Organic Chemistry In Biology And Drug Development
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Lecture - 38
Chemistry of Cofactors/Coenzymes (Contd.)

Welcome back to this course on organic chemistry in biology and drug development. In this session we will complete the remaining coenzyme chemistry and we will show the action of a metal ion as a cofactor also. Last session we ended up by saying few aspects of folic acid biochemistry. We have told you that folic acid is a part of the vitamin B group and it is bio synthesized by the bacteria.

So, bacteria can bio synthesize folic acid but we as humans cannot do that. We take the folic acid from the diet.

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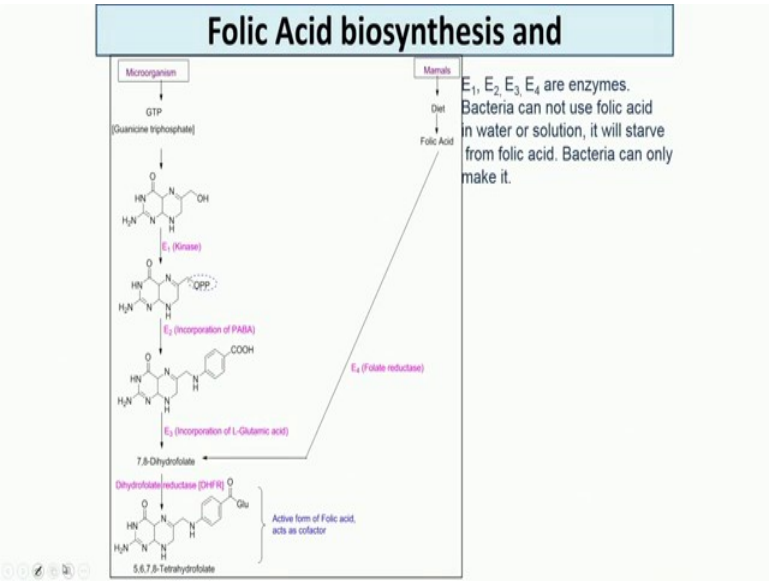


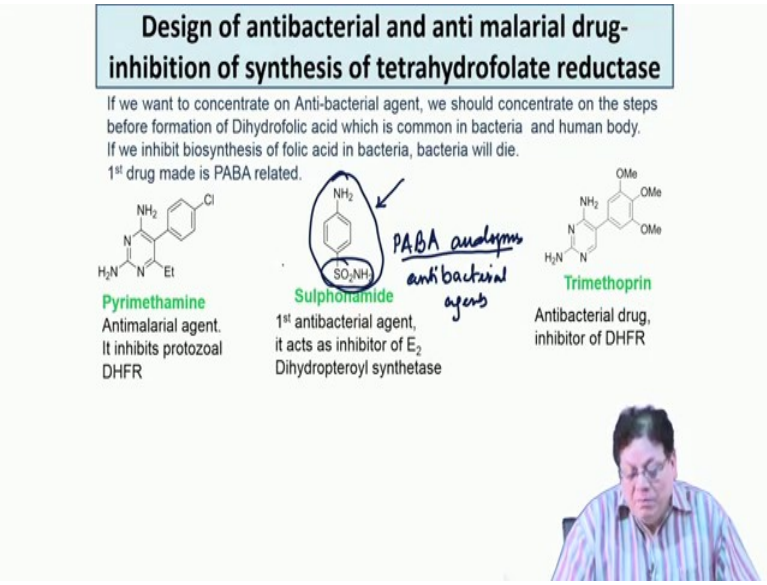
In case of bacteria, the biosynthesis of folic acid starts with GTP which undergoes rearrangement and form the 7,8-dihydropteridine nucleus. After that it undergoes phosphorylation by a kinase enzyme followed by displacement of the pyrophosphate by *p*-amino benzoic acid.

So, the amine acting as the nucleophile and the next step is that it is the glutamic acid which forms the amide bond with the carboxylic functionality of *p*-aminobenzoic acid. The *p*-aminobenzoic acid is now hooked up to a dihydro pteridine nucleus and then that

gives rise to 7,8-dihydrofolate. Now this 7,8-dihydrofolate ultimately goes to 5,6,7,8-tetrahydrofolate which is the active form. It is a coenzyme form of folic acid and in case of mammals like humans we get folic acid from the diet.

Remember folic acid has fully unsaturated pteridine nucleus and then this folic acid has to be reduced to the 7,8-dihydrofolate by an enzyme called folate reductase and followed by again the similar enzyme dihydrofolate reductase to go to the 5,6,7,8-tetrahydrofolate. Now the dihydrofolate reductase is little bit different although the dihydrofolate reductase isolated from bacteria or from parasite or from human they perform the same kind of reactions but their structures are slightly different.

They are so that gives an avenue to develop molecules which specifically inhibits the dihydrofolate reductase DHFR in bacteria/parasite/human. So, lot of chemistry has been developed based on the folic acid biosynthesis and this differential way of getting folic acid and utilizing it bacteria and the mammals.

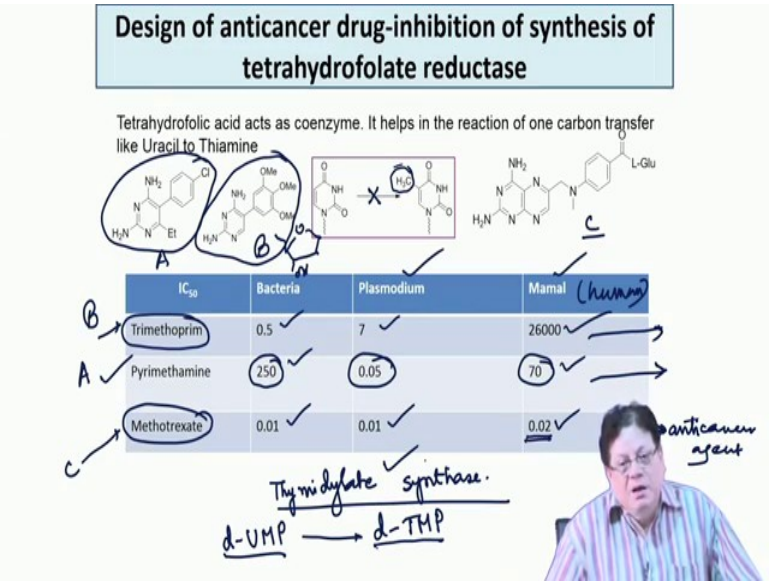


Now last time I also told you that the first type of drug that was developed was targeted to the enzyme which incorporates the para-aminobenzoic acid to the dihydropteridine nucleus.

So, para aminobenzene sulphonamide mimics the structure of the para-aminobenzoic acid and molecules which possess para amino benzene sulphonamide moiety can inhibit the enzyme which is responsible for in cooperation of the para-aminobenzoic acid. So, these are called PABA analogs and they are antibacterial agents.There are different

PABA analogs available, but the ultimate mechanism of action is that these PABA analogs ultimately release this para aminobenzene sulphonamide.When para aminobenzene sulphonamide is incorporated into the enzyme, the n enzyme thinks that this is the substrate in place of para-aminobenzoic acid and it takes a para aminobenzene sulphonamide. So, then it cannot hook up the glutamic acid which requires a carboxyl function at this position.

So, that was the first antibacterial agent which was based on the folic acid bio synthesis.



But then people realized about this dihydrofolate reductase. I told you already that there are differences in their affinity for the bacterial DHFR, parasitic DHFR and mammalian DHFR. Now because of there are these differences so you can specifically target one or the other DHFR and I have shown you here 3 molecules this is called pyrimethamine this one.

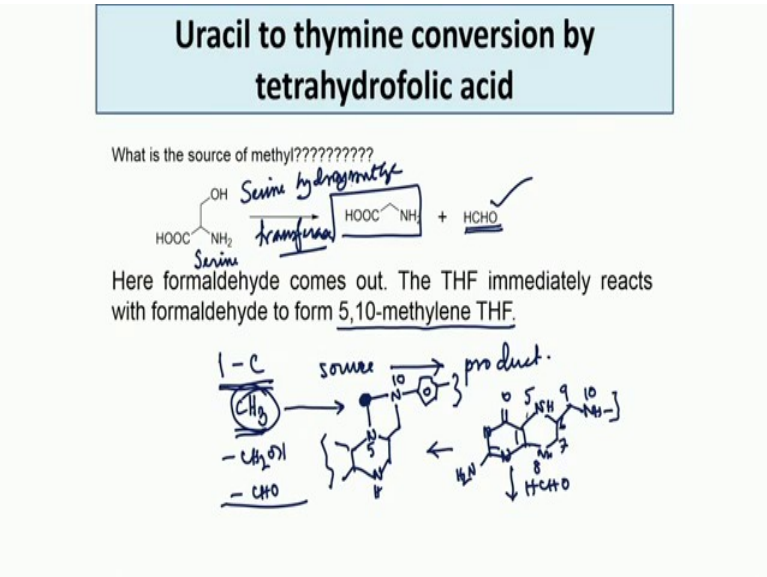
So, this is suppose A, this is A this is B. B is the Trimethoprim and C is Methotrexate that is this one which is very having great resemblance to the folic acid structure.

All these molecules are acting as drugs. For example, pyrimethamine acts on plasmodium and that is reflected in their IC_{50} values. IC_{50} is the inhibitory concentration required to inhibit 50 percent of the activity. So, IC_{50} value for plasmodium is 0.05 microgram per milliliter and for bacteria it is 250 and for mammal it is 70.

So, you see it is a extremely specific for plasmodium. So, Pyrimethamine is used as an anti malarial agent because plasmodium is the causative organism for malaria. So, this is an anti malarial agent. Trimethoprim is a bacterial dihydrofolate reductase inhibitor. The IC_{50} value for bacteria is 0.5, for plasmodium is 7. So, it is 10 about 14 times higher. Remember lower the IC_{50} value the stronger is the potency and for mammal it is totally ineffective because the IC_{50} value is 26,000.

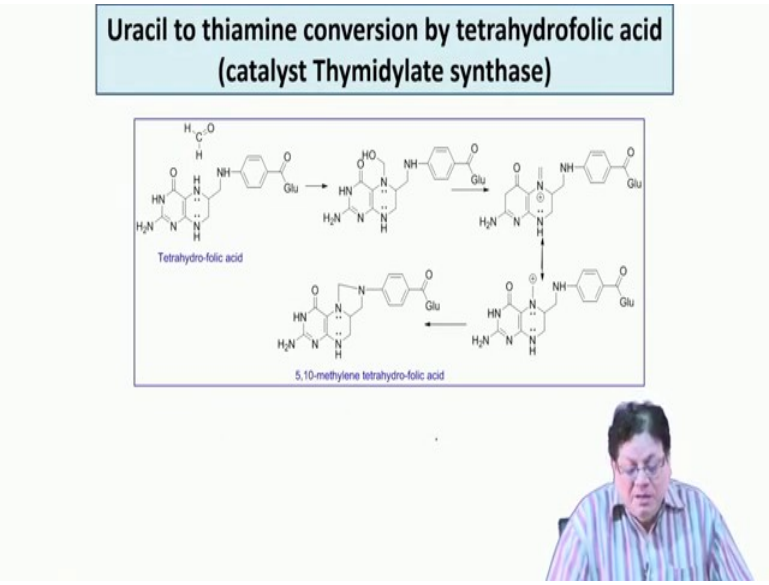
So, one can use this Trimethoprim as an antibacterial agent. For Methotrexate, IC_{50} value is 0.01 for bacteria and plasmodium and for mammal it is 0.02. So, it can act as an antibacterial agent, anti plasmodium agent and also it can act against dihydrofolate reductase of mammals. It kills the cells in the human body specially the cells which grows very fast *i.e.* the cancer cells.

So, Methotrexate is used as an anticancer agent. This is your antibacterial and that is the anti malarial. So, you see that you can target DHFR specifically and 3 classes of drugs have been developed. They are applied one to bacteria, another to this parasitic organisms and the third one is a human. All these 3 are available in the market.



Now, the question is that what will happen if you stop the dihydrofolate reductase from functioning; that means, if you stop the production of the tetrahydrofolate. It converts the dihydrofolate into tetrahydrofolate which is the coenzyme form of folic acid. I told you in the last session that tetrahydrofolate participates in the transfer of one carbon from some source to a product.

So, it transfers one carbon. One carbon means mostly it could be in the form of methyl or it could be in the form of CH_2OH.Here we will be discussing only the transfer of methyl because that is extremely important.



Thymine is present as the base in DNA and Uracil is present as the base in RNA.

So, this thymine is generated from uracil which is attached to the sugar and a phosphate and it is called the uridine mono phosphate. So, that is converted into thymidine mono phosphate. This methyl is the one which is transferred from a source to the uracil moiety and thereby resulting in the thymine. How can you stop this conversion? This can be stopped by inhibiting the DHFR because this is a DHFR catalyzed reaction and enzyme that carries out this reaction is called thymidylate synthase.

I will tell you what the genesis of the name is. This conversion is basically what you are having from uridine monophosphate to thymidine monophosphate. This is also can be abbreviated as d-UMP. Deoxy because this is the sugar with the 2 prime oxygen not there. So, this is d-UMP converting to d-TMP. This is also known as uridylic because the phosphate is actually an acid when it is attached to the 5 prime OH. It is phosphorous and there are 2 ionizable groups and so it is an acid. So, it is called uridylic acid and this is called thymidylic acid and since this reaction generates thymidylic acid which is present at the biological ph as thymidylate.

So, it is called thymidylate synthase. Now this is the enzyme which is prevented from acting properly; that means, the methyl transfer does not take place. How it is

specifically transferred by tetrahydrofolate? What is the mechanism of this reaction? Now before that we have to figure out what is the source of this one carbon unit. The source of the one carbon unit is this is serine amino acid.

So, serine if you remove the CH_2OH group in the form of formaldehyde then you get glycine and this is what will be called serine hydroxymethyl transferase. It catalyzes the conversion of serine to the glycine and formaldehyde. But this formaldehyde is very toxic. If formaldehyde is generated in the body it is toxic unless it reacts. So, what happens that you have this tetrahydrofolate, I just write the structure tetrahydrofolate here, this is carbonyl, this is in NH_2 and there is a double bond \and double bond there and then this is NH, this is NH and this is the CH_2 and then you have NH and subsequently the p-amino benzoic acid and those things. So, this is the tetrahydrofolate. I showed you numbering that this is your 1 2 3 4 5 6 7 8.

So, this is 5 this is 6 7 8 that is why it is called 5 6 7 8 tetrahydrofolate, but now you have to extend the numbering. Once the ring numbers are done then this will be called the next one 9 and this is 10. So, this tetrahydrofolate molecule reacts with formaldehyde and forms a derivative which is called 5, 10-methylene THF; that means, it goes into this structure and then you have this NH. Here is the other aromatic ring I am not drawing that and this becomes N and this formaldehyde is now hooked to act as a agent to link up these 2 nitrogens and then this is para amino benzoic acid moiety. So, this is called this is 5 and this is 10.

So, 5, 5,10-methylene tetrahydrofolate is the source of your one carbon unit, but this is not the end of the reaction. This is so first; it is a coupled reaction serine hydroxymethyl transferase breaks up into formaldehyde that formaldehyde reacts with the tetrahydrofolate forming 5,10-methylene tetrahydrofolate. I think this is the mechanism of formation of the 5,10-methylene.

So, the formaldehyde this nitrogen first will first react because this is more nucleophilic and then that attacks the carbonyl and that becomes CH_2OH. So, this is the first step. This loses the hydrogen and in the second step, lone pair goes here, the OH comes out as water and then you form this iminium ion and then the nitrogen attacks the terminal methylene carbon and this comes back to the nitrogen.

So, that nitrogen becomes neutral. You can draw a resonating structure like this and then also using the resonating structure you can add this nitrogen; the 10 nitrogen 10. If you do this reaction it actually violates Baldwin's rule because it is an endo cyclization which is unlikely. There are many ways to show the mechanism either direct displacement by this nitrogen lone pair and kicking out. So, that utilizes this type of resonating structure and bypass the Baldwin's rule problem, but this is the mechanism of formation of this methylene-THF.

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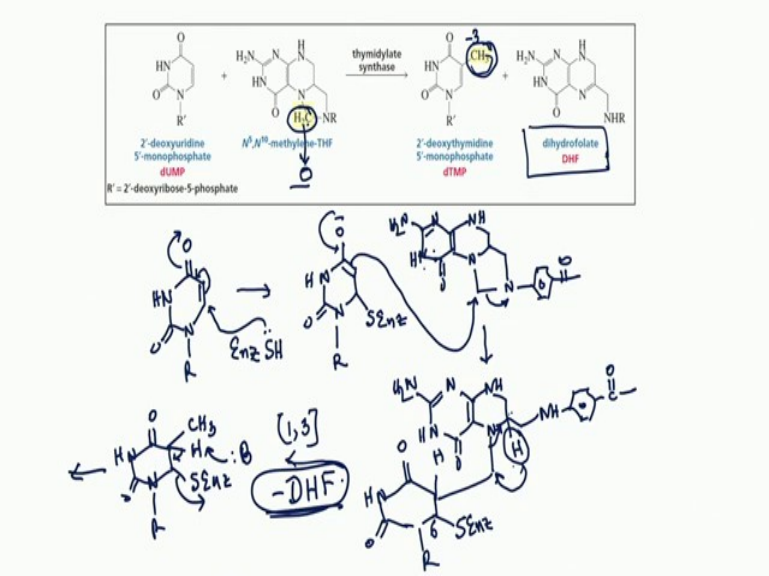

Now, this methylene-THF actually transfers this methylene into this uridine molecule and this methylene comes from CH_2 and in the process the tetrahydrofolate is reduced to dihydrofolate because this methylene is in the oxidation level of an aldehyde. Subsequently it is converted to a metal; *i.e.* this is reduced.

So, the tetrahydrofolate in the process is converted into dihydrofolate. It is a reduction reaction. Let me again just work it out this is a methylene which is at the oxidation level. Here the oxidation level of the carbon is 0 and here the oxidation level of this carbon is minus 3. So, it is a reduction here. So, in this case the tetrahydrofolate now is devoid of 2 hydrogens. So, that is now oxidized to the dihydrofolate. So, this is reduced and that is oxidized.

What is the mechanism? This is your uracil molecule and the enzyme thymidylate synthase has a active SH in the form of a histidine. So, it attacks here like in a Michael

type 1,4 addition. So, that is the first formed species. So, you get NH and then O minus and then you have this double bond and this N and the carbonyl and this is R and here you have S enzyme.

Now this methylene-THF. I will just write this part NH N. So, that is your NH_2, this is your carbonyl and this is your NH.

So, you do not have the other bonds. So, I have to erase this one and that one but the nitrogen is gone.

So, I have to write the nitrogen N and this is connected here. This is methylene-THF and it has been inverted by 180 degree and then you get this structure. So, it is N and then the benzene ring. So, this enolate comes back and this carbon becomes a good nucleophile. These now attacks this methylene and carbon nitrogen bond has to be broken because it stimulates the aromatic ring attached to the carbonyl. So, it can react to the carbonyl.

So, this is the one which is first broken and you get your NH_2, this is your carbonyl and that is NH then N and this now released as the *p*-amino benzoic benzoyl, then this is glutamic acid. So, this is attached to uracil moiety. This is double bond O, this is N, R and this is the scenario N R. So, you have a hydrogen here and your enzyme is hooked up at this position 1 2 3 4 5 6.

So, this is the 6th position. At the 6 carbon, the enzyme with the help of sulphur it is linked carbon sulphur bond. The first reaction is the Michael type reaction forming the enolate, then the enolate attacks through the reactive carbon and that makes an attack on the electrophilic methylene and resulting in the cleavage of this carbon nitrogen bond first. Then this hydrogen undergoes a 1 3 sigmatropic shift.

This is the other aromatic ring. This is N R, this is the NH and this is your double bond O. Now this is hydrogen. This is becoming a methyl now and basically you ended up with the uracil -the thymine has not been produced here but the methyl has been transferred.

So, now you have to expel S enzyme N, R double bond O, NH. Then the enzyme is released by a beta elimination. This hydrogen is very acidic. So, some base in the enzyme active site that abstracts this hydrogen and it goes off. Then you get thymidylic

acid. So dihydrofolate is released. The dihydrofolate will be reduced to the tetrahydrofolate and then that again participates in the reaction.

that the summary of the mechanism- first it is the Michael addition of the enzyme via the sulphur of a cysteine in the active site and then attacks the methylene resulting in the breakdown of the this carbon nitrogen bond followed by 1,3 migration of this hydrogen into the to the methylene. So, making it a methyl and this bond goes to make up the positive charge created here. Although this is pericyclic reaction-there is no positive negative charge. It is a cyclic transition state.

So, in one step dihydrofolate is coming out and you get this methyl. Then there is release of this enzyme by a base mediated elimination to give you the free enzyme to catalyze the next step of reactions. The dihydrofolate is again reduced by dihydrofolate reductase to tetrahydrofolate. However, there is one problem in this mechanism is that 1,3 shift is not allowed thermally. So, people objected to this mechanism.

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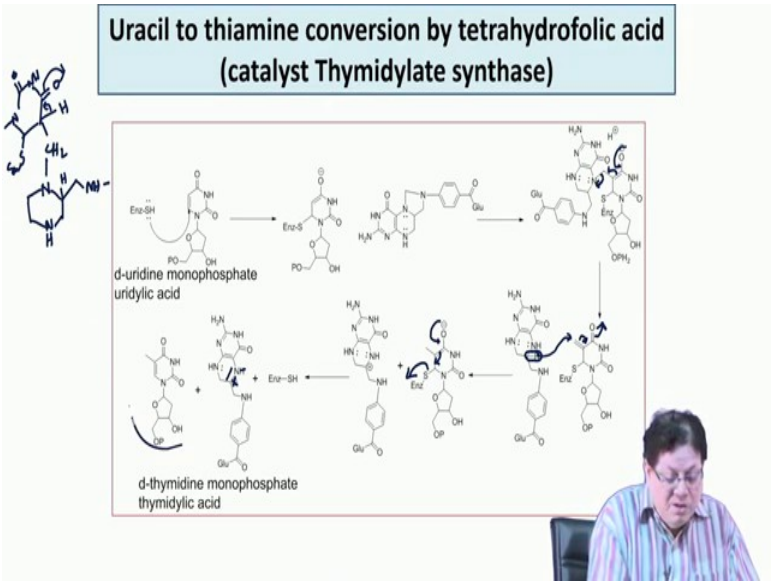

So, an alternate mechanism was proposed and I will show it quickly here. One more point is that when they put the deuterium in place of the hydrogen at that position then the deuterium ends up at the methyl. So, that will be CH_2D. So, there is no doubt that this hydrogen is migrating, but the mechanism seems to be little bit awkward. So, an alternate mechanism was proposed which is shown here. This is the first adduct that is formed when this enolate is attacking the methylene.

So, this carbon nitrogen bond has been broken. After the first reaction, you have CH_2 and then you have this uracil moiety N R. Here it is the S enzyme here, it is the double bond O and you have double bond O NH. So this is the intermediate and you have this *p*-amino benzoic acid attached.

So, earlier mechanism was hydrogen 1,3 shift migrating here, but now people have suggested that the first is basically formation of the enolate. So, this is the enolate and then this enolate comes here, this goes there and that comes to the nitrogen.

Basically you have an exocyclic methylene compound coming from the uracil moiety and hydrogen are very close by hooked in the active site of the enzyme. Now this hydrogen as hydride adds to these. You will get again an enolate, but this becomes a methyl. As the hydride attacks this as a nucleophile it becomes positive charge.

So, the nitrogen now loses the hydrogen and make a double bond here. Ultimately it should be double bond, the plus charge will no longer be there. This enolate is still hooked up to the enzyme. You bring that negative charge and kick out the enzyme.

In terms of mechanistic organic chemistry, earlier problem was thermal 1,3 hydrogen shift has to be antarafacial but antarafacial migration is not possible in a 1,3 system involving hydrogen. Remember we are not talking about carbon 1,3 shift that is possible but thermal 1,3 hydrogen shift is not allowed. So, this alternate mechanism has been suggested and most of the textbooks are showing this mechanism as the formation of the thymidylic acid.

So, this is your thymidylic acid synthesis.

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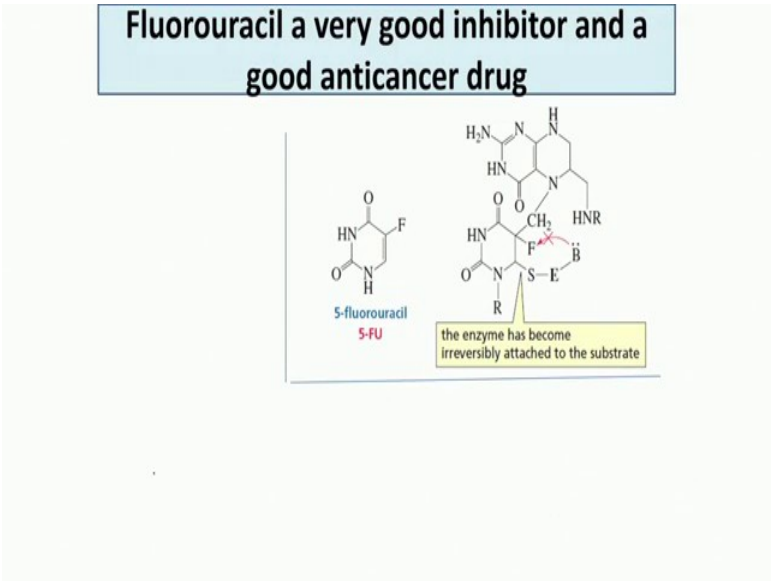

So, that just to finish up. Now if you have a fluorine instead of hydrogen it is called 5-fluorouracil. So, in the body it is attached to the deoxy sugar and then the phosphate. So, you will get fluoro uridylic acid instead of uridylic acid and this fluorouridylic acid we will have some problem. The problem is the second mechanism; that means, the correct mechanism what you have is the fluorine here instead of the hydrogen.

So, there is no possibility of enolization because there is no hydrogen. Here you have the fluorine and you cannot take up fluorine as a positive ion. Moreover this carbon fluorine bond is very strong. So, it is not possible to release fluorine as F plus not even also F minus because this bond is very strong. The whole thing is stuck at this stage. The coenzyme tetrahydrofolate is linked to this fluoro version uracil by the methylene. So, this is a stable species and this is an example of irreversible inhibition.

So, 5-fluorouracil is still used as a very good potent anticancer drug. So, there are 2 anticancer drugs one is Methotrexate and another is 5-fluorouracil. Methotrexate works by inhibiting the dihydrofolate reductase. It is a non covalent interaction, but the binding is very strong. It has a very high binding constant. The crystal structure of dihydrofolate and the methotrexate is known and it is said that the inhibition is essentially irreversible. Remember irreversible inhibition is formation of a covalent bond with the inhibitor and reversible is no covalent bond formation. It is all non-covalent interactions, but this methotrexate and dihydrofolate the binding is so strong. It is a collection of many weak

interactions which makes the binding very strong. So, this is called an essentially irreversible inhibitor.

So, DHFR methotrexate works by almost essentially irreversibly inhibiting the enzyme dihydrofolate reductase and on the other hand fluorouracil works by perfectly irreversibly inhibiting the thymidylate synthase.

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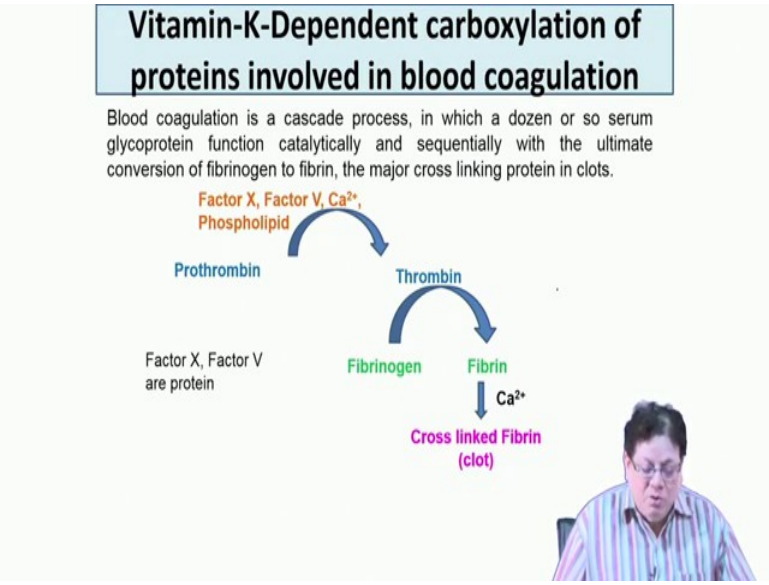

So, I think we have thoroughly discussed folic acid chemistry. This is very important as you see that so many drugs have been discovered based on the folic acid biochemistry.

Thank you.