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PROFESSOR: Today what I want to do within the lexicon is tell you about nature's most spectacularly beautiful cofactors. And these are formed from vitamin B-12, which you find in your vitamin bottle. OK.

So what is the structure of vitamin B-12, and why do I say they are spectacularly beautiful? So it's very hard to see, but if you look at the structure of this, where have you seen a molecule this complicated with five membered rings, each of which has a nitrogen in this? You've seen this when you studied hemoglobin, and you think about heme and proto protoporphyrin IX.

If you look at the biosynthetic pathway of heme, a branchpoint of that pathway is to make this ring, which is found in adenosylcobalamin and methylcobalamin, which is what we're going to be focusing on today. And this ring is called the corrin ring. So what I want to do is introduce you a little bit to this corrin ring and what's unusual about it compared to protoporphyrin IX that you've seen before.

So the vitamin, as in the case of all vitamins that we've talked about over the course of the semester, is not the actual cofactor used in the enzymatic transformation. The vitamin, which in this case would have this group replaced with cyanide, is vitamin B-12. The actual cofactors that bind to the enzyme have that cyanide replaced with either a methyl group-- and that's called methylcobalamin-- or they have it replaced with 5-prime-deoxyadenosine, and that's called adenosylcobalamin. And so the rest of the molecule is exactly the same. The only thing that's distinct is the axial ligands.

So you remember from your transition metal chemistry you had when you were freshman that here you have a cobalt III, and it's coordinated to four nitrogens. OK? So this would be the equatorial plane, and we're going to draw it like this, subsequently, because you can see how complicated this molecule is. And the middle sits right in this plane, but you can see that you don't have complete saturation of these pyrrole- type rings, so there's some pucker in contrast with hemes which are very flat. And then these are called equatorial ligands, and then you have axial ligands.

And so there are a number of things that again I wanted to point out that are unusual about B-12. One is the fact that the corrin ring again is much more reduced than a pyrrole ring. And so it's puckered. And if you look at the visible spectrum, or you use your eye and use your eyeball method, you see that cyanocobalamin is bright purple. If you replace this R group with a methyl group, this color turns out to be sort of yellow-orange. And if you replace this with 5-prime-deoxyadenosine, it turns out to be pink. So that's why I say that this is nature's most spectacularly beautiful cofactor.

So the other thing is you have two axial ligands. I just introduced you to these guys on the top face. But what you also see on the bottom face-- the bottom axial ligand-- is this unusual structure, which is called dimethylbenzimidazole. And it's attached to a ribose, but the unusual thing is in most nucleosides that you've encountered like ATP, this configuration is in the beta position-- it's on the top face of the sugar-- and here it's in the alpha position. So that's distinct. And the other thing that's distinct is the decorations around the side chain compared to what you see in porphyrins.

So this is the cofactor we're going to be talking about, and one of the questions that you're interested in is where do we find thes cofactors in metabolism. In a mammalian metabolism, the appearance of these cofactors is quite limited. So the only place you see it in metabolism in mammalian systems is you see methylcobalamin-- OK, where this is a methyl group-- in formation of the amino acid methionine. And so the methyl group from methionine-- I'll show you briefly at the very end of this little presentation-- comes from this methyl group.

You use adenosylcobalamin in odd chain fatty acid metabolism. So you have fatty acids that are either an even or an odd chain. When you break the odd chain ones down, you get proprionyl CoA. The proprionyl CoA, through a series of steps, is converted into malonyl CoA, which then gets converted to succinyl COA, which feeds into the TCA cycle. And in that pathway, you use an enzyme-- a mutase-- that uses a adenosylcobalamin. We'll talk briefly about the chemistry of a adenosylcobalamin; also methylcobalamin.

OK. So there were a few things that I wanted to say about-- some generalizations that I wanted to make about these cofactors. And at the first one is that again, the corrin ring is much more reduced than the pyrrole ring that you see in protoporphyrin IX, which you've seen in hemoglobin before.

The second thing is that you have this unusual dimethylbenzimidazole axial ligand, which you

see nowhere else in cofactor chemistry. It's only found in this particular cofactor. The second thing, which I think is the most amazing, is that what you see if you look back here is that you have-- if this is a methyl group, or this is this 5-prime-deoxyadenosine-- you have a carbon cobalt bond.

Well, this was discovered in the 1950s, and the first structure was solved of this molecule by Dorothy Crowfoot Hodgkin in 1964, and she won the Nobel Prize for this work. No chemist had ever seen a carbon cobalt blonde bond, and thought in fact biochemists were crazy that they even proposed such a structure.

So this structure-- and we'll see that this is where all the chemistry happens-- is completely unique. And people spent 25 years figuring out how this cofactor actually worked to do the transformations that I'll very briefly introduce you to.

So this is the first example of a carbon cobalt-- and will see that cobalt is in the plus 3 oxidation state bond-- so it's the first organo-metallic cofactor. And what people also found by studying this molecule is that the cobalt can actually exist in three oxidation states. It can exist in the cobalt I state, where in the d z squared orbital-- if you don't remember what a d z squared orbital is, you need to go back and look at your freshman chemistry-- you have two electrons. And it turns out that cobalt I is a super nucleophile. And we'll see that that plays a key role in the chemistry. And so again, this is the cobalt. The d. We're only looking at one of the orbitals of the cobalt.

On the other hand-- and this is found in methyl-- this is going to be used in methylcobalamin. So when you have a methyl as the axial ligand, you're going to use cobalt in this oxidation state, which is the plus one state. For almost all other B-12 dependent reactions, you have cobalt II, which has-- you've lost an electron, so you have only one electron by itself. And its d z squared orbital. And this really dictates the chemistry. And so this is found in adenosylcobalamin chemistry.

And then in the resting the state you have cobalt III. And cobalt III has none of the electrons in the d z squared orbital. And this is basically the resting state-- its most stable state-- where you find this cofactor. So in cyanocobalamin, which is vitamin B-12, the cobalt is in the plus 3 oxidation state.

OK. So we're going to see very briefly-- we're not spending much time on this-- is the cobalt I

state. It's a super good nucleophile. It affects the chemistry of the cobalt II state. It has one unpaired electron. You haven't been introduced to chemistry with one unpaired electron, which is radical chemistry-- that most people don't spend a lot of time talking about adenosylcobalamin because it's radicals, and they don't learn much about radicals in introductory organic chemistry. But I'll show you briefly how the enzymes work that use this cobalt II state.

The other thing I wanted to mention was the colors. And so if you want to understand how these cofactors work, the different number of electrons govern what colors you can end up seeing. And again I always use this is as an example on MIT'S campus in the spring. Cobalt II has spectacular orange color like the orange azaleas, and cobalt III is like the pink azaleas. And so they're really dramatically different. And this is why I say this is nature's most beautiful cofactor.

OK. So what I want to do now is briefly talk about mechanism. And I'm going to mostly talk about mechanism of the cobalt II state, and how adenosylcobalamin works, since that's the one that's most complicated. So what I'm drawing here is if we look at the structure of adenosylcobalamin, you see you have a cobalt with four nitrogen. These are the four nitrogens and these are the two axial ligands. So this is the abbreviation I'm going to be using in all the subsequent chemical descriptions of what I'm talking about, OK? So I'm not going to draw this structure out over and over again.

OK. So here's our adenosylcobalamin that we just talked about. And there's DMB. It's a dimethylbenzimidazole axial ligand, and then you have the 5-prime-deoxyadenosine in the top face, which is where all the chemistry is going to happen. So the business end of the molecule is going to be this part of the molecule. And the key to everything is this carbon cobalt bond.

Now, what's unusual about a carbon cobalt bond? It's very weak. If you go to 40 degrees, the bond breaks. Most things-- you can go to hundreds of degrees, and the bond is stable. So it's thermally labile.

And then the other thing that's unique about this cofactor is that it's light sensitive. So if you put adenosylcobalamin out on the bench top here, within two minutes the whole thing would be destroyed because you would break this carbon cobalt bond. OK? And that's the key to the chemistry of how this cofactor works. OK?

So I'm going to give you a generic mechanism, and then I'm going to focus on the mechanism

of methylmalonyl-CoA mutase, which you find in human metabolism. Before I get started, let me just show you what the reaction is and show you why it's unusual, and then we'll go back to the actual mechanism. OK.

So what does methylmalonyl-CoA mutase do? Again it plays a central role in odd chain fatty acid metabolism. OK, so this is CoA. You need to go back and look at your lexicon if you can't remember the structure CoA. But just remember it's a thioester. That's all you need to know.

OK. So this is the substrate, and this is called methylmalonyl-CoA. OK. So adenosylcobalamin catalyzes rearrangements. And this reaction has fascinated chemists for 30 years because there was no chemical precedent-- just like there was no chemical precedent for carbon cobalt bonds. When biochemists discovered this, there was no precedent for the reaction I'm going to show you.

So what is the reaction? It's a rearrangement. And it's a rearrangement because this hydrogen moves from this carbon to this carbon. And this whole group-- this thioester-- moves from this carbon to this carbon. So that's the actual reaction. It's reversible.

And so what you do is you generate succinyl CoA. OK so this hydrogen is moved over here. And this is succinyl CoA. And we haven't talked about it yet, but succinyl CoA plays a central role in metabolism in the TCA cycle.

OK. So the question that we want to focus on now is how do you catalyze this weird rearrangement. What is this cofactor? This big, huge molecule with this weak carbon cobalt bond. What does it do? OK. So I'm going to come back to this in a minute, but just let me show you what it does.

OK. So this is a generic mechanism, because while I said there's only one enzyme in humans that uses adenosylcobalamin, if you move into fungi, or you move into archaea, or bacteria, you find there are many B-12 dependent reactions that also do rearrangements, but different kinds of rearrangements. OK. So what's the generic mechanism? OK. So the generic mechanism is the following.

So here we have our adenosylcobalamin. Here's our substrate. OK. And the idea is we need to move the hydrogen from here to here. OK? Does it just jump through space? And the answer is no.

The cofactor adenosylcobalamin is going to remove the hydrogen, and then it's going to transfer it. You're going to generate a reactive speciea, and then it's going to transfer it back to form a new product.

So the cofactor-- and this took people a long time to figure out because there was no chemical precedent for this-- is mediating the hydrogen transfer. So that's what I'm going to show you-- how that actually works.

So here what happens is the whole key to the chemistry of adenosylcobalamin-- which again, most of you haven't seen something like this before because you're not exposed to radical chemistry-- is that you have homolysis of the carbon cobalt bond. So what does that mean? It means one electron goes to the axial ligand, and one electron goes to the cobalt. So the cobalt III is reduced from cobalt III to cobalt II, and what you're left with is this radical species of 5-prime-deoxyadenosyl radical.

OK. So this is the reactive species. Because what you're doing in these transformations is pulling off an amazingly non acidic hydrogen. And normal amino acid side chains cannot do that kind of chemistry. You have to go to these reactive radical species to be able to do this tough chemistry.

So here we generated a radical species. It is sitting right next to the substrate in the active site of the enzyme. And so what does it do? It removes a hydrogen atom-- a hydrogen with one electron. OK? And so again, this is free radical chemistry that most of you don't think about that much.

But what you do is the hydrogen from the substrate is now transferred to this axial ligand and that stays stuck in the active site. So we've seen this guy move over here. And now what you've done is transferred one radical from the cofactor into a second radical-- the substrate.

OK. So that's the key. Now it's carrying this hydrogen, and eventually it wants to put the hydrogen back on to form the product. OK? That's part of the rearrangement reaction.

So what happens now is-- and this looks like magic. I'm just showing that a substrate radical goes to product radical, and I will show you how this works in the case of methylmalonyl-CoA mutase in a minute, OK? So there's some kind of rearrangement. Remember we had two things rearranging the hydrogen, but we also had a second-- a thioester rearranging.

So we have a rearrangement reaction, and this is reversible in the reaction I'm going to be

talking about today. And so this is the same is this. Structurally these are the same. I've just written the methyl group. And so we have a substrate radical converting into a product radical. OK?

And now what we want to do is generate the product. And so the hydrogen from this carrier-- your axial ligand-- is now going to be transferred by hydrogen atom transfer back to p dot to form the product. So again, it's one electron chemistry, and doing one electron chemistry, the hydrogen is transferred back to p-- and then what do you do? You lose one radical, and you generate another radical. You regenerate the radical we started with-- the 5-prime-deoxyadenosyl radical on the axial ligand.

Now I have written here hydrogen in black and in red. Why is that true? Because here's a methyl group. And if you have free complete freedom of rotation around that carbon carbon bond, you can pull off either-- the methyl hydrogens become equivalent, so it can pull off a hydrogen red or a hydrogen black. OK? Can't distinguish in the active site of the enzyme.

So what you now generate is the same thing we started with, except we have a product. But we have 5-prime-deoxyadenosyl radical cobalt II. Here we have 5-prime-deoxyadenosyl radical cobalt II. And what you do is you re-form the carbon cobalt bond at the end of every turnover, and now you're ready to start all over again.

So that's the reaction. This 5-prime-deoxyadenosyl radical. This axial ligand sitting over here acts as a hydrogen atom transferring agent to remove a hydrogen from the substrate and to transfer it back to the product. OK?

I'm going to show you how this works now in the case of methylmalonyl-CoA mutase. So here's our methylmalonyl-CoA, and we're going to be converted into succinyl CoA. So this guy is migrating, and this guy is migrating there. That's the goal. And so what happens here is you cleave the carbon cobalt bond to form cobalt II and 5-prime-deoxyadenosyl radical. And now this 5-prime-deoxyadenosyl radical can remove a hydrogen atom from the substrate. So it leaves you with another radical. This radical goes away. You generate another radical. And now this hydrogen from the substrate is transferred to our cofactor. So it's the hydrogen transferring agent.

So now the question is, how does this weird rearrangement reaction end? How does this CoA migrate from one place to another? Again, there was no chemical precedent in the literature

for this. And the answer is we still don't know the answer.

So there are two mechanistic possibilities. One is that you go through a three membered ring-- cycle propane ring intermediate. So what you can picture happening is that one of the electrons from the carbonyl forms a bond with the unpaired electron on this carbon. And you generate this cycle propane intermediate. So you've gone from this radical to another radical. We haven't lost any radicals.

But now what we want to do is we want this group to migrate. So now what happens-- this intermediate can collapse. It can collapse back to form starting material and the 5-prime-deoxyadenosyl radical. Or you can break this bond, in which case you collapse to form the direct precursor to the product. So it can break down in either direction, and the reaction's reversible.

And if it breaks down in this direction, you form a new radical. This radical is distinct from this radical. And now what happens is that the hydrogen that you removed from your starting material can be returned back to the product. And what you generate then is 5-prime-deoxyadenosyl radical and cobalt II. And now when you transfer the hydrogen back, you form your product like I showed you in the previous slide. And now you re-form the carbon cobalt bond to re-form the active form of the cofactor.

So alternatively you can make this arrangement happen by another mechanism which is called a fragmentation mechanism. And I won't go through the details of this, but you can sit and look at this for those of you who are really interested in sophisticated proposals for the chemical mechanisms of the rearrangement.

So adenosylcobalamin chemistry was unprecedented every step along the way. And it's now known to be widely used, but not so much so in humans. But really humans are only a small part of the world. We have many, many more bacteria and archaea than we have humans. So this is a pretty important transformation.

So hopefully now when you see this again it won't be completely magic. But this is a challenging reaction that most of you haven't been exposed to before. But hopefully some of you get excited about radical mediated transformations.

Just let me close by saying-- from bioinformatic studies in the last five years or so, we now know there are over 50,000 reactions that use free radical chemistry, yet we don't talk about

radical chemistry in 507. So hopefully some of you will get interested enough in biochemistry to come in and start figuring out how all these radical dependent reaction occur, not in primary metabolism, but in many secondary metabolic pathways.

OK. Thank you.