9.14: SOME BIOLOGICAL CARBONYL CONDENSATION REACTIONS

OBJECTIVES

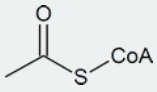
After completing this section, you should be able to

1. identify acetyl coenzyme A as an important biomolecule which undergoes carbonyl condensation reactions.
2. identify aldoases as enzymes that catalyze aldol reactions in biological systems.
3. identify the steps in which a carbonyl condensation reaction has occurred, given a general outline of a specific biosynthesis.

STUDY NOTES

Carbonyl condensation reactions occur in biological systems; for example, in the biosynthesis of citric acid.

You first met acetyl coenzyme A in Section 21.8. Again, we stress that it is not essential that you know the detailed structure of this compound (or other large biochemical stuctures discussed), but you should know that it may be represented as



and that it has the ability to behave just as any other acetyl-containing compound.

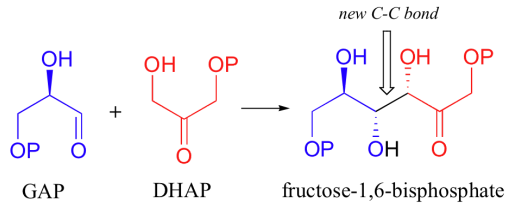
ALDOL REACTIONS IN NATURE

So far, we have examined the non-enzymatic reaction of an aldehyde or ketone with itself (a so-called 'self-condensation' reaction, where 'condensation' means the formation of one larger molecule from two smaller ones). However, aldol reactions occur in several biological pathways, most commonly in the metabolism of carbohydrates (sugars). The enzymes that catalyze aldol reactions are called, not surprisingly, '**aldolases**'. They occur in all organisms, but note that **Class I aldoases** are usually found in animals and higher plants, while **Class II aldoases** normally appear in bacteria and fungi.

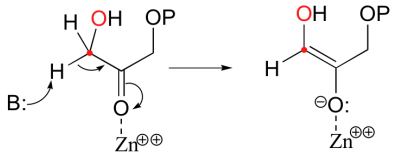
TYPICAL ALDOLASE REACTIONS - THREE VARIATIONS ON A THEME

The first step in an aldolase reaction is the deprotonation of an alpha-carbon to generate a nucleophilic carbanion. Nature has evolved several distinct strategies to stabilize the intermediate that results. Some aldolases use a metal ion to stabilize the negative charge on an enolate intermediate, while others catalyze reactions that proceed through neutral Schiff base or enol intermediates.

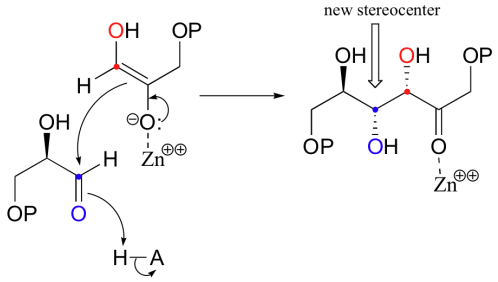
Let's examine first a reaction catalyzed by a so-called **'Class II' aldolase**, in which a metal cation - generally Zn^{2+} - bound in the active site serves to stabilize the negative charge on an enolate intermediate. Fructose 1,6-bisphosphate aldolase is an enzyme that participates in both the glycolytic (sugar burning) and gluconeogenesis (sugar building) biochemical pathways. For now, we will concentrate on its role in the gluconeogenesis pathway, but we will see it again later in its glycolytic role. The reaction catalyzed by fructose 1,6-bisphosphate aldolase is a condensation between two 3-carbon sugars, glyceraldehyde-3-phosphate (GAP) and dihydroxyacetone phosphate (DHAP), forming a six-carbon product (which leads, after three more enzymatic steps, to glucose).



In the first step of the condensation, an alpha-carbon on DHAP is deprotonated, leading to an enolate intermediate. The strategy used to stabilize this key intermediate is to coordinate the negatively-charged enolate oxygen to an enzyme-bound zinc cation.

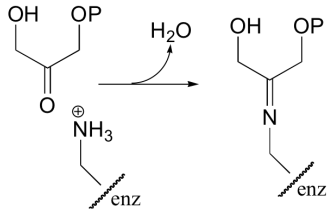


Next, the deprotonated a-carbon attacks the carbonyl carbon of GAP in a nucleophilic addition reaction, and protonation of the resulting alcohol leads directly to the fructose 1,6-bisphosphate product.

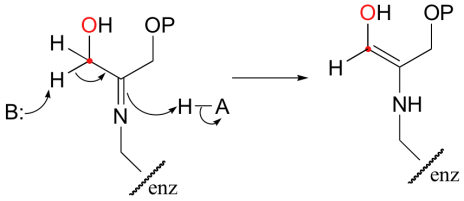


As with many other nucleophilic carbonyl addition reactions, a new stereocenter is created in this reaction, as a planar, achiral carbonyl group is converted to a tetrahedral, chiral alcohol. The enzyme-catalyzed reaction, not surprisingly, is completely stereospecific: the DHAP substrate is positioned in the active site so as to attack the *re* (front)face of the GAP carbonyl group, leading to the *R* configuration at the new stereocenter.

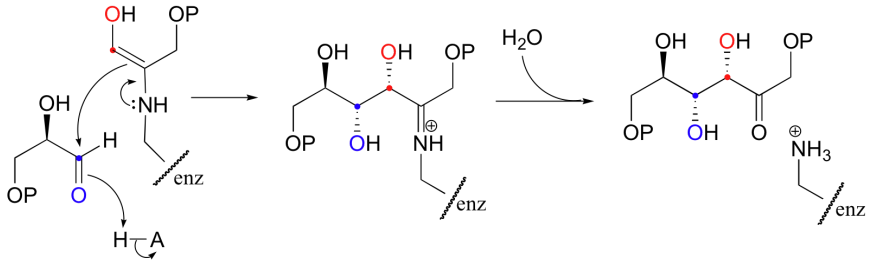
Interestingly, it appears that in bacteria, the fructose bisphosphate aldolase enzyme evolved separately from the corresponding enzyme in plants and animals. In plants and animals, the same aldol condensation reaction is carried out by a significantly different mechanism, in which the key intermediate is not a zinc-stabilized enolate but an enamine. The nucleophilic substrate (DHAP) is first linked to the enzyme through the formation of an imine (also known as a Schiff base) with a lysine residue in the active site.



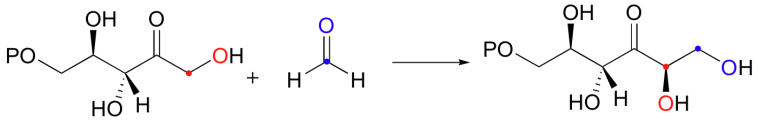
The alpha-proton is then abstracted by an active site base to form an enamine.



In the next step, the alpha-carbon attacks the carbonyl carbon of GAP, and the new carbon-carbon bond is formed. In order to release the product from the enzyme active site and free the enzyme to catalyze another reaction, the imine is hydrolyzed back to a ketone group.



There are many more examples of **'Class I' aldolase** reactions in which the key intermediate is a lysine-linked imine. Many bacteria are able to incorporate formaldehyde, a toxic compound, into carbohydrate metabolism by condensing it with ribulose monophosphate. The reaction proceeds through imine and enamine intermediates.

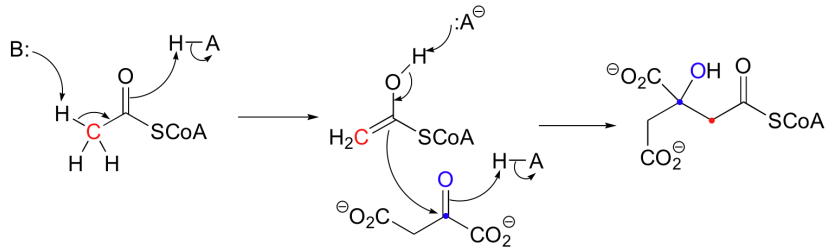


EXAMPLE 23.13.1

a. Propose a complete mechanism for the condensation reaction shown above.
b. Propose a complete mechanism for the conversion of hexulose-6-phosphate (formed from the condensation of ribulose-5-phosphate and formaldehyde) into fructose-6-phosphate.

Solution

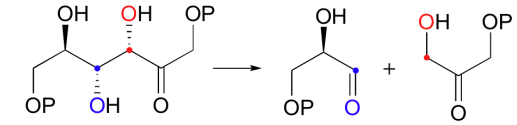
Along with aldehydes and ketones, esters and thioesters can act as the nucleophilic partner in aldol condensations. In the first step of the citric acid (Krebs) cycle, acetyl CoA condenses with oxaloacetate to form (S)-citryl CoA.



Notice that in this aldol reaction, the nucleophilic intermediate is stabilized by protonation, rather than by formation of an imine (as in the Class I aldolases) or by a metal ion (as in the Class II aldolases).

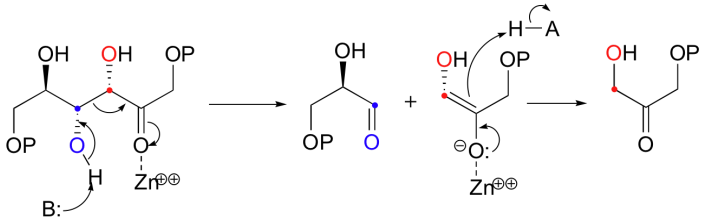
GOING BACKWARDS: THE RETROALDOL REACTION

Although aldol reactions play a very important role in the formation of new carbon-carbon bonds in metabolic pathways, it is important to emphasize that they are also highly reversible: in most cases, the energy level of starting compounds and products are very close. This means that, depending on metabolic conditions, aldolases can also catalyze **retro-aldol** reactions (the reverse of aldol condensations, in which carbon-carbon bonds are *broken*). Recall that fructose 1,6-bisphosphate aldolase (section 13.3B) is active in the direction of sugar breakdown (glycolysis) as well as sugar synthesis (gluconeogenesis). In the glycolytic direction, the enzyme catalyzes - either by zinc cation or by imine/enamine mechanisms, depending on the organism - the retro-aldol cleavage of fructose bisphosphate into DHAP and GAP.

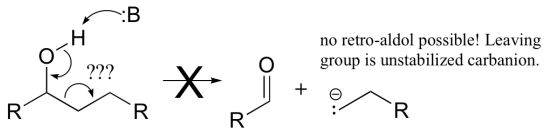


fructose-1,6-bisphosphate GAP DHAP

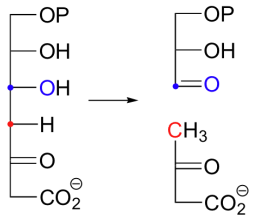
The mechanism is the exact reverse of the condensation reaction. Shown below is the mechanism for a Zn^{2+} - dependent (Type II) retroaldol cleavage. Notice that in the retroaldol reaction, the enolate intermediate is the *leaving group*, rather than the nucleophile.



The key thing to keep in mind when looking for a possible retro-aldol mechanism is that, when the carbon-carbon bond breaks, the electrons must have some place to go, where they will be stabilized by resonance. Generally, this means that there *must* be a carbonyl or imine group on the next carbon. If there is no adjacent carbonyl or imine group, the carbon-carbon bond is not free to break.



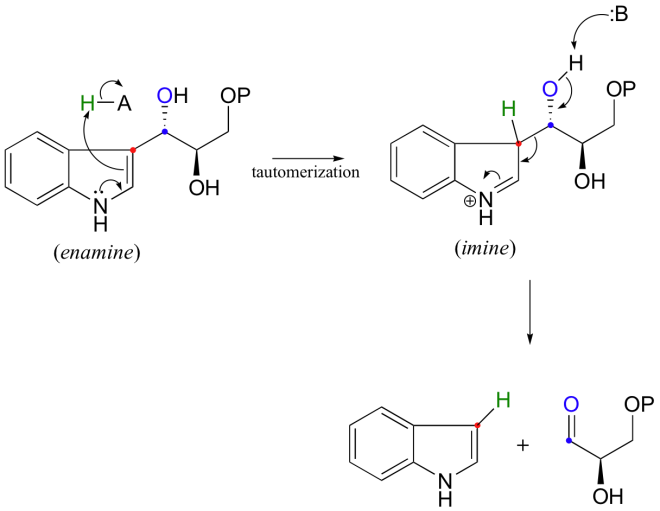
Here are two more examples of retro-aldol reactions. Bacterial carbohydrate metabolism involves this reversible, class I retro-aldol cleavage: (*Proc. Natl. Acad. Sci* **2001**, *98*, 3679).



EXAMPLE 23.13.2

Draw the structure of the enamine intermediate in the retroaldol reaction shown above.

Another interesting example is the retro-aldol cleavage of indole-3-glycerol phosphate, a step in the biosynthesis of tryptophan.



Look carefully at this reaction - how is the leaving group stabilized? There is an imine group involved, but no participation by an enzymatic lysine. The imine is 'built into' the starting compound, available from the initial tautomerization of the cyclic enamine group in indole-3-glycerol phosphate.

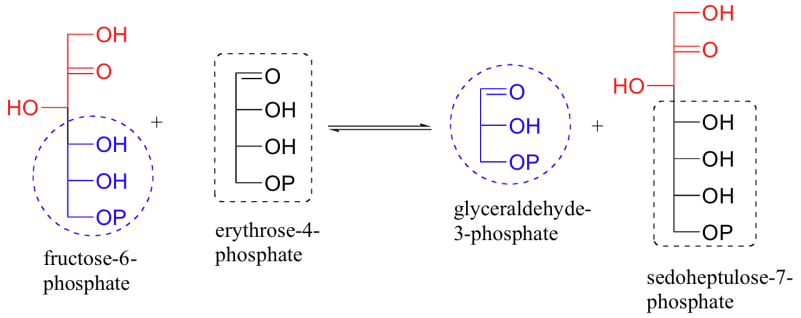
EXAMPLE 23.13.3

Draw the reverse (aldol condensation) direction of the reaction above.

Solution

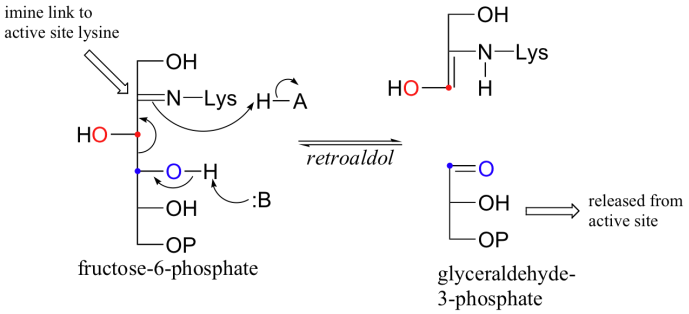
GOING BOTH WAYS: TRANSALDOLASE

An enzyme called transaldolase, which is part of the 'pentose phosphate pathway' of carbohydrate metabolism, catalyzes an interesting combination of class I aldol and retro-aldol reactions. The overall reaction, which can proceed in either direction depending on metabolic requirements, converts 3- and 7-carbon sugars into 6- and 4-carbon sugars. Essentially, a 3-carbon unit breaks off from a ketone sugar (ketose) and then is condensed directly with an aldehyde sugar (aldose).

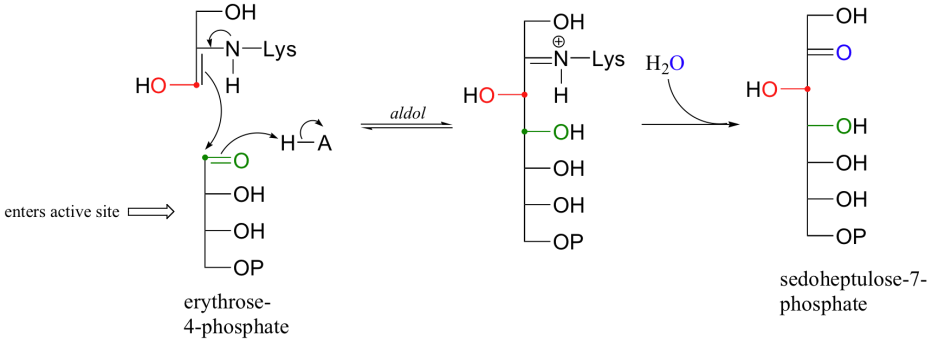


Let's follow the progress of the reaction in the left-to-right direction as depicted above. Because this is a class I aldolase, the first step is the formation of an imine linkage between the ketone carbon of fructose-6-phosphate (F6P) and a lysine group from the enzyme. The enzyme-

substrate adduct then undergoes a retro-aldol step to free glyceralde-3-phosphate (GAP), which leaves the active site.



The second substrate, erythrose 4-phosphate (E4P), enters the active site, and an aldol condensation occurs between E4P and the 3-carbon fragment remaining from the cleavage of fructose-6-phosphate.



The final step is hydrolysis of the imine and subsequent dissociation of sedoheptulose 7-phosphate from the active site.
