Organic Chemistry In Biology And Drug Development
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Lecture – 37
Chemistry of Cofactors/Coenzymes
(Contd.)

Welcome back. In the last session, we have showed you the structure of thiamine pyrophosphate which is the coenzyme form of vitamin B_1.

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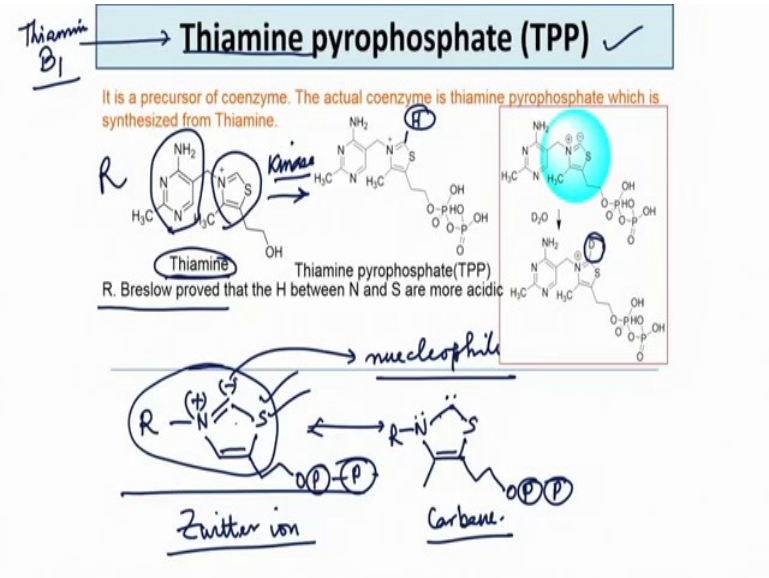

Thiamine has got a pyramide ring and thiazolidinium ring and it is present as the pyrophosphate in the coenzyme form. We have discussed the various structural aspects that it can exist in zwitter ionic form which is also stabilized by the 3d orbitals of the sulphur. Here you can also have the carbene form. So, these are the two forms. But this is the predominant form. Predominant form makes this carbon very nucleophilic. So, if there is an electrophilic carbon then it can react with that electrophilic carbon.

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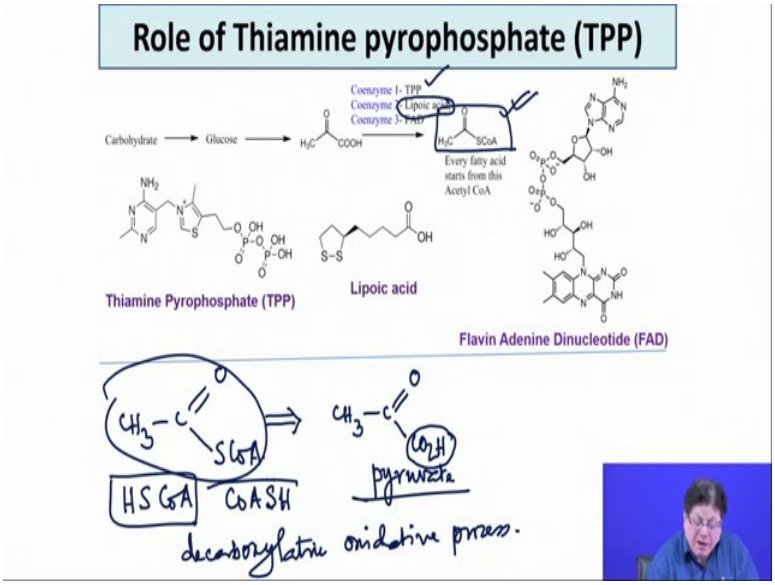

Thiamine pyrophosphate participates in many reaction. But one very important reaction which we are going to discuss is the formation of acetyl coenzyme A, $CH_3COSCoA$. Acetyl coenzyme A is the building block for biosynthesis of various long chain fatty acids. The building blocks are used for making the steroids like cholesterol and from cholesterol you get different types of hormones. So, formation of acetyl CoA is biologically very important.

Now, what is acetyl CoA? That is CH_3 structure is given C double bond O and this is SCoA. That means, it is a thioester of what? Thioester of coenzyme A, this is the structure of coenzyme A. You can write it CoASH. So, it ends up with a sulphur. So, it has got a sulphur SH arm and that is a very good nucleophile. So, it is a thioester of coenzyme A. Here the acid component is acetic acid. So, it is called acetyl coenzyme A.

Now, what is the starting point of acetyl coenzyme A? Now, this is the retrosynthesis or retro biosynthesis. What is the source of this, acetyl coenzyme A? The source is nothing but the product of the metabolism of glucose. Glucose is broken down to the final product pyruvate in glycolytic cycle. So, this pyruvate undergoes decarboxylation.

Decarboxylation should lead to the aldehyde stage, but it is not in the aldehyde stage. This carbon is in the acid stage, so it has to further oxidized. So, it is a decarboxylative oxidative process. This reaction needs actually various Coenzymes. Although the

mechanistically it is very simple organic chemistry, but there are 3 different different coenzymes are involved in the process.

First of all, first coenzyme that is required is called thiamine pyrophosphate. So, thiamine pyrophosphate first does the decarboxylation. Then there is another coenzyme which is called lipoic acid.

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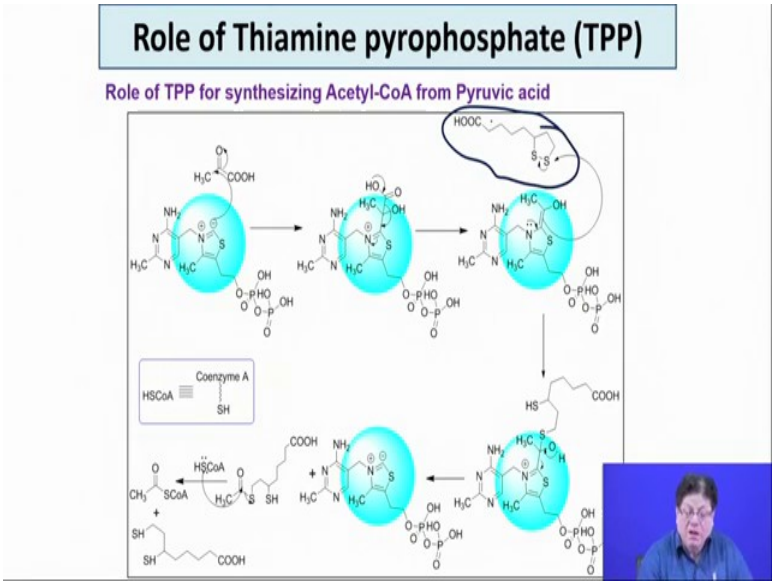

It is a C-8, 8 carbon fatty acid, but containing a disulfide bridge, it is a 5 member disulfide bridge. So, the second coenzyme that is required is what is the lipoic acid. The third coenzyme is called flavin adenine dinucleotide. All the structures are given here this is lipoic acid and this is thiamine pyrophosphate and this is flavin moiety . You know riboflavin is vitamin B_2.

So, that is converted into the active coenzyme form by the formation of a di-nucleotide because there is one type of base and then phosphate. There is this base this is a sugar also ribitol and the phosphate, so flavin adenine dinucleotide, FAD. So, 3 coenzymes are needed to carry out this reaction and I will show the mechanism slowly step by step.

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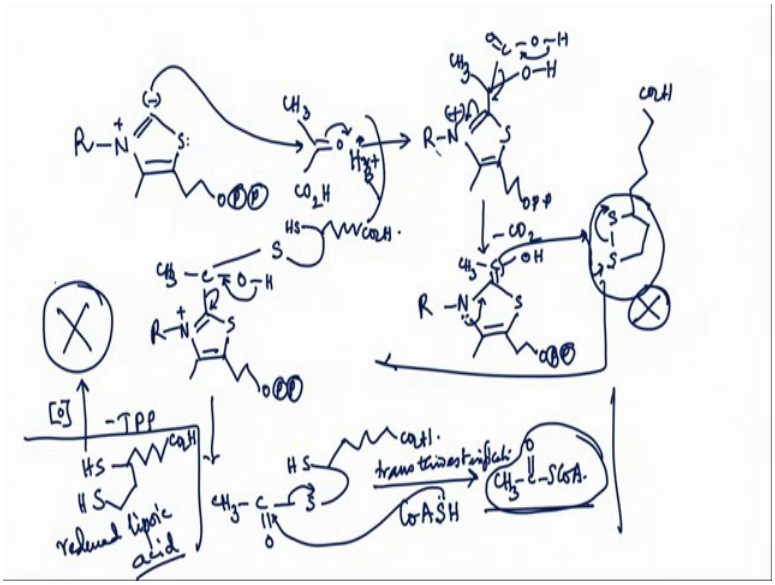

This is the structure of this TPP, thiamine pyrophosphate. We will simplify the writing of the structure. This is phosphate, this is methyl, and this is the minus and that is plus. Your substrate is pyruvate,

If there is a good electrophilic carbon then this can act as a nucleophile. Because this alpha carbonyl moiety in the pyruvic acid is very electrophilic because of the presence of the electron withdrawing carboxylic acid.

So, you have RN, then double bond, then S, then thisand then OPP. CH_3 and this takes up a hydrogen. It takes up a hydrogen most likely from the enzyme active site amino acid which is having a basic site. That is present in the conjugated acid form and then that can be donated. So, I am saying is t

hat BH plus, the B is having the plus charge so that takes the hydrogen and the B is released as the base. So, that becomes OH and then you have this C double bond O-OH.

Now, just like a scenario in pyridoxal phosphate it can act as an electron sink and after acting as electron sink in acts as a electron source. The same thing happens here. This is the alpha carbon with respect to the carboxyl, this is the beta carboxyl. You have this double bond N plus. It is a very good electron withdrawing group or electron sink. This type of process can take place.

This is the decarboxylation. You get minus CO_2. The nitrogen becomes neutral and this these are the substituents of thiamine pyrophosphate. Here you have double bond C methyl and then OH. Now, again what happens here? That nitrogen pulls up the electrons. This whole system loses the aromaticity because of the sulphur lone pair participation in this ring completing the 6 electron Huckel system.

So, it again once to regain the aromaticity making this carbon quite nucleophilic. If there is another electrophile, now this electrophile is basically in the form of lipoic acid. One more CO_2H is there in the form of lipoic acid. This sulphur and the disulphide bond is very vulnerable. You get the SH. SH means the hydride attacks this sulphur and this goes and take the hydrogen. So, this acts as a nucleophile and attacks the sulphur and then this one goes out.

So, the product is now N double bond S, this is the structure, this is R. Now, this becomes again plus and then this is methyl, OH and then you have this S and then I can just write this is SH and you have this carboxyl side chain. So, that is the fate of this reaction. But this is not very stable though it is aromatic. That is why this reaction is taking place. But now you can release it. It will be most stable if it is in the original zwitterionic form.

So, how it can do that? This which H is again released and this carbon carbon bond breaks, releasing the TPP. Because that is the original starting point TPP and in the process what you are getting is CH_3 and then CO, then you have SH and CO_2H. So, this is a thioester now. This thioester is from the reduced form of lipoic acid because now the SS bond is no longer there. So, now, there will be a trans-thioesterification.

What is that? You have coenzyme A which can displace the thiol that is coming from the lipoic acid. So, it is a trans-thioesterification step and that gives you this CH_3CoA. What is released here? Released is the reduced form of lipoic acid 1, 2, 3, 4, 5. So, this is the reduced lipoic acid. But you have got your product what you wanted. The acetyl coenzyme A is the biosynthetic block for synthesis of various other large molecules like fatty acids, steroid, hormones etcetera.

Now, the question is the lipoic acid which is released is in the reduced form. So, this lipoic acid molecule cannot participate in the next time because the participation has to be by the oxidized form of lipoic acid. Because, if it is not converted then the reaction

will need a stoichiometric amount of nucleic acid which is not desired in any catalyzed reaction.

Stoichiometric amount of the catalyst is required where the catalyst is basically enzyme plus coenzyme. So, there must be regeneration process of this reduced lipoic acid into the oxidized form of lipoic acid. Remember there were 3 requirements of 3 coenzymes, one is the TPP that does the decarboxylation, then the lipoic acid that does the thioesterification. Then it also does this trans-thioesterification by the coenzyme A. As coenzyme comes and then causes the trans-thioesterification releasing the acetyl coenzyme. In the process, the lipoic acid is reduced. So, it has to be oxidized. So, there is one more coenzyme requirement that is FAD. FAD is a coenzyme which participates in redox processes.

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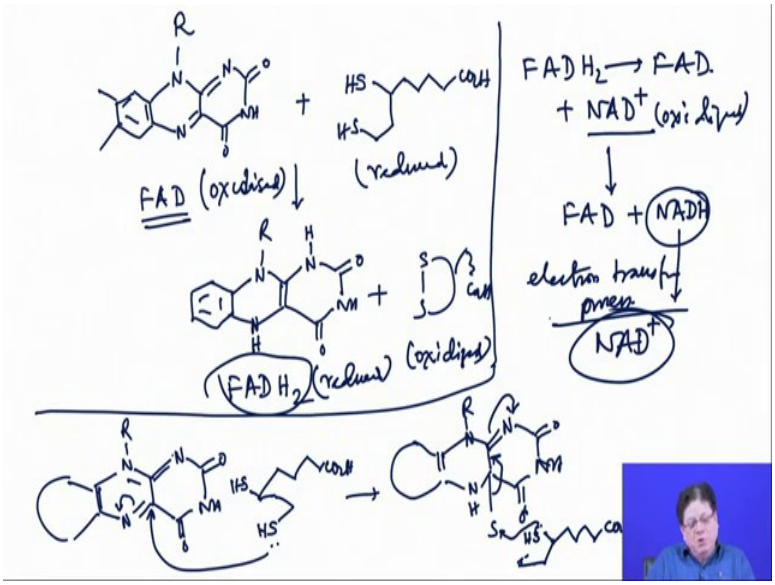

This is the FAD molecule, so there are two substitutes here. This is the R, R means that ribitol and then followed by diphosphate, then the sugar ribose, then added then the adenine. So, it is flavin adenine dinucleotide.

Now this has to be in the oxidized form. This is the lipoic acid SH 1, 2, 3, SH and then you have 1, 2, 3, 4, 5. This is the reduced form of lipoic acid. So, when the reaction takes place this goes to the reduced form, and the hydrogens are reduced form is addition of hydrogen.

So, the hydrogen are added to both the nitrogens and the double bond. So, basically this becomes $FADH_2$. How is it happening mechanistically? So, mechanistically this is happening in this way. We have NR and then double bond N. This is the aromatic ring here. Here you have again double bond NCONHCO and this. You have this SH and here you have this CO_2H. This SH first attacks this double bond making this nitrogen negative which then abstracts the hydrogen and that goes to the first part of the reaction.

So, one of the nitrogen is converted to the NH. This is N, that is R and you have NH here and double bond N here. So, that nitrogen has not got the hydrogen yet, but this nitrogen is already reduced to the NH. Now, you have S and then you have CH_2CH_2, then SH and you have this carbolic acid 1, 2, 3, 4, 5, CO_2H. Now, there will be an intra molecular disulfide formation. So, this attacks the sulphur, this breaks and this nitrogen takes up the hydrogen. So, if that happens you are getting this is the oxidized form of lipoic acid.

This is the oxidized form of lipoic acid and this is a reduced form of flavin. This is the simple mechanism. This is basically addition of this moiety, this one of the thiol and this intra molecular attack. Intra molecular nucleophilic attack leads to forming the disulfide resulting in reduction. Because the disulfide means this part is oxidized, so this part has to be reduced. So, that goes to $FADH_2$. That is the mechanism of flavin adenine FAD mediated reaction. This is the mechanism by which lipoic acid is again oxidized back to the active coenzyme form.

But this also gives rise to another question that is the next time when lipoic acid will be reduced next cycle of reaction. Who is going to oxidize that? Because the FAD is converted to $FADH_2$. So, now, there has to be another way of making this $FADH_2$ into FAD. There should be another oxidizing agent in the process, where $FADH_2$ is converted to a FAD and that is done by NAD^+ that is your vitamin B_3. NAD is nicotinic acid adenine dinucleotide. We will discuss that mechanism also.

This is basically an the acceptor of electrons because it is a positive nature species. That is the oxidized form of the vitamin of the coenzyme from vitamin D_3. Then the reaction takes place. NAD^+ brings the $FADH_2$ to back to FAD the oxidized form and in the process this goes to NADH. Again it will give rise to another reaction that what happens to the NADH, because unless you have NAD^+ your $FADH_2$ cannot be converted into FAD and unless you have FAD your reduced lipoic acid cannot be converted to the to

the oxidized form of lipoic acid. So, it is a cycle of effects. Finally, the NADH goes to the NAD plus by the electron transfer process.

So, just to summarize that what happens? At first the FAD goes to the the TPP, then it carries out oxidative decarboxylation of pyruvate, then the lipoic acid comes into play and converts the pyruvate into a thioester containing the reduced lipoic acid. In the process TPP is released, so that it can participate in the next cycle of reaction.

Now, this thioester of lipoic acid, acetyl thioester that will undergoes trans thiosterification with coenzyme A and then forms the acetylCoA and releasing the reduced form of lipoic acid. Then the reduced form of lipoic acid is converted to the oxidized form the disulphide form by a FAD and FAD in turn goes to $FADH_2$ and this $FADH_2$ is finally, oxidized back to FAD by NAD plus and NADH is then again converted into NAD plus by a separate process which is done by some electron transfer mechanism.

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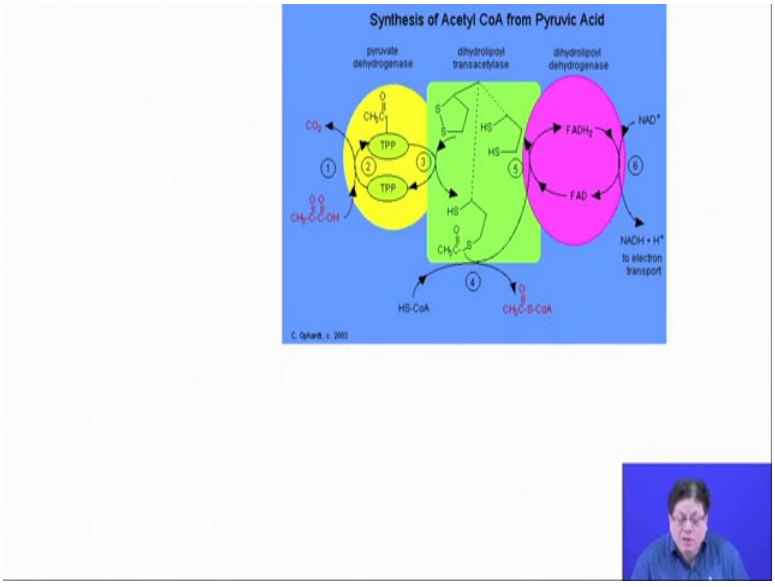

So, that is the chemistry of behind the formation of acetyl coenzyme and this has been summarized clearly here although it is little difficult because the colour combination is not very good. This is pyruvate. The first reaction is carbon dioxide goes TPP comes basically, this is a TPP immediate reaction and that holds of the acetyl group and then the acetyl the lipoic acid has reacted whatever I have said this is the thioester and then there

is a exchange that is the transacetylase reaction and it goes to the again the oxidized form. Who does that? FAD.

So, FAD goes to the $FADH_2$ and finally, $FADH_2$ is oxidized by NAD^+ and then NAD plus again finally, goes to the electron transport processes and then by which it can again be regenerated into NAD plus. So, that is the full mechanism. It is a very complicated process, but it is a very absolute requirement for the substance for the existence of the living system.

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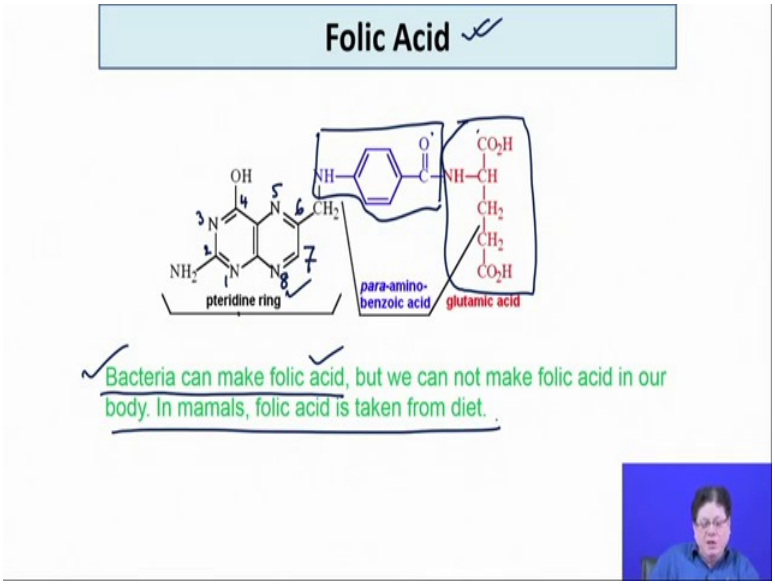

Next in the vitamin B group of coenzyme chemistry. Next vitamin that comes is folic acid. Now, folic acid again is a very obligatory coenzyme. It is absolutely required for the growth for maintaining the cellular division processes. We will go back, we will discuss the chemistry, but before that let us inspect the structure of folic acid. Folic acid looks bigger than the pyridoxal phosphate or thiamine pyrophosphate.

It contains a heterocyclic moiety which is called a pteridine ring. So, this is a pyramidine fused to 1,4 diazene, and then pteridine ring is attached by a CH_2 to para amino benzoic acid. Then this the acid group of para amino benzoic acid is attached by an amide bond into the glutamic acid moiety. So, this is basically a pteroyl para amino benzoyl glutamate or glutamic acid. That is what is folic acid.

Now, the numbering system goes like this is 1, this is 2, this is 3, this is 4, this is 5, this is 6, 7 and 8. Interesting fact about folic acid is that this is not the coenzyme form, this is the vitamin. So, this is the vitamin, this has to be transformed into the actual coenzyme form. Now, bacteria can make their own folic acid. In fact, it cannot take up any folic acid from outside. See if there is a exogenous supply of folic acid, it will not utilize that. It will always make in its own cell. It has got a biosynthetic machinery to make the folic acid.

On the other hand, we humans are part of the mammals. So, we cannot synthesize folic acid, but this folic acid has to be taken from outside; that means, through diet. So, through our diet we take the folic acid. So, there is a difference between the bacteria and the human.

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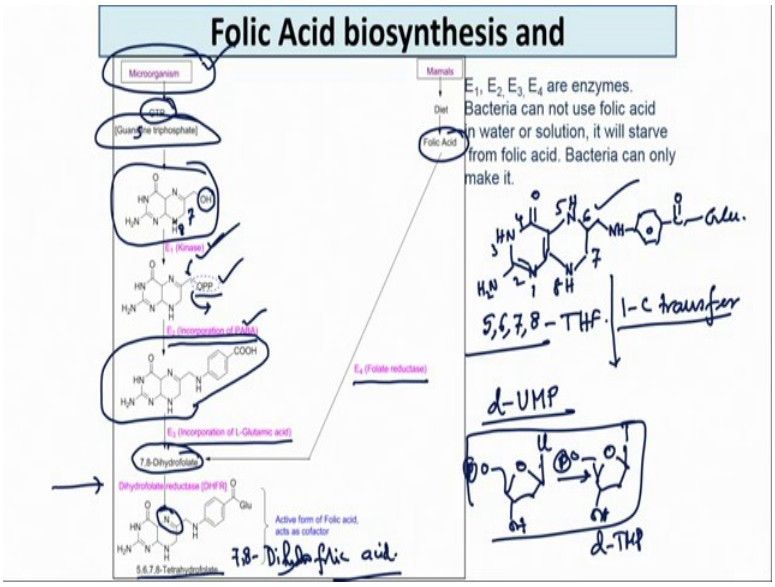

Now, what is the active form of folic acid? It is this one, that this is NH, this is NH, and then you have CH_2, this is NH, this is your para amino benzoic acid, and that is attached to glutamic acid.

So; that means, it is again I write 1, 2, 3, 4, 5, 6, 7, 8, so this is 5, 6, 7, 8; 5, 6, 7, 8 tetra hydrofolicacid that I can write THF. This is the active form of or active coenzyme form of folic acid. What type of reaction it does? It participates in reactions as a coenzyme supporting the actual enzyme to catalyze a reaction in which one carbon is transferred from a substrate into another substrate and resulting in the product. So, basically it

participates in one carbon transfer. So, one carbon transfer reactions are very important. I can give you an example that in RNA you have uracil and in DNA you thymine.

Now, uracil difference between thymine and uracil is basically one carbon. Thymine is derived from deoxy uracil monophosphate, that is called uridine monophosphate. UMP or uridine monophosphate means you have the uracil then the deoxy sugar and then you the phosphate. So, that uracil is converted into thymine deoxyribosyl phosphate; that means, that is called d-TMP. So, d-UMP deoxy uridine [noise] monophosphate; that means, here you have this OH, you have this phosphate and here you have uracil that is converted into the thymine, phosphate and here the OH.

So, that is the reaction. This is a very vital reaction because unless this d-TMP synthesized you cannot get the building blocks of your DNA. So, that is extremely important. That can give rise to anti-cancer compounds because you can stop the biosynthesis of DNA. If DNA cannot be made; that means, application cannot be done and your application cannot be done ultimately cell cannot divide and ultimately it has to die. So, there is no growth of the cell..

Just have a quick look how bacteria makes it is. Because we are not making the folic acid. We get the folic acid from diet. That chemistry is little bit easier, that folic acid has to be converted into the active coenzyme form. In the bacteria microorganisms there is GTP, the guanosine triphosphate.

First product in the biosynthetic pathway is dihydro pteridine nucleus. The second is this OH has to be replaced by para amino benzoic acid, but OH is the bad leaving group. So that has to be converted to a phosphate or a pyrophosphate. It is a kinase enzyme which does pyrophorylation. The pyrophorylation make it a good leaving group. Now, in presence of another enzyme, NH of the para amino benzoic acid and attacks here kicking out the pyro phosphate and this para amino benzoic acid now is attached to the pteridine nucleus.

So, this is not yet the folic acid. But remember you started all the time with 7,8-dihydro. So, this is one enzyme, that is another enzyme. First enzyme converts pyrophosphate; second enzyme diplaces the pyrophosphate by para amino benzoic acid. Then the third enzyme in this biosynthetic machinery is the enzyme involved incorporation of glutamic acid.

This is not tetrahydrofolic acid, this is the dihydro 7,8-dihydrofolicacid. Your active coenzyme form is this one, 7,8-dihydrofolate has to be reduced. This double bond has to be reduced in order to get this fully the saturated form here. So, that is done by an enzyme called dihydrofolate reductase.

So, these are the 4 important enzymes, one is the kinase, another is incorporation of paba, and then the third one is this incorporation of glutamic acid, and the fourth one is dihydrofolate reductase by which you will get 5, 6, 7, 8, tetrahydrofolate. What about in humans? The humans from diet you get folic acid. So, double bonds is here as well as here.

There is an enzyme called folate reductase. This folate reductase forms the dihydrofolate, 7,8-dihydrofolate. So, folate reductase can reduce 7,8 double bond in folic acid to form the 7,8-dihydrofolate. This is a human dihydrofolate reductase that converts the 7,8 dihydrofolate into the tetra hydro folate 5, 6, 7, 8 tetra hydro folate. Remember, this when it occurs in bacteria this is the bacterial dihydrofolate reductase. When it occurs in the human it is the human dihydrofolate reductase and when it occurs in a parasite, so that will be a parasitic dihydrofolate reductase.

Why I am spending so much time on this biosynthesis? Because, understanding the folic acid biosynthesis has given rise to different types of drugs, one is antibacterial drug because if you can stop producing this dihydrofolate from the in the bacteria. You will get antibacterial compound.

So, by selectively inhibiting parasitic dihydrofolate reductase you can get anti-parasitic compound like antimalarial compounds and if you can selectively elevate this dihydrofolate reductase in human you will get an anticancer compound. And if you can selectively block the dihydrofolate reductase in bacteria, you again get antibacterial compounds.

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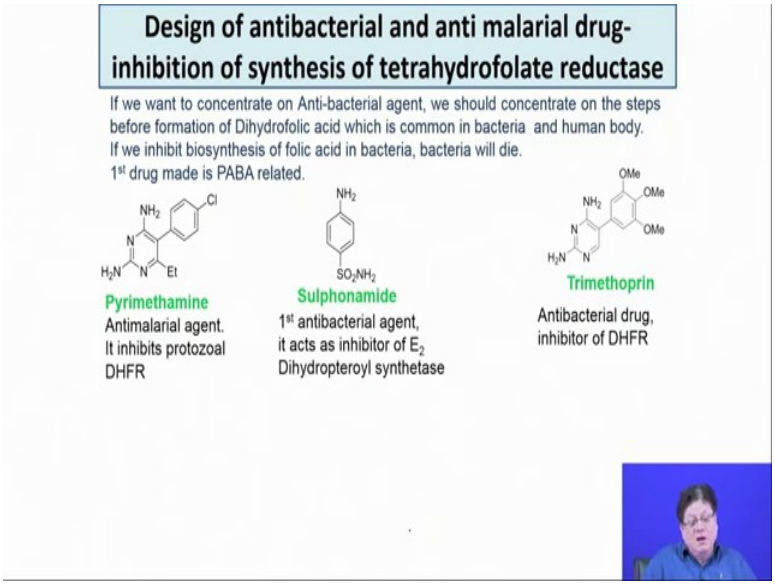

So, there are 3 different classes of drugs that you can get. Some names are given here. Pyrimethamine is an antimalarial agent, then sulphonamide which is a first antibacterial agent to be used. This is another drug called trimethoprim which is an anti-bacterial compound.

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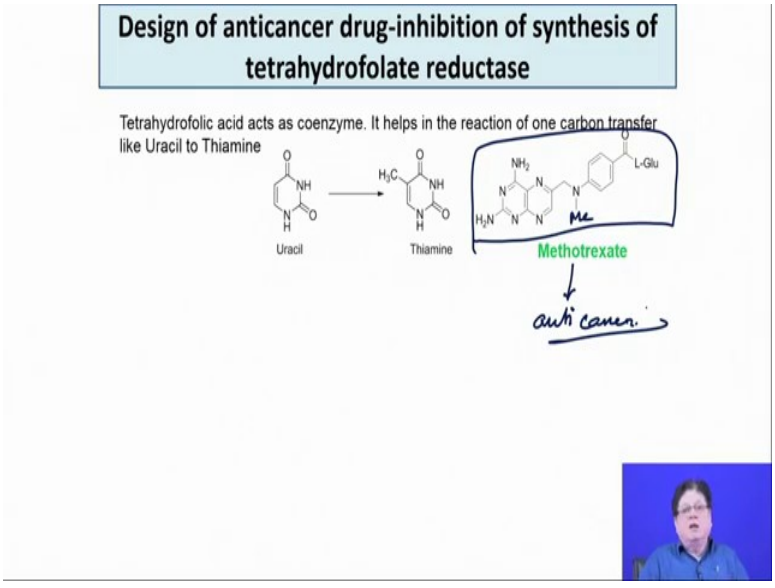

There is a one more compound anti-cancer compound, that is the called methotrexate and you will see methotrexate has a structure which is very similar to the folic acid. Folic acid as a carbonyl here, here it is the NH_2 and folic acid has here NH and instead of NH

there is a methyl here, but it is a very it is a structural mimic of your folic acid. It is a very famous anticancer compound.

In the next session, we will discuss the how these types of compounds have evolved and then finish of the coenzyme chemistry. Remaining coenzymes are the vitamin k, some other B group of coenzymes like cyanocobalamin that is the B_{12} and biotin.

Thank you.