BOGDAN FEDELES: Hello and welcome to 5.07 Biochemistry online. I'm Dr. Bogdan Fedeles. This video is about pyridoxal 5 phosphate, or PLP, an essential metabolism cofactor derived from vitamin B6. All animals are auxotrophic for PLP, meaning they need to supplement their diet with vitamin B6 in order to survive. PLP is one of the most ancient cofactors, and surprisingly, it can catalyze chemical transformation, such as a transamination even without an enzyme. PLP is actually involved in a staggering number of biochemical transformations. This video summarizes the most important reactions involving PLP that you will see in 5.07, and will also show you how to write the complete curved arrow mechanisms for these transformations.

Let's talk about PLP-catalyzed reactions. As we just mentioned, PLP is the cofactor derived from vitamin B6. This cofactor is very important for a number of reactions. In this course, we're going to look particularly at the transamination reaction. This is a crucial reaction for the metabolism of all amino the acids, and we're also going to encounter this reaction when we replenish the intermediates in the TCA cycle, what we call anaplerotic reactions. And we're also going to see PLP involved in reactions in the malate-aspartate shuttle that transfers redox equivalents, reducing equivalents, between mitochondria and cytosol.

Let's take a look at the structure of vitamin B6, also known as pyridoxine. This is the molecule that we ingest when we get our daily vitamin supplement. Now in the body, this gets oxidized to form intermediate called pyridoxal. Notice the aldehyde group here, which is going to be the business end of the molecule. Now, the active co-factor, PLP-- it's actually the phosphorylated version of pyridoxal. This requires one molecule of ATP and the enzyme pyridoxal kinase. And we get PLP, or pyridoxal 5 phosphate. The name PLP comes from the initials as outlined here.

Now, this nitrogen on the pyridine ring tends to be protonated because it's pKa, it's close to physiological pH, between 6 and 7. Now, for the rest of this presentation we're going to be abbreviating this phosphate group as such, and throughout the course.

Now, a related molecule is pyridoxamine 5 phosphate, which we'll see, it's an intermediate in the mechanism of PLP-catalyzed reactions. Also known as PMP. Now, notice the PMP has an

amine group here which replaces the aldehydic group, which is the business end of the molecule. Now, in all reactions with PLP, this co-factor is actually covalently bound to the enzyme that uses it. Typically, there's a lysine in the active site of the enzyme. As you remember, lysine has an amine group, and this can form a Schiff base with the aldehyde.

The reaction proceeds in two steps. First, we form a tetrahedral intermediate. As such. And then, we form the Schiff's base. So this will be the enzyme-bound PLP. And this is where all the PLP-catalyzed reactions start.

Let's take a closer look at the transamination reaction. Transamination reaction occurs between an amino acid and an alpha keto acid. We have here amino acid 1, where we highlighted the amine group, and alpha keto acid 2. As you can see, there's a keto group next to the carboxyl. Now, in a transamination reaction catalyzed by PLP, the amine group moves from the amino acid to the keto carbon of the alpha keto acid. And we obtain a new alpha keto acid, and a new amino acid. So in effect, the PLP-catalyzed transamination reaction facilitates the transfer of the amine group from an amino acid to an alpha keto acid.

Now, this reaction actually occurs in two steps. In the first step, the amino acid transfers the group to the co-factor itself. So if you remember from the previous slide, the PMP contains an amino group, and that will actually contain this amino group that was taken from the amino acid 1. Now in the second step, the PMP will transfer its amino group to a different alpha keto acid to generate a new amino acid. Now, there are enzymes for virtually every single amino acid that can accomplish this first transformation, where by using PLP, to transfer the amine group and form an alpha keto acid and PMP.

Now, in the second part of the reaction, however, the alpha keto acid 2 is typically alpha keto gluterate or oxaloacetate. So in this case, not any alpha keto acid can function. Alpha keto gluterate or oxaloacetate.

Now, let's take a look at an example. For example, glutamate. It's going to be our amino acid. And oxaloacetate is going to be our alpha keto acid. And in a PLP-catalyzed transformation, we will obtain the alpha keto acid corresponding to glutamate, which is alpha keto glutarate, and the amino acid corresponding to oxaloacetate, which is aspartate. This enzyme that catalyzes this transformation is in fact ubiquitous, and it's found both in the liver and the muscles, and it is in fact a-- we can call it, depending and the product, we can call it aspartate, transaminase or glutamate oxaloacetate transaminase. In fact, if we find this in the

bloodstream, this enzyme acts as a biomarker. And it tells us about some damage that might have occurred in muscle or liver, which forced the cells to spill out their contents. This biomarker is-- you'll often see as SGOT-- serum glutamate oxaloacetate transaminase. And this is just one of the biomarkers that are measured in blood tests that tells us about heart disease or liver disease.

Let's take a look at the mechanism of the transamination reaction. And in particular, we're going to take a look at part one, which as we discussed before, the amino acid reacts with PLP to form an alpha keto acid and PNP. Here is our co-factor PLP, covalently bound to the lysine in the active side of the enzyme via a Schiff's base. And here is our amino acid starting material.

So in the first step, the lysine that forms the Schiff base with the co-factor is going to be replaced by the amine functionality of the amino acid, and it will form a new Schiff base with the co-factor. This starts with the amine group attack on the pyridoxal carbon to form a tetrahedral intermediate. And following an additional proton transfer, the lysine can be kicked off to form the new Schiff base.

So far, we have started with the Schiff's base corresponding to the PLP bound to the enzyme and we now obtain a co-factor forming a Schiff base with the incoming amino acid 1. So this portion of the mechanism is called transamination, because we're starting with one amine and we're forming a different amine.

Now, let's take a look at the alpha proton attached to the alpha carbon, which we're going to highlight here. This proton is now in between two carbonyl-like groups. Here is the carboxyl group and here is the Schiff base group. So it becomes acidic enough that it can be removed by an active side base, for example the lysine in the active site. This will generate a carbanium alpha carbon. This carbanium can only form because it is resonance stabilized. And indeed, the PLP link system-- it's highly conjugated, and it's a good electron sink. For this carbanium, we can write, in fact, many different resonance structures.

Let's take a look at one of them. This symbol denotes resonance structures. Notice in this structure that the positive charge on the pyrodine nitrogen is now gone, and highlights the fact that this is a good electron sink. And the ring now looks more like a quinone. That's why we call this a quinoid structure, or intermediate. This quinoid structure shows us a glimpse into how the reaction will proceed, because the alpha carbon now-- it's doubly bonded to a

nitrogen, which anticipates how this alpha carbon will become a keto group and a product of the reaction will be an alpha keto acid.

What happens? The quinoid structure can be re-protonated, but at a different place. For example, on the aldehydic carbon of pyridoxal. To highlight that these facts-- these structures are in fact resonance structures, we're going to put them in brackets.

So let's take a look at what happened in this past couple of steps. So we had an alpha proton that was fairly acidic, it was able to be removed by the active site lysine. And then this proton came back to a different position. So all that's happened in just a couple of steps was a proton transfer. Now, looking at this intermediate, we can see that in fact the Schiff's base or the imine of the PMP form of the co-factor and the alpha keto acid corresponding to amino acid 1. So via a hydrolysis reaction, these two can come apart. So in the first step, an activated water molecule attacks alpha carbon, forming a tetrahedral intermediate. And one more proton transfer and we're going to kick off the pyridoxamine form of the co-factor. And notice we obtain PMP and the alpha keto acid corresponding to the amino acid 1. This last step is, in fact, just a hydrolysis reaction of a Schiff base.

Now, let's take a look at the second part of the PLP transamination reaction. The part one left us off with formation of PMP. So in this second part, PMP will react with a new alpha keto acid to regenerate PLP and a new amino acid. In fact, this part of the mechanism-- it's the exact reverse of part one. Here is PMP and our alpha keto acid. In the first step, we're going to form-- as we've gotten used so far-- to a new imine between the keto group of alpha keto acid and the amine group of PLP. As usual, first we're going to get a tetrahedral intermediate. And one more proton transfer, and we can kick off the OH group to form the imine. Now, this portion of the reaction is, in fact, imine formation, which is the reverse of the hydrolysis step that we saw in part one. Now, as you remember, there is an active site lysine which can act as a general base. And it's going to de-protonate one of these two protons on the pyridoxal ring.

The reason that we can form this carbanion here is because this negative charge is delocalized throughout the entire ring system. And let's show one important resonance structure. Which is none other than the quinoid structure we saw before. Just as before, the protonated lysine can now donate proton on a different position. For example, the alpha carbon of the alpha keto acid. As you can see here, now the proton is on the alpha position. And now where this starts to look more like the Schiff's base formed by an amino acid with the PLP version of the co-factor.

So from here on onwards, we're just going to substitute the PLP-- the amino acid bound to the PLP-- with the active site lysine in the transimination reaction that we saw before. So first, the lysine can attack this carbon, forming a tetrahedral intermediate. And then, following some proton transfer, we can kick off the amino acid and generate the Schiff's base corresponding to the PLP co-factor bound to the enzyme. So here we have PLP, enzyme bound, and the new amino acid 2.

We mentioned PLP is a very versatile co-factor, so let's take a look what other reactions besides transamination can PLP catalyze. One interesting reaction, used especially by bacteria, is a racemization. This involves taking an L amino acid, for example L alanine, and converting it via a PLP-catalyzed reaction to D alanine. This is an important reaction for bacteria, because they incorporate the alanine into the cell walls, which make it very hard to recognize by the immune system, and makes it very hard to digest by the host proteases.

Let's take a look at a key intermediate in the PLP-catalyzed reaction. As before, L alanine is going to react with the PLP bound to the enzyme, and it's going to form an amine. Here we're highlighting the stereochemistry of the alpha hydrogen. And here is our active site lysine. As we've seen before, this alpha hydrogen is acidic enough that it can be removed by the lysine. And it's going to form a carbanion at this position.

Now, this carbanion, as we've seen before, is able to form because the charge is, in fact, delocalized through the entire system of the pyridoxal ring. So for this structure, we can write a number of resonance structures, which we're not going to mention here. Now, this carbanion can be re-protonated. And here we had a-- the hydrogen was pointing up on the top of the plane of the page, but we can re-protonate it from the bottom, and that will change the stereochemistry of this carbon. See if that re-protonation happens from the bottom, we will obtain the Schiff base corresponding to the D alanine. So by being able to generate this carbanion at the alpha position, the PLP reaction and co-factor allows the inversion of the configuration and the alpha carbon, converting L alanine to D alanine.

Now, another interesting reaction that requires PLP is de-carboxylation. Here we're looking at an amino acid-- for example, glutamate. In a PLP-catalyzed reaction, you can lose this CO2 group and form this molecule, which is called gamma aminobutyric acid, or GABA. This is, in fact, a very important neurotransmitter and inhibitor, a neurotransmitter that is required in the brain.

Let's take a look how this de-carboxylation is catalyzed by PLP. As always, have we seen so far, the glutamate will react with PLP bound in the active site of the enzyme, to form a Schiff base. Here is the Schiff base. Like that. Now instead of de-protonating at the alpha position, the CO2 is activated to leave. Because it will leave behind the carbanion. Such as that. And as we've seen before, a carbanion formed at this position can de-localize throughout the entire pyridoxal ring, and therefore it stabilize and it can exist long enough. And we're not going to draw, but there-- you can imagine, there are a number of different resonance structures.

Now, this carbanion-- it gets protonated quickly by a general acid, and will generate this structure, which is just a Schiff base corresponding to gamma aminobutyric acid with PLP. And now, from here, a transimination where the active site lysine will remove the PLP and free up the GABA products of the reaction.

In this video we talked about PLP-catalyzed reactions. PLP is the co-factor that comes from vitamin B6. Here's vitamin B6, what we call pyridoxine, which is the molecule that we find in our vitamin pills. Now, in the body, pyridoxine formed pyridoxal, which is activated to form pyridoxal 5 phosphate, or PLP. And typically when it reacts, PLP is found as a Schiff's base bound in the active site via a lysine. PLP is very important for transamination reactions, which are essential for the metabolism of all amino acids.

We have seen the transamination reaction where an amino acid 1 reacts with alpha keto acid 2 and the PLP catalyzed reaction forms an alpha keto acid 1 and amino acid 2, essentially transferring the group-- the amino group from the amino acid to the alpha keto acid. The reaction occurs in two steps, where first the amino acid is transferred to PLP to form PMP. Then PMP then transfers this group-- the amino group-- back to a alpha keto acid to generate a new amino acid.

As we saw, the mechanism of transamination involves multiple steps. The first step is a transimination reaction where the Schiff's base that's formed between the active site lysine and the PLP becomes a Schiff's base between the incoming amino acid and PLP. Next, we have a proton transfer, which is allowed by the ability of the PLP ring to stabilize a negative charge, and move the proton from the alpha position to somewhere on the PLP ring via a quinoid structure. And finally, that the resulting Schiff base is hydrolyzed to generate PMP and an alpha keto acid. In the second part of the reaction, PMP now reacts with alpha keto acid to form a new Schiff base, and then the proton transfer happens in reverse, via, again, a quinoid structure, to generate the Schiff base corresponding to the PLP and the new amino acid 2.

Which, via a transamination reaction will generate amino acid 2 and the PLP enzyme bound.

Finally, we mentioned that PLP is very versatile, and it can catalyze other reactions, such as racemization, for example, switching the configuration of the alpha carbon from L alanine to D alanine, or de-carboxylation, generating alpha aminobutyric acid, or GABA, an important neurotransmitter from glutamate.