BOGDEN FEDELES: Hello and welcome to 5.07 Biochemistry online. I'm Dr. Bogden Fedeles. Despite the staggering biodiversity we see in nature, the types of chemical reactions employed are only but a couple of handfuls. And these are used over and over again very efficiently and with conserved mechanisms.

As you might recall from Organic Chemistry, one of the most versatile chemical groups is the carbonyl, C double bond O. Not surprisingly, carbonyl chemistry is well-represented in biochemistry. In fact, the carbonyl chemistry allows formation of carbon-carbon bonds.

It's one of the very few ways in which enzymes can start with small molecules and put them together into a macromolecule, or start with a macromolecule and break it down into smaller pieces during metabolism.

This video summarizes some of the most important carbonyl reactions you will encounter in 5.07. In this video, we're going to be talking about carbonyl chemistry. And as we will see, carbonyl chemistry is fundamental for some of the carbon-carbon bond formation and cleavage reactions.

As you recall from organic chemistry, carbonyl contains a C double bond O. And all the properties of the carbonyl derive from its ability to polarize this bond, so that we can draw a resonance structure where the carbon has a positive charge and the oxygen a negative charge.

As you recall, there are simple carbonyls, such as aldehydes and ketones. Also we have acyl derivatives, compounds in which the carbonyl is attached to a heteroatom. x can be oxygen, nitrogen, sulfur.

So here, respective, we have esters, amides, thioesters, and of course, we have an OH group here, carboxylic acid. Here is a summary of the reactions that we're going to be talking about. First, we're going to be discussing nucleophilic addition.

Here, the good nucleophile reacts with the carbonyl, adding to the carbon that the carbonyl can generate. This tetrahedral compound. Next we're going to be talking about enolization. This is the property of carbonyls that contain an alpha hydrogen, which can rearrange to form enol.

Next we're going to introduce the aldol reaction. This is the reaction in which a carbon-carbon bond is formed and occurs between a carbonyl that acts as electrophile and a enolizable carbonyl, which acts as a nucleophile. In the aldol reaction, a bond is formed between these two carbons, generating an aldol.

We're also going to see that the aldols can dehydrate. The aldols we saw above can lose a water molecule to form an alpha, beta-unsaturated carbonyl. Now about the acyl derivatives, we're going to be talking about acyl transfer reactions, where an acyl derivative can convert into a different acyl derivative with the appropriate nucleophile.

A variation of this reaction is Claisen reaction, where similarly to the aldol reaction, we have an enolizable carbonyl reacting with an acyl derivitive and generating a beta-keto carbonyl. This reaction also forms a carbon-carbon bond, which is right here.

Let's talk in more detail about the nucleophilic addition. The general reaction scheme is as we saw before. Here is a carbonyl compound reacting with a nucleophile and forming a tetrahedral intermediate that contains an alkoxide or an alcohol.

Now let's take a look at two different reactions. One is the reaction of alcohols with carbonyl compounds, where we form a compound that looks like this. This is called a hemiacetal. Now this reaction is reversible. And, in fact, it reaches equilibrium because delta G naught is approximately zero.

This reaction can be acid or base catalyzed. Let's take a quick look at that mechanism. If it's based catalyzed, the base will first deprotonate the alcohol, which will form the alkoxide, which is then a very good nucleophile to attack the carbonyl, which forms this alkoxide version of the hemiacetal, which can be then protonated.

In acid-catalyzed mechanism, we have to activate the carbonyl first, so the protonation of the carbonyl is the first step.

All right, so this activated carbonyl can then be attacked by our alcohol. Which, this product is just one proton transfer away from our hemiacetal.

All right, the second reaction I want to include here is the formation of Schiff bases which is the reaction of a carbonyl with an amine. Similarly to the hemiacetal formation, this reaction generates first a tetrahedral intermediate, which is, however, unstable, and loses water to generate the imine, with a Schiff base.

Let's take a look at the mechanism. As you notice, the reaction-- because the amine group is a good nucleophile, the reaction can occur even in neutral conditions. We don't need, necessarily, acid or base catalysis. The first step, the imine attacks the carbonyl, forming this compound with split charges. Now proton transfer happens to generate our intermediate. Then water is eliminated. And this is the imine. You'll notice the imine nitrogen can also be protonated, to generate this iminium ion, which, as we will see in other situations, it's an activated version of the carbonyl group.

From these two examples, we can get some idea of how the nucleophilic addition occurs. So let's take a look at what kind of nucleophiles we can add to the carbonyl group. We have some good nucleophiles. And here we have things with negative charges, such as alkoxide, or hydroxide. I have the thiolates. And other things such as amines. And we also have some OK nucleophiles. And here we have alcohols, even water, and thiols.

As you saw in these couple of mechanisms, the OK nucleophiles don't react very well, unless they are deprotonated to form good nucleophiles, such as the alcohols. Or the carbonyl gets activated, either by protonation in a strong acid, as we saw here, or it becomes an activated carbonyl, for example, in an iminium ion. Another important nuclear force that we're going to see is the, what we're going to call, a C minus. Basically a carbanion In our case it's going to be enolates, which can also add to the carbonyls. And these will form the basis for the aldol reaction.

The second reaction we're going to be talking about is enolization. Here, a carbonyl that contains an alpha hydrogen can rearrange to form an enol. We're going to call this the keto form and this the enol form. An equilibrium between a keto and an enol form is called tautomerization. And this is a very important reaction in many biochemical systems. Turns out, the delta G, for the reaction as written, it's very high, 30 to 50 kilojoules per mole. That means that equilibrium strongly favors the keto form. However, in certain cases, the enol can form and get stabilized.

The mechanism of enolization, it's very straightforward. All we need is a decent base that can remove the alpha proton. And it will form this enolate. Now, enolate is able to form because it has resonance stabilization. We can draw another resonance structure, as such, where we see the negative charge is on the carbon. So it is in fact a carbanion. We're going to call it a disguised carbanion.

As the carbon is not very electronegative, having such a high electron density on the carbon would make it a very good nucleophile. And in fact, this enolate is the nucleophile that executes reactions such as the Aldol reaction and the Claisen reaction.

Something to keep in mind, well, how acidic is this alpha hydrogen? We can compare it with a hydrogen in an alkyne. The pKa of such a hydrogen is close to 50. It's extremely hard to remove a proton. Now if we look at an alpha hydrogen next to a carbonyl, the pKa is 18 to 20. So it's 30 orders of magnitude more acidic, and this is because, as we saw, when we removed this hydrogen, we formed the enolate anion, which is resonance stabilized.

The more extreme case of this, if we have two carbonyls, alpha to the same proton, the pKa drops even further, around 9 to 11. This is because we can draw even more resonance structures to the enolate that's formed. This is one. This is another. And another. As we saw before, the charge here is delocalized between the oxygens and the alpha carbon. So it is this beta keto carbonyl in its enolate form will behave as a carbanion and it can act as a good nucleophile.

The Aldol reaction. This is a very important reaction in biochemistry because it allows formation of carbon-carbon bonds. Or, if the reaction runs in reverse, cleavage of the carbon-carbon bonds. The Aldol reaction is the reaction between an enolizable carbonyl, as we show here, a carbonyl that has an alpha hydrogen, and another carbonyl. And what happens is, a new carbon-carbon bond forms between alpha carbon and the carbonyl carbon.

The product of the Aldol reaction, it's called Aldol as a contraction between aldehyde and alcohol, as in some cases this carbonyl will be an aldehyde and this would be Aldol. It's essentially a beta hydroxy of carbonyl. Now, this reaction has a delta G naught close to 0. That is, it reaches equilibrium. And it can be catalyzed by acid or by base.

Let's take a quick look at the mechanism. Given the previous mechanistic insights-- we looked at the nucleophilic addition, and enol formation, then the mechanism of the Aldol reaction should be fairly straightforward. If it's base-catalyzed, the base is going to help us form the

enolate, as such. And as we discussed previously, the enolate is a good nucleophile, and can react via nucleophilic addition with the other carbonyl. And one proton transfer to generate the Aldol product. The reaction can also be acid-catalyzed.

Again, formation of the enol in acid catalysis involved first protonation of the carbonyl. Now this activated carbonyl, it's a much better electron sink, and stabilizes the enol formation. Now, in the second step the enol can react with the other carbonyl, to generate a protonated version of the Aldol, which is one proton transfer away from the Aldol product. In biochemical systems, the enzyme that catalyzed the Aldol reaction is called aldolase.

And there are actually two kinds of aldolases. Class one, and class two. The distinctive feature of these enzymes is the way they catalyze the reaction. Class one uses an active site lysine to form a Schiff base with the carbonyl, which activates the carbonyl, and allows for the enol formation. Class two uses a metal ion, such as zinc, to accomplish the same thing.

So here is how the mechanism for the class 1 aldolase would look. So here is our enolizable carbonyl, and here is our active site lysine. As we saw before, an amine reacting was a carbonyl will give us a Schiff base. The reaction goes via a tetrahedral intermediate, which we're not going to draw here, but what we form is this iminium ion. Now the carbonyl is activated enough that an active site base can remove an alpha hydrogen to form the enol. Which is now well-positioned to attack the other carbonyl. This generates the Aldol product, in its imine form, still attached to the enzyme. And now the hydrolysis of imine is going to release the Aldol.

Now, class two enzymes use a zinc ion. As the ion approaches the carbonyl, it's going to draw some of the electrons from the carbonyl, and make the proton in the alpha position a lot more acidic. So you can imagine, some of these electrons get de-localized. So that a base can remove the proton and form the enolate. Which, in the second step, it reacts with the carbonyl, which will generate the Aldol product in the active site of the enzyme, still bound to the zinc, and now which can dissociate, and generate the final-- and release the product.

Now, a very important consideration for the Aldol reaction is that it can occur in the reverse fashion. For example, to cleave a carbon-carbon bond. So the bond that will be cleaved, as we see here, is the bond that got formed, which is the bond between the alpha and beta carbons. The aldolase is one of the key enzyme in glycolysis, that allows us to break a six carbon sugar into two three-carbon sugars by cleaving a carbon-carbon bond via the Aldol reaction.

As the mechanism catalyzed by the aldolase, we can see that the reverse pathway is pretty straightforward, where the Aldol binds to the enzyme, say in class one, forms an active site, covalent attraction, a Schiff base with the lysine, from which the chemistry occurs to break the carbon-carbon bond, and leads to the release of one carbonyl molecule, and then the other one will be still bound to the enzyme as a Schiff base and hydrolyzed. For the class two, the Aldol will interact with the enzyme by forming an interaction with the zinc, and this activated carbonyl allows the chemistry to occur exactly in the reverse manner, as shown here.

One other reaction involving Aldols is Aldol dehydration. Here's an Aldol, beta hydroxy carbonyl. Now, if an Aldol has an additional alpha hydrogen, it can lose a water molecule to form an alpha beta unsaturated carbonyl. Now this reaction is favorable thermodynamically. The delta G naught is approximately 0. And this is a reaction we're going to see in a lot of biochemical pathways, for example, in the biosynthesis of fatty acids, going left to right, or in the catabolism of fatty acids, going right to left.

Here's a quick insight on the mechanism. Once again, it can be base- or acid-catalyzed. This reaction works because the alpha hydrogen here is next to a carbonyl, and therefore can form an enol. So if a base can remove this hydrogen to form the enolate, then we can envision how this electronic movement will allow for a water molecule to be eliminated, forming our alpha beta unsaturated carbonyl.

The acid-catalyzed mechanism goes along the same lines. As you remember, in order to form the enol in an acid-catalyzed context, first we have to protonate the carbonyl. All right. Now a base can remove our alpha hydrogen, forming the enol, which can kick off a water molecule, generating these pieces, which is just one proton transfer away from our final product.

So let's talk now about acyl derivatives, and acyl transfer. As we mentioned, acyl derivatives have a carbonyl attached to a header atom. And this header atom can be oxygen, nitrogen, sulfur. As all these header atoms contain a lone pair of electrons, one of the key properties of the acyl derivatives would be resonance between the header atom and the oxygen.

Now the properties of the acyl derivatives will be dictated by how easy or how difficult it is to adopt this minor resonance structure. In other words, how likely is it for the header atom to participate in these electron conjugations.

Let's take a look at a couple of acyl derivatives. This is a carboxylate. If the header atom a

nitrogen, we have amide. If the header atom is oxygen, we also have esters, or carboxylic acids. And when the header atom is sulfur, we have thioesters.

The order in which I wrote them here is not actually random. It turns out for the carboxylate, because it has already a negative charge, the ability to adopt this resonance is greatly increased. So it's very well resonance-stabilized. The ability to form these resonance structures, it's also great for amides, and this dictates the chemistry and the biochemistry of the amide bond, which is explored in greater detail when we talk about protein. Esters can also adopt these resonance structures.

However, thioesters, because the sulfur is a third-row element, so the p-orbitals of sulfur are much bigger, they don't overlap very well with the p-orbitals of the carbon, the ability to adopt these resonance structures is greatly diminished. Therefore, thioesters behave a lot more like ketones, where the electrons of the carbonyl bond are localized between the carbon and oxygen, and not so much between the carbon and sulfur. So therefore, thioesters are the least resonant. And this is the trend. And this trend inversely correlates with the reactivity. Carboxylates are least reactive, whereas thioesters are the most reactive.

Now, when we talk about acyl transfer, we talk about the reaction between an acyl derivative with another nucleophile, which will replace the x header atom with the y header atom. So this reaction always occurs via a tetrahedral intermediate. When both substituents are attached to the carbon. Now from here, this tetrahedral intermediate can fall apart by kicking off the YR to regenerate the starting material, or it can kick off the XR group, to generate a new acyl derivative.

Let's now talk about the Claisen reaction. This is a very important reaction in biochemistry, related to the Aldol reaction, in which we form or cleave carbon-carbon bonds. The Claisen reaction happens between an enolizable carbonyl and an acyl derivative. Let's pick in this case an ester. And during this reaction, a carbon-carbon bond is formed between the alpha carbon of the enolizable carbonyl, and the keto carbon of the acyl derivative. The product of the Claisen reaction is a beta keto carbonyl.

Let's look at the mechanism. As with all carbonyl reactions, when we form a carbon-carbon bond, we need to form an enolate. So this is the first step. A base will form, remove the alpha proton, and form the enolate, which is now poised to add to the acyl derivative in an acyl transfer reaction, forming first a tetrahedral intermediate, which can spontaneously fall apart

by eliminating the header atom group, to form our beta keto carbonyl product.

Now, in biochemistry a preferred substrate for Claisen reactions is a thioester. One of the most common thioesters we're going to encounter in this course is acetyl-CoA. CoA, or coenzyme-A, it's a thiol that can form thioesters with a lot of acids, for example, acetic acid here. Acetyl-CoA can undergo a Claisen reaction with itself, and therefore acts both as an enolizable carbonyl and as an acyl derivative.

From when we were talking about thioesters, because of their limited conjugation with the carbonyl, they are very reactive, and they allow the formation of the enolate. Here is the acetyl-CoA enolate, which can react with another acetyl-CoA molecule. It will generate a tetrahedral intermediate. Let's draw this molecule first. Which can lose one of the CoA molecules, to generate this beta keto thioester, acetoacetyl-CoA.

As we will see later in the course, this is a precursor to formation of ketone bodies, one of the ways in which acetyl-CoA can be used to store energy.

Now, what is coenzyme-A, often abbreviated CoA? We mentioned it's a thiol. That means it has an SH group, which it turns out, is on a very long linker. There you go, this is coenzyme-A. You might recognize this part of the molecule as being adenine bound to a ribose bound to two phosphates. It's essentially ADP. But notice there's another phosphate in the three prime position, so it's an ADP with a three prime phosphate.

This portion of the molecule, If we squint, resembles the amino acid cysteine, but without the carboxyl group. And this middle portion of the molecule, it's something that looks very difficult to synthesize. Notice this carbon that has two methyl groups attached, and two other carbons attached to it. So it's like a tetravalent-- a carbon attached to it, four other carbons, that's it. Fairly rare sight in biochemistry. This portion of the molecule is called pantothenic acid. Pantothenic acid is an essential nutrient, also known as vitamin B5.

In this video we talked about carbonyl chemistry. Carbonyl is the C double bond O, and a lot of its properties are due to the polarizability of this bond, where the carbon has a partial positive charge, and oxygen a partial negative charge.

We talked about reactions to simple carbonyls, such as nucleophilic addition, enolization, Aldol reaction, and the Aldol dehydration. And acyl derivatives, where the carbonyl is next to a header atom, such as oxygen, nitrogen, or sulfur. And we mentioned the acyl transfer

reaction, and the Claisen reaction.

We saw in this video the nucleophilic addition, where a nucleophile attacks the carbon of carbonyl to add and form a tetrahedral product. For example, alcohols can add to carbonyls to form a hemiacetals, and amines can add to carbonyls to form imines, or Schiff bases.

And we reviewed that good nucleophiles are the ones like alkoxides, thiolates, amines, or C minus enolates. Whereas OK nucleophiles like alcohols and thiols, they need to be activated first to undergo nucleophilic addition.

We also talked about enolization, the ability of a carbonyl with an alpha hydrogen to rearrange into a hydroxyl bound to a double bond, which we call an enol. Now this equilibrium, called tautomerization, favors strongly the keto form. However, it does form to a sufficient extent to allow chemistry to happen. For example, when we remove the alpha hydrogen, we form an anion called enolate, which is a disguised carbanion which is a very good nucleophile.

Next, we discussed the Aldol reaction, a very important carbon-carbon bond formation or cleavage reaction in biochemistry. This reaction happens between an enolizable carbonyl and the regular carbonyl, and a new carbon-carbon bond is formed between the alpha carbon and the keto carbon, as shown here. The mechanism can be both base-catalyzed and acid-catalyzed. And the enzymes that catalyze this, called aldolases, use either a lysine in the active site to form first a Schiff base, or they use a zinc in the active site to polarize the carbonyl and allow for the enol formation.

We also saw that Aldol products can dehydrate to form alpha beta unsaturated carbonyls. The mechanism could be both acid- and base-catalyzed, and involves in both cases formation of an enol.

Next, we also talked about acyl derivatives, and acyl transfer. As we show here, the resonance in the acyl derivative dictates there how well they react. Carboxylate and amine are the most resonant stabilized, and therefore are the least reactive, whereas esters, especially thioesters, are the least resonance stabilized, and therefore most reactive.

Finally, we discussed the Claisen reaction, a reaction similar to the Aldol, between an enolizable carbonyl and an acyl derivative, which generates a beta keto carbonyl. We introduced the acetyl-CoA, a very important thioester, that can undergo Claisen reaction with itself to form acetoacetyl-CoA. And we also introduced the structure of CoA, which is built

around vitamin B5, an essential nutrient.