or trypsin actually are present in an inactive form called chymotrypsinogen. So, as soon as I take the food the system gives a signal that chymotrypsinogen has to be broken down into the active chymotrypsin.

So, that is nothing, but a proteolytic cleavage of one portion in the long polypeptide. You take out some of the portion which is making it inactive. That portion is chopped up and you will get the active form of the protein. This breakage is nothing but a protease reaction. You are basically breaking the peptide bond. It is peptidase or protease. So, this fibrinogen to fibrin conversion is catalyzed by an enzyme which is called thrombin. Now the question is who synthesizes the thrombin.

It is something called prothrombin. Prothrombin by the action of many species like factor 10, factor 5 (these are all glycoproteins), calcium and phospholipid. If all these are present then prothrombin goes to the thrombin, the actual enzyme which catalyzes the conversion of fibrinogen to fibrin. Now before that prothrombin there are other reactions.

The previous cascade reactions because I told you there are at least dozen of cascade reactors. These are the final steps of blood coagulation. Prothrombin goes to thrombin by the action of this factor 10 factor 5 calcium and phospholipid and the thrombin that is

formed that hydrolyzes fibrinogen to fibrin and fibrin in presence of calcium forms a cross link. So, we are going to discuss this step where vitamin K has a role prothrombin to thrombin.

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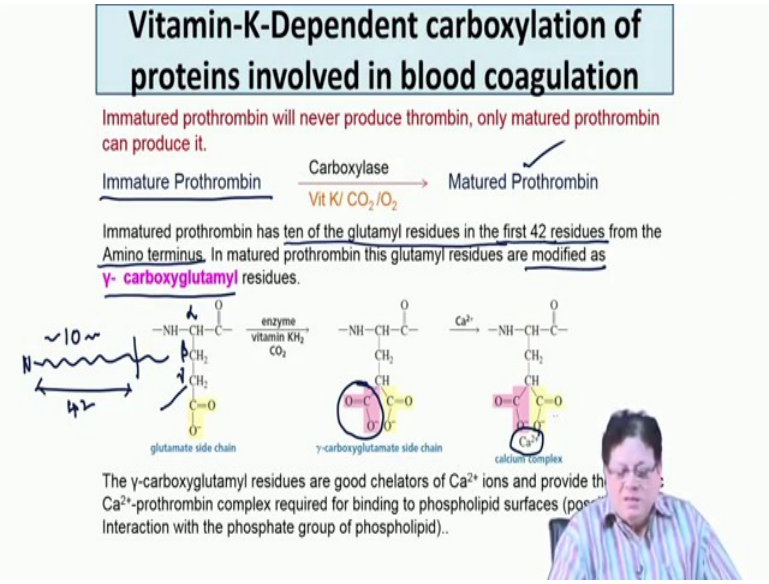


When we had the prothrombin before that there is immature prothrombin.

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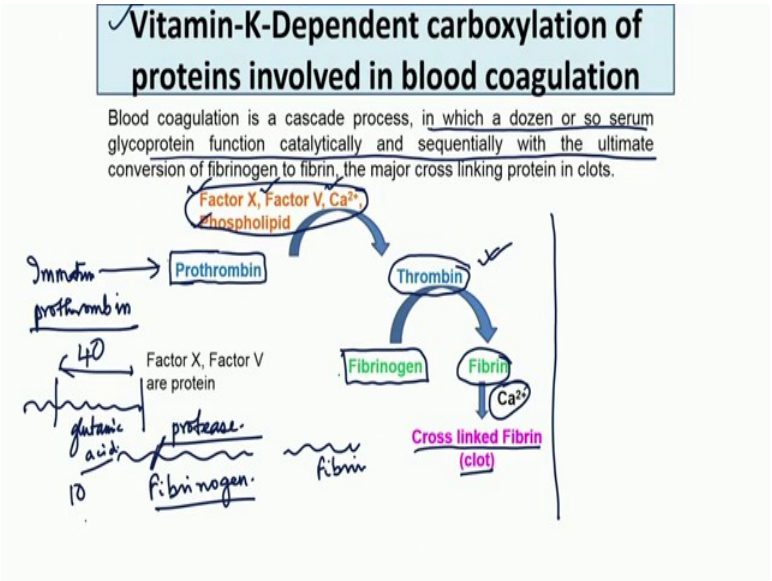


So, this prothrombin is generated from immature prothrombin. What is immature prothrombin? Immature prothrombin is that this if you look at the protein the amino acid

sequence in this protein. Immatured prothrombin has ten of the glutamyl residues in the first 42 residues from the amine terminus. In matured prothrombin this glutamyl residues is modified as **γ- carboxyglutamyl** residues.

The γ-carboxyglutamyl residues are good chelators of $Ca^{2+}$ ions and provide the specific $Ca^{2+}$-prothrombin complex required for binding to phospholipid surfaces (possibly *via* Interaction with the phosphate group of phospholipid). The cleavage in vivo to thrombin splits off the **γ- carboxyglutamyl** containing fragments, it does not appear in thrombin. The carboxylation process is Vitamin K dependent. It acts as cofactor of carboxylase. Source of carboxylation is $CO_2$.

Why you need this gamma carboxyglutamyl residues because they form complex with the calcium and that is necessary condition for the prothrombin to carry out the next reaction. In the next reaction, this prothrombin goes to thrombin. So, unless this carboxylation happens this immature prothrombin cannot be converted into the thrombin.

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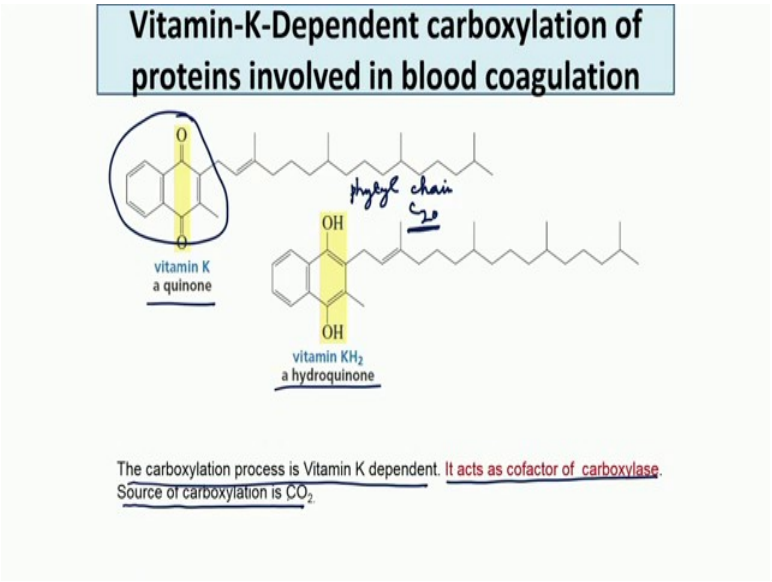


Let us see what is the structure of vitamin K? Vitamin K has this quinone moiety and attached to a phytyl side chain. It is a $C_{20}$ phytyl side chain. $C_{20}$ made of 20 carbon atoms. So, this vitamin K is the oxidized form *i.e.* quinone and in the reduced form it is a hydroquinone (that you can have OH and OH).

The carboxylation process is vitamin K dependent and it acts as a cofactor for the carboxylase. So, let us see what the mechanism of that reaction is.

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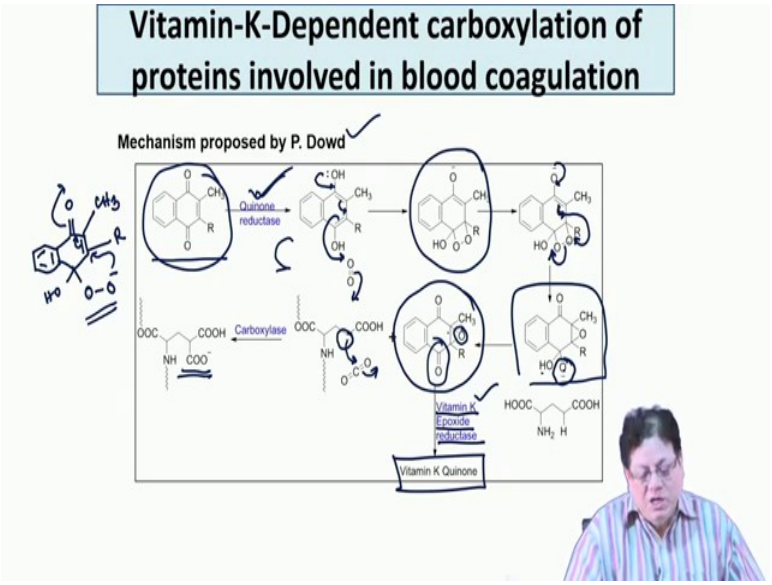


Now the most accepted mechanism is was proposed by the famous organic chemist Paul Dowd and there are many evidences to support this mechanism. Here first this quinone form of vitamin K is reduced to the quinol by an enzyme called quinone reductase.

It goes to the quinol state and then quinol means it becomes again the electron donor system. This lone pair can come here and this goes here and that attacks the oxygen and these goes here. The earlier mechanism proposed before 1980s, did not involve oxygen. Everybody thought that the oxygen does not play any part here, but later on it was found that oxygen is required to do this carboxylation. Then Paul Dowd did some experiments to show the involvement of oxygen in this way.

So what is happening here? You have this double bond O and then a double bond here and you have a OH here and this will become O O minus. Now you can say that actually 2 electron transfers are prohibited in case of the normal oxygen which is in the triplet state. So, you can do the mechanism by single electron transfer and you will get the same thing. Precisely this should be triplet oxygen. So, these type of transfer should be one electron one electron means like this so, but ultimately you will get either here dot and a dot here. For ionic mechanism, you will get O minus.

You can show this mechanism that I am showing with an assumption that this is in the singlet state. So, this can come back. There are 2 substituents. Remember this is R that is the phytyl chain and this is the methyl and that goes here and this goes. This is dioxane intermediate. So, basically it is a 4 membered ring with dioxygen-actually this is called dioxetane intermediate. You get the original state what I have showed to you. This attacks the oxygen because the O O bond is unstable that can easily break. If it goes here then you will get the previous intermediate.

So, now, it is attacking this oxygen to break the oxygen peroxo bond which is very weak. You get this another intermediate where there is an epoxide here and this is O minus, but these O minus is what an alkoxide is. So, that will be extremely basic alkoxides. So, this alkoxide now can abstract the hydrogen at the gamma position of the glutamic acid residues.

So, all these are required because this hydrogen has to be abstracted and that requires a strong base. Through this oxygen mediated pathway you get this vitamin K epoxide and an alkoxide. The alkoxide now takes the hydrogen from here. It becomes OH and you know 2 OH s in one carbon means basically it is a carbonyl. So, that goes to carbonyl with loss of water and this glutamic acid residue becomes the anion and this anion now reacts with carbon dioxide and that is how this carboxylation takes place.

Now this vitamin K is basically the oxidized form and moreover it is having a epoxide here. So, this is called vitamin K epoxide.

In order to have the second cycle of reaction by the same vitamin K molecule, you have to reduce it now. So, that is called vitamin K epoxide reductase and you get first vitamin K quinone and now these enzymes are already there. So, the quinone reductase now will again take it forward from the next cycle of reactions. So, vitamin K epoxide reductase.

It takes the oxygen off and puts a double bond here. This is very important enzyme because if it can inhibited then what will happen your next cycle of reactions. Next reactions will not take place and ultimately the clotting will affect the formation of the matured prothrombin and may affect mature prothrombin. It is a problem because mature prothrombin will not be converted to prothrombin that will be converted to the thrombin and if it there is no thrombin then fibrin cannot be formed from fibrinogen.

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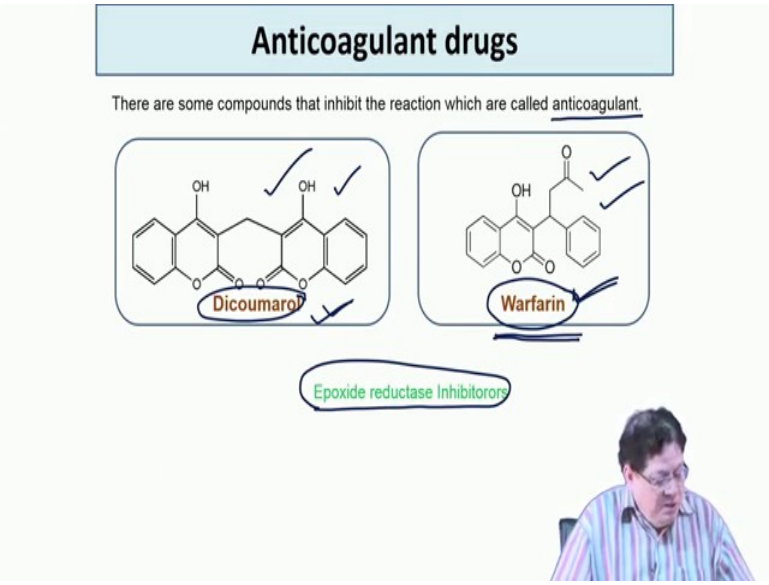


So, there are molecules which are called anticoagulant. Anticoagulant actually does not allow the blood to clot readily. You might say that for what reason we want to stop the clotting of blood. If there is a clot then it is required for people who are suffering from hypertension due to the narrowing of the blood vessels.

If the blood vessels become narrow then there is a lot of stress that is involved in the movement of the blood. Blood clotting means you are increasing the viscosity or density of the blood. You can stop this coagulation process because many of the heart attacks are basically catalyzed by this formation of the clots, the solid precipitate.

They can block the valves in the heart or brain. If there is block in brain then it calls the cerebral attack. So you need blood thinners. Blood thinners does not allow the blood to make clot. There are compounds like dicoumarol and warfarin whose structure is shown here. They are used as blood thinner, but they can be also used to kill the pesticide or insecticide. Basically warfarin is a rodenticide. Rodenticide kills rodents. Rodents like the mice and all these things will destroy the crops in the agricultural field or in household. There are lots of big grasshoppers and they destroy the agricultural field. To prevent grasshopper you can use warfarin. Basically people who are prone to heart attack or cerebral they use this dicoumarol or warfarin and even they also use aspirin as a blood thinner.

Dicoumarol and warfarin are inhibitor of the vitamin K epoxide reductase. So, it inhibits that enzyme and cuts of that catalytic cycle.

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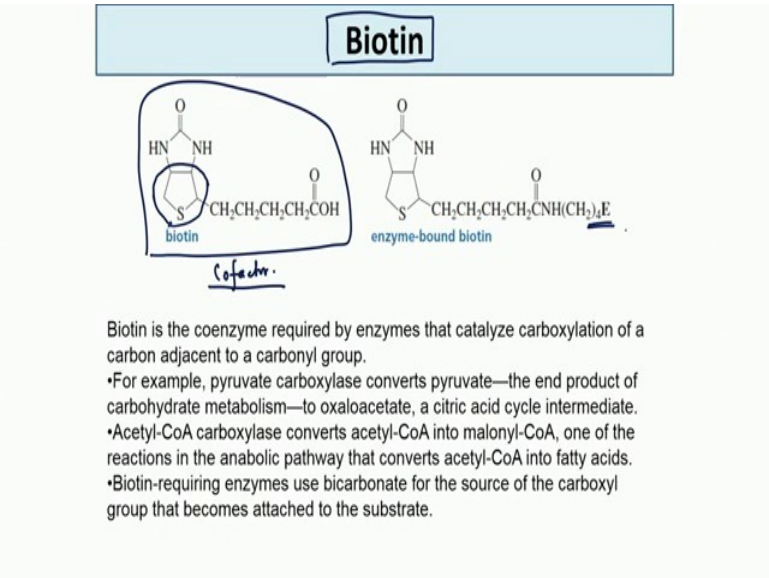


Anticoagulant drugs are very effective. Vitamin K is over now. We have discussed many of the other vitamins like thiamine pyrophosphate, pyridoxal phosphate, folic acid, flavin adenine dinucleotide and the lipoic acid biochemistry.

The next one is what is called biotin. Now biotin was originally called vitamin H, but now it is again grouped into vitamin B. Now structure of biotin is shown here and the biotin is basically a bicyclic compound and this is a urea like moiety NH CO NH, a 5 membered ring this is 1,3-triazole, a tetra hydro thiophene moiety is attached to a long chain carboxylic acid.

That is biotin and this is the cofactor. Vitamin k that is the quinone form has to be reduced in order to form the coenzyme and in this case biotin it is attached to the enzyme as a prosthetic group that can itself act as the coenzyme.

You do not need in any transformation. If it is given from outside then it will be attached to the enzyme by a lysine moiety because this is a carboxy. So, you make a peptide from here by an amide bond to the lysine and then will be called a prosthetic group. So, this is the enzyme bound biotin. So, in what type of reaction it participates.

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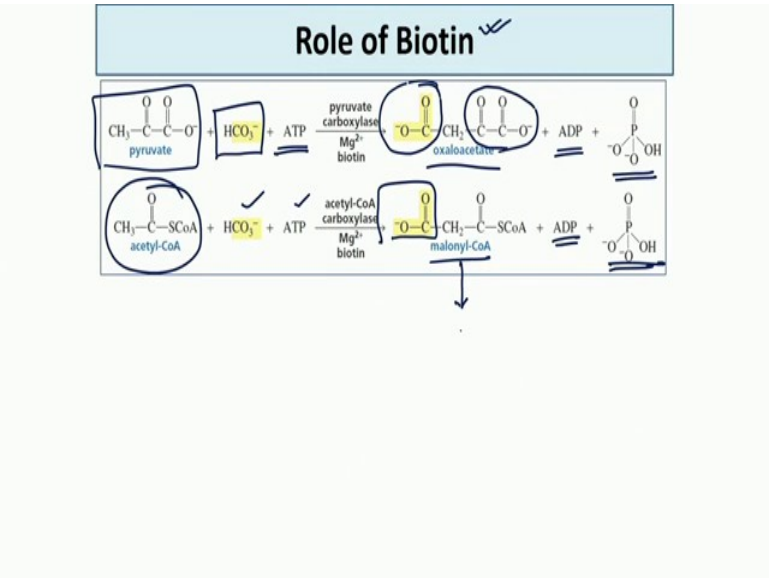


Vitamin are very important because they are coenzymes and coenzymes are responsible for various metabolic activities in the body which are absolutely essential. Now the biotin does carboxylation, but the role of vitamin K is to form this alkoxide in presence of oxygen and remove the hydrogen from the gamma position of the glutamic acid.

So, that is a separate class. Now we are talking about biotin. Biotin take pyruvate and again the source of carboxylation is carbon dioxide. In case of biotin, it is in the bicarbonate form and in presence of ATP it forms this $CH_2CH_3$, 1 hydrogen is replaced by $CO_2$ because that is carboxylation.

So, you get oxaloacetate. This is the oxalyl group and this is the acetate moiety. You will get ADP and a phosphate that is ATP is hydrolyzed. The energy of is utilized to do this reaction oxaloacetate and then acetyl coenzyme A. We have read the formation of the acetyl coenzyme A by the thiamine pyrophosphate, lipoic acid and FAD with cascade of reactions. So, here is bicarbonate and again ATP. So, it again does carboxylation here.

You have a carboxylation here that is called malonyl coenzyme A and then ADP plus a phosphate. This malonyl coenzyme is also a very important metabolite for making the different fatty acids in our body that is obtained from malonyl coenzyme A together with acetyl coenzyme A.

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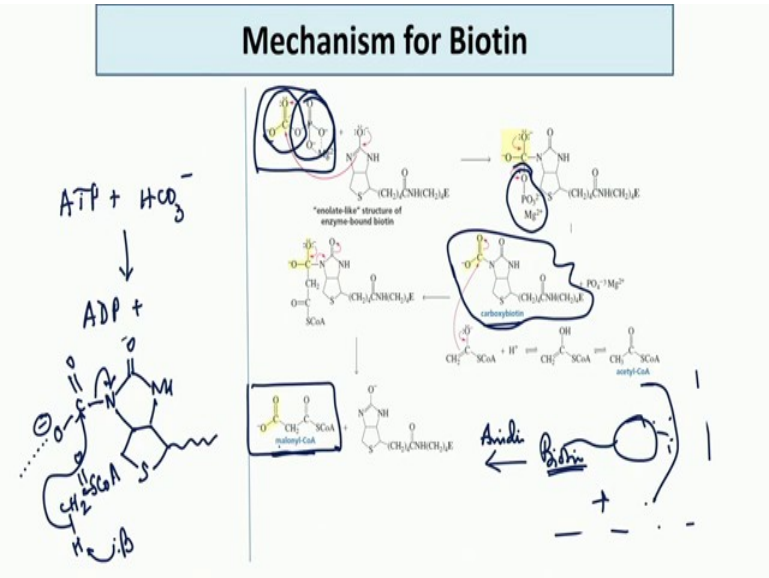


The mechanism of this reaction is - biotin is your NH, but you can write the enolate form if there is a base. The base can abstract the hydrogen, it goes to the enolate. You have this ATP. The first reaction that happens is ATP plus your bicarbonate and that goes to ADP plus this species. This is what a phosphate linked to a carbonate.

A carboxyl basically carbo this is what is carbonate. This negative charges are taken care of by magnesium. So, that can come and attack the carbon in the carboxyl. This is carbon in the carbonate species and forms this O minus. It is attached to this O phosphate and magnesium. In the next reaction, that comes here and the phosphate goes out. So, basically earlier ADP was there.

Now, phosphate goes. You have N carboxy biotin intermediate. You have this N carboxy biotin, it is N double bond O, then NH, then this sulphur. You have this CO and O minus N carboxy biotin. Now obviously, this will be has to be taken care of if you want to attack by a nucleophile. It is the chain attached to the enzyme. Your acetyl coenzyme A SCoA. There is the base in the enzyme that takes up, this goes here and that is kicked out.

So, you get $CH_3CO$ SCoA. We have written the CH here just to show the abstraction by the base. So, base abstracts the hydrogen and that goes here and this comes out. This is a very good leaving group because now negative charge on nitrogen is stabilized by the carbonyl. You get carboxylated acetyl coenzyme A. It is basically nothing, but malonyl

coenzyme A. A very similar reaction will be there for conversion of the oxalo acetate from pyruvate. I think you will be able to draw that.

It is the same mechanism you first form the carboxy biotin, the mixed anhydride of carbonate and phosphoric acid and carbonic acid and phosphoric acid and then you have a nucleophilic attack on the carbon forming the carboxylated biotin. Then that carboxylated biotin releases the carboxy group and it carboxylate the alpha hydrogen in acetyl coenzyme A and in this case it will carboxylate the hydrogen beta to the carboxy. This is alpha keto acid. That is the beta carbon. So, the beta carbon hydrogen is also very acidic.

That will be abstracted and the carboxy group will be put on the beta carbon and in all these reactions the biotin is again released and biotin can again participate in the next cycle of reactions in the similar way. There is a protein called avidin. The avidin and biotin complex is one of the strongest complex - biotin is a substrate and avidin is the protein.

So, biotin is utilized as a medium for affinity chromatography because it has very high affinity.

If you attach a molecule to biotin this molecule interacts with some enzyme. So, if it is done in presence of various other proteins only the protein which has the small molecule attached to biotin will come out and that will be pulled down from this mixture of proteins. So, that is called affinity guided separation.

So, that is the utility of biotin in protein purification, but here we have talked the utility of biotin in as a cofactor. Malonyl CoA is extremely important because that is the starting point is acetyl coenzyme A. But then acetyl part of the acetyl coenzyme A is converted to malonyl coenzyme A and then they do react with each other and form a 4 carbon acid and then slowly increases by 2 carbons. Ultimately palmitic acid or stearic acid or oleic acids are formed. This is the biosynthesis of fatty acids.

So, it takes care of another B group of cofactor that is biotin. We have discussed TPP, we have discussed PLP, we have discussed the FAD, we have discussed lipoic acid, and we have discussed folic acid, now we have discussed biotin.

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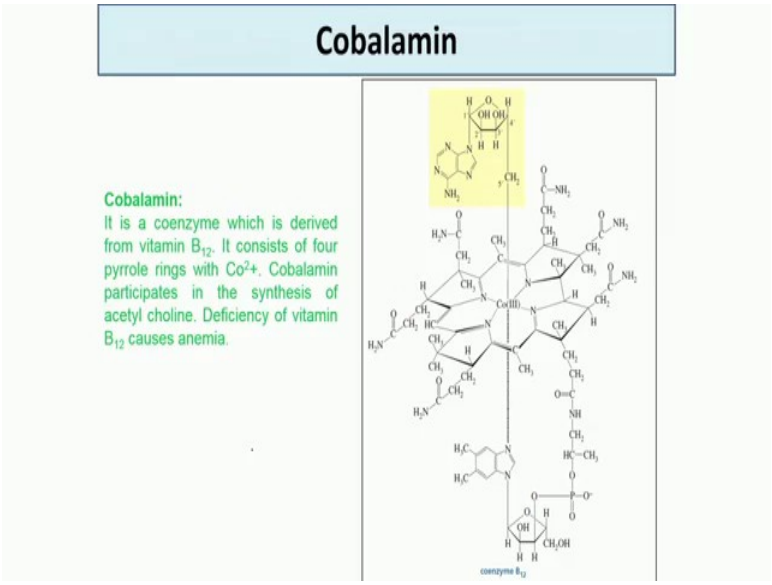


So, next remaining is called cobalamin that is vitamin $B_{12}$ group.

So thank you.