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**BOGDAN  
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Greetings, and welcome to 5.07 Biochemistry online. I'm Dr. Bogdan Fedeles. Let's metabolize some problems.

Today we're going to be talking about problem one of problem set seven. Now this is a problem we are chasing labels through biochemical pathways.

Although it sounds funny, it's actually one of the established ways through which we can test whether the mechanism we propose for these pathways is in fact consistent with what we observe inside the cells.

In part a of this problem, we're going to be looking at glycogen, and try to figure out which carbon's in glycogen end up being lost as CO<sub>2</sub> in the pyruvate dehydrogenase step of the metabolism.

Here is a shorthand representation of glycogen. As you know glycogen is a polymer formed of glucose monomers. Here is a cyclic form of glucose, and in glycogen we have these one four linkages as shown here.

Occasionally we'll have one six linkages, as in the case for branches. But for simplicity we're not going to represent them here.

Now we want to figure out which one of these carbons in glycogen-- we can label them starting from here. One, two, three, four, five, and six. Which one of these carbons is going to be lost as CO<sub>2</sub> in the pyruvate dehydrogenase step.

Now let's take a look at the pyruvate dehydrogenase reaction. As you remember, one of the endings of glycolysis is the pyruvate dehydrogenase reaction, in which pyruvate loses a CO<sub>2</sub> molecule and forms acetyl CoA which later can enter the TCA cycle.

Now this carbon in the carboxyl or group of pyruvate-- I'm going to label with a red dot. This is the carbon that is being lost as CO<sub>2</sub>.

So we want to find out which of the carbons in our glycogen molecule ends up being this red dotted carbon that's being lost as CO<sub>2</sub>. To figure this out we have to backtrack from pyruvate all the way to the beginning of glycolysis to figure out where this carbon is coming from.

Shown here is a layout of the entire glycolysis pathways starting from glycogen. Let me walk you through it very quickly.

Glycogen, shown here-- we've shown only a couple of monomers attached to the glycogen and protein. It's going to get hydrolyzed by glycogen-phosphorylase to form glucose-1-phosphate, which then mutates to glucose 6-phosphate shown here.

And then becomes fructose-6-phosphate, fructose 1,6-bisphosphate. Then the aldolase reaction splits it into dihydroxyacetone-phosphate and glycerol-dihy-3phosphate, or GAP. Then the gap dehydrogenase converts it to 1,3-bisphosphoglycerate. And then it would go down, 3-phosphoglycerate, 2-phosphoglycerate, phosphopyruvate, and finally, pyruvate. And here, I've also written the pyruvate dehydrogenase reaction. Pyruvate becomes acetyl-CoA by losing the CO<sub>2</sub>.

Once again, this carbon that's lost, the CO<sub>2</sub>, is the carbon that we want to track. So we're going to put a red dot on it. And as we just said, this carbon is the carboxyl group in the pyruvate.

So the way to solve this problem is basically go backward through the pathway and figure out where this carbon is coming from in the original glycogen molecule. For these couple of steps, it's pretty clear. It's going to be the carboxyl in each one of these molecules all the way to 1,3-bisphosphoglycerate right there.

So now this 1,3-bisphosphoglycerate is coming from GAP. So this carbon is, in fact, the aldehyde carbon in GAP. Now, as you guys know, triose isomerase interconverts between dihydroxyacetone phosphate and GAP. So this carbon in dihydroxyacetone phosphate is actually this carbon. Because the phosphate group is going to stay unchanged, and then the carbonyl group at C2 can interconvert with C1 to form an aldehyde here. So any of these two carbons, if they were labeled, they would end up being lost as CO<sub>2</sub> in the pyruvate dehydrogenase step.

Now, if we go backwards in the aldolase reaction, GAP and DHAP, when put together, these carbons are going to be carbons 3 and 4. So counting here, 1, 2, 3. This is a carbon that comes from dihydroxyacetone phosphate. And this is the carbon that comes from GAP, so carbons 3 and 4.

And obviously, they're going to be staying carbons 3 and 4 all the way back to glucose, 1, 2, 3, and 4 right there. And also in glucose, 1 phosphate, and consequently in glycogen as well.

So to answer part one, we can now write here that carbons 3 and 4 of glycogen are going to be lost at the pyruvate dehydrogenase step as carbon dioxide.

Part B of the problem deals with the metabolism of glycerol. As you know, glycerol is formed by the hydrolysis of triacylglycerides. Now, we are asked to trace a label from the C2 carbon of glycerol all the way to the first step, in which this carbon is lost as carbon dioxide.

Let's first take a look at the metabolism of glycerol. As I've shown here, triacylglycerides can be hydrolyzed to form glycerol, which is 1, 2, 3 propane triol. Now, as you know, glycerol is metabolized in two steps.

First, we have a glycerol kinase that's going to form a glycerol 3-phosphate, as shown here. And then, we're going to use a dehydrogenase that uses NAD to oxidize the second carbon of glycerol to dihydroxyacetone phosphate. Then it can enter glycolysis very conveniently right here. And then it's going to continue getting metabolized towards pyruvate and acetyl-CoA, as we've seen before.

Now, the second carbon in glycerol is C2 right here. I'm going to mark it with a blue square. So this carbon is right here, and it's going to end up right there.

Now, this second carbon in dihydroxyacetone phosphate is going to be the second carbon in GAP and then second carbon here, here, here. Isn't this fun? Then the second carbon in pyruvate.

Now, once the pyruvate decarboxylates, then it's going to be the carbonyl of acetyl-CoA. It's this carbon right here. Now, so far, this carbon has followed the metabolic pathway, but it has not left yet as CO<sub>2</sub>.

Now, what happens to acetyl-CoA? It's going to enter the TCA cycle. Now let's take a look at the TCA cycle.

As we just said, we're looking now at the carbon, the first carbon in acetyl-CoA here, the carbon that has the carbonyl group on. So as I've shown here in the TCA cycle, the two carbons in acetyl-CoA are marked with this red line. And they will enter and combine as oxaloacetate to form citrate. Then citrate isomerizes to isocitrate. Then we're going to lose a CO<sub>2</sub> molecule, which is this middle guy, to form alpha-ketoglutarate.

But notice, the two carbons from acetyl-CoA are still in the molecule. Then we're going to lose another CO<sub>2</sub> with this bottom one to form succinyl-CoA. But once again, the two carbons that came from acetyl-CoA are still here.

So in the first TCA cycle, none of these CO<sub>2</sub> will contain the label that came from the glycerol. Now, as we go through the TCA cycle, we reach this step where it's succinate.

Now here, I stopped putting this red mark, because succinate is a symmetric molecule. So therefore, if these two carbons were coming from acetyl-CoA, at this point they will scramble. So we won't be able to tell whether it's these two carbons or these two carbons.

Now, let me backtrack and put in the labels. So acetyl-CoA, we have this carbon came from the C2 of glycerol. So we'll find it in this carbon, this carbon, this carbon, this carbon.

Now, we get to the succinate step, and we said, well, it was here. This would be the carbon that corresponds to succinyl-CoA. But because this molecule's symmetric, by the time we get to the malate step to add this hydroxyl group, it's going to be to the carbon next to the label or the carbon further from the label.

Now, because the molecule is symmetric, we can't-- the fumarase enzyme cannot tell which carbon was labeled and which wasn't. Therefore, malate is going to be-- half of the molecules is going to have the label on this carbon, and half is going to have the label on this carbon. So I'm going to write like 1/2 square and 1/2 square.

Similarly, when we get to oxaloacetate, the label is distributed 1/2 on one carboxyl group, and the other 1/2 is going to be on the other carboxyl group.

So we've gone through the TCA cycle once, and we have not lost the carbon that came from the glycerol. But look what happens when we continue the TCA cycle a second time. So now let's say we combine with an acetyl-CoA that doesn't have any label at all.

Now, these two carboxyl groups are going to be these two carboxyl groups in citrate. And as we discussed, both of these two groups are going to be lost as CO<sub>2</sub> in these two steps. First is the middle carboxyl group that's being lost here. And this other carboxyl is going to be lost at the alpha-ketoglutarate dehydrogenase step.

So the second time we go through the TCA cycle, we lose half of the label at the isocitrate dehydrogenase step and half of the label at the alpha-ketoglutarate dehydrogenase step. So I'm going to circle these.

So to answer part B, the C2 carbon of glycerol is going to be lost as CO<sub>2</sub> in the TCA cycle. But we have to go once through the cycle first, through which none of the label will be lost. And then the second time, first in the isocitrate dehydrogenase, then at the alpha-ketoglutarate dehydrogenase, we're going to be losing the CO<sub>2</sub> that came from the C2 carbon of glycerol.

As you might imagine, glycerol can also be used to produce energy. In fact, some bacteria can grow on glycerol using no other carbon source. Now, in part C of the problem, we're going to explore how much energy we can get from one molecule of glycerol. Let's first review the metabolism of glycerol.

As we just discussed, glycerol enters metabolism with glycerol kinase, which then in two steps becomes dihydroxyacetone phosphate, which can enter glycolysis all the way to pyruvate, and then acetyl-CoA. Here, the pyruvate dehydrogenase allows us to lose one CO<sub>2</sub>, and then acetyl-CoA will enter the TCA cycle, where within one cycle, we're going to lose two more CO<sub>2</sub>s. So that's a total of three carbons that we lose. And that's exactly how many carbons we have in the glycerol.

Now, what we need to keep track in order to evaluate how much energy we get from one molecule of glycerol is, whenever we need to, use ATP. For example, we need to put in energy, or whenever we generate NADH or FADH<sub>2</sub> molecules, then we can then take to the electron transport chain and generate ATPs out of them.

So I've put together a list of the steps in the pathway where the energy balance is affected, either we need to use energy or we are generating energy in the form of ATP or in the form of redox cofactors, such as NAD and FAD. So the first step, glycerol kinase, we're going to need to spend one molecule of ATP. So I'm going to put a minus 1 here for ATP equivalents.

Now, in the glycerol 3-phosphate dehydrogenase step, which is shown right here, we are

generating one molecule of NADH. So plus 1 NADH. Now, for the purpose of this problem, we're going to use the convention the 1 NADH is worth about 3 ATP equivalents.

Now, later on in the pathway, we're going to get to the GAPDH step where we generate one more NADH molecule. So GAPDH, another NADH molecule. That's equivalent to 3 ATPs.

So moving ahead, we have the phosphoglycerate kinase step, where we generate 1 ATP, and then we have the pyruvate kinase step where we generate 1 more ATP. I have these written here. So plus 1 ATP, and plus 1 ATP.

Now, finally, we know that pyruvate, it's going to be decarboxylated by the pyruvate dehydrogenase, and here, too, we're generating 1 NADH. So plus 1 NADH, that's going to be equivalent to 3 ATPs.

And finally, the TCA cycle. Now, we have one molecule of acetyl-CoA that enters the TCA cycle. As you guys know for every molecule of acetyl-CoA that enters the TCA cycle, we're going to be generating 1, 2, 3 NADHs, 1 FADH<sub>2</sub>, and 1 GTP. So the tally is 3 NADH, 1 FADH<sub>2</sub>, and 1 GDP. Now, GDP is equivalent to an ATP. FADH<sub>2</sub> counts as 2 ATPs, and NADH counts as 3 ATPs. So that's a grand total of 12 ATPs.

So putting all of this together from one molecule of glycerol, when fully metabolized to CO<sub>2</sub>, we get 22 ATP equivalents. So that's the final answer for part C. From one molecule of glycerol, we get about 22 ATP equivalents.

In part D of the problem, we're tracing the same labels we had in part A, but instead of tracing them to CO<sub>2</sub>, we're going to trace them to the amino acid alanine. As you know, one way to produce alanine is by transamination from pyruvate. Since we already tracked the label to pyruvate, we need to know how do we convert pyruvate into alanine. Let's take a look at that reaction.

As you know, alpha-keto acids, such as pyruvate, can be converted into amino acids by a transamination reaction. Here, we're going to use another amino acid to donate the amino group to the alpha-keto acid pyruvate to form alanine. Now, all these transamination reactions are catalyzed by PLP or pyridoxal 5-phosphate, which is a cofactor derived from vitamin B6.

So when it's left to right, in this transformation is the other pair of amino acid-- alpha-keto acid. So basically, where is this amino group coming from? Typically for most transaminases the other pair is glutamate alpha-ketoglutarate.

So I'm going to have glutamate is going to donate the amino group, and in the process is going to become an alpha-keto acid alpha-ketoglutarate. So in this way, pyruvate becomes alanine.

Now, we were tracking the label from this carbon, so the carbon that will be lost as CO<sub>2</sub> in the pyruvate dehydrogenase reaction. So that is this carbon right here in pyruvate. So in the transamination reaction, this carbon becomes the carboxyl carbon of alanine.

So if you were to start with a glycogen that was labeled at the 3 or 4-- the carbons 3 and 4, that label would be lost as CO<sub>2</sub> in the pyruvate dehydrogenase reaction that we saw in part A. But also, that label would be incorporated in alanine at the carboxyl group of this amino acid.

Parts E and F of the problem deal with tracing labels to the amino acids glutamate and aspartate. Now, both of these amino acids have their corresponding alpha-keto acids as part of the TCA cycle. So let's first take a look at this TCA cycle.

Here, we have the TCA cycle where I highlighted alpha-ketoglutarate going into glutamate through a transamination reaction. Alpha-ketoglutarate and alpha-keto acid can undergo a PLP-catalyzed transamination to form glutamate. And similarly, oxaloacetate, another alpha-keto acid can transaminate to form aspartate.

Now, in part B of the problem, we were looking at the label present at carbon 1 in acetyl-CoA. And we said that this label will stay inside the intermediates of the TCA cycle for the whole round.

Now, when this label gets the alpha-ketoglutarate, it's going to be on this carboxyl group. So in the transamination reaction, the label is going to end completely on the furthestmost carboxyl in glutamate. So that takes care of part E.

Now, if we continue chasing this label, once we get to the succinate, the label is going to split half and half between these two carboxyl groups, because we cannot tell which one-- because the molecule is going to be symmetric. Similarly, for fumarate and in malate as well.

So in oxaloacetate, the label is going to be on both of the carboxyl group. Half of the molecules will have it one. Half of the molecules will have it on the other. So therefore, the aspartate is going to mirror that label distribution, 1/2 label on one carboxyl, 1/2 label on the other carboxyl. So that should answer the final part of this problem.

Now, I hope this problem gave you a better understanding of what it means to chase labels through biochemical pathways, and also that chasing labels can help us better understand the mechanisms of biochemical transformations.