Organic Chemistry In Biology And Drug Development
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Lecture – 49
Chemistry of Penicillins

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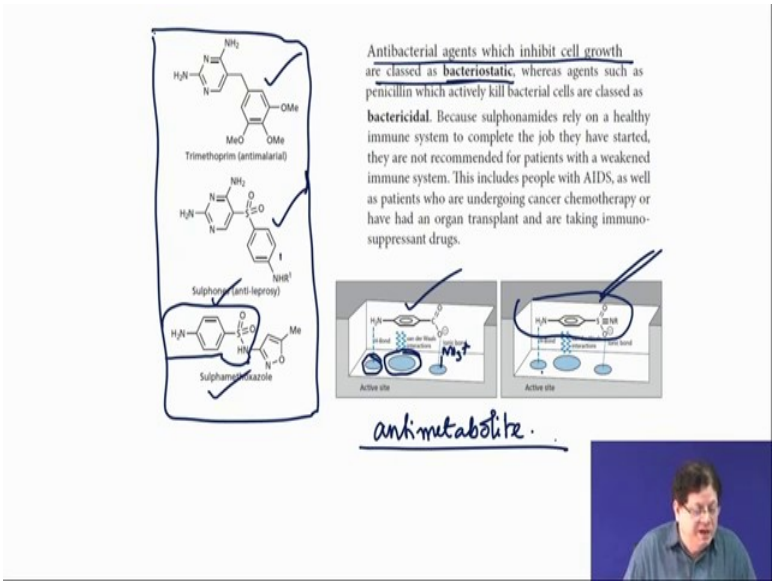

Welcome back, now we are going to take the individual antimicrobial compounds and discuss their chemistry and biochemistry. We will just recapitulate; we started with these compounds. This is the structure of trimethoprim, an antimalarial compound; this is *para* amino phenyl sulphone; that is an anti-leprosy compound, this is sulphamethoxazole which is an antibacterial compound.

You see here it is written bacteriostatic; that means, these are the antibacterial agents which inhibit the cell growth. Why these are anti-bacteriostatic? Because these are the compounds which stop the folic acid biosynthesis which is one carbon transfer; it is a very important conversion that is uracil to thymine and that was ultimately the consequence of perturbing the folic acid biosynthesis.

So, one of this compound is like sulphamethoxazole or this anti-leprosy compounds this is little bit different because that inhibits the DHFR (dihydrofolate reductase) of microorganisms; . And these two are nothing, but you can write them as SO_2NR; so *para* amino SO_2 and then NR. It mimics the *para* aminobenzoic acid which is a component of

the folic acid. So, *para* aminobenzoic acid has to be incorporated into the dihydro pteridine nucleus. If you remember, it is the 7, 8-dihydro pteridine that is formed.

So, that reacts with this *para* aminobenzoic acid and the enzyme that does that it is called a pteroate synthase. Now, this pteroate synthase must be such that there is hydrogen bonding; it goes to the active site of the enzyme and this is the NH_2 which forms the hydrogen bond with some amino acid here.

There is an aromatic amino acid somewhere here, it must be so that there is π-staking interaction with this benzene ring. And then there must be some ionic interaction; that means, there must be some sites like NH_3 plus which forms an electrostatic interaction here. So, for the *para* aminobenzoic sulphanomide, NH_2 forms this hydrogen bond, this is the aromatic ring and instead of this CO_2 minus you have SO_2 minus; so that forms a ionic bond.

So, this is a very good competitive inhibitor of *para* aminobenzoic acid; these compounds are also called antimetabolite. What is antimetabolite? Compounds which interfere with the metabolic processes that are that are going inside the microorganism or any species. And since this is now inhibiting the incorporation of *para* aminobenzoic acid; that means, it disturbs the normal metabolic process; so initially this was considered to be a very good antibiotic.

But today the bacteria has developed resistance against the sulphonamides by producing more of *para* aminobenzoic acid and you know that in competitive inhibition, if you increase the concentration of the substrate, you can overcome the inhibition; that is the nature of competitive inhibition. So, now sulphonamides are virtually not working against any microbial infection.

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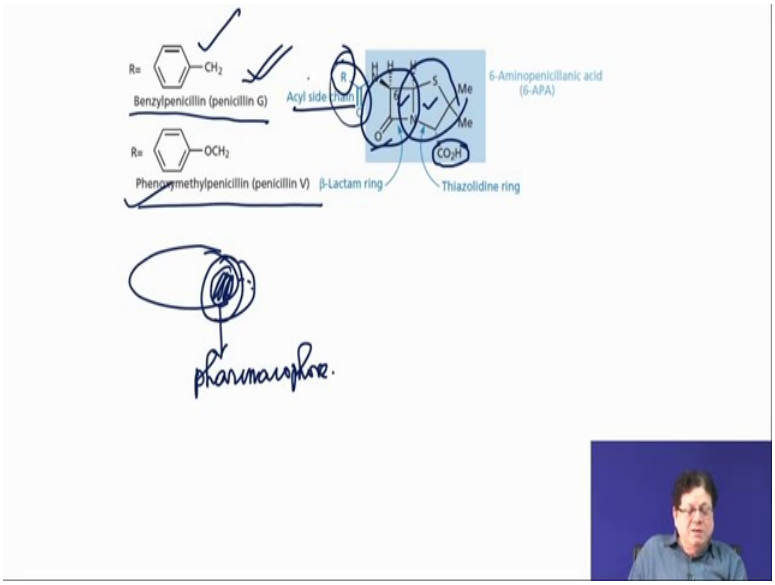

Now, let us come to the chemistry of penicillin. So, this is the structure of penicillin; what is the pharmacophore of penicillin? Pharmacophore is the molecular entity present in the entire molecule; it is the essential part of the molecule which is interacting with some enzyme or any nucleic acid, whatever is the target.

So, basically what we are saying that is that if I have a big molecule like this and if I see that only this part is the one which is interacting with the enzyme, then this part is what is called the pharmacophore. So, the pharmacophore of penicillin is this part which is put as light blue; so that part is the pharmacophore. Now, what is there? There are two rings which are fused to one another.

Now, this is called a β-lactam ring which is fused with this thiazolidine ring; that is the name of the nitrogen and sulfur containing heterocycle; then there is the substituent amine here which is acylated. So, you have an acyl side chain here and you have a α-carboxy group attached to the thiazolidine ring.

Now, this is the minimum; so for any penicillin, the variation that we see in different penicillins; you know the names of different penicillins, ampicillin, amoxicillin, cloxacillin, methicillin; whatever name any penicillin the difference is only in this R group. So, you have different R groups and you get different types of penicillins. The initial penicillin that was made was benzylpenicillin called penicillin G and another one

was called penicillin V that was phenoxymethylpenicillin; phenoxymethylpenicillin also called penicillin V. In fact, penicillin G is still available in the market.

It is usually now prescribed for rheumatoid arthritis; usually for this, benzylpenicillin was used. Otherwise, for other bacterial infections, respiratory infection usually this penicillin G is not used. But historically these two compounds are important because the first penicillins that were introduced into the market where this benzylpenicillin and this penicillin V, penicillin G and penicillin V.

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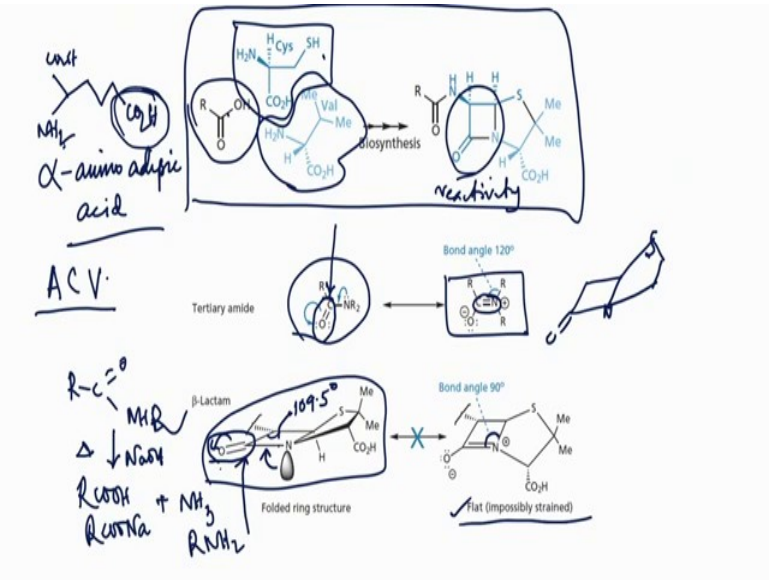

We will talk about this biosynthesis later on because we know that penicillin is made by the microorganism that is why this is an antibiotic. Now, that means, the microorganism has a biosynthetic machinery to make this penicillin; enzyme systems which starts form building blocks and join them together and final get to the penicillin.

We will discuss this formation of penicillin in a much elaborate way, but here just to let you know that it is a very simple, these three simple building blocks which ultimately combine and make the penicillin; one is this variable amino acid, then this is cysteine and this is valine. So, cysteine, valine and another amino acid which I am not naming, actually this amino acid is alpha aminoadipic acid which is not a protein amino acid.

So, basically this α-aminoadipic acid combine with cysteine and that also combines with valine to make what will be a tripeptide; tripeptide then cyclizes and you have this penicillin molecule.

Remember, the α-aminoadipic acid it is the ε-carboxylic acid that reacts with cysteine and cysteine reacts with valine to make a tripeptide which will be called A C V; Adipoyl Cysteinyl Valine that cyclizes to give the penicillin. Now, another interesting aspect of the structure of penicillin for which the activity is there is the reactivity of this β-lactam ring.

Reactivity to what? Reactivity towards nucleophile because cyclic amide carbonyls are susceptible to ring opening by hydrolysis; like if you take an amide $RCONH_2$ and if you heat it with alkali you get RCO_2H or RCO_2Na because you are using alkali say NaOH and you get the ammonia. And if it is NHR; then you will get RNH_2 the amine, but in order to do this you have to heat it with alkali.

It does not work at room temperature or if you want to do it at room temperature; then you have to wait for months and months so that the hydrolysis will take place. Now, why amides are difficult to hydrolyze? Because of this resonance; where nitrogen lone pair is resonating with the carbonyl; so you have a resonating structure where the nitrogen is planer and this bond is also becoming very rigid; so that will not allow you allow the bonds to rotate. This is exactly what happens in peptide bonds when we discuss the structure of peptides; we have seen that the amide bond in this nitrogen carbon framework has a significant amount of double bond character and that stops the rotation.

So, in the normal amides, because of this resonance, this carbon is losing the electrophilicity. Because if there is a only carbonyl then the oxygen will pull the electrons towards itself by electromeric effect and then this will have a positive charge, but here nitrogen lone pair is there that will neutralize the positive charge on the carbon.

So, nucleophilic attack on the amide requires higher temperature or higher activation energy. On the other hand, in penicillin what happens? This is the structure of penicillin; see it is not a planar molecule, it is like this is a planar β-lactam ring; this 4-membered ring is almost planar and then you have this 5-membered ring which is going upwards. So, basically it is like an open book type. So, this is the 5-membered ring sulfur; this is the carbonyl, this is the nitrogen.

So, it is like an open book type of or a butterfly type of structure. So, it is not a planar molecule. In acyclic amides; this lone pair is entering into resonance with the carbonyl that is actually not permissible in β-lactam. Because if the lone pair participates in resonance then this angle becomes 120°; that means, the angles strain in a four membered ring; the angle is virtually 90°.

So; that means, you have already deviated the angle quite much from 90°; but actually the hybridization demands this angle to be 109.5°. So, if you have a resonance where the angle is still increasing further here; so that will not be allowed. So, this will be flat and it will be highly strained. So, this type of resonance is almost not possible and that is spectroscopically reflected; if you take the IR spectroscopy, then you will see that if there is resonance then the carbonyl stretching frequency will be low.

And if there is no resonance; the carbonyl stretching frequency will be higher. So, in penicillin the carbonyl stretching frequency is about 1770 cm^{-1}. On the other hand, in the amide carbonyl it is around 1680 cm^{-1}. So, that shows that it is a very highly strained molecule and the carbonyl is virtually present as a separate carbonyl; there is no such assistance from the nitrogen lone pair adjacent to it. So, that makes its carbon highly electrophilic; that makes this carbon highly susceptible to nucleophilic attack. So, that is one of the major reason why this molecule acts as an antibacterial agent.

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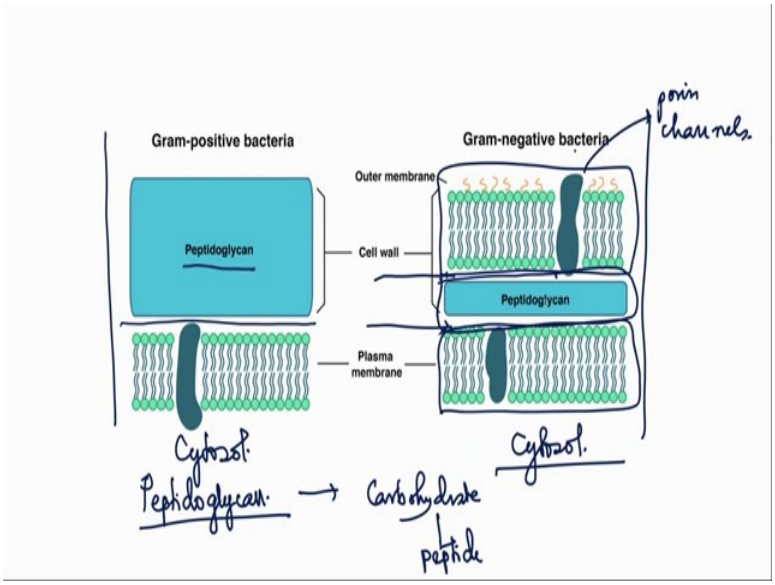

We will slowly go back to that, but again before I go any further, I should tell you that whatever mechanism that we are discussing today that this is the way the penicillins work; that is what is called post facto analysis.

That means it is after the fact that penicillin is a very good antibiotic, it kills the microbes; it kills the bacteria and once that is known at that time Fleming or Florey or Chain or Dorothy Hodgkins or even Woodward, Robert Robinson; they did not know how it is working only thing they knew that it was a very good antibacterial agent.

Of course that is what ultimately matters. Some people might say that I do not care how does it work, but it is working nicely to cure me of the infection. However, science can't stop there, science has to progress you have to know how penicillins work. Because until we know that we cannot device or rationally design new antibiotics based on the mechanism of action of penicillin because today we are living in an era where most of the earlier penicillins are not working.

So, now how to make new penicillins and new type of antibiotics that depends on our understanding of the antibacterial activity of penicillin. Just to make it very clear that whatever we are discussing now this is after the discovery of penicillin and after the penicillin entered into the market. So, the mechanism was established. Now, what is the mechanism? I told you one thing that penicillins works against the bacterial cell wall. So, we have to now know what is a cell wall in bacteria; that is so unique for them.

Cell wall is a rigid wall that protects the cytosolic material inside the bacteria. Now, there are two kinds of bacteria; one is gram positive, another is gram negative. So, gram positive bacteria have a thick cell wall structure and it is made up of peptidoglycan; what is a peptidoglycan? Peptidoglycan means you have peptide units as well as glycan units, glycan means sugar units.

So, basically you have carbohydrate which is the part of the sugar and you have peptide. So, that is why this is called peptidoglycan. In gram positive, you have the peptidoglycan as the outer wall and then you have the membrane like we have; this lipid bilayer membrane and in that membrane there will be definitely channels because bacteria has to communicate with the outside world.

Some molecules will come inside; there are carrier molecules which will carry the outside molecules that are needed. So it has to be controlled like ion channels. Bacteria have these pores also; so through that pores, materials enter or go out, ok.

So, this is your peptidoglycan and that is quite thick in gram positive bacteria. In gram negative bacteria what happens? First it has an outer membrane which is a lipid bilayer and then there are these pores these are called porin channels. Then there is a much thinner peptidoglycan that was earlier for the gram positive it is outside, then little bit inner; that means, this is protected by a lipid bilayer membrane and then you have another membrane, an inner membrane and then the cytosol.

So, there is a distinct difference and you can now say that penicillins work against the gram positive very well because in the gram positive the peptidoglycan is exposed outside. So, penicillin comes and destroys the peptidoglycan, but here the penicillin has to initially traverse through barrier.

Here these spaces are by the way called periplasmic spaces and this spaces where the peptidoglycan is there. So, then it has to come inside here and then act on the synthesis of the peptidoglycan. So, it will be difficult to kill the gram negative bacteria by this type of mechanism, but anyway let us see what the other problems in gram negative bacteria are.

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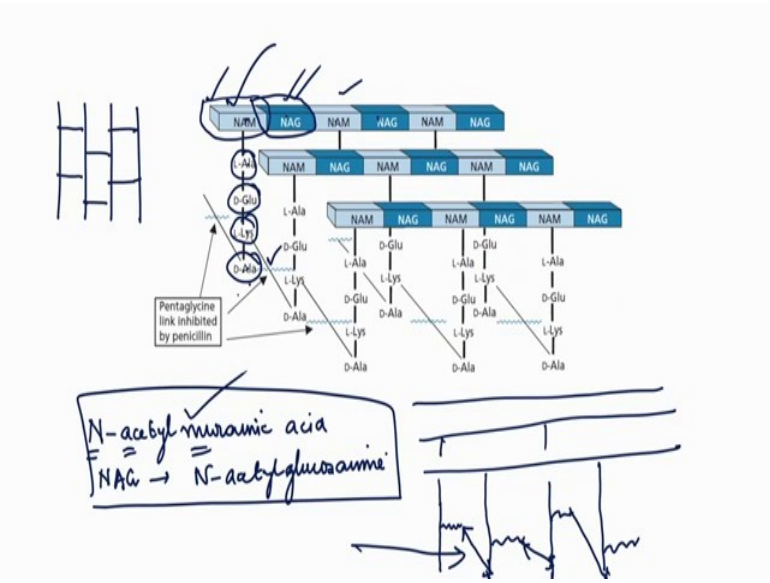

Gram negative bacteria have other sorts of problem; so, we will discuss that. Let us inspect it at the molecular level the peptidoglycan. Here this is the schematic diagram you see this is one; one sugar unit that is called NAM. What is NAM?

N-acetyl muramic acid is NAM. And the other is called NAG; NAG is *N*-acetyl glucosamine. So, these are the two sugar units; they are joined one after another, NAM then NAG, *via* glycosidic linkages. NAM, NAG; NAM, NAG NAM NAG; so this way some chains of sugar are there, then another chain of sugar is there, then another chain of sugar is there.

In each strand of sugar polymer; in the NAM (*N*-acetyl muramic acid), there is a peptide which is attached to one of the hydroxy. So, the first amino acid is L-alanine, then D-glutamic acid then L-lysine, then D-alanine. And actually there was a another D-alanine, but by reaction while forming this a cell wall, one of the D-alanine goes out.

This is the structure of the cell wall when the matured cell wall is there. But we are discussing that just before the formation of the matured cell wall; what is the status? The status is that that all these peptide bonds are there and the amino acids are there including the last D-alanine.

So, this is a pentapeptide; they are hanging from one strand. And in adjacent strand, there is again those five amino acids which are hanging.

Now, each peptide has a side chain also which is the reactive arm. Like this one has a side arm then there is another one, suppose here which has got a side arm and then there, this has got a reactive side arm. Now; there will be a reaction between this strand and the side arms. So, there will be a attachment like this; then there will be an attachment like this.

If that happens, then the bacterial cell becomes very rigid. Suppose you have lot of bamboo rods and then you have to put to make a fence. If you want to do that by only putting these bamboo rods; the bamboo poles will not be sufficient to protect. So, in order to make them very rigid, you do this type of cross-links to make it very strong.

That is to reinforce the structure. So, to reinforce the structure, this cross-link is required. So, basically now what we have learnt? That there is this glycan units glycan polymer

that is NAG NAM NAG and NAM NAG; in each of these, NAM is attached to a pentapeptide and then there is cross-link between the adjacent pentapeptides. So, we will look very carefully at what are these reactions; how these cross-link is formed.

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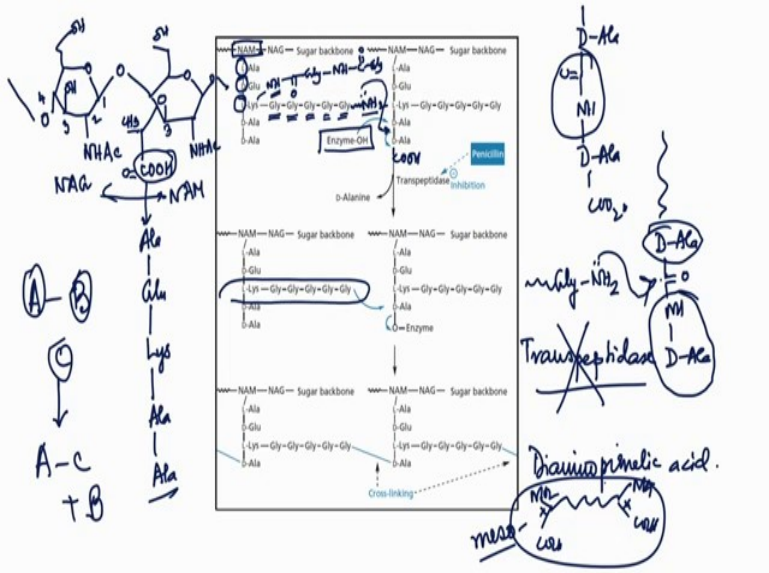

Here it is now written in little bit better way; let me write the structure of the sugar. So, this is the glycoside linkage, this is *N*-acetyl glucosamine means NH. Glucosamine means in glucose only one hydroxyl is replaced by an amine which is acetylated. You know in glucose what happens? This is α then the next one is β, the third one is this is number 1, this is number 2, this is 3rd, this is number 4 carbon OH is alpha and then you have this CH_2OH.

So, the glycoside linkage is made between C1 and C4; that means, this is attached to another sugar. So, this is your NAG; *N*-acetyl glucosamine; and what is your NAM? The NAM is like this, NHAc; *N*-acetyl that is there and this is attached to another sugar unit via glycosidic linkage and here the OH is β. So, that is attached to a lactic acid this is CH_2OH.

So, this lactic acid moiety is attached by an ether linkage. So, this is your NAM and this is your NAG; so this is how it is done. So, this is NAG attached to a NAM. NAM has a carboxy which is attached via a lactate side chain and this carboxy is the one which is used to make the peptide. So, it will be CO; then there will be alanine, then there will be

glutamic acid, then there will be lysine, then there will be alanine, there will be alanine. I hope this is clear.

So, every NAM is attached to alanine glutamic acid lysine alanine alanine. Also look at the configuration of this alanine this is L-alanine, this is D-glutamic acid; I told you that in bacteria only, D-amino acids are present; where from this D amino acids come? By isomerisation of the L-amino acids.

But this lysine side chain amine is attached to 5 glycine units. So, what is the end point here? Is it the *N*-terminus or *C*-terminus that you have to be clear now; lysine ends up with NH then you have a glycine; so NHCO glycine. Then glycine ends up with NH_2 that is attached to another glycines; so NHCO. If that be the case, you can say that it ends up with an amine.

Lysine starts with NH. So, NHCO; then ultimately it does not end up with a CO_2H, it ends up with NH_2 and then you have D-alanine. And in the adjacent NAM NAG; you have the same chain alanine D-glutamic acid lysine that has got penta glycine; and then D-alanine, D-alanine.

Now, the reaction that takes place in cross linking is that this side chain reacts with the primary chain that is hanging here; these D-alanine D-alanine; again you have to be sure that what ends up here; you started with NAM; NAM is this one. So, this is CO then NH. So, alanine NH is on this side and CO_2H on this side; that means, this will end up with a CO_2H.

So, this is a carboxy end here. In between D-alanine D-alanine, you have a CONH. So, you have a D-alanine here that is the end point and this is the CO_2H and this D-alanine is attached to lysine.

So, now the reaction that takes place is basically that the glycine NH_2 attacks this amide bond and kicks out the last D-alanine. So, what will happen now? This is the chain that is now cross-linked with this chain and the result is a new peptide bond between the two peptide chains.

Suppose this is glycine that has got NH_2 and then you have D-alanine D-alanine. So, D-alanine then CO, then NH, then D-alanine and this was going to the top attached to a

NAM and now the reaction is basically this NH_2 is attacking here that goes out; that makes a cross-link. What is the nature of this reaction how can you classify this reaction?

This is nothing, but look at this D-alanine this was connected to another amino acid D-alanine earlier. Now what you are doing? You are replacing this D-alanine by glycine. So, this can be called a transpeptidation reaction; that means, your peptide was having another amino acid that is replaced by a new amino acid.

So, what is transpeptidation? That if you have a peptide like this and if you have another amino acid and if you get A-C plus B that is a transpeptidation; these are all amino acids. So, this peptide bond is replaced by a new peptide bond; so that is why this is called transpeptidase. So, there is an enzyme to do this; otherwise this reaction will not take place, this enzyme is a serine based enzyme.

So, there is an enzyme it is written here enzyme OH. So, initially enzyme OH like chymotrypsin what happens when you hydrolyze a peptide bond; you attack the peptide bond with the serine OH, replace at that time you break that other amino acid and then this serine is released again when the water comes and attacks. So, in this case; the enzyme attacks the carbonyl forming the tetrahedral intermediate, the D-alanine leaves and then instead of water now the adjacent glycine amine attacks and releases the serine OH.

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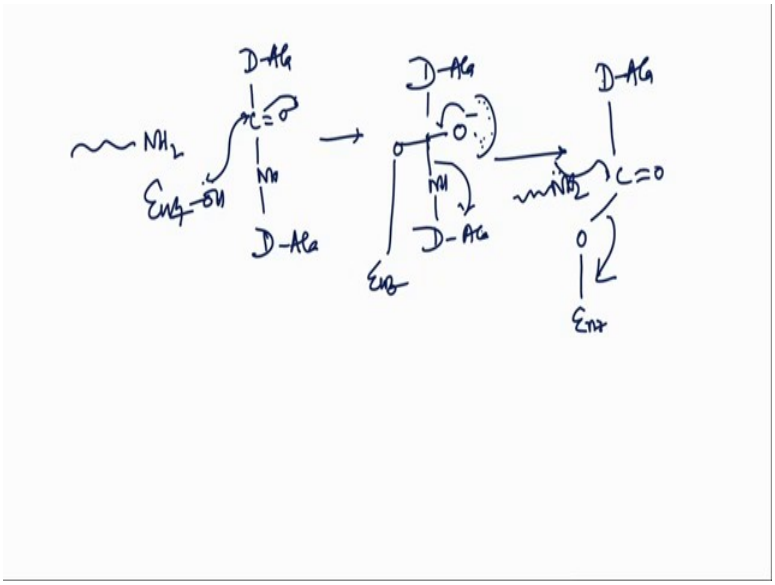

I can show it again in an even better way that basically what we are talking about CONH; D-alanine and this is D-alanine and on this side there is this glycine NH_2. So, initially the enzyme which is having a serine OH that attacks here; forms the tetrahedral intermediate; so D-alanine CO minus and then NH then D-alanine and this is the serine enzyme. Now what will happen? So, there must be some oxyanion hole here which will stabilize this O minus.

Now, this comes back; that goes out; so D-alanine has gone out. So, D-alanine and then CO and that is attached to the serine and now the glycine which was waiting for this to happen, comes attacks breaks this bond. So, you get the link between the glycine and the D-alanine. Look at the work load on the bacteria; the work load on the bacteria is that it has to synthesize the NAM, it has to synthesis the NAG and then combine NAM and NAG form the glycoside bond and make the polymer, make another set of polymer.

All these rods which are made up of NAM NAG; NAM NAG; then it have to form the peptides. And this is the last reaction in the synthesis of the bacterial cell wall; so the last reaction is this transpeptidation reaction that is somehow stopped by penicillin.

So, how penicillin works? It stops the enzyme transpeptidase from working, but its a beautiful way to really harass the bacteria. Because you are not killing the bacteria or stopping the bacteria from the very beginning; you have done enormous amount of work; you have prepared for an exam reading 24 hours a day and on the final day you could not answer anything because the questions are so hard.

So, basically that is the case; the bacteria have worked to make all the cell walls; all the components assembled together; but stopped in the last stage. Like in the marriage ceremony, if the priest does not arrive the ceremony cannot take place. So, it is basically there at the terminal point you are stopping the bacteria from forming the cell wall. And if that cross-link does not happen; the cell wall is very fragile, water will now go through the cell wall inside the cell and then the cell burst open and that is what is called lysis.

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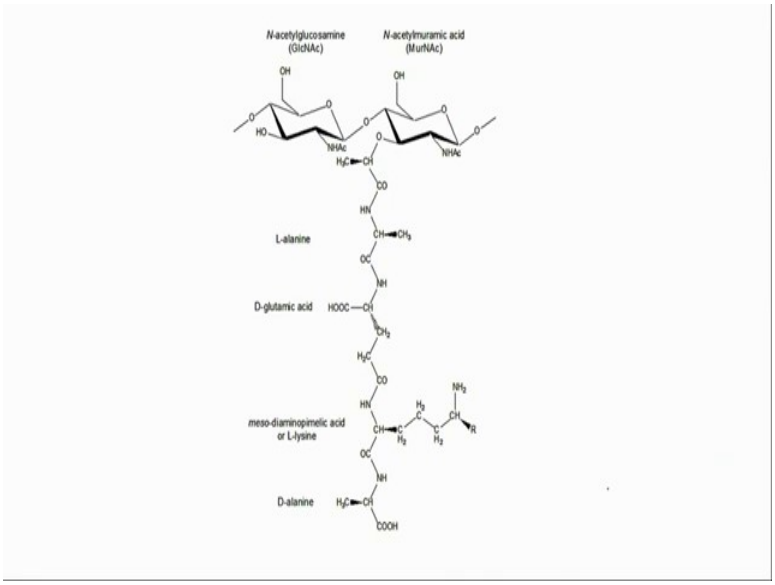

Now, the question is why penicillin will stop this? Why will it stop this cell wall formation at the terminal stage? What is the similarity of the reaction or the structure? But before that I may mention that this is the structure that I gave you; that lysine, connected to five glycine units; this is usually present in gram positive bacteria; in gram negative bacteria like E. coli, this lysine is replaced by an amino acid which is called diaminopimelic acid; Pimelic acid is 7-carbon dicarboxylic acid HO_2C-$(CH_2)_5$-CO_2H.

So, you have CO_2H; NH_2 and then all these and then another NH_2 and CO_2H. So, this diaminopimelic acid you might see in different books. The mechanism is same in that case the amine side chain of the diaminopimelic acid will react with that. And incidentally this is the meso diaminopimelic acid because this has got two stereogenic centers; if there is opposite configuration that becomes meso. It is the meso diaminopimelic acid in E. coli that replaces the lysine.

This is normally written in the medicinal chemistry books; where the mechanism remains the same. Now, let us see what is it is there; a meso diaminopimelic acid or lysine any one of this, then penta glycine has to be attached; it is through the glycine side chain.

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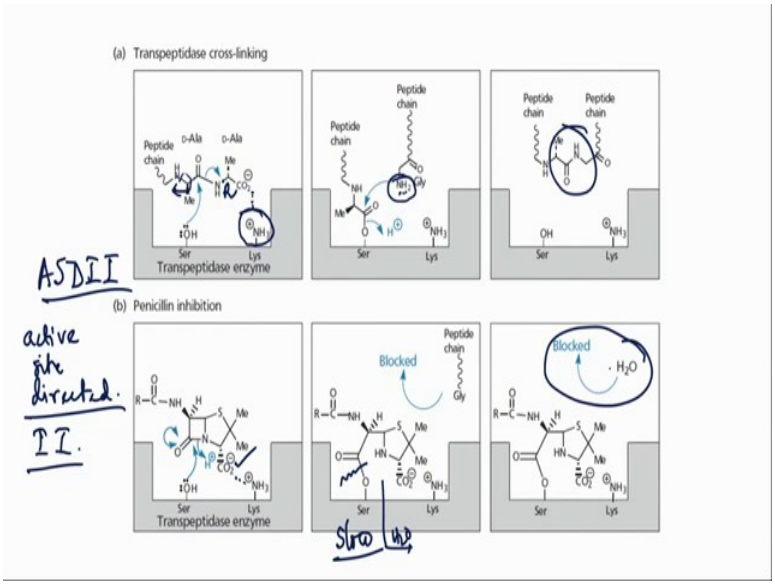

This is the mechanism which I have already discussed. Penicillin is an inhibitor of this transpeptidase cross-linking reaction. If this is the transpeptidase enzyme, then this is the D-alanine D-alanine remember this is D; so, that has to be of configuration R; so 1, 2, 3. So, that is R and this is also 1, 2, 3 but the methyl is α; so this is also R. So, this is the D-alanine and D-alanine; so what is the reaction? Why this D-alanine is binding to the enzyme; there must be positively charged species like lysine. So, that forms the salt bridge.

So, this goes and binds and then this serine serine OH attacks, forms the acyl-enzyme complex and now, the glycine NH_2 attacks and forms the cross-link. In case of penicillin, it has been found that instead of D-alanine; D-alanine penicillin comes; bacteria thinks that penicillin is my substrate and penicillin has got CO_2 minus that binds with the lysine and this β-lactam moiety is a very reactive ring which can open up immediately.

So, serine OH attacks and *via* the formation of the tetrahedral intermediate, it goes back and kicks out this. So, this is the situation now; so the serine is acylated by the penicillin and now the serine can be regenerated provided water comes and breaks this bond, so that the enzyme is freed again but this is a very slow reaction.

If it is a very slow reaction by the time this hydrolysis happens, the bacteria cell is already lysed. Bacterial cell will not wait for such long time that the enzyme is free

again. So, basically it is an active site directed irreversible inhibition. What is active sight directed? That it goes straight away and attacks the enzyme and stays there.

Formation of covalent bond means it is a irreversible inhibition and then after the reaction, no further reaction happens to produce a more reactive species. So, this is nothing, but an active site directed irreversible inhibition ASDII. So, this is the mechanism; again this is written here; water attacking; this is blocked; water cannot come and hydrolyze this one, ok. So, that is the mechanism of penicillin.

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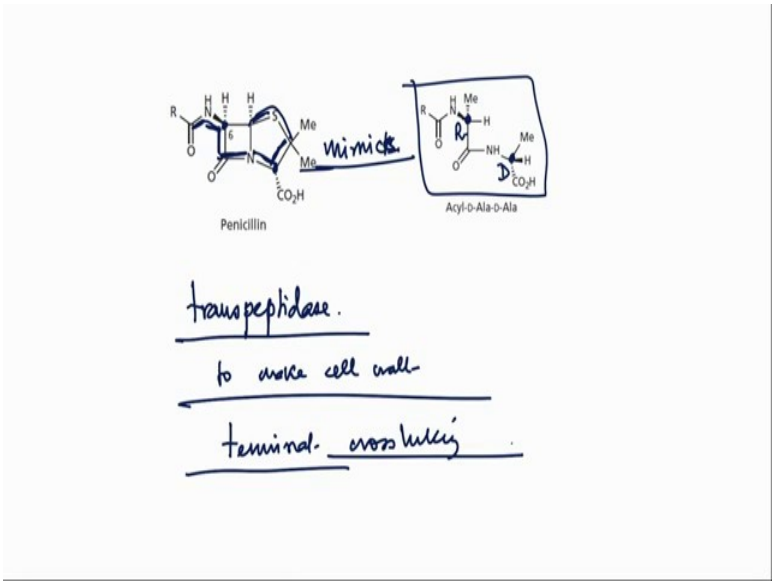

Now, the last thing that is remaining is why penicillin is so selective; why bacteria thinks that this is my substrate. Now, compare the two structures; this is again another way of writing D-alanine D-alanine. See this is the methyl; you see this has to be R configuration because the methyl is α. So, it has to be R now; so that is D and this is also D. So, I have written this structure D-alanine D-alanine in this fashion.

And look at the structure of penicillin; see this is same; if you look at the back bone only, you will see the similarity of penicillin and D-alanine D-alanine. So; that means, penicillin mimics D-alanine D-alanine in structure; it is only D D; it is not L L.

If bacteria had used L L, penicillin would have been ineffective; this streochemistry is also matching; only thing penicillin has some extra portion. So, it is even better that it is a conformationally constrained system which resembles D-alanine D-alanine. Of course,

D-alanine D-alanine must be having different types of confirmation, but in one confirmation you see that it resembles the penicillin.

So, now in short what is the mechanism of reaction of penicillin? It is a transpeptidase inhibitor. What is transpeptidase? Transpeptidase is an indispensible enzyme to make cell wall. What it does? The terminal step again I repeat now the bacteria has made these cell wall premature cell wall, it is like making premature or immature mRNA.

And then suppose you stop now the splicing with the spliceosome; so your mRNA will be ineffective. So, similarly the bacteria makes everything except the cross-linking. So, the terminal-cross linking is inhibited and that is how penicillin works. I think that is all for this session; we will talk about the bacterial resistance in the next session.

Thank you.