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ESSIGMANN:**

We're now at storyboard 19, panel B. In the last lecture, I mentioned that we're going to be looking at several special cases with regard to the metabolism of fatty acids. The first special case concerns the metabolism of fatty acids that already contain double bond. You remember from last time that a double bond with a trans configuration forms naturally during beta oxidation.

In the special case we're going to look at now, the double bond has a Cis configuration. Fatty acids with a Cis-double bond typically come from membranes. One of the ways nature plasticizes our membranes is to make them more fluid by putting in Cis-double bonds that bend the fatty acid molecule and reduce stacking. Ultimately, this reduction in stacking results in a lowering of the overall melting temperature of that part of the membrane.

The example I'm going to use is oleyl Coenzyme A. This fatty acyl Coenzyme A comes from Oleic acid, which also has the shorthand notation (18:1)  $\Delta^9$ . That is, there is a double bond nine carbons in from the carboxylate. The reaction I've shown in panel B goes through three rounds of beta oxidation resulting in a Cis  $\Delta^3$  enoyl Coenzyme A.

The enzyme oleyl Coenzyme A isomerase will then remove the acidic hydrogen from the 2 carbon, repositioning the double bond, and then putting that double bond into trans configuration, which is now amenable to further hydration and beta oxidation. Overall, the isomerase has taken the Cis-double bond and repositioned it to make it a trans double bond that is now able to undergo further oxidation by the classical beta oxidation pathway.

Let's turn now to panel C of storyboard 19. The second special case that I'd like to deal with concerns the way that we metabolize fatty acids that have an uneven, that is odd, number of carbons in them. One example would be the C15 fatty acid Pentadecanoic acid, which we get from milk fat. In this case, six rounds of beta oxidation will release six acetyl Coenzyme A's. But note that we're left with a 3 carbon residue, or carboxylic acid called propionyl Coenzyme A.

Nature doesn't like to throw anything away. It's going to take this three-carbon molecule, propionyl CoA, that doesn't easily integrate into any other biochemical pathways. Nature's going to add a carbon to it, hence making it into a four-carbon molecule. As we'll see, that four-carbon molecule will integrate seamlessly into the TCA cycle.

The conversion of the three-carbon compound into a four-carbon compound begins with the enzyme propionyl Coenzyme A carboxylase. In a few minutes, we'll look at the fine details of how this enzyme works. For now, however, what it does is use the cofactor biotin in order to introduce a carbon dioxide, or CO<sub>2</sub>, into the 2 carbon of the propionyl Coenzyme A. The 2 carbon is the middle carbon of the propionyl group. This is now a branched molecule that's called methylmalonyl Coenzyme A.

Next, the molecule is subjected to a carbon chain rearrangement that takes the branched molecule and linearizes it to form succinyl Coenzyme A. Succinyl Coenzyme A will then integrate directly into the TCA cycle to allow its carbons to be metabolized fully to CO<sub>2</sub>.

In summary, nature puts a CO<sub>2</sub> into the three-carbon propionyl CoA, forming a four-carbon branch structure. Then, in an amazing rearrangement that we'll look at later, the branched molecule is made linear, forming succinyl CoA, which is a TCA cycle intermediate. So the otherwise useless C-3 molecule, propionyl Coenzyme A is converted to something of value.

Lastly, let's turn to panel D. I'm going to deal more with the details of this reaction in a few minutes. But for now, what I'd like to say is that there are many sources of propionyl CoA, not just from odd chain fatty acids in the diet. Secondly, the introduction of succinate into the TCA cycle results in an increase in the amount of carbon going through the TCA cycle. That is, this is truly an anapleurotic reaction. This is a reaction that we can use to increase the overall rate of carbon metabolism in the TCA cycle.

We're now going to turn to storyboard 20. Let's look at panel A. In the last lecture on fatty acid metabolism, I talked about propionyl CoA carboxylase, which uses biotin to add a CO<sub>2</sub> moiety to the middle carbon of propionyl Coenzyme A. This lecture is a chemical interlude, in which we're going to look at propionyl CoA carboxylase and several other carboxylases that are used in biochemistry.

One of these is acetate CoA carboxylase. This carboxylase puts the CO<sub>2</sub> group onto acetyl CoA to make malonyl CoA, which is the precursor to fatty acids during the fatty acid biosynthesis reactions.

Let's also look at panel B. We're also going to be looking at pyruvate carboxylase, which adds a carbonyl group to the pyruvic acid molecule in order to make oxaloacetate. This is one of the anaplerotic enzymes that can increase the quantity of carbon going through the TCA cycle. That is, it facilitates anapleurosis in the biological system.

Let's now look at storyboard 21, panel A. The carboxylases all use carbon dioxide as their carboxylating reagent. CO<sub>2</sub> itself is not very soluble in water, so cells use carbonic anhydrase in order to hydrate it to form carbonic acid. The biotin carboxylase subunit will then phosphorylate the carbonic acid in order to form a chemically-reactive intermediate known as carbonyl phosphate or carboxy phosphate.

Now, carbon dioxide by virtue of its structure-- that is, a carbon with two electronegative oxygens pulling on it-- is pre-activated for nucleophilic attack. And as I said a few minutes ago, CO<sub>2</sub> is just not present at high enough concentration in the cell for it to be able to add effectively to nucleophiles. A nucleophile such as the amide nitrogen of biotin would be an example.

So what the cell does is use ATP to make carbonyl phosphate, which is a very water-soluble molecule. It's a very efficient delivery vehicle for carbon dioxide into the active site of an enzyme.

Now let's look at panel B. The biotin carboxylase subunit of pyruvate carboxylase has a biotin cofactor at the end of a 14-Angstrom-long swinging arm. This subunit of the enzyme breaks the bicarbonate phosphate bond and releases carbon dioxide in high concentration in the active site. Biotin on the swinging arm then attacks the high concentration of carbon dioxide in order to become N-carboxy biotin as shown in this figure. The N-carboxy biotin takes advantage of its swinging arm to move away from the active site at which it acquired the CO<sub>2</sub> and then move to a second site on the enzyme at which the arm releases carbon dioxide, once again at high concentration.

But this time CO<sub>2</sub> is released at high concentration in the vicinity of the substrate, pyruvate. Pyruvate, in order to be a good nucleophile to acquire the CO<sub>2</sub>, probably exists as shown in the figure as the pyruvate enolate.

Let me review panel B at this point. Overall, this reaction has resulted in putting a carboxylic acid residue on the 3-carbon of pyruvate. And this results in the formation of oxaloacetate.

Thus, activation of pyruvate carboxylase will result in taking a molecule of pyruvate and converting it to oxaloacetate. This anaplerotic reaction gives the ability to increase the volume of carbon that's cycling in the TCA cycle, thus allowing you to be able to increase their overall rate of respiration.

And as I've said several times in the past, oxaloacetate is present at rather limiting concentrations within the mitochondrial matrix. So this is a very useful biochemical reaction.

At this point, let's move on to story board 22 and look at panel A. Let me loop back now and give some bigger picture points with regard to carboxylase chemistry. Pyruvate carboxylase and the other enzymes of this class have two active sites. One of them is the biotin carboxylase domain, where a biotin moiety covalently attached to the enzyme will acquire CO<sub>2</sub>. Let's call this step 1 of the reaction scheme.

Once the biotin on the swinging arm has been N-carboxylated as in step 1, the N-carboxy biotin moves to site 2, where the substrate is. The second site is the biotin transferase domain. In the example I just used, the substrate was pyruvate. But it just as easily could have been another quote unquote "carboxylation substrate," for example propionyl CoA.

In the case of pyruvate, as we just saw, it's the pyruvate enolate that becomes carboxylated in site 2 in order to form the final product oxaloacetate. This carboxylation of substrate is the second and final step of the overall reaction.

Take a look at panel B. Now let me give you a second example of carboxylase chemistry. In this case, our substrate will acetyl Coenzyme A. We're going to use the same chemistry we described a minute ago for pyruvate, but in this case it is another carboxylate-- acetyl CoA carboxylate-- that picks up the CO<sub>2</sub> residue at its carboxy transferase domain. In step 2, which occurs in the carboxy transferase domain, the substrate acetyl CoA picks up CO<sub>2</sub> on its 2-carbon-- the one distal to the Coenzyme A group-- to form the three-carbon product malonyl Coenzyme A.

We'll see later that the carbon dioxide that's been added to acetyl CoA to form malonyl CoA is an excellent leaving group. Malonyl CoA is the fundamental precursor to all fatty acids through the fatty acid biosynthesis pathway. Again, we'll see the details of this reaction scheme later, but keep in mind now that this is a biotin-requiring reaction, just as the one we just looked at with pyruvate carboxylase.

Let's look now at panel C. My last example of carboxylase chemistry will be propionyl Coenzyme A carboxylase. I mentioned earlier that the breakdown of odd chain number fatty acids results in the formation of a 3-carbon linear fatty acid called propionyl CoA. I also said that nature doesn't want to waste any of the carbons. What she is going to do is add a carbon dioxide or a CO<sub>2</sub> to the central carbon of the propionyl group.

As well as fatty acids with odd numbers of carbons, certain amino acids-- such as isoleucine, methionine, valine, and threonine, for example-- also break down to form propionyl CoA. So what I'm going to do now is describe a fairly general method of metabolism that involves restructuring of the carbon chain subsequent to the carboxylase reaction. In this overall reaction, a carboxylase is going to carboxylate the middle carbon of the propionyl group. Then, following the carboxylase reaction, there's going to be a skeletal rearrangement to form succinyl CoA, which will then enter the TCA cycle.

As shown in the reaction scheme, propionyl Coenzyme A carboxylase carboxylates propionyl CoA to form a very specific stereoisomer, (S)-methylmalonyl Coenzyme A. Please note the position of the carbonyl group that's highlighted in blue. This carboxylate in blue is attached to a methylene carbon that has an inverted triangle on top of it. Epimerization about the carbon with the triangle results in the blue carboxylate pointed down the way I've drawn it.

The resulting epimer is (R)-methylmalonyl Coenzyme A. In the (R)-methylmalonyl Coenzyme A molecule, I put a little lasso around the hydrogen atom at the methylene moiety of the molecule. I've also lassoed an electron in some of the atoms of the Coenzyme A moiety. A vitamin B12-dependent enzyme called methylmalonyl Coenzyme A mutase, which contains an adenosylcobalamin functional group, will now act on this molecule.

In this enzyme, a cobalt residue is going to enable the formation of an adenosyl radical. Look at the book for the detailed mechanism. But for now, it's sufficient to say that this adenosyl radical is going to facilitate the homolytic scission of the bond between the carbon and hydrogen of the (R)-methylmalonyl Coenzyme A. That is, you're going to get a free radical formed at the carbon that has the small blue o written over it. That molecule will lose its hydrogen atom. That hydrogen atom will migrate to the carbon that has the small blue triangle.

Coordinately, the carbon with the small box that's part of the Coenzyme A carbonyl group will migrate to the position originally occupied by the hydrogen atom on the carbon with the small o over it.

The skeletal re-arrangement is shown here in panel C, and it results in a linearization of the original methylmalonyl Coenzyme A. If we redraw it as in the box, you'll see that you form succinyl Coenzyme A. Succinyl Coenzyme A will then feed directly into the TCA cycle. This is an anaplerotic reaction. That is, it will increase the volume of carbon that's rotating in the TCA cycle.

Overall, in the way of a review, the three-carbon compound propionyl Coenzyme A, by way of the carboxylase reaction, is converted to a four-carbon branched molecule called (S)-methylmalonyl Coenzyme A. It's epimerization forms the (R)-methylmalonyl Coenzyme A, which is the substrate for a third enzyme, methylmalonyl Coenzyme A mutase, a vitamin B12 enzyme. This enzyme catalyzes a free radical-mediated rearrangement of the side chain. Specifically, one hydrogen and one Coenzyme A functionality switch positions. This rearrangement linearizes the molecule forming, ultimately, succinyl Coenzyme A, which then flows into the TCA cycle.