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JOANNE STUBBE: Hi. Today, what we're going to do is focus on yet another cofactor that you get out of your vitamin bottle. And today we're going to be looking at vitamin B6. And vitamin B6 is the cofactor that you use whenever you want to metabolize amino acids.

Where do you get amino acids? We all eat proteins. The proteins get degraded to amino acids. And we can use the energy released. And trap that energy to do bio synthesis. And we use amino acids, convert them into central metabolism, and use them to make fats, or sugars, depending on what the environment is telling us we need to do.

So let me introduce you to this cofactor, the vitamin itself, as you have seen now, over and over again, is not the actual cofactor. It's Pyridoxine. And so that's what you eat out of the vitamin bottle. And as in the case of all vitamins, inside the cell it has to be converted into the active form of the cofactor. And the cofactor we're going to be talking about, the active form is Pyridoxal phosphate, which is called PLP. And the structure of pyridoxal-- there two main structures of the pyridoxal phosphate cofactor, one of them is involved in 80% of all the chemistry. And the one we're going to talk about today, uses both forms of the cofactor. That fits into central metabolism. I'll show you how that fits in a minute.

So pyridoxal phosphate has this structure. You have a pyridine ring, the pKa. The pyridine ring is 6 5, so it may or may not be protonated. And the key part of the cofactor-- one of the key parts of the cofactor, is this aldehyde. So pyridoxal means that this is an aldehyde.

The second form of the cofactor is pyridoxamine. So this aldehyde is somehow-- and we will look at how this happens-- is converted from this aldehyde, into an amino group. And this is called PMP, or pyridoxamine phosphate. Now the other thing I wanted to show you, before we move on to look at more details of pyridoxal phosphate, is-- while this is the form of the vitamin. You almost never see this form inside the cell. It is always bound in the active site of the enzyme. And it binds in the active site of the enzyme-- so here's your enzyme-- through the epsilon amino group of a lysine. So you never really, when you purify the protein, you

never isolate the aldehyde.

So this is the aldehyde. And I won't draw out all the rest of the structure, but what you have is an amino group, that's attached to a lysine, in the active site of your enzyme. And what we're going to do is convert this ketone into an imine. So this is chemistry you see over and over and over again. You've seen it already with carbonyl chemistry, with carbon-carbon bond forming reactions, with peptide bond hydrolysis, that we've already talked about. But what you do is, you're going to convert this aldehyde into an imine. You need to go through a tetrahedral intermediate. And the carbonyl is polarized, delta plus, delta minus. You spend a lot of time practicing this kind of chemistry to form a tetrahedral intermediate.

And I'm going to have a proton transfer. So one of the things that's tricky about this chemistry is that, if you count the number of protons they move around a lot in the active site. And the fact is, that you want to protonate something to make it into a better leaving group, you want to deprotonate something to make it function more like a nucleophile, or as a base, and nature has figured out how to orchestrate all the residues in the active site to do this. In most cases, we don't understand all of the details. And so I'm not going to focus on proton transfer, but you're going to see many proton transfers within the active site.

So this gives us a tetrahedral intermediate, or transition stage. You've seen that over and over again. And now what will happen is that you want to lose a molecule of water. So again, you need to pronate that, and what you form then is a new carbon doubly bonded to a nitrogen, rather than doubly bonded to the oxygen, that's covalently bound to the enzyme. And so, this is called an imine. And because it's an imine of an aldehyde, it's called an aldimine. So whenever you isolate your enzyme, whenever you isolate your enzyme, the pyridoxal is always covalently bound. OK, so, this bond is chemically easy to hydrolyze, but it's always covalently bound.

OK. So what I want to do now, is make a few generalizations about pyridoxal, but the first thing is, that in all cases, whenever you metabolize-- I want to metabolize an amino acid. Pyridoxal phosphate requiring enzyme is going to be the key player. OK. So here's an amino acid. OK, here's the alpha position, the beta position, and the gamma position. Well what's so amazing-- I remember first hearing about this-- is that you can do chemistry at all these positions. And the way nature figures out how to do this is by orchestrating the active site, with acid based catalyst sitting around in the right place, to allow you to do the chemical transformation that this protein has evolved to do.

So let me to show you what the alpha position, so this is the alpha position, you can cleave this bond. I'll show you how this happens. You can do all this chemistry with a few simple chemical transformations, takes practice, but once you sort of get what these transformations are, it's amazing what you can get this cofactor to help you do. And I'll explain that in a minute.

So you get cleavage of the carbon carbon bond that's loss of CO2 that's a decarboxylation reaction. We don't talk about that in 507, but that kind of reaction generates all of our neurotransmitters. You're going to cleave this carbon-hydrogen bond. Remember, amino acids are in the S configuration-- but for example in cell wall, in bacteria, they can be either the S or the R configuration, so you can cleave that bond, and put the proton back on the other face, that's a racemization reaction. You can cleave this carbon-carbon bond between the alpha and the beta position, that's a reaction-- remember, we talked about carbon-carbon bond formation-- this is the reverse aldol reaction. And the one where we're going to focus on today, which is the one that fits best into central metabolism, is what happens to this carbon-nitrogen bond.

So we have an amino group of our amino acid is going to get converted into a ketone group. So, this group, is going to get converted into this group. And to do that, we're going to use the imine of pyrodoxial phosphate that we have in the active site of the enzyme. So this is sort of like a carbonyl and that's going to get converted into the amine, the pyridoxamine. So these are the two forms of the cofactor. So what are we going to be focusing on, is how this reaction, actually, happens, and this is the most complicated of all the pyridoxal prostate dependent reactions. So that I'm going to come back to this in a minute, but what I want to do is make a few generalizations about where you're going to see pyridoxal phosphate chemistry in primary metabolism.

So what we're going to see is that the TCA cycle, tricarboxylic acid cycle, or the Krebs cycle, plays a central role. It's found in the mitochondria. You're going to see this over and over again, over the course of the semester. Things feed in and out of the cycle. A cycle means it goes around and around, and if you remove something from the cycle and don't put anything back into the cycle, the cycle stops. And you're in serious trouble.

So one way-- one thing-- one way to feed in and out of this cycle is through amino acids. So this reaction, which we're going to call the transemination, or a transamination, is metabolism of amino acid, we'll see into an alpha keto acid. So if you look at the TCA cycle and we look at

this reaction, what you'll see is you have this compound, called alpha ketoglutarate. OK, so alpha ketogluterate and that going to interconvert with the amino acid and so this ketone is going to be converted into an amino group, and so we're having the amino group converted into a ketone group. And pyridoxial is going to be converted into pyridoxamine. So we're going to have PLP converted into PMP. I'm going to show you how that works.

So, if you feed in glutamate from your diet, it-- by this pyridoxal phosphate dependent reaction-- can feed into the Krebs cycle. If you want to make amino acids, on the other hand, you can suck some of the alpha keto acid out, and convert it into amino acids. And you have to have a way of controlling whether you feed in, or you, actually, remove your metabolites from the cycle. If you come up here and look at oxaloacetic and aspartic acid-- So oxaloacetic acid is also an alpha keto acid, OK. And the amino acid-- can you see? I'm probably too close to the edge-- OK, and so here we have the amino acid. So here we have amino acid alpha keto acid, amino acid alpha keto acid.

If you go further up and go pyruvate feeds into the TCA cycle-- pyruvate comes from the glycolysis pathway, which again is breakdown of sugars, pyruvate is an alpha keto acid. So this is a CH3, so this is the simplest, and this can get interconverted into alanine. So what you see is this same theme-- amino group ketone, amino group ketone. I'm being sloppy, here. Most amino groups are protonated, because the pKa, you should remember, is around 9. So they're, mostly, protonated in solution. I should protonate this over here, as well. And so you have a ketone group and amino group. So this is called anaplerotic pathways where things can feed in and feed out of central metabolism. And this is the only time, during the course of 507, that you're introduced to amino acids, and how they're metabolized.

So what I want to do now is then, briefly, show you how this transformation, actually, works. OK. So I'm going to give you some general rules. So the transformation I just showed you-- an amino acid into an alpha keto acid-- looks to be complicated. And, I told you, the cofactor changes its structure from an imine into an amino group. And the question is, how does that happen? So I want to show you a bunch of simple, basic rules that allow you to think about all pyridoxal phosphate requiring chemistry. So I'm going to write down the rules, and then I'm going to show you how it works on the reactions we were just looking at this these transeminations, or transamination reactions.

So how do we think about the mechanism of these PLP enzymes? So the first thing is-- the first step is-- so you're going to start out with an imine bound to the active site of your pyridoxal

phosphate and an amino acid. I'll abbreviate it aa. And the first thing you do is, you're going to, remove this imine and form a new imine with the amino acid. So that's called a transimination reaction, OK. So what we're going to do is form a new imine. And so the imine from the amino acid, and I'll show you how this happen, is going to switch with this one. And so what you're left with, in the active site, is the amino group of the lysine. OK. So this lysine-- nature has figured out how to minimize the numbers of acid and base groups constrained in the region, the active site, where all the chemistry happens. And she uses this lysine, which initially is holding covalently the cofactor, in the active site. She then uses this lysine to do general acid, general base catalysis. And she uses it over and over and over again. Now every pyridoxal enzyme is distinct and has additional groups in the active site. But we know a lot about this chemistry. We even know what the groups are, but we're going to look and talk about generalizations.

So the first thing, we have to do is, we need to free up our general acid base catalyst, lysine, and we need to covalently bind the amino acid, which is a substrate, into pyridoxal. So that's the first step. And I'm going to come back to this in a minute.

The second step in all of these reactions is-- all amino acids have an alpha hydrogen, that alpha hydrogen has a very high pKa. It's very hard for a normal amino acid side chain, in the active site, to remove that proton, because it's not acidic enough. So what I'm going to show you is pyridoxal increases the acidity of that alpha hydrogen, making it easier to do the chemistry. So the second step in almost all pyridoxal reactions, is removal of the alpha hydrogen. And we can do that, because PLP makes the hydrogen more acidic. And I'll show you why that's true in a minute.

And then, the third thing is, once you remove that hydrogen, then you get a look at the chemistry you want to catalyze. And we're going to be talking about this transimination transamination reactions. But remember I told-- you can do all this chemistry at alpha, beta, gamma. So you need to assess what the substrate is, and what the product is, and then, within the active site, you're going to have to do a lot of manipulation with acid and base catalysts to get you into the final stage, where you can release the product you want to release.

So the last step in this reaction-- in all pyridoxal reactions-- so we do some chemistry in here. And I'll show you what the chemistry is with transamination reactions. The word transimination reactions-- now I keep saying transamination and transimination. That's because most textbooks call it transamination, because they think about pyridoxal as the aldehyde. But, in

reality, pyridoxal is always in the imine, covalently bound. And so that's why it's that transimination, rather than a transamination. So the last step then, is, hydrolysis. And I'll show you how that happens, or transimination to reform this structure. So we get ourselves back to where we started. So there's three simple steps, and in the middle, depending on what the reaction is, you have to do additional manipulations. But all pyridoxal enzymes go through these three, general steps.

The first step, I told you, in all these reactions is transimination. OK. So here's our Schiff-base, and it's your pyridoxal. It's covalently bound to the lysine in the active site. Now one thing that students often find confusing is the protonation state of this imine. And that's because it's right around neutral pH, pH 7. So depending on what's in the active site, it could be protonated, or not protonated, if it's protonated of course it enhances reactivity for nucleophilic attack. So you want it to be protonated. So the active site is going to manipulate itself to put in the protonated state.

So here you have an amino acid, and here we have a protonated imine, and so this is the nucleophile, and it can attack the carbon of the imine to form a tetrahedral adduct. So that's what we're doing here. So, this, is going to attack, this, to form this tetrahedral adduct. And, you've seen again, the tetrahedral chemistry over again, when I showed you how you formed this imine in the first place. So tetrahedral chemistry, tetrahedral intermediates, transition states, which collapse to form back imines, or carbonyls, happens over and over, and over again in pyridoxal chemistry.

So now what happens is we have this tetrahedral intermediate, or transition state-- I have it in parentheses, because it's a high energy intermediate, it doesn't sit around, and let us look at it. Most of the time you can never see it. It's very high on a reaction coordinate. This, collapses then, and when it collapses, what do you generate? You generate the lysine in the active site. So now we have generated a residue in the active site that can function as a general acid, or general base, catalysis through the rest of the chemical transformations. And what've we done, is we've converted this imine with lysine, now to an imine of the amino acid. So that's what transimination is, one imine to another imine. The imine that's covalently bound to the protein through pyridoxal, to an amino acid imine. OK, so, that's the first step.

The second step is that ultimately, in almost all pyridoxal reactions, you want to remove this alpha hydrogen. And that alpha hydrogen, again, is extremely non-acidic but by complexing the amino acid to pyridoxal-- this is what the function of the cofactor is-- you are enhancing the

acidity of this alpha hydrogen. You're making it easier for a group in the active site, a general base catalyst, like lysine, can now pull off this proton to generate this intermediate. Now why is this hydrogen more acidic? Well, if you look at the structure, you can draw all kinds of resonance structures which shows that this carbanion is more stabilized, because it's attached to the pyridoxal cofactor. So if you look at this, you can draw this resonance structure, which is shown here, and you can draw 20 other resonance structures. OK, so, the key here is you can remove the alpha hydrogen, because you're able to delocalize these unpaired electrons on this carbon over the entire system. So let me show you what that looks like.

So if I draw-- this is called Dunathan's hypothesis-- so here's our pyridine ring, here's our imine, and here's our amino acid. Here's the carboxal-- here's the side chain in our group and here's a carboxylate, OK. So the idea is, you have like a benzene ring-- if you haven't seen pyridine rings-- but you have a pi cloud that delocalizes-- where these electrons are completely delocalized over the aromatic ring-- but here, you also have a pi cloud and these things are close enough so you can delocalize. Now what you're going to do-- and the way nature decides what chemistry happens is since we want to cleave, in this case the carbon-hydrogen bond, she places that carbon-hydrogen bond-- by complexing the carboxylate and complexing whatever the R group is-- she places that carbon-hydrogen bond perpendicular to this plane of the pi aldimine system. So what you're doing then is the lysine, that we just liberated through doing the transimination reaction, you now generate an empty P orbital with unpaired electrons in it, generate the carbanion and now this system can completely delocalize. So, again, this pyridine ring is planar. So you can, completely, delocalize the electrons over this whole system. This is a pi cloud. And now this is already set up so that it can delocalize over this whole system. So what you've done then is because of the ability to stabilize this carbanion you're making that hydrogen more acidic.

So if you wanted to say, for example, cleave-- remember, I told you at the very beginning, you might be able to decarboxylate-- that enzyme, would place that CO2 perpendicular to the plane of the pi aldimine system and use the same strategy to stabilize the resulting carbanion intermediate. We're not going to talk about that chemistry in 507. So what you've done then-- what the beauty of pyridoxal is that she's increased this acidity, and allows you a great deal of flexibility. Because once you generate that carbanion-- those of you who've had 513-- you'll, immediately, recognize if you have a leaving group on a carbon adjacent to this carbon, you can do an elimination reaction. So it sets up a whole series of transformations.

For today, were only focusing on the transimination reaction. How do we convert this to an alpha keto acid, and the pyridoxal to pyridoxamine? That's the question we're asking. So we do this chemistry. And now what we need to do, we can use this resonance structure, we want to ask the question, what is the product? Well, we want to get to an alpha keto acid. OK, and if you look at this structure, this molecule is an imine of an alpha keto acid. So this is, exactly, the state we want to be in, but then we have all of this-- we have this reactive intermediate here. So what we want to do is protonate some place here to generate this state. So that the last step in all pyridoxal reactions is hydrolysis. And now we're set up with the hydrolysis reaction to generate the alpha keto acid.

So, we then want to ask the question, where can we protonate? And, so, again, we have this lysine which we now have used to pull off the alpha hydrogen. It's now protonated. So now instead of being a general based catalyst, here, it's functioning as a general acid catalyst. And so now what can happen is you can pick up a proton from this lysine, it's supplying it with that proton, to generate this structure and regenerate lysine that can function now as a general base catalyst. So it's toggeling between general acid and general base catalysis. And now remember, what we want to get in the end is this pyridoxamine, and we want this alpha keto acid, and now we're set up to rapidly go to an alpha keto acid. Where have you seen chemistry like this before? You've seen it in the aldolase reaction that we talked about with carbon-carbon bond forming reactions. So the last step in all PLP dependent transaminations is hydrolysis.

So here we have the lysine acting as a general base to activate water for a nucleophilic attack on this imine, which is activated to have water add, and now you form again, your tetrahedral transition state. I have all of these unstable species in brackets. We really-- sometimes we see them, sometimes we don't. But you have to work hard to see them. And now this simply, the tetrahedral adduct, collapses to form pyridoxamine and forms the alpha keto acid. So that's where we wanted to get. But now what happens, is we're in the form of the cofactor-- instead of being in the aldehyde form we're in the imine form. So what we want to do now is reverse this reaction using a different alpha keto acid, and we will generate a different amino acid. So now what can happen-- so in their pyridoxamine form, we then can bind a different alpha keto acid-- remember, in the first slide, I showed you three different alpha keto acids-- oxaloacetic acid, alpha-ketoglutarate, pyruvate-- you can reverse this whole process. And, in the end, what you end up with is a different amino acid and you regenerate your imine of pyridoxal.

So out of all-- the only pyridoxal phosphate requiring enzyme that goes from the aldehyde, or imine, to the pyridoxamine are these transamination reactions. And, so, this is the most complicated. Normally, at the end of your reaction you wind up in this state. And this probably seems extremely confusing to most of you, but after you solve three, or four, problems where you have to look at the actual transformations, and think about this tetrahedral chemistry, imines, and amino groups, and alpha keto groups, you will, I think, be able to actually easily see how ingenious nature has been to actually design pyridoxal phosphate. And, I think, the most amazing thing, of course, is that pyridoxal phosphate without any enzyme-- I told you can catalyze many, many reactions you do all the reactions with the en-- it does it spontaneously, at room temperature, at pH 7. What the enzyme does, is only allows one of these reactions by having everything positioned exactly the right way in the active site. So this is one of the cofactors, that I thought was amazingly cool when I was in graduate school, that made me want to become a biochemist.