BOGDAN FEDELES: Hello, and welcome to 5.07 Bio Chemistry Online. I'm Dr. Bogdan Fedeles. Let's metabolize some problems.

Today we're going to be talking about Problem 2 of Problem Set 4. Here we're going to be discussing in detail the mechanism of HMG-CoA synthase, a key enzyme in central metabolism responsible for making the five carbon building blocks from which all sterols, such as cholesterol and steroid hormones are made.

HMG-CoA synthase catalyzes the following reaction. It takes acetyl-CoA, which we're going to encounter a lot in the central metabolism, and combines it with acetoacetyl-CoA, another thioester. In this process, the one molecule of CoA is lost, and we get the product hydroxymethylglutaryl CoA or HMG-CoA, as you notice the initials H, M, and G are part of this name. To help us understand the mechanism, a crystal structure of this HMG-CoA synthase is provided in this problem, and it's shown here.

Question 1 of this problem is asking us to provide a detailed curved arrow mechanism of the reaction catalyzed by the HMG-CoA synthase. And for that we're going to use the information provided in the crystal structure. And we are also given an additional hint and that is the acetyl CoA substrate provides the CH2CO2 CO2 minus moiety in the HMG-CoA product. Let's take a look.

So the hint is telling us that these two carbons, which I'm going to label in blue from acetyl-CoA, are, in fact, these two carbons, CH2 COO minus in hydroxymethylglutaryl CoA. That means that the other four carbons in the product must be the carbons from the acetoacetyl-CoA. Let's label these red.

So now since we have a thioester functionality in both cases, these are probably the same, so the carbons from a acetoacetyl-CoA are as follows. So notice the HMG-CoA synthase, it accomplishes the formation of a carbon-carbon bond, and that is this bond right here. It's between the CH3 carbon acetyl-CoA and the carbonyl carbon of acetoacetyl-CoA. And in the

process, this carbonyl will become a hydroxyl group.

Now, let's take a look at the crystal structure and see what information we can gather there. Now this is a picture of the active site of the HMG-CoA synthase. We notice the acetyl-CoA moiety is actually now bound to this C111. As you know C is a cysteine-- so it's actually covalently bound to a cysteine-- in the active site.

Now, notice this is the other substrate, which is the acetoacetyl-CoA and bound in the active site. So as we've discussed before, we're going to make this carbon-carbon bond, and that's going to happen between this carbon here and this carbon here. So this bond needs to be formed.

However, let's take it one step at a time. The reaction will start by forming this thioester between the acetoacetyl-CoA carbons and the cysteine in the active site of the enzyme. In many reactions that use a cysteine in the active site, which is used to form a covalent bond to the substrate, we first need a base to deprotonate the cysteine and make it a really good nucleophile, which will subsequently attack the substrate and form a covalent bond.

Now, in a lot of these cases, the base is a histidine. Let's take a look if we see a histidine in our structure. There is a histidine in the structure, H233. But if you look at this histidine, it's quite far from our cysteine here. Well, obviously, because this is a projection, this is a two-dimensional structure so that we only see here a projection of a three-dimensional structure, it's very hard for us to tell if this histidine is, in fact, close enough to deprotonate this cysteine.

So even though we have this crystal structure, from this one picture, we do not have enough information to figure out, well, what is the base that will deprotonate cysteine before it reacts with acetyl-CoA.

Remember this is often the case with crystal structures that because of the resolution which we can collect them, we cannot see the protons. And if we cannot see the protons, it's very hard to tell which amino acid residues are protonated and can serve as a general acid or general bases.

A lot of the researcher's intuition comes into play to draw these kind of structures. And it's only with collecting many different kinds of experimental evidence that we can put together a more definitive mechanism. Therefore, we might not know for sure from this one picture, which is the general base that deprotonates the cysteine at 111. So we're not going to assign it for

now.

Let's try to write the mechanism for that part. Here is the acetyl-CoA substrate, and here is the active site cysteine 111 with its thiol group. And again, we're not going to assign it, but there will be a base in the active site of the enzyme that will need to deprotonate this cysteine before it can react.

Therefore, the reaction first involves activation of the cysteine, is deprotonated, and then cysteine can attack the thioester and form a tetrahedral intermediate. As we've seen it before, for a lot of these reactions involving thioesters. In the first step, we will form four bonds to this carbonyl carbon, and we have formed a new bond between the thiol of the cysteine 111 and the starting material, acetyl-CoA.

Of course, there is a negative charge here. And our base in the active site of the enzyme will now be protonated. Now that the tetrahedral intermediate is going to fall apart by kicking off the CoA moiety. Of course, this will get protonated presumably by the same base. Now it's a general acid that will protonate a CoA.

So we obtain the thioester with the cysteine in the active site and one molecule of CoA is going to be leaving. And here is our base.

Notice the tetrahedral intermediate that we're forming here. We have an oxygen that develops a negative charge. And this is very similar to the kinds of intermediate you've seen in the serine protease mechanism. Now in that case, such an intermediate was stabilized with hydrogen bonds from the backbone of the protein in a structure that was called an oxyanion hole.

Now let's take a look at the crystal structure of HMG-CoA to see how a tetrahedral intermediate might be stabilized. Here is the acetyl of the acetoacetyl-CoA substrate. The acetyl now is bound to the cysteine 111 here in the active site.

Now, let's take a look if there are any other residues close enough to this oxygen. One of them that's shown here is this NH, which belongs to an amide bond, the backbone amide, and that's part of the amino acid serine 307. So this distance here, C3.06 Angstrom, it's actually close enough for a fairly good hydrogen bond.

Presumably, this interaction will stabilize the binding of acetyl-CoA in this region of the active site and may also be involved in catalyzing the reaction with the cysteine by stabilizing the

tetrahedral intermediate. In that case, this distance will have to become even smaller, that is to form a really good hydrogen bond on the order of 2.6, 2.7 Angstrom. Since this is only one snapshot of the reaction, we don't have enough information to tell if this is a key catalytical interaction.

Once acetyl-CoA has reacted with the enzyme, hence, formed the thioester with the cysteine 111, we're now ready to proceed with the reaction and form a carbon-carbon bond. Let's take a look.

So in the next step of the reaction, we want to form a carbon-carbon bond between this methyl group here and this carbon of the carbonyl of the other substrate. So first of all, we need to deprotonate this methyl group. We're going to form an enolate. Then the enolate is going to attack this carbonyl.

Once again, we look for a suitable base, and we see, for example, this glutamate 79 may be, in fact, serving as a general base to deprotonate the methyl group here and form the enolate. Once the enolate is formed, it's going to attack this carbonyl, and this oxygen is going to start developing a negative charge, which needs to be compensated by a general acid. And it looks like this histidine 233 is close enough to donate a proton and generate a hydroxyl group here.

All right. So let's try to write a mechanism based on what we just said. Here is the thioester between cysteine 111 and acetyl. And let's show the general base. It's going to be glutamate 79. Because it's a base, I'm going to put a negative charge on it. There you go.

And this will serve to deprotonate the methyl from the acetyl, moiety here, and generate an enolate. So it's picking up a proton, electron move, and it's going to generate, as before, a negative charge on the oxygen, which may be stabilized by that interaction with the [INAUDIBLE] hydrogen that we just discussed.

Now, let's try this again. And here is our enolate. In the next step, the enolate now is going to attack the carbonyl of the acetoacetyl-CoA and form the carbon-carbon bond.

All right. This is the acetoacetyl-CoA, and we discussed that next to this oxygen there is our histidine 233, which we're going to show it as being protonated. Therefore, the reaction proceeds as follows.

Here we're forming the carbon-carbon bond. Then the oxygen is going to pick up the proton

from the protonated histidine and it's going to form a hydroxyl group. Therefore, we get cysteine 111 is still attached to what is known the HMG-CoA product.

So this is our carbon. This is the new carbon-carbon bond. We have here a methyl group. We have the new hydroxyl, which were formed here, and the rest of the molecule. And our histidine, it was protonated here, now it's going to be deprotonated.

So we just saw how the carbon-carbon bond is formed in the course of this reaction. And now we're left with a six-carbon thioester with a cysteine 111 in the active side of the enzyme. Therefore, the last step of the reaction would involve hydrolysis of this thioester to free the product and regenerate the system.

So for the last step, we need to hydrolyze this thioester, and for that, we're going to need to activate a water molecule. Once again, we don't know-- here is the water molecule-- we don't know what's the general base, the residue in the active site, which removed this proton from water to allow it to attack the carbonyl of the thioester. So we're going to call it a general base attached to the enzyme.

So this base is going to pick a proton from water, and then the water is going to add to the carbonyl and once again generate a tetrahedral intermediate. Say, this the OH from water, and this is the rest of the molecule. Once again, this is a tetrahedral intermediate, very similar to the one we saw in the first step when we formed this thioester, and presumably, it's going to be stabilized in a fairly similar manner.

Let's also show that this base attached to the enzyme is now protonated, and it would probably donate this proton to reprotonate the cysteine and reform that thiol group. So in the second step, thiol takes the electrons, which picks up the proton from the general base in the active site.

So at this point, we're just going to release the thiol of the cysteine 111, and the rest of the molecule is exactly our product, which is the HMG-CoA.

The curved arrow mechanism we just wrote answers part 1 of the problem. Second question of this problem is asking about the stabilization of the tetrahedral intermediate, which actually we have just discussed. But let me reiterate as you guys saw in the serine protease mechanisms, whenever we're forming this tetrahedral intermediate, they tend to be stabilized in an oxyanion hole, which is basically a structure of the enzyme, which can form hydrogen

bonds with the partial negative charge or full negative charge that develops in a tetrahedral intermediate.

Presumably, similar structure exists for this HMG-CoA synthase, but because we're only given one snapshot, one crystal structure, that is not sufficient information to say for sure which are the key interactions to stabilize these tetrahedral intermediates. A lot more work, a lot more experimental data is necessary to figure out which are the key hydrogen bonds and interactions that stabilize the tetrahedral intermediates.

Question 3 is asking us to review the mechanisms by which enzymes can achieve their amazing rate acceleration, which is on the order of 10 to the 6 to 10 to 15 times over the uncatalyzed reaction and of course, to point out these mechanisms in the context of the HMG-CoA synthase.

As you have seen over and over in this course, the three general mechanisms by which enzymes accelerate reactions are binding energy, general acid/general base catalysis and covalent catalysis. Now let's take a look at our structure and figure out how each one of these mechanisms might be operating.

To start off, covalent catalysis, it's pretty obvious here the acetyl-CoA substrate first reacts and forms a covalent bond with the cysteine of the HMG-CoA synthase. So this covalent attachment to the enzyme allows this residue to be positioned just right so that it can react with the other substrate.

Now, general acid/general base catalysis, we've seen all these residues that participate. Obviously, in order for this to react, we need a base to deprotonate the cysteine. Then we need a base, presumably, this glutamate 79, to deprotonate the methyl group here to form the enolate. And we need an acid to stabilize and form this hydroxyl group that will be developing on this oxygen. So covalent catalysis and general acid/general base catalysis, that's pretty obvious.

Now when it comes to binding energy, this is not something that we can obviously derive from the structure, but we can postulate the number of ways in which binding energy contributes to this reaction. First of all, both of the substrates need to bind to the enzyme. In order to do that, they need to be desolvated, that is to remove all the water molecules that surround them. So that by itself requires energy.

The binding energy is also derived by when we align the substrates in the active site of the enzyme, we align them so closely the right geometry and within a few tenths of an Angstrom so that the right orbitals overlap and allow the reaction to happen. So also the ability to align this residue so closely that also contributes to the binding energy.

And finally, the binding energy also manifests when we're stabilizing, for example, the transition state of the reaction relative to the binding of the substrates. So if, for example, for our tetrahedral intermediate that will form here, if that transition state or the tetrahedral intermediate is stabilized more than the substrate, then the reaction is accelerated and proceeds towards that pass.

Part 4 of the problem is asking us to look up the structure of coenzyme A, or CoA and then contrast the reactivity of say, acetyl-CoA, the thioester with CoA, with the reactivity of a thioester with a much smaller thiol group. Let's see what the coenzyme A looks like.

Here we have the structure coenzyme A. The business end of the molecule is this thiol group, which is attached to a substantially long arm. And at the end here, we have, as you can recognize, two phosphates, the ribose and the base adenine. This is a nucleotide is ADP. There's also another phosphate here.

But the business end of the molecule is this thiol group, and we're asked to contrast whether a thioester form with coenzyme A would behave similarly to a thioester form with this right-hand portion of the molecule, which I wrote here. So it's a much shorter thiol.

Now, it turns out these thioesters will be very, very similar because really it's only the thiol moiety that we need to form thioester. So those thioesters will behave very, very similarly.

Now, the advantage of having such a long arm for the coenzyme A is that it provides a way to insert the substrate, which will attach here, to say, acetyl-CoA, to insert acetyl-CoA in very deep into the active site of the enzyme. Having a long arm to guide the thioester may be important. As you will see later in the course, fatty acid synthases, which are these mega dalton complexes, have multiple active sites.

So the fatty acid attaches a thioester to coenzyme A allows it to be moved through different active sites and through a same kind of chemistry in multiple steps over and over again. The advantage of having a coenzyme A thioester is that it may provide some additional binding energy. That nucleotide portion of coenzyme A may interact specifically with the enzyme near

the active site, whereas, a much smaller thiol like the one we just saw will not have that kind of interaction available.

Since we've been talking about thioesters throughout this entire problem, the last question is asking us to rationalize why we're seeing thioesters in metabolism as opposed to oxygen esters. Now, the key message you need to remember here is that the resonance that we observe in oxygen esters is almost completely absent in thioesters. And this fact makes the thioesters less stable and therefore, more reactive. Not only their carbonyl group behaves more like a ketone group, but their alpha hydrogens are more acidic. Let's take a look.

Here is an oxygen ester that shows a proton in alpha position. And as you know, the lone pairs on this oxygen can conjugate with the carbonyl group and form certain resonance structures, one of which being this one.

So the electrons can move like this, and then we're going to have a negative charge here and a positive charge here. And this is possible because the electrons on both oxygens are found in orbitals of comparable energies. By contrast, in the case of a thioester, we have a sulfur.

Now, the electrons on the sulfur are in orbitals that are not in comparable energies with the ones on the ketyl group and therefore, this kind of conjugation does not, in fact, happen. And because this doesn't happen, therefore, the electrons on the carbonyl stay localized, and that makes the carbonyl a better reactive site. So it's a better electrophile to react as nucleophiles behaves more like a ketone.

For the same reason, when we are to deprotonate the alpha position on a thioester, the density on the carbonyl is there to stabilize the enolate much more so than it would be for an oxygen ester. Therefore, the pKa, the acidity constant for this hydrogen on the thioesters is close to 18, which is smaller, therefore more acidic than the pKa of the alpha position in oxygen esters, which is 22.

This sums up Problem 2 of Problem Set 4. I hope you now have a much better feel of how we can use crystal structured data to propose a reasonable mechanism for enzyme catalyzed reactions. Keep in mind that writing mechanisms on paper is relatively easy. But to truly confirm that that mechanism is taking place in real life inside the cells, it takes a lot more experimental work and evidence.