Organic Chemistry In Biology And Drug Development

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Lecture - 36
Chemistry of Cofactors/Coenzymes
(Contd.)

Welcome back to this course on Organic Chemistry in Drug Design and Development. In the last session we have discussed the some of the Chemistry of Coenzymes and we defined what are coenzymes and what are cofactors and, then we have seen that the most of the coenzymes are derived from vitamins. In fact, vitamins are the pro-coenzymes; that means, they have to be transformed into a species which finally, acts as the coenzyme.

We have seen that vitamins have been classified as a lipid soluble vitamins and water soluble vitamins. Lipid soluble vitamins are A, D, E, and K. We have discussed the chemistry of A then followed by D and we have also covered E. Vitamin A is actually a coenzyme which is involved in the chemistry of vision, D is a hormone and E is an antioxidant.

Vitamin K has a function in blood coagulation. We will discuss the chemistry of Vitamin K after discussing some of the water soluble vitamins, mainly the B group of vitamins and C that is ascorbic acid.

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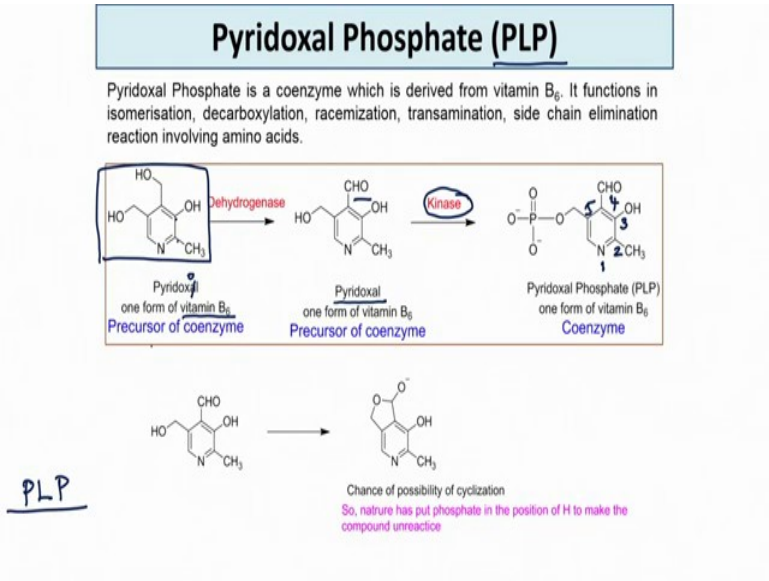

So, let us start with a water soluble vitamins. The first one we want to discuss is what is known as Pyridoxal Phosphate or abbreviated as PLP. PLP is the final coenzyme form, this is not the vitamin. The vitamin is pyridoxol that is the alcohol. So, it is a pyridine framework with 4 different substitutions at the 4 position- two hydroxyl methyl at 4 nad 5 position, one phenolic OH a 3 position, a methyl at 2 position.

The pro-coenzyme form is the vitamin B_6. Now, in the biological system this pyridoxol is converted to pyriodoxal by a dehydrogenase enzyme. Then this pyridoxal is converted to phosphate at carbon 5 by kinase enzyme and this is the final coenzyme form of vitamin B_6.

Why nature has selected to protect this 5 hydroxyl methyl? Remember there is an aldehyde at the 4 position. There is very well known in sugar chemistry that a hydroxy aldehyde can form a hemiacetal linkage provided the ring size is either 5 or 6 membered (furanoid structure for 5 membered ring and pyranoid structure for 6 membered ring). So, in order to suppress this 5 hydroxyl methyl is protected as the phosphate. If it is not protected then the aldehyde cannot show its full functional activity because it will form the hemiacetal.

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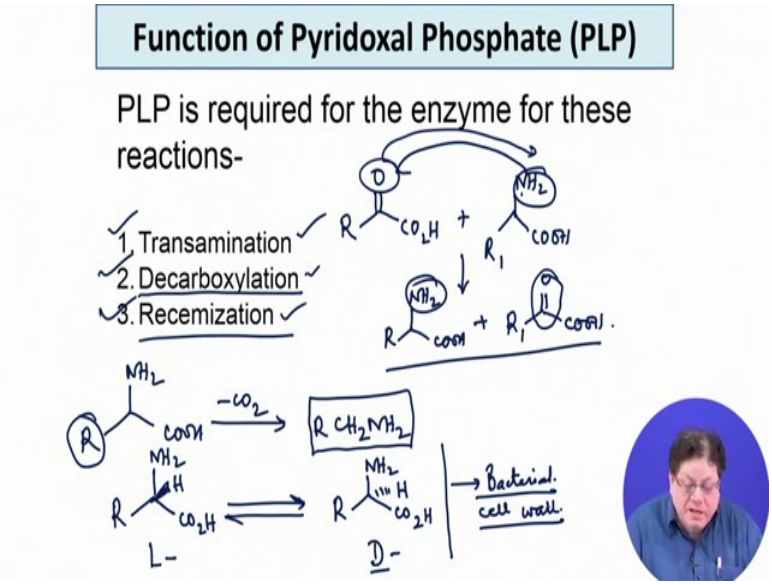

Now, let us see in what type of reaction pyridoxal phosphate(PLP) is involved as a coenzyme. One reaction is called the transamination reaction.

What is a transamination reaction? That is basically transfer of an amino group from an amino acid into an alpha keto acid. In this reaction, the starting alpha keto acid becomes the amino acid and on the other hand, the amino acid becomes the alpha keto acid. For transformation of amino acid to alpha keto acid, oxygen is resulted from water.

So, by transamination reaction one can synthesize new amino acids utilizing whatever existing amino acids that we have. PLP is also required for decarboxylation of amino acids. Now, here amino acids means alpha amino acid i.e protein amino acids. So, decarboxylation will give you RCH_2NH_2 and this is done by an enzyme called decarboxylase.

The transamination gives a route to new amino acids which are not present in the body. So, you can make that and then decarboxylation allows you to make a compounds which are having primary amino groups. Now, the compounds containing these primary amino groups act as neurotransmitters.

Now, the third reaction is called a recemization reaction and the enzyme is called racemase. All the protein amino acids belong to the L configuration or if you translate it

into R S configuration, they are S configured amino acids except for cystine. Cystine is the L cystine corresponds to D.

PLP dependent racemase enzyme will convert the L amino acid into D amino acid. Now, we generally have the impression that all the protein amino acids or amino acids present in the in the in the living system belong to a L configuration. But there are a few a few exceptions.

The hydrogen is beta in order to make it L and the hydrogen is alpha in order to make a D (refer vide 29:47).

Now, this transformation is very important. Recemization is always a reversible reaction. If you do it chemically then you will get a 50-50 mixture. But if you do enzymatically then you will get one predominant product as the enzyme is chiral.

D amino acid like D alanine is building block of bacterial cell wall. So, this is very important for survival of bacteria.

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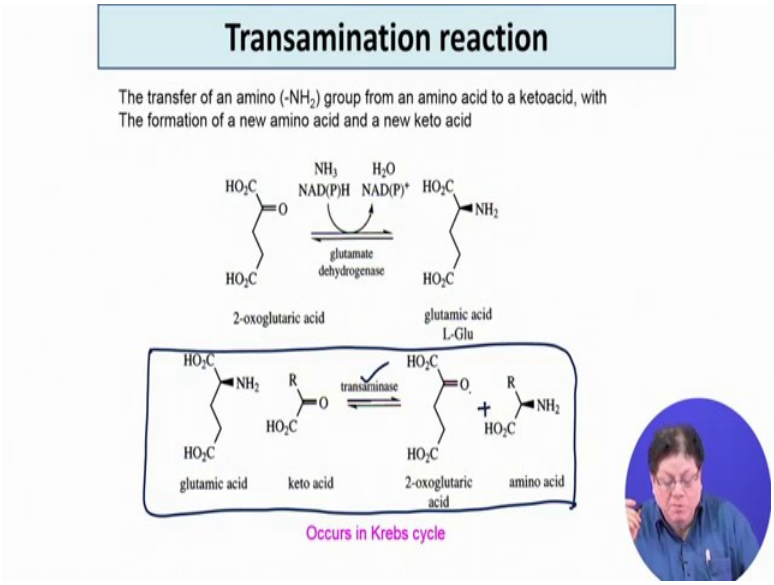

We will discuss mechanisms of these different reactions. So, let us consider the transamination reaction catalyzed by a transaminase.

We can call this enzyme a transaminase because switching takes place between glutamic acid and then another alpha keto acid. The glutamic acid goes to the keto acid that is 2-

oxoglutaric acid also known as alpha ketoglutaric acid or alpha kg and the in turn the starting keto acid goes to the amino acid. So, this will be called a glutamate transaminase; that means, your glutamate is the amino acid part. So, we want to discuss the mechanism of this reaction.

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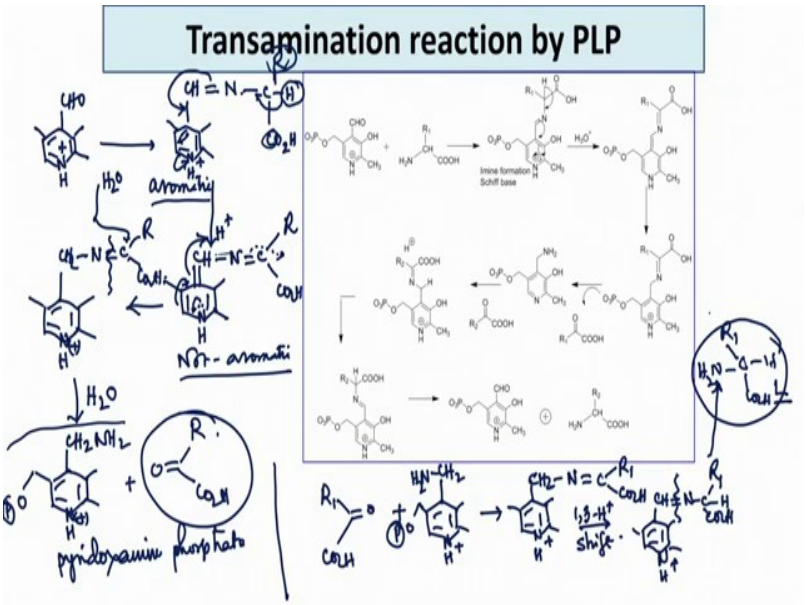

The mechanism of this reaction is shown in this slide, but I will explain it little bit elaborately. We again write the structure of this pyridoxal phosphate; CHO we are not writing these substitutes that are. So, these are the 3 substituents here. Now, this group this pyridoxal phosphate in presence of in presence in the biological medium, where the pH is normally kept usually maintained at 7.2. So, this nitrogen because its pKa is more than 9 so, that will be mainly in the protonated form.

So, if it is in the protonated there is a pressure on this molecule. Here, a positive charge is on electronegative nitrogen, so it will like to have a neutral nitrogen. It can do so by drawing the electrons towards itself, the bond pairs specially the pi bonds are the vulnerable bonds.

So, we can say that this is kind of an electron sink because it pulls electron. Here we are talking about a reaction where a keto acid is converted to a amino acid and the amino acid is converted to keto acid.

It is a two substrate reaction. First, the amino acid containing substrate that first reacts with the goes and binds. Pyridoxal is already bound to the enzyme.

You will have this CH that is the alpha carbon, CO_2H and R attached to the carbon. Now, let us inspect this molecule a little bit in detail because this is an electron sink. So, it wants to pull the electrons towards itself. If it does that that this electron pair, the pi bond goes to the nitrogen. So, that creates a positive charge here in the carbon. So, the carbon gets neutralized, it neutralizes the charge by pulling the next adjacent double bond, if it does that the positive charge comes here.

So, ultimately then this double bond will be pulled towards this self to this carbon-carbon bond, nitrogen becomes plus, but nitrogen also because the whole genesis is the plus charge of nitrogen. So, this nitrogen also does not want to be positively charged. It will pull or break some of the; because there is no double bond now. Now it wants to pull some of these breaks, some of these bonds in order to neutralize the positive charge of the nitrogen.

So, what happens? So, there is a C-H bond that can break, there is a C-C bond, but this C-C bond belong to a carboxyl group and this is a C R bond that is a C-C bond. But, this is not this could be either methyl in case of alanine, in case of glutamic acid this is CH_2 CH_2CO_2H.

So, it is a when basically carbon attached to an aliphatic carbon, that is a very strong one carbon-carbon bond. This is also a carbon-carbon bond, but this is attached to the carboxyl group. So, now it has got two options: one is that the hydrogen can be lost like this.

So, there is a relay process whereby the electrons that is released by the hydrogen that goes ultimately to the nitrogen in the pyridinium ion. Why it happens; because this is an electron sink, the nitrogen wants to neutralize its positive charge. The other option is the carboxyl group also can be this C-C bond can be can be broken, but that will lead to the second type of reaction that is called the decarboxylation, we will come to that.

So, first let us consider that this hydrogen can be lost. So, if this hydrogen can is lost then what you get is a system of NH double bond. You have a double bond here CH and then N double bond C and have CO_2H and these are the three substituents. Now, what happens? This system has paid the price of acting as an electron sink. The price is that this was aromatic to start with, now this is not aromatic.

So, if it is not aromatic, it wants to regain the aromaticity. So, again you have to utilize this nitrogen, bring it here, this bond goes here. This goes this has two options either it can take up the proton at this point. It can go further down and then take this wherever that carbon was attached to a hydrogen, it can go up to the up to that point also. Basically in this slide I can explain all the three reactions. First of all these hydrogen can be lost. Why is this lost? Because, this is acting as a sink that is the driving force.

Instead of hydrogen you can have decarboxylation that gives the family of decarboxylase enzymes and then when it comes back. There is a price as there is this aromaticity loss. So, it wants to regain the aromaticity, when it regains aromaticity the electrons just fly in the in the opposite direction.

So, the nitrogen lone pair comes here, this goes here and now it has there are two options. Either the hydrogen can be taken up by this carbon or if it goes further here in this carbon nitrogen bond, then this has to take the hydrogen.

Now, remember when the hydrogen is lost, this carbon has lost its stereo chemical integrity. It is no longer a stereogenic or chirality center. So, when the chirality is lost and then regain at that point it can go from L to D. Then the enzymes which allows it to abstract the hydrogen here, it goes to the D. It has the option to form the D-Amino acids and if it goes up to here then actually that is the mechanism for transamination. I will just continue NH and now it has become the positively charged nitrogen.

This becomes CH_2 and then N double bond CR and CO_2H. So, now what will happen? The first part of the reaction is over, now water comes and attacks this carbon and hydrolyzes the imine. Imine hydrolyzed means it will from the amine and the and the carbonyl. So, back to the amine carbonyl stage. So, what is the amine and what is the carbonyl species? The amine is basically now this will be $CH_2 NH_2$. Now, this is what is called pyridoxamine phosphate.

Remember this CH_2OH is actually attached to a phosphate. And what is the other product? The other product is your RC double bond O and CO_2H. So, now the amino acid has been converted into the keto acid alpha keto acid.

Now this pyridoxamine is held as a in the active site and then now the other keto acid; other keto acid means namely R_1, suppose another alpha keto acid R_1CO_2H. Now, that reacts with this pyri doxamine phosphate.

Pyridoxamine means NH_2 will form the imine. The new alpha keto acid that was your substrate your starting material. So, this will form CH_2 and then N double bond CR_1CO_2H that is your starting R_1. It comes from the starting alpha keto acid. Now, what will happen there will be a tautomeric shift, this one of the hydrogen here goes to the carbon.

So, that is a 1,3- prototropic shift and if that happens then you get an imine. But the double bond is now between the pyridoxal carbon and the nitrogen and not the alpha carbon and the nitrogen.

So, then it goes to that one CO_2H. You have this NH^+. I think now it is easy, this is R_1, now there will be a hydrolysis here. So, if there is hydrolysis so, what will happen? You get the R_1, this is there is a CH that is what is missing; so, NCR_1CO_2H and there is the H.

Now, this tautomeric shift is at enzymatically controlled. So, what you get is it is the L configured amino acid. It is an enzymatic reaction and it is a kind of asymmetric synthesis.

Finally, you will get only the L configured compound plus enzymes are chiral reagent.

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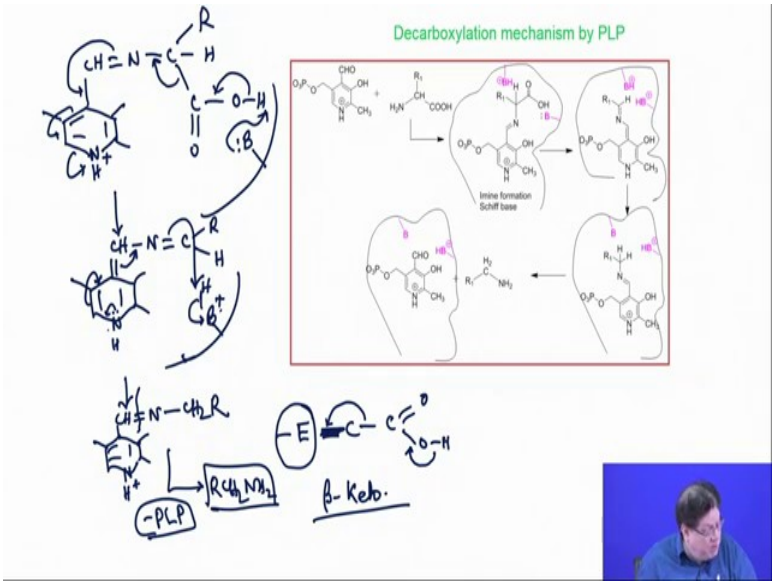

So, mechanism of decarboxylation is now easy. Basically everything is an electron source and electronsink. Initially electron source in transamination was the amino acid itself. When it forms the imine, the hydrogen breaks in transamination and then ultimately that amino acid is released as keto acid.

Then the other substrate keto acid comes and joins with the forms the amine, then there is a tautomeric shift. The double bond shifts from one position to the other resulting in release pyridoxal phosphate. That means, release of the same coenzyme and then the amino and the keto acid in the turn is converted to L alpha amino acids. Now, there is breakage of the carbon carbon bond.

The carbon basically involving the one of the carbon belongs to the carboxyl carbon of the amino acid. So, what happens here? That you have seen this is your imine so, the initial intermediate is same for all these cases, that is the amine and then now instead of the hydrogen, the enzyme must be having a some basic group and that abstracts the hydrogen. These goes here and that releases the that breaks the carbon dioxide releases the carbon dioxide and so this is plus.

This is as a result of the electron withdrawing power of this pyridoxal unit because, you know that this any carboxylic acid. If it has an electron withdrawing group attached here. Then electron withdrawing group is at this point. So, that goes there because it is electron withdrawing. So, the electrons now flow to this is acting as the electron sink.

We know that this is beta keto systems but there could be other functionalities. Now at it is not necessarily keto, it could be other functionality. Like it could be imine or it could be the pyridoxal mediated homologous imine. So, basically electron sink character promotes this decarboxylation and after that it is easy that you get the loss of aromaticity by doing this and the next step will be regaining of aromaticity.

So, instead of hydrogen CO_2 is lost now and then now the again it is the reverse direction, the electron starts to flow. Why it will flow; because to regain the aromaticity, now it is acting as electron source. As soon as it finishes it achieves the electron sink character, it becomes an electron source.

So, nitrogen lone pair again flies back in the opposite direction and this takes up the hydrogen. The base is already having that hydrogen. So, the base is released at the active

site. The product is again the imine but here the pyridoxal carbon is attached to the nitrogen by the double bond.

So, it is CH_2R now. Now, there will be hydrolysis and finally, what you get is RCH_2NH_2. The free amine you will get and you release the PLP. PLP can participate again in the reaction; another substrate goes and binds everything is intact. The base is also released at the active site whatever the amino acid side chain and the pyridoxal is also released as the aldehyde.

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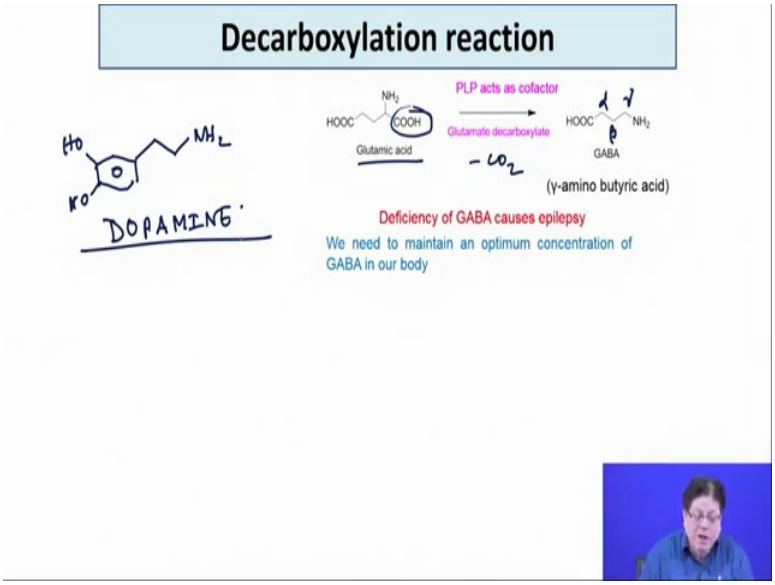

So, that is the mechanism of decarboxylation. What is the some of the decarboxylation reactions are extremely important, usually amino acids are the starting materials for decarboxylation. It has to be because the mechanism says that you have to form the imine with the pyridoxal making it an electron sink.

If you take glutamic acid, there are 2 carboxylic acids but it will only be the this carboxyl. The amino acid carboxyl will be decarboxylated.

If it is lost then what you will get is called GABA gamma aminobutyric acid. So, this is gamma amino butyric acid. This is a very important neurotransmitter. Many of these neurotransmitters which are essential for keeping our mental health in the perfect form.

So, this optimum concentration of GABA gamma aminobutyric acid is necessary. Similarly, you have other compounds like dopamine. Dopamine is also a very important neurotransmitter.

Neurotransmitters are basically released from the neuron and in the medicinal chemistry we will discuss. So, it brings about certain reactions and then it create certain signals, but you have to maintain an optimum concentration of these neurotransmitters in the central nervous system or in the brain. If there is a disbalance then the different types of mental diseases that occur. So, this is the very important target for drug development.

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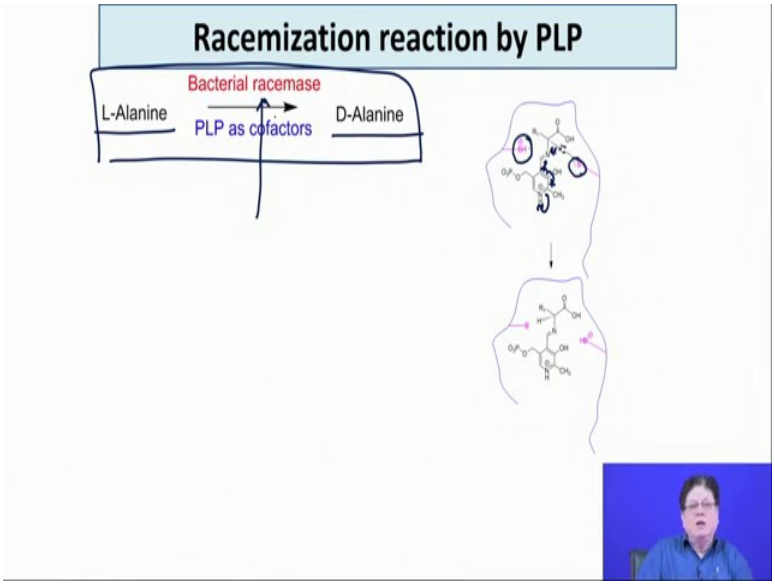

In case of racemization, it will take the amino acid forms the imine and then the hydrogen is lost. So, there are two sites; one is a base another is the conjugate acid form of the base and then this is projected upwards and that is projected downwards.

So, these base abstracts the hydrogen because this is alpha. It is closer to this B. So, that abstracts the hydrogen and then the same thing happens, electron sink character of this pyridinium ion is demonstrated.

As soon as that forms, it again comes back and then takes the hydrogen. Now it has the option by taking the hydrogen from this base which is having the hydrogen

It can take the hydrogen from the conjugate acid that is actually projected upwards.

So, if the hydrogen is delivered from the top and then you will get the D form of the amino acid. So, that is at the very simple mechanism. Its again the loss of hydrogen like the transamination but here again the hydrogen is regained. But, in the intermediate the stereochemistry is lost and in the final product the stereochemistry is again regenerated.

So, that is why the L amino acids can be converted to D. D-alanine is a constituent of bacterial cell wall. So, this isomerization is very vital for the bacteria to survive because they have to make the cell wall.

If if you can stop generate an inhibitor which inhibits the cell alanine into D-alanine process. Then you will get here what you will discover is an anti-biotic or an antibacterial agent3

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We will talk about this all these things during the later half of this course, that is the when we talk about take up the medicinal chemistry part.

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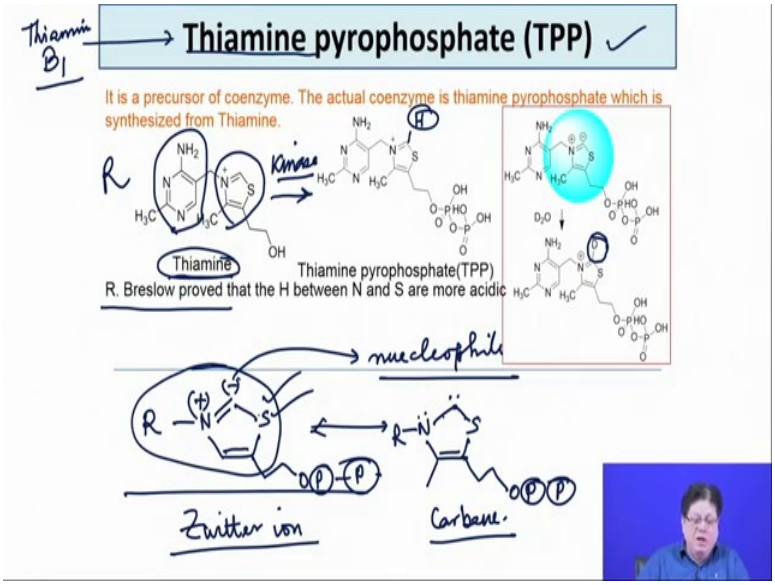

We have considered the vitamin B_6, we have discussed that three types of reactions. It catalyzes or it takes part along with the enzyme or it takes part to help the enzyme to catalyze the reaction and, the next one is what is called thiamine pyrophosphate. Remember the spelling thiamine; in DNA one of the base is called thymine. So, you must recognize the difference, this is thiamine pyrophosphate also the starting

compound. This is not the coenzyme form, the starting compound is known as thiamine only.

The structure is given here, this is what is thiamine. It has got a pyrimidine nucleus and it has got a thiazolidine which is present in the in the salt form N plus and then it has got a sidechain hydroxy ethyl and a methyl. So, this is the vitamin form or the pro-coenzyme form and the actual coenzyme becomes a diphosphate or a pyrophosphate.

So, this OH is converted to a pyrophosphate by the kinase enzyme. You get the coenzyme form of TPP. This hydrogen is quite acidic because of the positive charge on the nitrogen.

So, it wants to pull the electrons towards itself, thus making it the hydrogen very labile. Now, Ronald Breslow of Columbia University in the 1950s, he did an experiment. He took this thiamine pyrophosphate, dissolved it in D_2O and took an NMR of that and slowly what he found that this hydrogen is replaced by the deuterium.

So, the deuterium where from it is coming or the existence of deuterium or the exchange of hydrogen by deuterium shows, that this hydrogen is very acidic. If this hydrogen is very acidic, Breslow proposed a structure which looks like this for the thiamine pyrophosphate. This is a minus; the hydrogen is there I just represent this by R.

So, that is R, nitrogen is plus and double bond is here and that is the OPP . So, this is the thiamine pyrophosphate in aqueous solution at pH say 7.2.

So, it exists as a zwitter ion, it exists as a zwitter ion and you can draw another resonating structure of this. That nitrogen withdraws, the culprit for this formation of release of the hydrogen is the nitrogen with a positive charge.

So, nitrogen can we draw this electrons towards itself thus making it neutral, thus making it neutral but what happens to the carbon? The carbon now is the carbon is basically having a lone pair but it is not having any charge but the thing is that it has got 6 electrons.

It becomes a 6 state of electrons. So, again I go back to this so, this is the structure of this other form these substituents. So, the substituents are here so, that is OPP.

So, this is the neutral form, but this is also called the carbene because, the nitrogen is in the having a 6; the carbon is having a having 6 electrons around surrounding it; 2 electrons here 2+2+2=6. So, that is carbene, carbene is the neutral carbon bivalent carbon.

So, it has got a carbene structure, it has got a zwitterionic structure. But this structure; obviously, predominates because all the nitrogen's all the atoms are fulfilling the octet. Here the octet is not fully filled for the carbon, but anyway this is the predominant form, this carbon is now a nucleophile. In fact, Breslow's work on thiamine pyrophosphate has given rise to a new type of chemistry in synthetic organic chemistry that is the use of N heterocyclic carbene. These are N heterocyclic carbenes.

So, those are synthetic compounds you can have another nitrogen here, but nature has picked up sulfur because sulfur can have this empty 3d orbitals.

So, this anion can also delocalize with the sulfur d empty 3d orbitals, thus giving greater stability of the zwitterionic structure. So, this session concludes with the chemistry of pyridoxal. We have probed the structure of thiamine pyrophosphate which is the coenzyme form of vitamin B_1 i.e thiamine.

Thank you.