Organic Chemistry In Biology And Drug Development
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Lecture - 52
Non Beta – Lactam Antibiotics

Welcome back. In this session, we will discuss other antibiotics which are not β-lactam antibiotics and study their mechanism of action.

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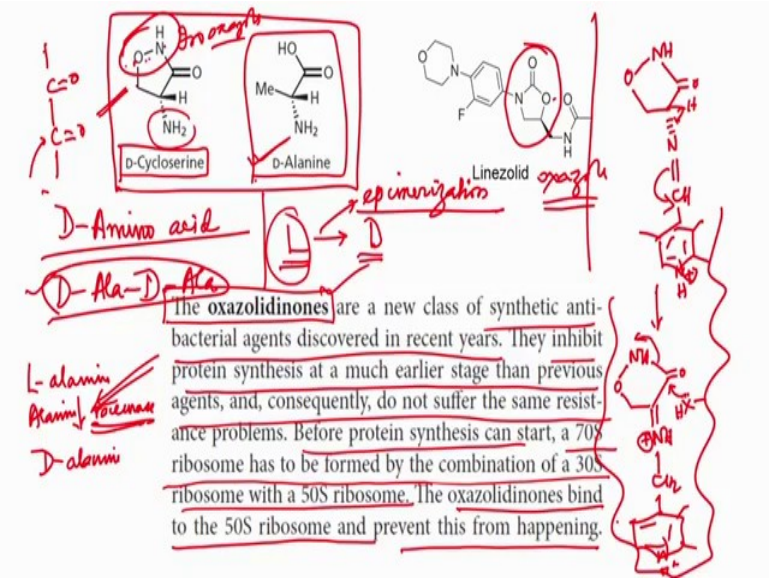

We raised that point that in the cell wall biosynthesis, what happens? You require D-amino acids like D-glutamic acid or D-alanine-D-alanine, that is very important for the cell wall biosynthesis. Now the question is from where this D-alanine is coming.

The D-amino acid is coming from L-amino acid because in the biological system, the amino acids belong to the L-configuration; I am not talking about plant origin; usually all are belonging to the L-configuration. So, the source of D-amino acid is nothing, but the L-amino acid.

So, in order to convert the L to D, what you need is epimerization. In organic chemistry, we call that as epimerization; if you remember now your biochemistry, the first part of this course, that epimerization is carried out by a PLP-dependent enzyme; PLP means the vitamin B6, the enzyme is the vitamin B6.

So, PLP-dependent isomerisation takes place when you convert L to D. We do not need any D-amino acid in our body, the host does not need. At least to this day, it is not known that there is the requirement of any D-amino acid; that means, this alanine racemase is the enzyme which racemizes alanine from L-alanine to D-alanine. This is done by alanine racemase.

The enzyme will be called alanine racemase. We human beings do not require this alanine racemase. So, if you can target the alanine racemase; that means, that will be a very good and very selective because we do not require this enzyme. Now there is an inhibitor for alanine racemase and that is called D-cyclo serine. What is cyclo serine? See on this side I have written the D-alanine. This is the structure of D-alanine; what is D-cyclo serine? You see it resembles the alanine very much. Instead of the methyl, you have CH_2; here the configuration is ok.

This is carbonyl instead of OH, you have nitrogen and then that is connected *via* this oxazole ring. So, basically now it resembles the D-alanine. So, it can now react with the alanine racemase and then inhibit the alanine racemase and the mechanism that is given in textbooks are like this that you have this CO. The first step of any PLP-dependent enzyme is the formation of the imine. Again just to brush up that earlier thing that this is present as a plus and in the plus form it is an electron sink.

And then what happens? This hydrogen is lost. So, this goes up to that point, nitrogen; then it comes back and stays at the CH_2 level. So, you have NH O and then CH_2, then you have double bond NH CH_2 and then the pyridinium ring. So, this is the situation now. Now this becomes plus; see PLP is attached to the enzyme do not forget the enzyme because everything is happening inside the enzyme.

So, now the enzyme nucleophile (any nucleophilic amino acid) that now attacks because this has a carbonyl, this is a very well-known fact in organic chemistry that if there are two carbonyls sitting side by side, then one of the carbonyl is very reactive. So, same thing is there; one is iminium, another is a carbonyl. So, this carbonyl is now very reactive. So, in the enzyme, any nucleophilic amino acid is going to attack here and opens this up; that means, you are forming a covalent bond, an acyl-enzyme covalent bond and that stays there.

So, this is the mechanism of D-cycloserine. So, I think this is used as the second line of defense in tuberculosis; second line, not the first line. First line of molecules are different; isoniazid, ethambutol, rifampicin etc; however, the second line of defense includes this D-cycloserine and it resembles D-alanine in structure. So, it is accepted by the enzyme and the mechanism is that it forms the imine, then there is a basically a tautomeric shift, it forms this imine which was earlier with the aldehyde carbon.

Now, it is with the cycloserine carbon and then that activates this carbonyl. So, the nucleophilic amino acid attacks and opens on the cycloserine. So, you can call it as a suicide inhibition; because there are changes that takes place in the molecule. There is another class of molecules which is quite recent, but that is entirely synthetic antibacterial compound, they are called oxazolidinones.

I think they are one of the modern discoveries of antibacterial agents. 1, 2 is isoxazole. 1,3 is oxazole. So, this is oxazolidinones because the double bond is not there.

So, oxazolidinones; that functionality along with these attachments can also be a an antibacterial agent discovered in recent years; they inhibit protein synthesis; protein synthesis means the translation. So, they inhibit protein synthesis. A 70S ribosome has to be formed by the combination of 30S and 50S ribosomes.

Again you remember that first the two sub units have to join together. Now this oxazolidinones bind to the 50S ribosome and prevent this 30S to bind to the 50S ribosome to make the whole complex that is the 70S ribosome. So, it stops the 30S from binding to the 50S; that is the oxazolidinones. So, that is their mechanism of action. So, it is basically a non-covalent interaction.

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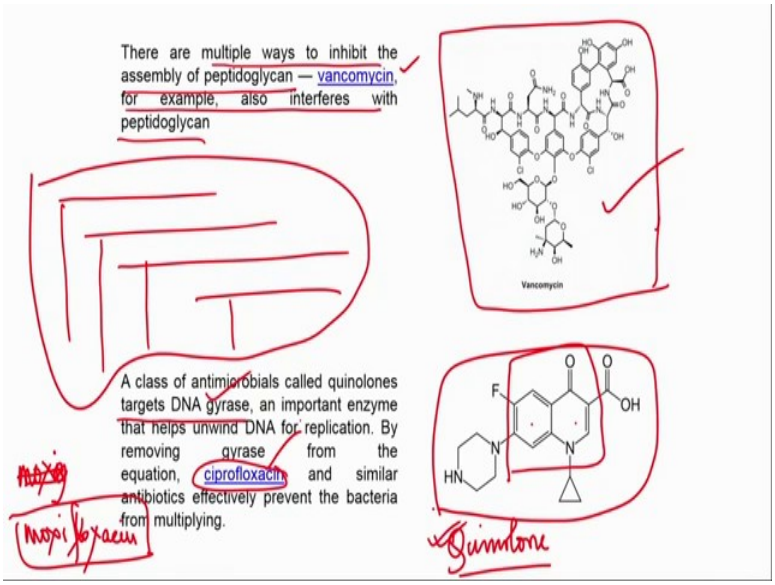

Then you have the vancomycin; one of our last line of defense. Vancomycin also stops the assembly of the peptidoglycan unit. So, the peptidoglycan has to assemble hanged from the glycan units, the peptide. Assembly means see you have the glycans like this and then you have this peptides hanging from this; so, that assembly has to be perfectly positioned.

So, that is inhibited by vancomycin; it's a big molecule, but that is again an antibiotic produced by a microorganism. Then you have another class of molecules which are called quinolone class of molecules; see quinoline is only 6 and another 6 with a nitrogen, but it is a quinolone with a carbonyl.

So, these quinolones are also very good antibiotics; at some point of time, when they came into the market, they worked extremely well. Their mechanism of action is to target the DNA gyrase; you know DNA gyrase helps in unwinding of the double helix during the replication. So, this quinolone is an inhibitor of the DNA gyrase; it uses non-covalent interactions it binds to the DNA gyrase and that stops the replication.

One of the famous quinolone compound is ciprofloxacin. Now this ciprofloxacin or norfloxacin, are the different generations that have come. Ciprofloxacin was the starting one, then came norfloxacin, then came levofloxacin, then gatifloxacin. Now today bacteria are getting resistant to them by reflex mechanisms.

So, ultimately now the best drug what is coming out in the market is moxifloxacin; let us see moxifloxacin. This is given for uninary tract infection; moxifloxacin works very well; ciprofloxacin or norfloxacin is now given to prevent diarrhea; if somebody is having diarrhea then this ciprofloxacin is given.

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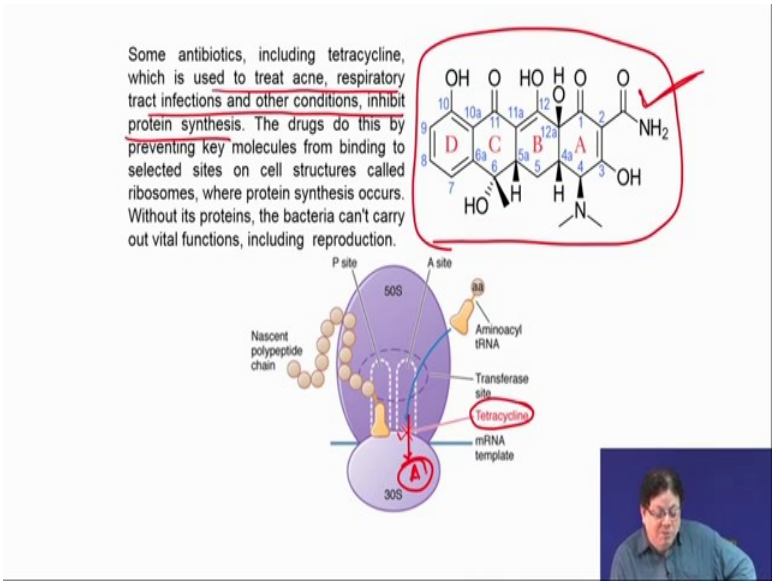

So, we should know some of these molecules; how they work; at least in terms of interactions because there is no covalent interaction here. So, no arrow pushing here. Then you have another group of molcules called tetracycline; it is a very broad spectrum antibiotic. However, the problem with all these molecules, specially this quinolone is that it is targeting DNA gyrase which is also present in human.

So, that is why they have much more toxicity then your vancomycin or your D-cycloserine or the penicillins because they have a different target which is not present in the host. So, now, what tetracycline does? Remember again the protein synthesis; you have a 50S ribosome and a 30S ribosome, you have this P site and the A site in the P site.

You have this growing peptide chain A site, the tRNA comes with the particular aminoacyl depending on the codon here. The tetracycline sits at the A site and blocks the tRNA from binding there. So, it stops the protein synthesis by binding to the A site. Tetracycline is used to treat acne, respiratory tract infection, other conditions and one another important use is that when there was this problem of plague outbreak in Surat, about say 10-15 years back, then the only drug that used to work was tetracycline.

So, people bought this tetracycline and stored it in their home for backup. So, tetracycline is very cheap; one of the cheapest antibiotic that is available in the market, but how does it work? It binds to the A site of the ribosome and then stops the protein synthesis.

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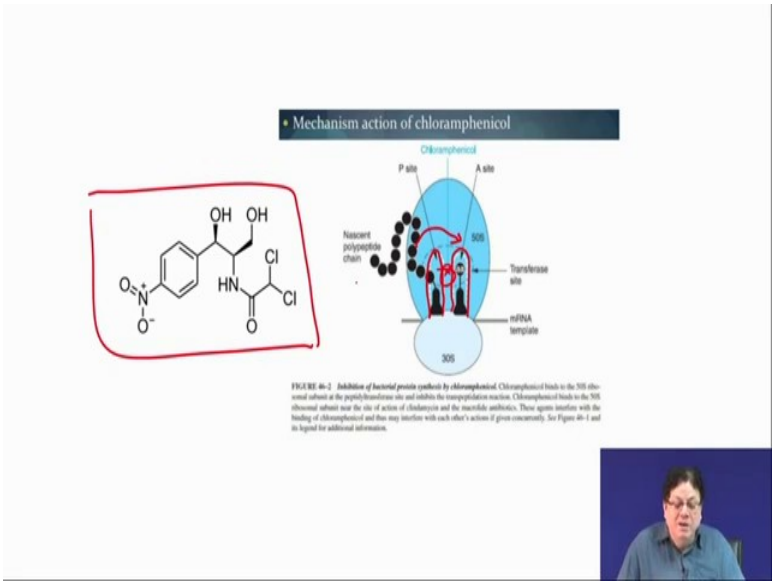

Then you have chloramphenicol. Chloramphenicol is given in typhoid; however, again I should remind that because these molecules are targeting some system which is also present in human so, they are bound to be toxic and that is indeed true, they are toxic.

So, you have to take only the prescribed dose. Chloramphenicol is rarely used these days, but it is one of the best medicines for typhoid and how does it work? You have this A site, you have this P site, the chloramphenicol is in between here. So, what happens? Now when the peptide synthesis takes place, first this growing peptide chain is transferred to this side and then it shifts, but the chloramphenicol is sitting right here.

So, it does not allow the shifting. So, the growing peptide chain cannot exit as it stops the exit pathway of the growing peptide chain. So, these are some of the other important antibiotics that I thought that you should know.

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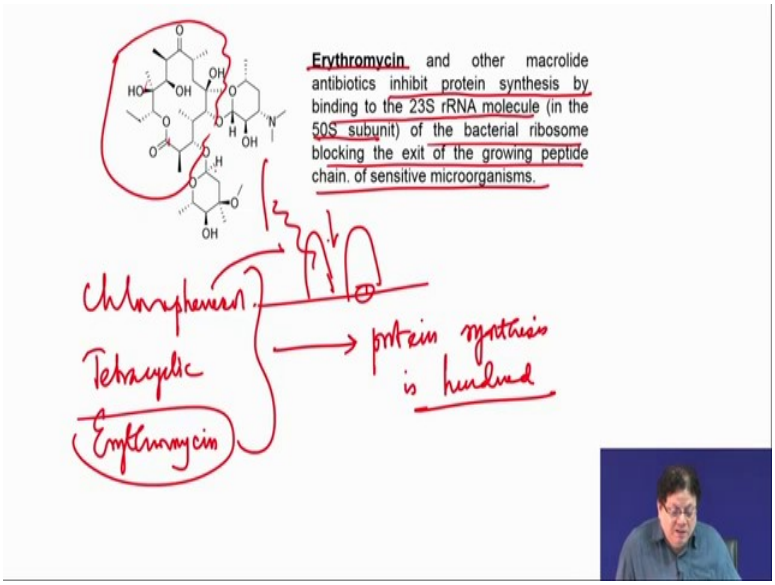

Another one is erythromycin. These are called macrolide antibiotics because they have a big macrocycle, you see they have this big macrocycle. So, they are called macrolide antibiotics. How do they work? They also inhibit protein synthesis by binding to the 23S rRNA.

So, they bind to the ribosomal RNA in the 50S subunit of the bacterial ribosome blocking the exit of the growing peptide chain. So, they are very similar in their mode of action; tetracycline binds to the A site, then chloramphenicol binds in between the A and the P site and erythromycin blocks the exit of the growing peptide chain. So, these are the three important ones and that is how they work. The consequence of their action is basically that the protein synthesis is stopped; only difference is that their binding pattern is different. Chloramphenicol binds in between the two sites; then tetracycline binds in the A site and erythromycin binds somewhere in that ribosomal subunit which does not allow the growing peptide chain to exit. So, those are the mechanisms.

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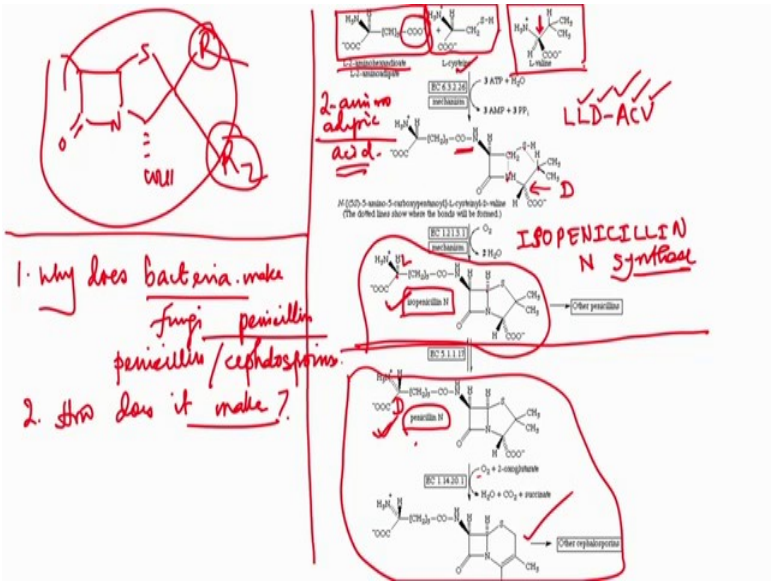

Now, let us come back to more organic chemistry and I will show you the power of organic chemistry in making newer antibiotics and specially, I will focus only on the penicillin and cephalosporin. Since these are still the major antibiotics that are used today. Now remember I told you that in penicillin, it is extremely difficult to substitute these methyls.

See if you can develop a method where these can be substituted with different groups, then what happens? Actually the bacteria are exposed to penicillin, they know the structure of penicillin, and they remember that. So, when the next time you give penicillin, they know that there will be two methyls here, and they immediately know that this is penicillin.

But now if you can have a method by which you remove the methyls and put some other group suppose some other R R_2. So, now, the bacteria becomes a little bit perturbed, as that is not the same penicillin it was used to having destroying that penicillin; but now there is a new molecule.

So, the bacteria may be killed by the process before it can realize what is coming here; however, to make different R-C-R is extremely difficult, but possible. And I will show you how it has been done, but in order to go into those details, what we need to answer the two very basic questions. Firstly, why bacteria make penicillin? Why does bacteria or fungus whatever or fungi make penicillin?

I think I gave you the answer here because like us the bacteria also are constantly bombarded or constantly attacked by other bacteria. So, it has to make some molecules to kill the surrounding bacteria. So, these are defense molecules, these are their weapons - basically penicillin, cephalosporin. So, they are their weapons. Secondly, how does it make the penicillin? What are the starting points? What are the building blocks?

I told you when the penicillin was introduced to you that this penicillin is made by a very simple sequence of steps. There are three amino acids; this is L-system, this is L-valine and this is L-2-amino adipic acid, L-cysteine. So, these three amino acids get combined to form a peptide; amino acids combining through carboxy and amine means they are forming peptide bonds. So, three amino acids combined means you will get a tripeptide.

So, this will be the tripeptide that you get. Now there are few questions here. See the configuration of the valine here, L-carboxy is up, hydrogen is down and when it makes the tripeptide what is the configuration here. Now it is D; the carboxy down hydrogen up. So, this molecule while making this tripeptide, there must be an enzyme which is doing some racemization here L to D, but when people thought that instead of L-valine, what if I give a D-valine. So, that the bacteria accepts the D-valine and makes this tripeptide, but it does not accept D-valine; it will only accept L-valine and then it will epimerized it into the D.

So, that is one interesting aspect. The other is that these two α-amino adipic acid, it is the side chain carboxy which participates in the peptide bond formation. So, now, you have this α-aminoadipoyl cystinyl valine and that is abbreviated as LLD-ACV; L for this A, L for the C, cystine, and D for V; full name is L-alpha aminoadipoyl-L-cystinyl-D-valine.

But you cannot write all the time this big name. So, LLD-ACV; now once the tripeptide is formed, there is an enzyme which is called isopenicillin N synthase; synthase means it does not require any ATP to make the molecule. What it does? It first forms this nitrogen-carbon bond and it forms this sulfur-carbon one and in one step you get this molecule.

Now this is what is called isopenicillin N not penicillin N; because earlier this molecule was discovered where this is having a D-configuration and that was named penicillin N. So, the scientists have no other option but to call it as isopenicillin N. So, penicillin N was known earlier, but then they got this L-configured α-aminoadipoyl system. So, they

had to call it isopenicillin N and that is why the enzyme which makes the penicillin from this tripeptide is called isopenicilin N synthase because this is known as isopenicillin N.

And now in some organisms it stops here, but for some organisms, this is further converted into penicillin N; that means, an epimerization has taken place and then another enzyme comes and converts this penicillin into cephalosporin. So; that means, penicillin and cephalosporins are two sisters or brothers; whatever you say; and then they belong to the same class and they start from the same molecule.

So, that is the tripeptide which is formed from three amino acids; only interesting part is that L-valine gets isomerized to D-valine. So, LLD-ACV if take, then IPNS, (isopenicillin N synthase) is added, then this is nothing, but a double oxidation; nitrogen-carbon bond formation and sulfur-carbon bond formation and that ultimately goes to isopenicillin N.

For the time being we just exclude this part that how the cephalosporin is obtained, but just to know that cephalosporin is just a further downstream process from penicillin; you can get cephalosporin and that is what happens in microorganisms, ok.

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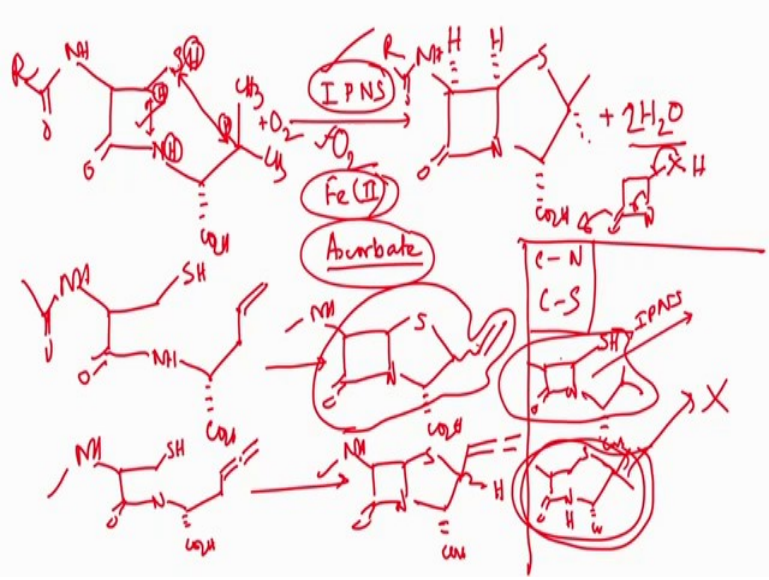

Now, this is this very interesting chemistry; so, we are saying that first these three amino acids combined to form this tripeptide methyl SH, that is cysteine, and you have NH and

this is the adipoyl side chain I am not writing that. So, there is an enzyme called IPNS. Remember that we are studying now the biosynthesis of penicillin.

How penicillins are made in the microorganisms; that is what is called biosynthesis like if you say how taxol is made in the plant that is biosynthesis of taxol. So, this is biosynthesis of penicillin. So, one enzyme does two reactions at the same time; one is this formation of this carbon-nitrogen bond, another is formation of this carbon-sulfur bond.

And while doing that, you have to remove this hydrogen; and you have to remove one hydrogen from here and the NH hydrogen from here; that means, four hydrogens are removed. So, what is the fate of the hydrogens? The fate of the hydrogens is 2 molecules of water. So, from where the oxygen comes? Actually this reaction requires oxygen. These reactions requires iron as a cofactor, ferrous and you know any ferrous requiring enzymes need vitamin C; the ascorbate to keep the iron in the plus 2 oxidation state.

Otherwise, if there is no ascorbate, it will go to the ferric. So, that means, oxygen is a co-substrate that is converted to water. IPNS is the enzyme; ferrous is the cofactor and ascorbate's function is to keep the ferrous iron in the plus two states. So, this is the reaction. Now suppose this tripeptide is accepted as the substrate for IPNS.

Now, if you take another tripeptide, suppose this, because tripeptides are easy to make; NHCO and then you put some groups suppose I put an allyl group here, change those two methyls, and put an allyl group. If this is accepted as a substrate then I get a new penicillin which is entirely different from the penicillin that bacteria used to see. So, you will get a vinyl penicillin now. This work was carried out by Sir Jack Baldwin.

He made various penicillins, he first showed that IPNS can be used in this fashion. I think in his lab, at least 100 new penicillin molecules have been made. But these penicillins where the skeleton pharmacophore is changed basically, you are replacing the methyls; now that is synthetically is not possible, it is very difficult. So, if you take this allenic amino acid; now you get this allenyl penicillin; but this was possible only when the biosynthesis was known, this enzyme was characterized.

So, basically why do you study biosynthesis? Because that is one of the ways by which you can help the drug discovery processes. And from organic chemistry point of view,

they are extremely interesting like if I ask you that how many bonds are formed in this reaction; two bonds one is this carbon-nitrogen bond, another is the carbon-sulfur bond. Which bond is formed?

In mechanistic organic chemistry, the first question is which bond is formed first because if this carbon-sulfur bond is formed first; that means, you get a macro cycle in the beginning like this and then the carbon-nitrogen bond is formed. The other is that carbon-nitrogen bond is formed first; that means, question is which is the intermediate for this reaction?

If this is the intermediate; that means, carbon-nitrogen bond is formed first. On the other hand if you get this, then you can say that the carbon-sulfur bond is formed first. So, people tried to see which intermediate is formed, but they could not find any intermediate. Then they did a reversal of their strategy; what they did is that they made this macrocyclic compound and give it to the enzyme and checked whether the enzyme converts it into the penicillin or not.

And what happened? This was not converted into the penicillin. Now it was pointed out that possibly, this is the intermediate that is formed first. So, they wanted to make this molecule and feed it to the enzyme and they thought that they will immediately get the penicillin. However, the problem was that this molecule had a very short half-life; within 15 minutes, it is destroyed because you cannot have a β-lactam where there is a XH here. Immediately ring opening reaction will occur.

So, this thiol nobody could make; that means, nobody could demonstrate that this is the one which is the intermediate; that means, nobody could demonstrate it directly that the carbon-nitrogen bond is formed first. See his is a negative experiment that this is not converted into penicillin.

So, that is not a direct proof; you cannot just say immediately; that means, that the carbon-nitrogen bond is formed first that is not so because that is a negative way of saying this; you have to show it that the carbon-nitrogen bond is formed first. How do you do that? A very simple experiment was done.

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This is an indirect experiment because directly you cannot do it as you cannot make that intermediate. So, this tripeptide was made where all the positions are occupied by the lower isotope; that means, the normal hydrogen, then this molecule was also made with proper stereochemistry, two deuterium here.

And then NH CO R and a third molecule was made; SH which had a deuterium at this position. Suppose this is A molecule, this is B molecule and this is C molecule and then A and B are mixed together. So, now, you have two test tubes; here it is A plus B. Suppose just for assumption, think that this is 1:1 and in another test tube, you will mix A plus C that is also 1:1. Now you add your IPNS, you add the iron, whatever requirements are there ascorbate, all these things.

So, the reaction started because you have given the tripeptide. So, it will form penicillin. So, in the experiment what was done, they took some aliquot from here, put it into the mass spec. So, what will happen in the mass spec? After 5 minutes, there will be lot of A and B still present. So, what will happen now? You will see two peaks one for A and one for B, what is the difference between A and B in mass units? So, this is M this is M plus 2; and what you do for the other test tube, you do the same thing. Start the reaction and check the mass spec; that means, check the ratio of B and C.

Check whether the ratio changes in any of these test tubes. It was a very interesting experiment because it contained physical chemistry also. So, you get M and M plus 1

because it has got only one deuterium. Now what happened? What was the result? I do not show the result first; let us just analyze that suppose this bond is formed first. So, when there is A and B, then what will happen? There is this deuterium here, you know the deuterium loss is more difficult, you get a huge isotope effect.

So, initially the enzyme will preferentially pick up this and not the other one. So, if that be the case; that means, A and B ratio will change. And if this sulfur-carbon bond is formed first, then there will be no isotope effect here.

So, both will be transformed into the intermediate and then later on when it finds deuterium; it can throw it off, but this will be processed to the intermediate and that will be processed at the same rate to the intermediate provided the carbon-sulfur bond is formed first. Because there is no isotope effect here as this is also hydrogen that is also hydrogen.

On the other hand, if you take A and C, what will happen? If this bond is formed first then both will be accepted as a substrate with equal preference, but the second step will be a problem. So, the conclusion is that if this is the first step that is formed then A:B is going to change and if this is the bond that is formed first then A:C is going to change.

Because; that means, you have to see where is the isotope effect; isotope effect will be shown in the first step because that will give you the ratio of A and B. Energetically, it is like this see if it is A and B if there is no isotope effect here; that means, if the there is no deuterium then all A and B will come here, but those are not A and B, those are transformed A and B. If A and B are processed not at the same rate, then there will be accumulation of one of these especially the deuterated ones. What was the experimental result?

The experimental result was that the experimental result was that the ratio changed, the ratio changed here, but on this side the ratio remains the same. So, what does it prove? If the ratio changed here; that means, A and B the first step you face the face the isotope effect. In the first step, you, face the isotope effect only when this carbon-nitrogen bond is formed first. If it is the second, then A is to C should have changed.

So, this got changed; as the time goes on you will see that when it is less; B will be only processed when all are consumed then only the B will be taken up. So, you have to study

this at initial points that what is the ratio of A:B and what is the ratio of A:C and that experiment was done and it was proved that this is the one which is the first step in the process of making the penicillin.

Interestingly the question can be asked that if the enzyme can make it stabilized and then form the next reaction; chemically when we make in the solution this does not stay; however the enzyme has some way to stabilize this thiol. I told you that some people try to make this compound and give it to the enzyme, but they never succeeded in that because it has got a very poor half-life especially at pH 7.2 where this IPNS works, it does not stay for a minute even only at lower pH, 20 minutes is the half-life.

I think so, that we will we can discuss in the next class we will discuss the mechanism of formation of this; that means, the cofactor chemistry; that I think that will take more time.

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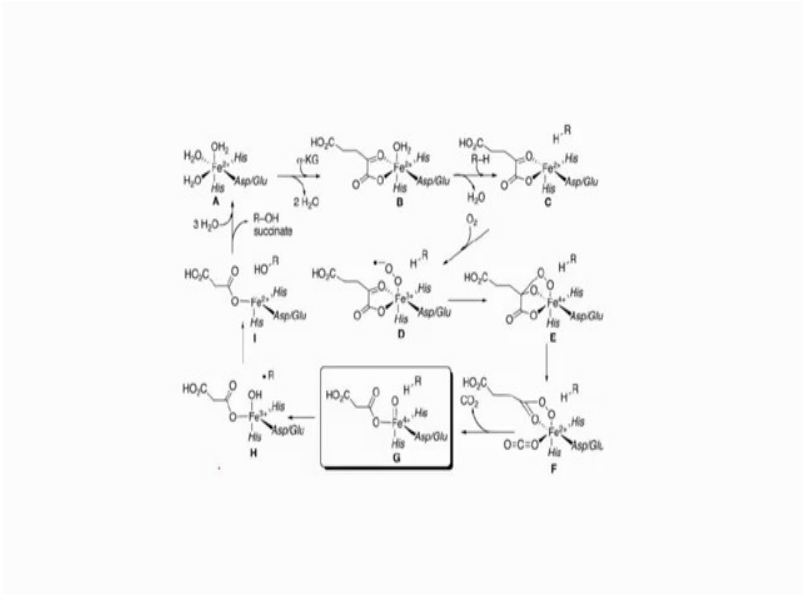

So, we will consider these biosynthetic mechanisms for penicillin formation as well as erythromycin information and in the next class, we will also show how you can make different types of penicillin, different types of erythromycin by what is now called the genetic engineering or protein engineering.

Thank you.