Chapter Zero

Setting the Stage for Biochemistry

A Review of Essential Concepts from General and Organic Chemistry

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Thanks to my 2006 Biochemistry students for spotting errors and for many helpful suggestions. PLEASE help me to make this a better learning tool by sending your suggestions and corrections to rhodes@usm.maine.edu. Last revision 2006/09/19.

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ARE YOU PREPARED FOR BIOCHEMISTRY?

I presume that you have successfully studied general chemistry for one year and organic chemistry for one year. If you have not, you are not prepared for this introductory biochemistry course. You should acquire the necessary background before continuing. Biochemistry, as most texts present it, is Organic III, a continuation of organic chemistry. A biochemistry course is in essence an advanced organic chemistry course that features the molecules and chemical processes of life. As you study it, you will draw heavily on what you learned in organic chemistry, as well as general chemistry. These courses are not merely paper prerequisites for biochemistry, they are knowledge requirements.

INTRODUCTION

This chapter serves two purposes: 1) to help you review useful concepts from your previous chemistry courses, and 2) to give you a setting in which to think about the whole of biochemistry as you learn its parts. You will accomplish both purposes by looking at the chemical details of digestion and breakdown of fats, from the hydrolysis of triglycerides into fatty acids and glycerol in the small intestine, through the absorption of fatty acids into cells and their complete oxidation to carbon dioxide and water. As you study this sequence of reactions, you will review many concepts from your general chemistry and organic chemistry courses. In addition, many areas of biochemistry that are new to you will become familiar territory, and then, as you move on and start learning the details of biochemistry, you will return to this territory repeatedly to help keep your bearings and try out your new knowledge.

This chapter contains questions and problems to solve as you proceed. They are signaled by lines that look like this:

> Question: How are esters formed?
>
> _____
>
> Answer: An ester is the product of condensation reaction between a carboxylic acid and an alcohol.

When you see such questions, cover the page below the solid line to hide the answer. Try to answer the question fully without reading on, consulting only material that has come earlier in this chapter. If you cannot answer a question, consult your organic-chemistry textbook, or if appropriate, your general-chemistry textbook. What? You sold these texts back to the bookstore? Very big mistake. It will be useful, sometimes essential, to refer to general and organic texts throughout this course. If you do not have them, buy old texts from used-book stores (or check Amazon.com) or find reliable websites for chemistry content. An excellent reference for organic chemistry is Professor Thomas Newton's **O=CHem**, at this address:

http://www.usm.maine.edu/~newton/Chy251_253/Welcome.htm

If you have taken all prerequisites for biochemistry, but still find the questions in this reading difficult, try reading Chapter 1 in *The Biochemistry Student Companion*, by Allen J. Scism (Pearson Prentice Hall, any edition), before you tackle this chapter. I also

wrote that chapter, and I had the the same goal as in this one, but with a different style. In Chapter 1 of the *Companion*, I present the same sort of review as here, but with examples, rather than questions and problems. So that Chapter 1 is a passive learning tool. This Chapter Zero is tool for active learning.

What is biochemistry? Any interesting biological question—like "How does a hormone produce chemical signals inside a cell?"—eventually becomes a chemical question, requiring the researcher to understand the relationship between structure of molecules and their biological function. ***Establishing structure-function relationships is the central goal of all chemistry****. Biochemistry entails applying the tools of chemistry (and physics and mathematics) to answer the pressing questions of biology at the deepest possible level, the molecular level.*

To help you recall terms from earlier chemistry courses, and recognize new biochemical terms, I will mark each clearly, as follows. Review terms appear first in **bold**, followed immediately by examples or questions. New biochemical terms appear first in *italics*, followed immediately by brief definitions. You will encounter more complete definitions of these terms as they arise in your detailed study of biochemistry.

On the next two pages are sequences of reactions that form the subject of this review.

Figure 1

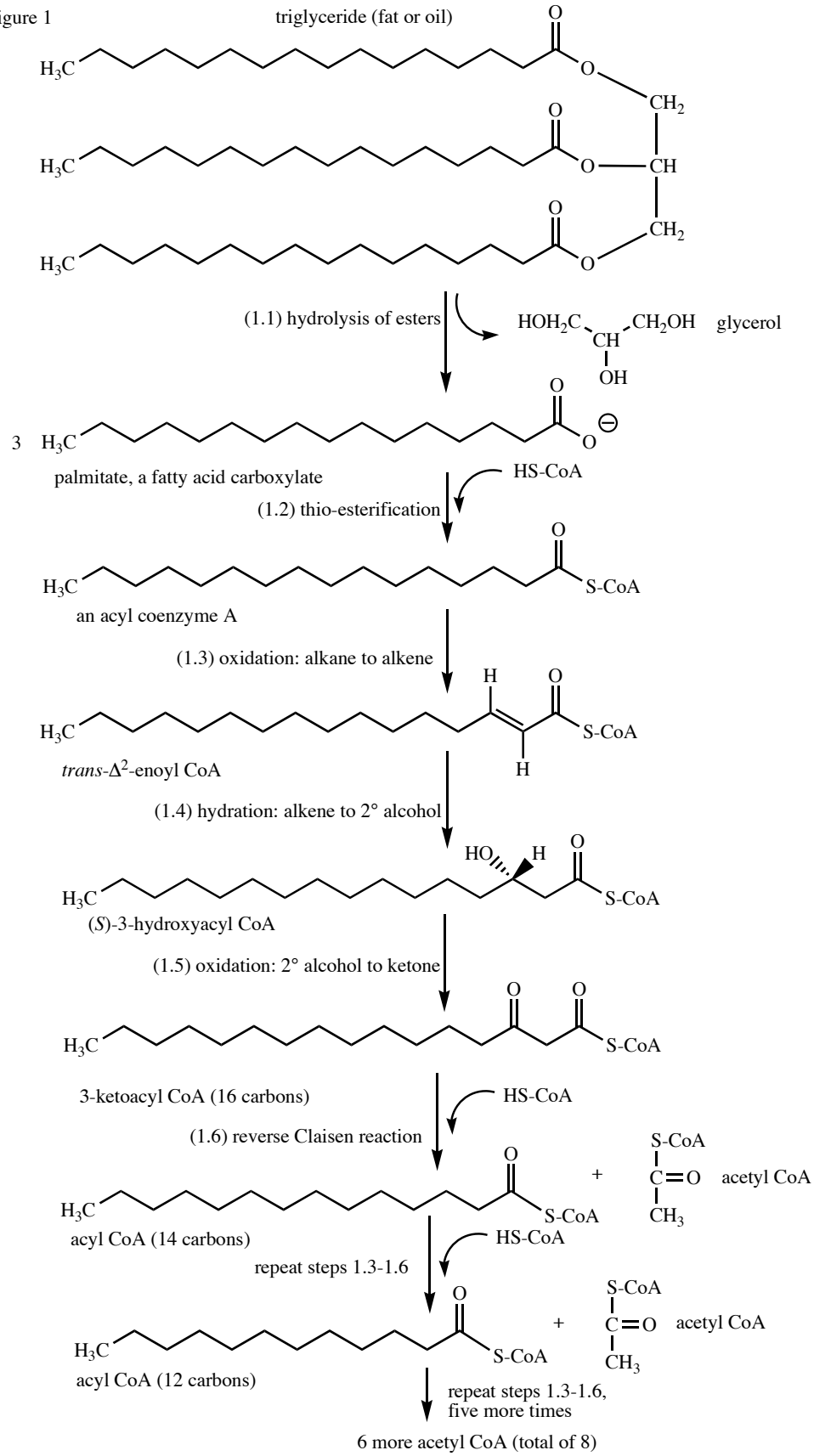
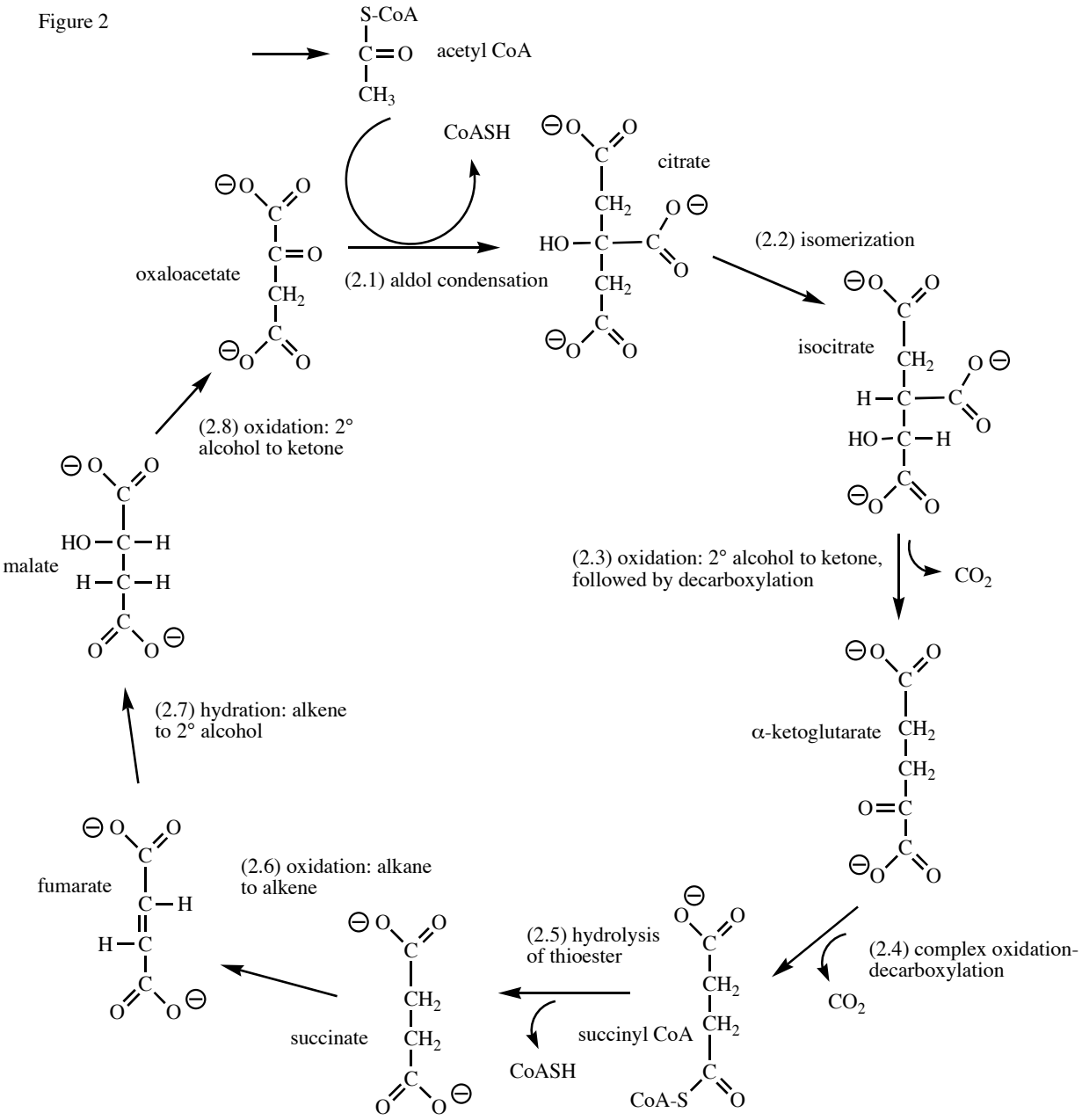

triglyceride (fat or oil)
(1.1) hydrolysis of esters
glycerol
3
palmitate, a fatty acid carboxylate
HS-CoA
(1.2) thio-esterification
an acyl coenzyme A
(1.3) oxidation: alkane to alkene
trans-Δ2-enoyl CoA
(1.4) hydration: alkene to 2° alcohol
(S)-3-hydroxyacyl CoA
(1.5) oxidation: 2° alcohol to ketone
3-ketoacyl CoA (16 carbons)
(1.6) reverse Claisen reaction
acetyl CoA
acyl CoA (14 carbons)
repeat steps 1.3-1.6
acyl CoA (12 carbons)
repeat steps 1.3-1.6, five more times
6 more acetyl CoA (total of 8)

Figure 2


S-CoA
acetyl CoA
CoASH
citrate
oxaloacetate
(2.1) aldol condensation
(2.2) isomerization
isocitrate
(2.3) oxidation: 2° alcohol to ketone, followed by decarboxylation
CO2
α-ketoglutarate
(2.4) complex oxidation-decarboxylation
CO2
succinyl CoA
CoA-S
(2.5) hydrolysis of thioester
CoASH
succinate
(2.6) oxidation: alkane to alkene
fumarate
(2.7) hydration: alkene to 2° alcohol
malate
(2.8) oxidation: 2° alcohol to ketone

GET AN OVERVIEW

Begin by studying the sequence of reactions in Figures 1 and 2. These charts show a sequence of biochemical reactions by which your body derives energy from dietary fats and oils. The process begins with their initial breakdown into fatty acids and glycerol, which occurs in the stomach and small intestine. The process concludes with the oxidation of the fatty acids to carbon dioxide and water, which begins in the cytoplasm and ends in the mitochondria of cells. By this process, cells extract and conserve a large amount of free energy that your cells use in many ways: to move muscles, to synthesize complex biomolecules, and to move nutrients across membranes, among other functions.

Biochemists refer to sequences of reactions like this as *metabolic pathways*, and to the sum of all such reactions in living organisms as *metabolism.* I will use these reactions to help you recall what you learned in general and organic chemistry, to introduce you to biochemical terms and concepts, and to provide examples of new concepts and principles. Along the way, you will learn whether you have the proper preparation to prosper and learn in a biochemistry course.

First, take an overview of the reactions. In reaction 1.1, dietary fats are taken apart into long-chain carboxylic acids, called *fatty acids*, and the three-carbon compound **glycerol**. These reactions occur in your stomach and small intestine. In reaction 1.2, which occurs in the *cytoplasm* of cells, and 1.3–1.6, which occur in *mitochondria*, fatty acids are broken down into **acetyl** groups, joined to a carrier molecule called *coenzyme A*. In reactions 2.1–2.8, which also occur in mitochondria, *acetyl coenzyme A* is broken down into carbon dioxide and water, completing the oxidation of fats. Mitochondria are the cell's primary energy producers, extracting most of the energy that is available from fuels by oxidizing them with oxygen.

Next, focus on the structures of the intermediates in this pathway. Look for familiar **functional groups** from organic chemistry. Find **esters**, **carboxylic acids**, **alcohols**, and other familiar groupings of atoms. What is the **geometry** around each central atom in these structures—which are trigonal, and which are tetrahedral? Are any of these functional groups actually hybrids of **resonance contributors** that are not shown? Focusing on the **stereochemistry** of compounds, do you see **chiral** or **prochiral** compounds, including ones that could exist as **enantiomeric** pairs, or as groups of **diastereomeric** structures? Do you see molecules that might exist in equilibrium with alternative forms, such as **tautomers**, or molecules in which two functional groups might undergo **intramolecular** reaction to produce a new compound?

Finally, focus on the reactions. In a biochemical pathway, each compound, which is called an *intermediate*, is both a product of the previous reaction and a reactant of the next reaction. Curving arrows above or below the reaction arrows indicate reactants consumed or products produced in addition to the intermediates. As you consider the reaction that turns one intermediate into the next, can you find examples of **oxidation** or **reduction** by finding atoms whose **oxidation numbers** are different in the reactant and the product? Given the **reduction potentials** of **oxidant** and **reductant**, could you

calculate the **free-energy changes** or **equilibrium constants** for these reactions? Can you spot **addition, substitution** or **elimination** reactions? And finally, can you write **mechanisms** for these reactions, depicting with curved arrows the movement of electrons to make and break **covalent bonds**, and assuming **catalysis** by hydrogen ions or hydroxide ions?

Now you know some of the concepts from general and organic chemistry that you will need to use in biochemistry. Let's begin a detailed review by looking at these structures and reactions more closely.

STRUCTURES

Lewis Models and Geometry

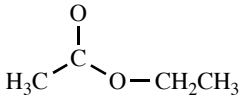
The first structure in Figure 1 is called a *triglyceride*. It is a familiar type of compound to anyone who has studied organic chemistry.

Question: What organic functional group occurs three times in a triglyceride?

———

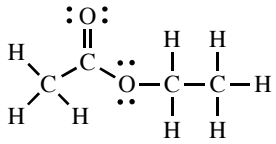
Answer: The **ester** functional group occurs three times in this structure.

Question: Draw a complete **Lewis diagram** of the simple ester ethyl acetate. Here is the skeleton of the molecule:



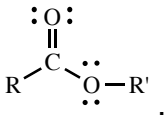
———

Answer: Here is the fully expanded structure of ethyl acetate, showing all bonds and unshared electrons:



If you are unable to produce this structure from the incomplete structural formula provided, you should review the chapter on the Lewis model in your general chemistry text. As was true in organic chemistry structural formulas are often shortened, and unshared electrons are frequently not shown. But you must know they are there in order to know the chemical potential of a compound depicted by a shorthand structural formula.

A triglyceride like the first compound of Figure 1 is an **ester**, a compound with the general formula



In this formula, R- and R'- represent unspecified groups of atoms, such as **methyl** (CH_3-) or **ethyl** (CH_3CH_2-) groups. The atoms joining R and R' constitute an **ester** linkage**.**

Questions: What are the geometries and hybridizations of the carbon and the oxygen in an ester? What kinds of bonds (π or σ) join these atoms?

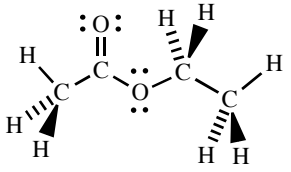
———

Answer: The carbon of the ester is **trigonal** and ***sp*²-hybridized**, while the oxygen linking the ester carbon with R' is **bent** and ***sp*³-hybridized**, with sigma (σ) bonds to C and R' using two of its sp^3 orbitals, while the other two each carry one unshared or non-bonding pair of electrons. The carbonyl oxygen is hybridized sp^2, with each unshared electron pair in a nonbonding sp^2 orbital. One of the two carbon-oxygen bonds is formed by σ overlap of sp^2 orbitals from the carbon and the oxygen, and the second bond by *pi* (π) overlap of *p*-orbitals from each atom. In elements from the second row of the periodic table, multiple bonds consist of one σ bond involving hybrid orbitals, and the remainder are π bonds involving unhybridized *p* orbitals.

Question: Make a full perspective drawing of ethyl acetate, showing bond angles realistically, and using wedges and dotted lines to show bonds not in the plane of the paper. Hint: Start with as many of the non-hydrogen atoms as possible in the plane of paper.

———

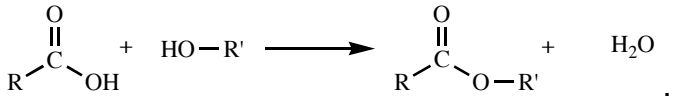
Answer:



You can draw all carbons and oxygens in the plane of the paper, along with two hydrogen atoms. All remaining hydrogens lie above or below the plane. In such drawing, you apply the principle of **valence-shell electron-pair repulsion**, the simple assumption that independent groups of electrons repel each other and take up orientations that minimize repulsion.

Structure and Properties

But a triglyceride is not a simple ester, it is a **triester**, containing three ester linkages. Recall from organic chemistry that esters are the product of **condensation** reactions (reactions that produce water) between **carboxylic acids** and **alcohols**, as follows:



Question: What starting materials would an organic chemist need to make the tryglyceride shown in Figure 1 by a condensation reaction?

———

Answer: To make the tryglyceride shown in Figure 1, the starting alcohol would be the **triol**, glycerol (1,2,3-propanetriol), which provides three alcoholic -OH groups, while the three molecules of carboxylic acid would be hexadecanoic acid, $CH_3(CH_2)_{14}COOH$, commonly known as *palmitic acid* (from palm oil, a rich source of tryglycerides containing this acid).

Reaction (1.1) is the reverse of a condensation reaction. It is **hydrolysis** of the three ester links, which produces free glycerol and three molecules of palmitic acid, a *fatty acid* (more about reactions later). Fatty acids are long-chain monocarboxylic acids. If the hydrocarbon chain is all **methylene** groups ($-CH_2-$), the fatty acid is referred to as *saturated*, meaning that it has the maximum number of hydrogens. *Unsaturated* fatty acids contain one of more double bonds.

Question: Why are the products of this hydrolysis negatively charged?

———

Answer: In the slightly basic medium of the small intestine, carboxylic acids are found in their **ionized** or **conjugate-base** form, as shown in the figure. In this case, the product is *palmitate ion*, or $CH_3(CH_2)_{14}COO^-$, usually referred to simply as *palmitate*.

Question: If the pK_a value of the carboxylic acid is less than 4.0, in what pH range will it exist in its conjugate-base form?

———

Answer: At any pH value larger than the value of the pK_a (or as chemists commonly say, "When pH is greater than pK_a"), acids exist predominately in their conjugate-base forms, and carboxylic acids exist as carboxylate ions. In biochemistry, we often refer to acids by their conjugate-base name (palmitate, not palmitic acid), because the carboxylate form is the predominant form of a conjugate acid-base pair under physiological conditions (pH near 7.0).

Question: Carboxylic acids are ionized at pH 7.0, but alcohols are not. Why the difference?

———

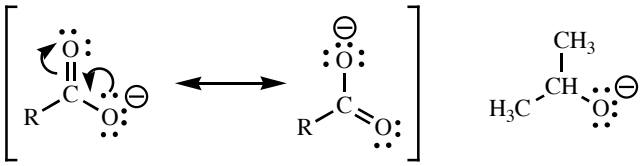
Answer: The carboxylate ion, product of loss of H^+ from a carboxyl group, is stabilized by **resonance**, whereas the **alkoxide ion**, from loss of H^+ from an alcohol, is not.

Question: Draw the two **resonance forms** or **resonance contributors** of a carboxylate ion, to show how resonance stabilizes the conjugate base of a carboxylic acid. Use

curved arrows to show how you generate the second resonance form from the first. Also draw the conjugate base of an alcohol, and show why it is not stabilized by resonance.

———

Answer: Resonance forms differ only in the positions of π-bonds and unshared electrons. Here are the two resonance forms of a carboxyl group, alongside the conjugate base of an alcohol, an alkoxide ion. Also shown are curved arrows that show how to derive the second resonance form from the first.



carboxylate alkoxide

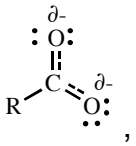
Notice that there are no π bonds adjacent to the negatively charged oxygen of the alkoxide ion, so it is not possible to move π-bonds and unshared electron pairs to draw additional resonance forms. This means that the carboxylate ion is stabilized by resonance, which delocalizes the negative charge, while the alkoxide ion enjoys no such stabilization. Resonance stabilization of the carboxylate ion is the main reason that carboxyl groups are acidic, but alcohols are not. The two resonance contributors to the carboxylate hybrid are identical, which implies great resonance stabilization.

Question: Are resonance forms different structures, that is, different compounds?

———

Answer: No. A set of resonance forms represent a single structure. We write resonance forms when no single structural drawing is an adequate description of a structure. We say that the "real" structure is a **resonance hybrid** or composite of the resonance forms. It does not equilibrate between them. Instead, its properties are the average of the expected properties of the resonance forms. For example, each oxygen of the carboxylate ion carries one half of a negative charge, the average charge of each oxygen in the two resonance forms. Each C-O bond in the carboxylate ion is neither single nor double, because both bonds are single in one contributor and double in the other. If we say that a single bond has a **bond order** of 1.0, and a double bond a bond order of 2.0, then the C-O bonds in the carboxylate ion have bond orders of 1.5, the average of the bond orders in each contributor.

A resonance hybrid like a carboxylate ion does not spend half its time as one contributor and half its time as the other. It spends all its time as an ion whose properties are the average of those of its contributors. The brackets enclosing the contributors emphasizes that they represent one, not two, structures. Another way to represent the carboxylate resonance hybrid is like this:



with ∂- representing partial negative charge. The full set of resonance contributors is more informative, allowing bond orders to be estimated more precisely.

Functional Groups and Isomerism

Question: What type of compound is the product of reaction (1.2)?

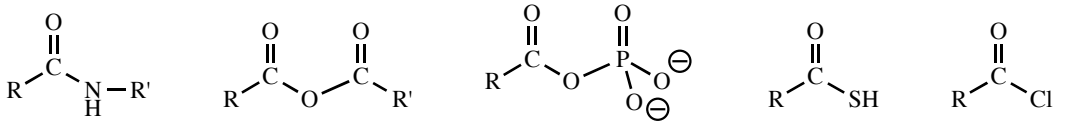
____

Answer: The product of reaction (1.2) is a **thioester**, an ester formed by condensation of a carboxylic acid with a **thiol**, R-SH. The thioester is one of several common **derivatives of carboxylic acids**.

Question: List five other derivatives of carboxylic acids.

_____

Answer: Carboxyl derivatives include **amides**, **anhydrides**, **phosphoanhydrides**, **thioacids**, and **acyl** chlorides (below), in addition to esters and carboxylate ions.



In Figure 1, the names of derivatives of palmitic acid are simplified. For example, instead of palmityl CoA, the thioester is referred to as a generic “acyl coenzyme A”. Any saturated fatty acid could undergo the transformations of Figure 1, so such general names are frequently used.

Question: Reaction (1.3) produces what kind of compound? Give the **IUPAC** name of the compound.

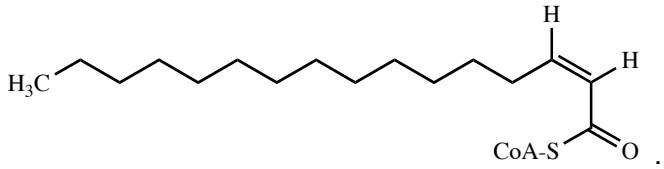
_____

Answer: Reaction (1.3) introduces a carbon-carbon double bond. Palmitate has become *trans*-2-hexadecenoate, which is still a carboxylate ion, but now also an **alkene**, specifically, a *trans*-alkene. In standard organic nomenclature, the carbonyl carbon of carboxyl derivatives is C-1, and the adjacent carbon is C-2, and so forth. Older, but still common, nomenclature refers to C-2 as the α-carbon, and successive carbons as β, γ, and so forth. So this new compound contains a 2,3- or α,β-double bond. Recall that substituted alkenes having two different groups on each double-bonded carbon are found in either *cis*- or *trans*- forms. In simple cases, if the two carbons have one group in common (the –H in this case), then the form with the like groups on opposite sides of the planar double bond is *trans*-, like the product of reaction (1.3).

Question: Draw and name the geometric isomer.

———

Answer: The geometric isomer of *trans*-2-hexadecenoate is *cis*-2-hexadecenoyl CoA:



Note that the only product of this reaction receives a new double bond at C-2, and not at any other chemically similar carbons. So this reaction is **regiospecific**: occurring at only one of what appear to be numerous chemically similar sites. In addition, only the *trans*-double bond forms. So this reaction is also **stereospecific**: producing only one of two or more possible stereoisomers. Regiospecificity and stereospecificity are common aspects of biological reactions that distinguish them from most common chemical reactions. Specificity arises from the nature of enzymes, the biological catalysts that promote these reactions.

Question: What type of compound is produced by Reaction (1.4)? Classify the new functional group as **primary**, **secondary**, or **tertiary**.

———

Answer: The product of reaction (1.4) is a **secondary alcohol**, (*S*)-3-hydroxyacyl CoA. Alcohols and other organic compounds with simple, single-bonded functional groups, like alkyl **halides** (R-Cl) or **mercaptans** (R-SH, also called **thiols**), are designated primary, secondary, and tertiary according to whether they have one, two, or three carbons attached to the carbon carrying the functional group. This alcohol has its hydroxyl on C-3, which is attached to two carbons, C-2 and C-4, so it is a secondary alcohol.

Question: In the name of this compound, what does the (*S*) mean?

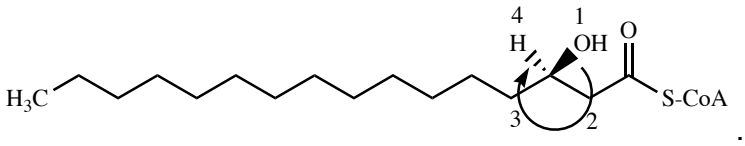
———

Answer: The (*S*) designates the **configuration** of C-3, which is a **chiral carbon**. This alcohol is the first structure in Figure 1 that contains a chiral carbon atom. Chiral carbons are tetrahedral carbons with four different groups attached. There are two possible arrangements of four different groups around a central tetrahedral atom, and they constitute a pair of **enantiomers**.

Question: Give a general definition of the term **enantiomer**, without referring to chiral carbons.

Answer: Enantiomers are stereroisomers that are non-superimposable mirror images of each other. Not all enantiomers contain chiral carbons, but in organic chemistry, most of them do. If enantiomerism is due to a single chiral carbon, we can designate the two

enantiomers as (*R*) or (*S*), according to the convention of Cahn, Ingold, and Prelog (CIP). In Figure 1, we find the (*S*-) form. For comparison, the other enantiomer is



.

To prove this statement, we number the four groups around the chiral center by CIP priorities. With H, the lowest-priority group, pointing away from the viewer, the curving arrow traveling through groups 1, 2, and 3 points clockwise, so the isomer is designated (*R*-).

Question: What type of compound is produced by Reaction (1.5)?

———

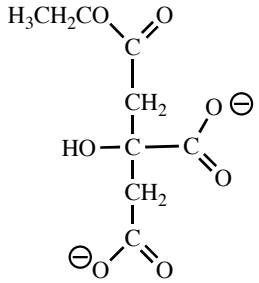
Answer: Reaction (1.5) produces a **ketone**, in this case, a 3-ketoacyl CoA, and the products of reaction (1.6) are a new fatty acyl CoA, which is shorter than palmitate by 2 carbons, and a 2-carbon compound, acetyl CoA. I will discuss these and the other reactions of Figure 1 in the next section.

Now look at the structures of Figure 2. There are no new classes of compounds in this figure, but some more structural matters worthy of review. Consider citrate, the product of reaction (2.2). Citrate is the conjugate base of a citric acid, which contains three carboxyl groups, making it a **tricarboxylic acid**. The cycle of reactions in Figure 2 involves a menagerie of di- and tricarboxylates.

Question: Find a **prochiral** or **meso** carbon in citrate, the product of Reaction (2.1). Give a general definition of the term **prochiral**.

———

Answer: Prochiral carbons carry two identical groups (in this case, carboxymethyl groups, $–CH_2COO^–$, and two different additional groups (–OH and $–COO^–$). Replacement of one of the two identical groups with a new group that is different from the other three would turn the prochiral atom into a chiral atom. In this case, alteration of either of the identical carboxymethyl groups, such as converting one to an ethyl ester, would make C-3 chiral:



Question: Designate this compound (*R*) or (*S*).

———

Answer: This new chiral compound is the (*S*) form. Converting the lower carboxyl instead to the ethyl ester would produce the (*R*) form. The two are enantiomers.

Question: Find the chiral centers in the product of Reaction (2.2).

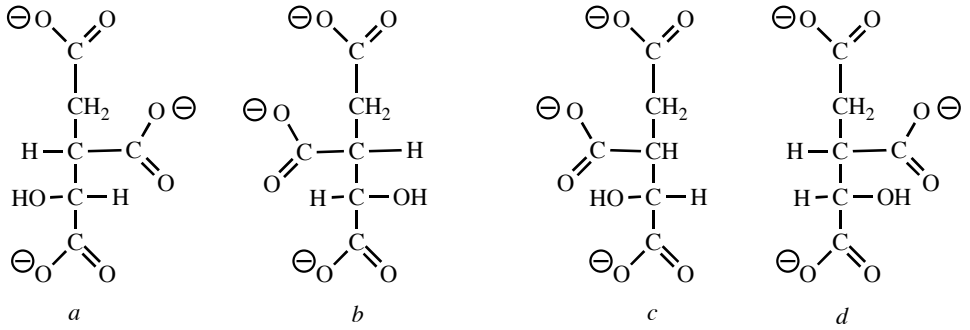
———

Answer: The product of reaction (2.2), isocitrate, has <u>two</u> chiral centers, C-2 and C-3.

Question: Draw all possible stereoisomers of isocitrate, and describe the stereochemical relationships among them.

———

Answer: In addition to the structure shown in Figure 2 (below, *a*), there are three other stereoisomers of isocitrate: the mirror image (the enantiomer) of the product of (2.2), shown below as *b*, and another enantiomeric pair, *c* and *d*. The pair *c* and *d* are **diastereoisomers** of the pair *a* and *b*. Diastereoisomers are non-superimposable non-mirror images of each other. By this definition, *cis*-2-hexadecenoate and *trans*-2-hexadecenoate are also diastereoisomers.



a *b* *c* *d*

All of the compounds shown in Figures 1 and 2 are produced only in the stereochemical configurations shown. As mentioned earlier, control of stereochemistry—stereospecificity—is a hallmark of biochemical reactions. When you learn about enzymes in your biochemistry course, you will learn how enzymes control both stereospecificity and regiospecificity.

Question: In Figure 2, can you find other chiral compounds besides isocitrate? Other prochiral compounds besides citrate? Other compounds capable of geometric isomerism? Compounds that are simple structural isomers of each other?

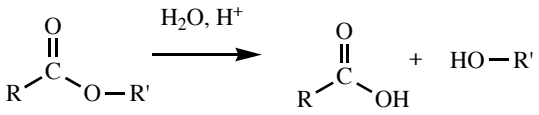
———

Answers: Malate is chiral. Every one of methylene carbon atoms (-CH_2-) in Figure 2 is prochiral. Fumarate is *trans*-2-butendioate, and has a geometric isomer, *cis*-2-butendioate, also know as maleate. Citrate and isocitrate are structural isomers, having the same chemical formula, $[C_6H_5O_7]^{3-}$, but different structures.

REACTIONS

Mechanisms: The Basics

Recall from organic chemistry that an ester undergoes **hydrolysis** (cleavage with addition of water) under acid or base catalysis to produce a carboxylic acid and an alcohol, as follows:



Hydrolysis of one mole of the triglyceride produces three moles of carboxylic acids and one mole of glycerol. The product carboxylic acid molecules, $CH_3(CH_2)_{14}COOH$, are hexadecanoic acid, better know as *palmitic acid* (found in palm oil). Long-chain carboxylic acids like this are called *fatty acids*. As mentioned earlier, in the slightly basic medium of the small intestine, carboxylic acids are found in their **ionized** or **conjugate-base** form, in this case, *palmitate ion*, or $CH_3(CH_2)_{14}COO^-$, and usually referred to simply as *palmitate*.

Let's review the hydrolysis of simple esters. Consider the mechanism, shown in Figure 3, of hydrolysis of ethyl acetate under basic conditions, promoted by hydroxide ion.

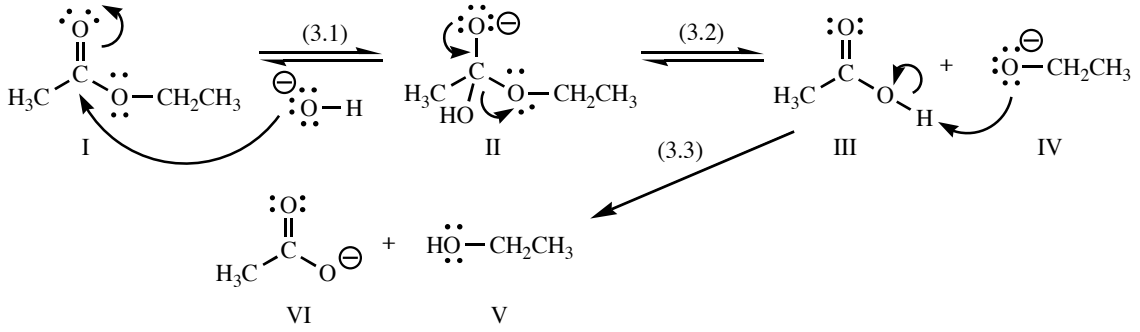


Figure 3. Base-catalyzed hydrolysis of ethyl acetate

The first step (3.1) is nucleophilic addition of hydroxide ion to the electrophilic carbonyl carbon of the ester, giving a tetrahedral intermediate II. This intermediate decomposes (3.2) by re-establishment of the carbonyl π-bond and loss of a leaving group. Two potential leaving groups are present, hydroxyl and ethoxyl ($-OCH_2CH_3$). Loss of hydroxyl as hydroxide ion is the reverse of the first step. Lost of ethoxyl leads to formation of the weak acid CH_3COOH and ethoxide ion, a strong base. Ethoxide is then shown taking a proton from acetic acid. In fact, ethoxide will probably first hydrolyze water to form hydroxide ion, and acetic acid will lose a proton to the first hydroxide ion it encounters, giving the same net result as shown in step (3.3). So the reaction consumes hydroxide ion, which is why I referred to the reaction as "promoted", rather than "catalyzed", by hydroxide ion. Notice that the first two steps are reversible equilibria, but the last step, reaction between a strong base and a weak acid, goes to completion. The resulting acetate ion is unreactive toward hydroxide, ethoxide, or any

other nucleophile, so this reaction will go to completion without need for such measures as removal of products as they form.

Question: What principle am I applying when I say that removal of III reaction (3.3) will drive steps (3.1) and 3.2) to the right?

———

Answer: **Le Chatlier's principle** states that when an equilibrium system is disturbed (by addition or removal of reactants or products), the system will shift to a new equilibrium position that reduces the effect of the disturbance. In Figure 3, removal of III lowers the concentration of the product of (3.2), causing the reaction to shift toward the right to a new equilibrium position. This in turn lowers the concentration of II, causing (3.1) to shift to the right. If molecules III are always removed as soon as they are formed, both reactions (3.1) and (3.2) will be driven to completion.

The mechanism of triglyceride hydrolysis in the small intestine is similar to the mechanism in Figure 3, but a digestive enzyme called a *lipase* catalyzes the reaction.

Question: What do the curved arrows on the structures mean?

———

Answer: Curved arrows show **electron movement**. They do not show the movement of atoms, which you must infer from the electron movement. Each curved arrow starts at a pair of electrons, either a bond or lone pair (unshared electron pair), and ends in the new location of that electron pair. If the arrow points from a lone pair to an atom (example: the large curved arrow in structure I), the lone pair is forming a new bond between that atom and its current atom, in this case, between the oxygen atom of hydroxide ion and the carbonyl carbon atoms of ethyl acetate. If the arrow points from a bond to an atom (example, the small curved arrow on structure I), the bond is breaking and the electrons are becoming a lone pair on the atom pointed to, in this case, the carbonyl oxygen. If an arrow points from a single bond to another single bond, a sigma bond is becoming a π bond, producing a double bond (no examples of this in Figure 3). Notice in Figure 3 that in each step, the curved arrows on the reactant show bonds that break and form to produce the product of that step. In a sense, the curved arrows are instructions for drawing the structure of the product from that of the reactant.

Question: Write a mechanism for the hydrolysis of the ester ethyl acetate ($CH_3COOCH_2CH_3$) with specific-acid catalysis (meaning catalysis by hydrogen ion). Note that **catalysis** means that the reaction does not change the net number of hydrogen ions in the solution—any hydrogen ions consumed in your mechanism are later released.

———

Answer: Figure 4 shows the mechanism requested.

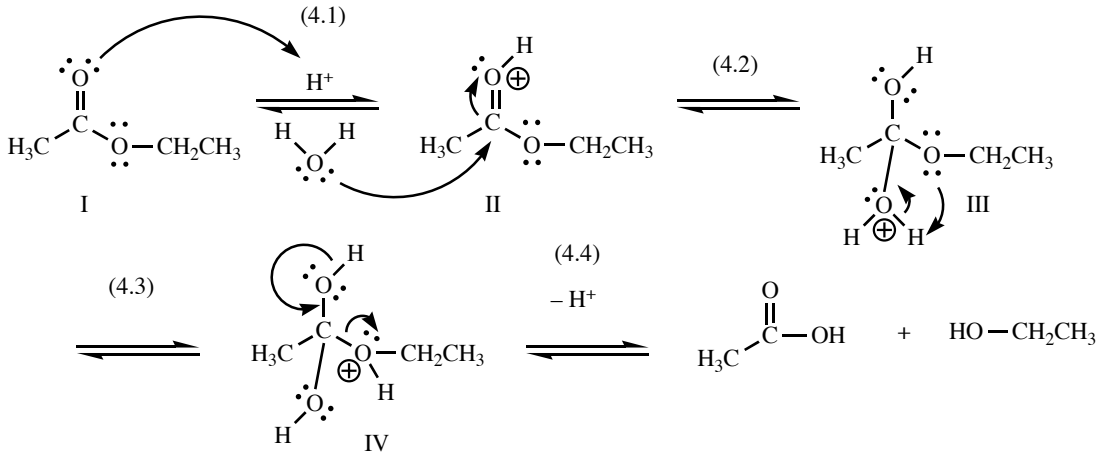


Figure 4. Acid-catalyzed hydrolysis of ethyl acetate

Step (4.1) is protonation of the carbonyl oxygen. This process initiates the reaction by polarizing the carbonyl group, making its carbon a stronger **electrophile**. Step (4.2) is nucleophilic addition of water, a weak **nucleophile**, to the strongly electrophilic carbon atom of the protonated carbonyl group, producing the tetrahedral intermediate III. Step (4.3) is a proton transfer, simply an equilibrium between two different protonated forms of the tetrahedral intermediate. In Step (4.4), a proton is lost as the carbonyl bond π reforms, and ethanol is displaced. Notice that one proton is consumed in step (4.1) and one is produced in step (4.4). So no protons are consumed, and protons are acting as catalysts in this reaction. Also note that all steps are equilibria. To drive this reaction to completion requires removal of one or both products.

If the curved-arrow formalism and reaction mechanisms like those in Figures 3 and 4 are not familiar to you, you may need to review them in your organic chemistry textbook.

Notice that I refer to the nucleophile water as <u>adding</u> to the carbonyl, not <u>attacking</u> it. The “attack” terminology so common in texts is quite misleading. Nucleophiles do not seek out and attack electrophiles or *vice versa*, like robins pouncing on beetles. Molecules simply collide with each other, purely at random. Nucleophiles and electrophiles contain centers of opposite charge, which increase the probability of their collisions, and thus increase the probability of exchanges of electrons (bond formation) when such collisions occur. You will have a clearer picture of how molecules react if you picture vast numbers of them dizzily dashing around at random, colliding at random, with the occasional productive collision in which nucleophile and electrophile, or anion and cation, combine to produce something new. The rate of movement and collision (at room temperature, about 10^8 or 10^9 collisions per second between molecules at a concentration of 1.0 mole/L) is so high, that in an unimaginably short time, molecules “try everything”, or collide in every possible way, and are thus transformed into the most stable products. This characteristic rate of collision, 10^8 or 10^9 collisions/M•s, is called the **diffusion limit**, and is simple a function of how fast molecules move at a give temperature. Because reactions result from collisions, no **bimolecular** reaction can proceed faster than the diffusion limit.

Meanwhile, back at fatty-acid metabolism, the fatty acids released by hydrolysis of triglycerides in the small intestine are transported to many cells in the body, by a variety of carriers. Triglycerides themselves are also found in the blood stream, usually bound to carrier molecules, and some cells take them up and hydrolyze them to produce fatty acyl carboxylates, which are rich sources of energy. If fatty acyl carboxylates are to be used as energy sources, they are first converted to thioesters of coenzyme A (Figure 1, reaction (1.2), in the cytoplasm of cells.

Reaction (1.2)

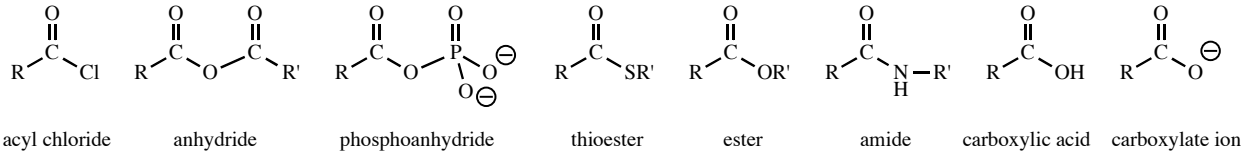
Coenzyme A is a complex biomolecule, whose structure you will learn later. For now, all you need to know is that it is a **thiol** (R-SH), and that its R- group is recognized by enzymes that carry out step (1.2). Recall that the thioester is among the derivatives of carboxyls that were mentioned earlier.

Thermodynamics and Kinetics

Question: Rank carboxyl derivatives in order of decreasing reactivity in hydrolysis reactions, most reactive first.

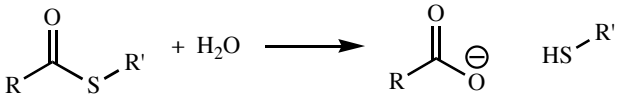
———

Answer:



This is a rough ranking, because the R- groups can have modest effects on reactivity. But in general, conversion of any derivative to one that lies to its right on this chart is a **spontaneous** process under standard conditions ($\Delta G° < 0$, and $K_{eq} > 1.0$). Conversion of any derivative to one that lies on its left is non-spontaneous, and can only occur with an input of energy.

Question: A particular thioester is hydrolyzed to carboxylic acid and thiol.



The equilibrium constant for the process is 120,000. What is the **standard free-energy change**, $\Delta G°$, for this process? Is the reaction spontaneous under standard conditions?

———

Answer: The equilibrium constant is related to the standard free-energy change by this relationship:

$$\Delta G^0 = -RT \ln K_{eq},$$

in which *R* is the gas constant, 8.314 x 10^{-3} kJ/mol-K, and *T* is the kelvin temperature, which at standard conditions is 298 K. So the standard free-energy change for the process is

$$\Delta G^0 = -(8.314 \times 10^{-3} \frac{\text{kJ}}{\text{mol} \bullet \text{K}})(298\text{K})[\ln(120{,}000)] = -29\text{kJ / mol}$$

Notice that the temperature units (K) cancel out in this calculation, leaving only kJ/mol, units that make sense for a free-energy change. The value of ΔG° is negative, implying that the reaction is indeed spontaneous under standard conditions.

Question: If we start this reaction at standard condition, which means all reactants and products at concentrations of 1.0 M, will the reaction go to the left or to the right to reach equilibrium, or is is already at equilibrium under standard conditions?

———

Answer: The reaction will proceed to the right to reach equilibrium. The large value of K_{eq} tells us that, at equilibrium, the molar concentrations of products (carboxylate and thiol) will be much higher than the molar concentration of the reactant thioester. In fact, practically no thioester will remain when the system reached equilibrium.

Question: Because ΔG° is large and negative, meaning that the reaction is strongly spontaneous, will the reaction therefore go very rapidly?

———

Answer: NO. The magnitude of the free-energy change for a process tells us nothing about its rate. A strongly spontaneous process might be fast or slow. The rate is governed not by the energy difference, ΔG, between reactants and products, but by the magnitude of the energy barrier, called the **activation energy** (sometimes called ΔG‡) between them. This diagram allows you to distinguish between the free-energy change, ΔG for a reaction, and its activation energy, ΔG‡.

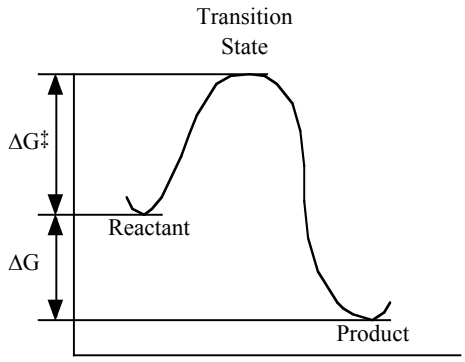
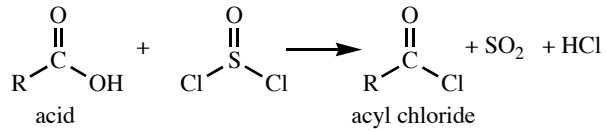


Figure 5. Progress of a one-step chemical reaction. ΔG is the free energy available from the reaction; if ΔG is negative, the reaction goes forward spontaneously. ΔG‡ is inversely related to the rate of the reaction. If ΔG‡ is large, the reaction will be slow, regardless of whether ΔG is small or large, positive or negative.

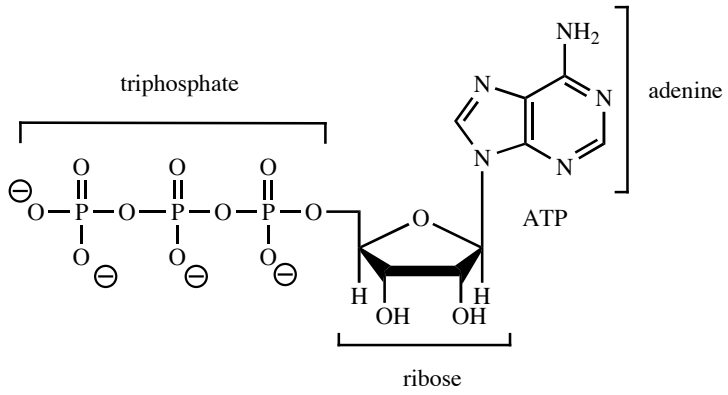
Activating Agents: Pushing Reactions Uphill

Because hydrolysis of a thioester is strongly spontaneous, you can clearly see that the conversion of a fatty acyl carboxylate to its thioester is a non-spontaneous process. How then does the cell do it? When organic chemists want to make a thioester from a carboxylic acid, they cannot do it by reacting a thiol with the acid, a non-spontaneous reaction. They first activate the acid, by converting it to a derivative that is more reactive than a thioester. For example, treatment of an acid with thionyl chloride ($SOCl_2$) produces an acyl chloride, one of the most reactive of the carboxyl derivatives. The acyl chloride will react spontaneously with R-SH to form the thioester (thioesters lie to the right of acyl chlorides in the reactivity series of carboxylate derivatives). In this case, thionyl chloride, the activating agent, is the energy source that allows organic chemists to make more reactive derivatives from less reactive ones.



So as you might guess, the cell uses an activating agent to make fatty acyl thioesters. In this case, the cell uses one of its most common energy sources, ATP. Like all the reactions in Figures 1 and 2, this one is catalyzed by an enzyme, but in this chapter, we will just look at the reactions themselves, and learn about the roles of enzymes later.

First, here is ATP, which is composed of the nitrogen-containing base adenine, the sugar ribose, and a triphosphate group:



The “A” stands for adenosine (adenine plus ribose), and “TP” stands for triphosphate, referring to the three linked phosphoryl groups on the left. The links between them are phosphoanhydride links, so this is a thermodynamically reactive molecule. It can transfer phosphoryl groups to other molecules, such as carboxylates, to activate them toward substitution. That’s precisely the role of ATP in producing fatty acyl CoA derivatives, as shown in Figure 6, which gives an overview of the formation of palmityl CoA.

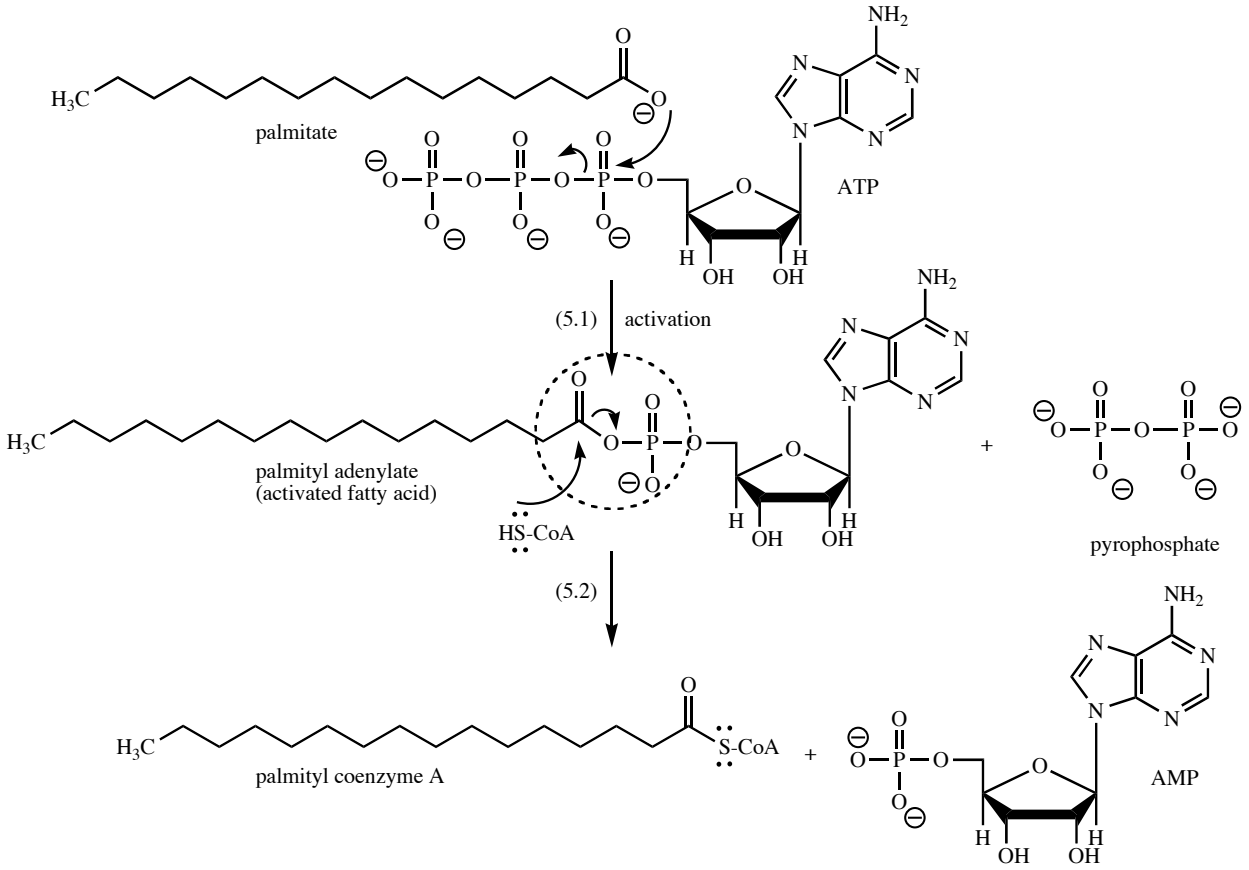


Figure 6. Role of ATP as activating agent in thioesterification of fatty acid.

In step (5.1), the carboxylate oxygen of palmitate displaces pyrophosphate from ATP, producing palmityl adenylate, an activated intermediate. Notice that the carboxyl-phosphoryl link (circled) is an anhydride, which is quite reactive toward displacement of AMP from the carboxyl carbon. This intermediate is far more reactive toward substitution than the starting carboxylate ion, in part because AMP is a much more stable (less basic) leaving group than the negative oxygen of palmitate. In step (5.2), the thiol group of acetyl CoA displaces AMP to produce palmityl CoA. Overall, an unreactive carboxylate has been converted to a much more reactive thioester, but at the expense of a phosphate anhydride linkage. This type of activation/displacement is a common means, in the cell and in the lab, of producing reactive products from unreactive reactants.

Question: Step (5.2) shows displacement of AMP as a single step. Is this correct?

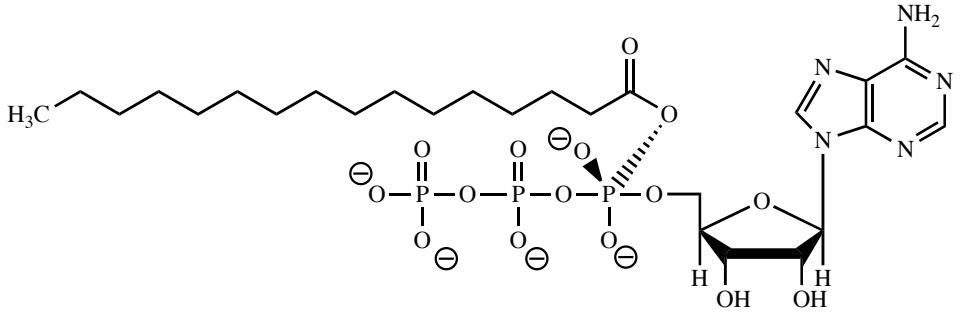
———

Answer: No. The reaction would proceed through a tetrahedral intermediate, like structure III in Figure 4.

Question: Step (5.1) also proceeds through an analogous intermediate, with a pentavalent phosphate atom. Draw this intermediate.

———

Answer:



The intermediate forms by addition of the carboxylate oxygen to the phosphorus nearest the ribose of ATP. In this intermediate, one of the two negative oxygens on the pentavalent phosphorus can re-establish a double bond to phosphorus and displace a leaving group, in this case, AMP. Displacement reactions at phosphoryl groups always proceed through pentavalent intermediates like this one, just as displacement reactions at carboxyl derivatives proceed through tetravalent intermediates like III in Figure 4. Note the regiospecificity of reactions with ATP. A nucleophile could in theory add to any of the three phosphorus atoms, but enzymes direct them to specific ones.

Beta-oxidation of Fatty Acids: Principles of Redox Reactions

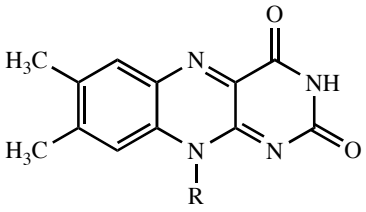
Production of fatty acyl CoA derivatives, called *activation of fatty acids*, occurs in the cytoplasm of cells that use fatty acids as energy sources. The CoA derivatives are transported into mitochondria, where the remainder of the reactions in Figures 1 and 2 occur. The remaining steps in Figure 1, from palmityl CoA to 8 molecules of acetyl CoA, are collectively called β-oxidation of fatty acids.

Reaction (1.3)

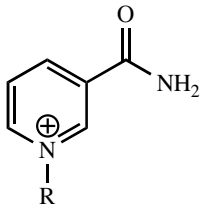
Question: What kind of reaction is step (1.3), and what kinds of reagents do organic chemists use to promote such reactions?

———

Answer: Step (1.3) is **dehydrogenation** of the palmityl chain, the removal of 2 hydrogen atoms and two electrons. The reaction produces a 2,3- or α,β-unsaturated acyl CoA. Organic chemists use such reagents as metal catalysts to cause dehydrogenation, but living organisms frequently use organic oxidizing agents based on flavin or nicotinamide. Flavin is the oxidant in such agents as FMN and FAD, while nicotinamide is found in NAD^+ and $NADP^+$. The flavin and nicotinamide moieties of these agents are shown here:



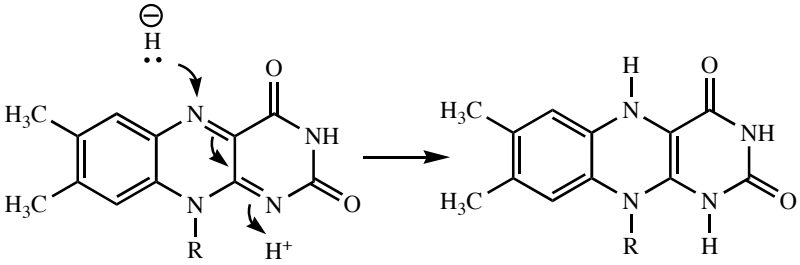
flavin, as in FAD



nicotinamide, as in NAD^+

We will learn more about the derivatives FAD and NAD^+ later. In this chapter, we will examine the chemistry of flavin and nicotinamide.

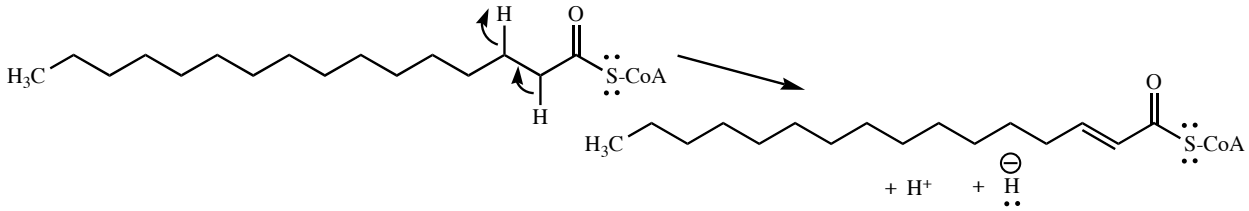
Let's look first at flavin, because its derivative FAD is the oxidizing agent in reaction (1.3). Flavin can accept two hydrogens and two electrons. We can think of this formally as one proton, H^+, and one hydride ion, H^- .



oxidized flavin, as in FAD

reduced flavin, as in $FADH_2$

We can think of the proton and hydride ion coming from the α and β carbons of palmityl CoA:



When we see how an enzyme catalyzes this reaction, we will see that the hydrogen and hydride are transferred directly from palmitate to flavin. Reactive species like hydride ion never exist free in aqueous solution, rapidly reacting with water to form molecular hydrogen, H_2, and hydroxide ions.

Question: Why is it reasonable to imagine the proton coming from C-2 and the hydride from C-3, instead of the other way around?

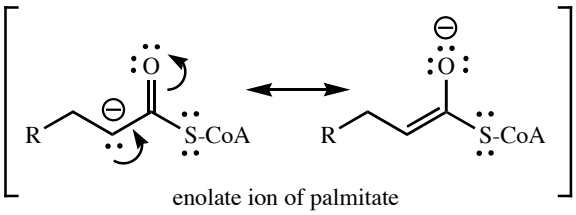
———

Answer: Loss of proton from C-2 develops negative charge that can be stabilized by resonance with the thioester carbonyl. So the carbonyl assists in proton loss, as in formation of enolates in ketone and aldehyde chemistry. Developing negative charge at C-3 is not assisted by resonance.

Question: Draw the resonance contributors for the enolate derived from palmitate after proton loss, and before hydride loss.

———

Answer:



enolate ion of palmitate

Because (1.3) is a redox reaction, we can calculate ΔG° from the **reduction potentials** of the participating redox couples. The appropriate reduction **half-reactions** and potentials are as follows:

a) FAD + 2 H^+ + 2 e^- ---> $FADH_2$ E° = – 0.22 V

b) alkene (RCH=CHR) + 2 H^+ + 2 e^- ---> alkane (RCH_2–CH_2R) E° = + 0.03 V

Question: Use this information to calculate the standard free-energy change ΔG° for a reaction like (1.3).

———

Answer: This problem, a review of basic electrochemistry calculations from general chemistry, requires three steps: 1) Combine the half reactions to derive a balanced equation for the reaction. In doing so, you learn how many electrons are transferred during the reaction. 2) Calculate the standard cell potential (called $E°_{cell}$ or $\Delta E°_{cell}$) from the reduction potentials for each half reaction. 3) Compute the standard free-energy change G° from $\Delta E°_{cell}$.

Step 1) An oxidation like reaction (1.3) is the summation of half reaction a) going forward, as a reduction, and half reaction b) going in reverse, as an oxidation:

reduction: FAD + 2 H^+ + 2 e^- ---> $FADH_2$

oxidation: RCH_2–CH_2R ---> RCH=CHR + 2 H^+ + 2 e^-

net: RCH_2–CH_2R + FAD ---> RCH=CHR + $FADH_2$ (electrons and protons cancel)

The result shows that two moles of electrons are transferred. This number is needed in step 3.

Step 2) $\Delta E°_{cell} = E°_{red} - E°_{ox}$ = (– 0.22 V) – (+ 0.03 V) = – 0.25 V.

The sign conventions used in this calculation are the same as those in most biochemistry text books, and may differ from those you used in general chemistry. Consult your biochemistry text to learn the conventions your authors use.

Step 3) $\Delta G° = - nF\,\Delta E°_{cell}$ = – (2 mol e^-)(96.48 kJ/V-mol)(– 0.25 V) = + 48 kJ/mol.

Question: Is this reaction spontaneous or non-spontaneous under standard conditions?

———

Answer: Non-spontaneous. The value of ΔG° for the process as written (sum in step 1) is positive.

Question: Yet this reaction proceeds in the forward direction in the cell. How might that be possible?

———

Answer: The actual free energy ΔG change for the reaction in the cell depends on the cellular concentrations of reactants and products. In this case,

$$\Delta G = \Delta G^\circ + RT \ln\left(\frac{[\mathrm{FADH_2}]\bullet[\mathrm{RCH=CHR}]}{[\mathrm{FAD}]\bullet[\mathrm{RCH_2-CH_2R}]}\right)$$

Even if ΔG° > 0.0 kJ/mol, the reaction can be spontaneous (ΔG < 0.0 kJ/mol) if the concentrations of products are kept low and the concentrations of reactants are kept high. Indeed, the ratio of reactant to product in all metabolic reactions (like [RCH_2–CH_2R]/[RCH=CHR] here) are kept high by the consumption of the product by the next reaction in the metabolic pathway. In addition, in mitochondria, the ratio [FAD]/[$FADH_2$] is kept very high by the continual re-oxidation of $FADH_2$ to FAD at the expense of oxygen (O_2), which is a strongly **exergonic** process (has a large, negative ΔG). These two aspects of mitochondrial metabolism makes the second term in the ΔG equation large and negative, contributing to a negative ΔG and a spontaneous reaction.

Oxygen is the ultimate acceptor of the electrons transferred from fatty acyl CoA molecules to FAD (or to nicotinamides). In mitochondria, $FADH_2$ is reoxidized to FAD, and the electrons, after passage through a series of carriers, reduce oxygen to water. The half reaction for the oxygen/water redox pair is

$$\tfrac{1}{2}\ O_2 + 2\ H^+ + 2\ e^- \text{ ---> } H_2O \qquad\qquad E^\circ = +\ 0.82\ \mathrm{V}.$$

The reoxidation of flavins and nicotinamides by oxygen is a major source of energy for production of ATP from ADP and phosphate ion, which maintains high ATP levels in support of energy-requiring functions like making thioesters of fatty acids. The standard reduction potential of the oxygen-water redox couple is quite high, so transfer of electrons from $FADH_2$ to O_2 is strongly exergonic: – 201 kJ/mol (can you verify this figure?).

Question: Calculate ΔG° for the transfer of electrons from fatty acid (alkane) to oxygen.

———

Answer: We can combine the alkane oxidation and the $FADH_2$ oxidations as follows:

a) alkane + FAD ---> alkene + $FADH_2$ ΔG° = + 48 kJ/mol (calculated above)

b) $FADH_2$ + ½ O_2 ---> FAD + H_2O ΔG° = – 201 kJ/mol

(net) alkane + ½ O_2 ---> alkene + H_2O $\Delta G° = -153$ kJ/mol (sum of a and b)

This is an application of **Hess's law**, which states that if a chemical equation is a sum (the combined or net reaction) of other chemical equations (the component reactions), then the change in free energy (or any state function) for the net reaction is the sum of the changes for the component reactions.

Question: If the production of ATP from ADP and phosphate ion requires 29 kJ/mol under standard conditions, how many molecules of ATP can be produced per pair of electrons transferred from alkane to oxygen?

———

Answer: Divide the total free-energy change available from oxidation of the alkane by the free-energy change needed to make one mole of ATP:

(153 kJ/mol)/(29 kJ/mol) = 5.28,

or about 5 ATP per pair of electrons. In fact, mitochondria obtain about 2 molecules of ATP per pair of electrons transferred to oxygen as a result of oxidation of $FADH_2$. This amount to *conserving*, in the form of ATP, about 38% of the free energy available from the alkane oxidation. This statement is based on standard free-energy changes, not the actual cellular free-energy changes, which depend on cellular concentrations of reactants and products. The actual efficiency of energy conservation is somewhat higher.

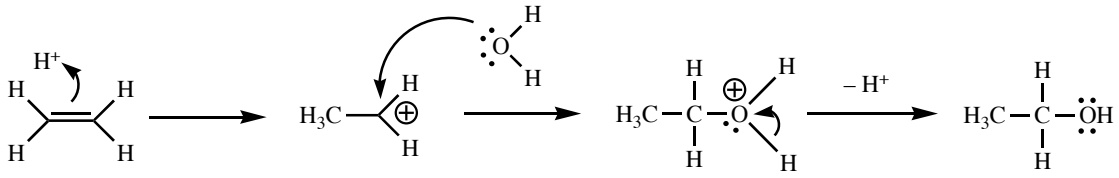
Reaction 1.4

The next step in β-oxidation is hydration of an alkene, a reaction familiar to any student of organic chemistry.

Question: Write a mechanism for hydration of ethylene (CH_2=CH_2) with specific acid catalysis (that is, catalysis by hydrogen ion), to produce ethanol.

———

Answer:



Note the details of electron movement in this reaction. In the first step, protonation of the alkene, the arrow shows movement of the π electrons of the double bond to form a bond to the proton, which becomes one of the hydrogens in the methyl group of the first intermediate, a carbocation. Next, water and the carbocation combine, as a lone pair of the oxygen of water forms a bond between oxygen and the cationic carbon atom, The result is protonated ethanol, a strong acid. Finally, protonated ethanol loses a proton to produce ethanol. Note that the final arrow does not follow the hydrogen away from

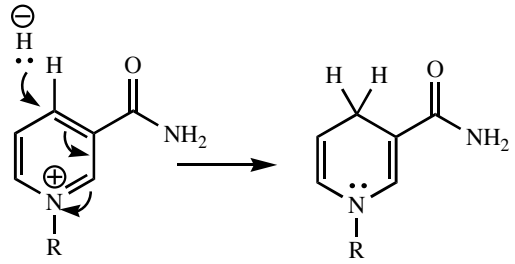
oxygen, but instead follows the H–O bonding pair as it becomes a lone pair on oxygen. Curved arrows always show the movement of electrons, not atoms. Atom movement is inferred from arrows that show electron movement. Understanding these principles is crucial to understanding the details of chemical reactions.

The product of reaction (1.4), a 3-hydroxyacyl CoA, is a chiral compound. Under specific acid catalysis, the reaction would produce an enantiomeric mixture. Like the vast majority of biological reactions, however, this one is stereospecific, yielding only the enantiomer shown in Figure 1.

Reaction (1.5)

The next reaction is oxidation of a secondary alcohol to a ketone. Organic chemists use oxidants like permanganate ion or CrO_3 for such oxidations, but in cells, the usual oxidant for polar bonds like the C-O bond here is nicotinamide. [Flavin is more common in oxidation at nonpolar bonds, like C-C, as in reaction (1.3).]

Nicotinamide oxidizes by accepting a hydride ion, which satisfies the positive charge on nitrogen in the aromatic ring:



Question: Write the mechanism for abstraction of a proton from the hydroxyl group, formation of a C–O double bond, and loss of hydride from the alcohol carbon.

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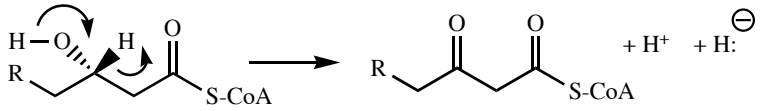
Answer:



As in biological oxidations involving flavin, those involving nicotinamide entail direct transfer of hydride from the substrate to the acceptor.

Now let's review another way of looking at oxidation.

Recognizing Oxidation and Reduction

Question: Prove, using oxidation numbers, that C-3 of the fatty acyl chain is oxidized during this reaction.

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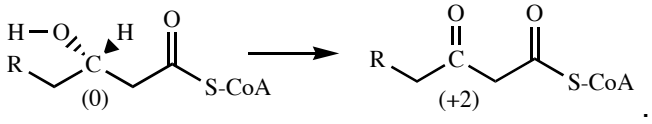
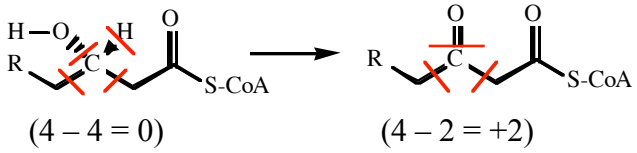
Answer: Oxidation of an atom constitutes an increase in its oxidation number. Oxidation numbers at C-3 are shown here for reactant and product:



To compute the oxidation number for an atom X in a structural formula, assign electrons in each bond around atom X as follows: assign unshared electrons on X to X; assign bonding electrons around X to X if its electronegativity is higher than that of its bonded neighbor, assign them to the neighbor if its electronegativity is higher, or split them if X and the neighbor have the same electronegativity (such as when they are the same element). After assigning electrons, calculate the oxidation number of atom X as follows:

oxidation number = (group number of atom X) – (# electrons assigned to X).

Oxygen is more electronegative than carbon, which in turn is more electronegative than hydrogen. In the structures shown again here, red lines show electrons assigned to C-3 in reactant and product. A red line next to C means that electrons in that bond are assigned to its neighbor. A red line crossing a bond means that one electron is assigned to C-3 and one to its neighbor.



Oxidation numbers provide a quick way to find oxidants and reductants in chemical reactions.

Breaking and Forming Carbon-Carbon Bonds

So far in our examination of fatty-acid metabolism, we have found only relatively simple and familiar organic reactions: hydrolysis of ester, activation and esterification of carboxylic acid, dehydrogenation of alkane to alkene, addition of water to alkene, and oxidation of secondary alcohol to ketone. Reaction (1.6) may look more complex at first, but it is also a reaction discussed in any organic chemistry course. Its nature is concealed because it is the reverse of the reaction as usually presented. The reaction is an acetoacetic ester condensation or, for name-reaction fans, the Claisin condensation, an important for forming new carbon-carbon bonds during chemical synthesis.

Question: Give the product of condensation of two molecules of ethyl acetate, promoted by ethoxide ion.

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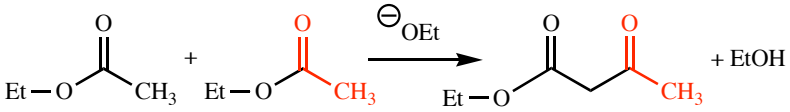
Answer:



I have used color to distinguish carbons from the acetyl groups of the two molecules of ethyl acetate, so that you can see their fate in the product. This should help you with the next question.

Question: Write the mechanism of this reaction.

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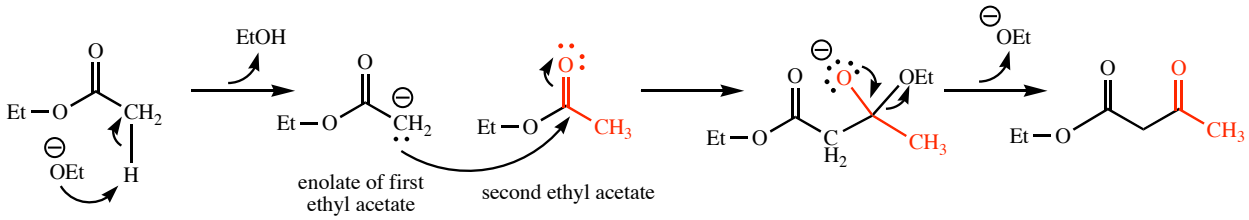
Answer:



Now think about the reverse of this reaction, in which an ethoxide ion adds to the red carbonyl carbon, and so forth, to produce two molecules of ethyl acetate. This is similar to what is happening in reaction (1.6), with the thiol of HS-CoA playing the role of the negative oxygen of ethoxide ion.

Question: Write a plausible mechanism for reaction (1.6).

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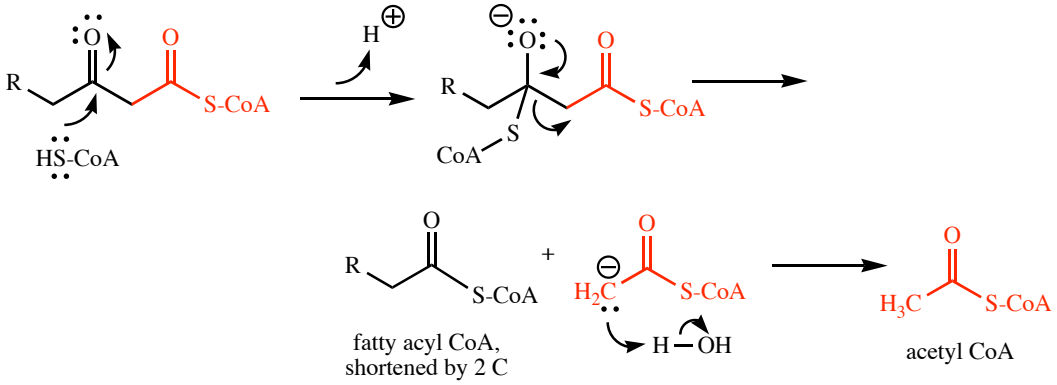
Answer:



The thiol of coenzyme A initiates the reaction by nucleophilic addition to the β-carbonyl carbon atom. The resulting tetrahedral intermediate dissociates, displacing the enolate of acetyl CoA, a strong base that finds the nearest proton, perhaps from a water molecule, to form acetyl CoA.

Reactions (1.3) through (1.6) constitute one round of β-oxidation of palmitate. The reaction sequence removes two carbons, as acetyl CoA, from a fatty acyl CoA, and

leaves a new fatty acyl CoA that is two carbons shorter than the starting material. This new fatty acyl CoA can now go through reactions (1.3) through (1.6) again, again losing two carbons as acetyl CoA.

Question: For palmitate, a sixteen-carbon fatty acid, how many cycles of β-oxidation will turn the whole molecule into acetyl CoA units?

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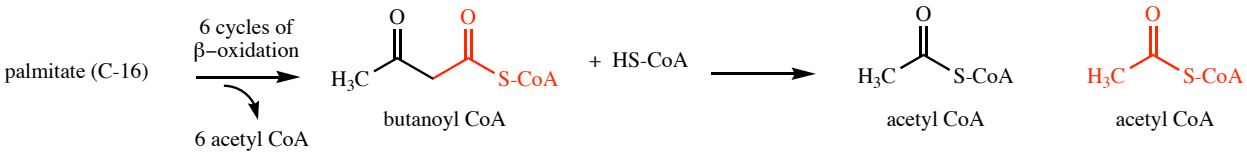
Answer: It takes only seven cycles, not eight, to do the trick. The acyl CoA that enters the seventh cycle, butanoyl CoA, gives acetyl CoA as both products.



As mentioned earlier, all of these reactions are catalyzed by specific enzymes, each of which is a complex protein made by the cell at great energy cost. But there is a great economy in this pathway, which uses the same four enzymes repeatedly to reduce long-chain fatty acids to acetyl CoA. As long as the fatty acid is saturated and contains an even number of carbons, it can be converted completely to acetyl units by the four enzymes of β-oxidation.

Question: If a fatty acid contains an odd number of carbons, what are the products of the final cycle of β-oxidation?

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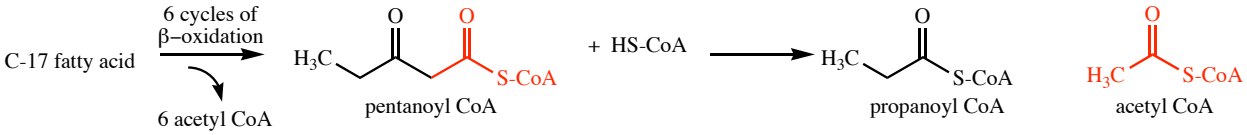
Answer: The last cycle of β-oxidation will begin with pentanoyl CoA and produce propanoyl CoA and acetyl CoA, as follows:



Additional enzymes are necessary for degradation of propanoyl CoA.

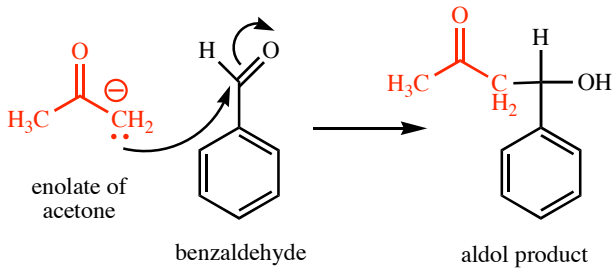
The Fate of Acetyl CoA: Parallels with Beta-Oxidation

Fatty acids are not the only nutrients that are broken down to acetyl CoA. Sugars, from the digestion of carbohydrates, and amino acids, from the digestion of proteins, also contribute at least some of their carbons to energy metabolism in the form of acetyl CoA. This little two-carbon molecule is thus a central actor in the derivation of energy from dietary fats, carbohydrates, and proteins.

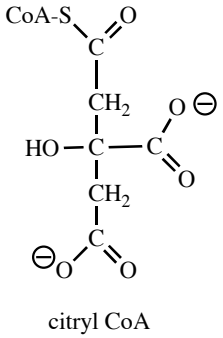
Figure 2 shows how acetyl CoA is oxidized to carbon dioxide, in a cyclic process that goes by several names: citrate cycle, tricarboxylic (TCA) cycle, or Kreb's cycle. Each acetyl CoA molecule reacts with the four-carbon dicarboxylic acid oxaloacetate to form the six-carbon tricarboxylate citrate [reaction (2.1)]. There follows a sequence of chemical reactions whose overall effect is the oxidation of two citrate carbons to carbon

dioxide and the regeneration of oxaloacetate, which can accept another acetyl CoA and repeat the process. The citrate cycle is, in a sense, a catalytic process for oxidizing acetyl units to carbon dioxide. Each cycle requires one oxaloacetate molecule, but regenerates it at the end.

In the citrate cycle are a few more opportunities to review organic reactions. First, examine the citrate-forming reaction, which is listed on Figure 2 as an aldol condensation. Aldol condensations entail addition of an enolate with the carbonyl of a ketone or aldehyde, shown here as a addition of the enolate of acetone to benzaldehyde:



In reaction (2.1), the enolate of acetyl CoA adds to the ketone carbonyl of oxaloacetate to give citryl CoA, which is then hydrolyzed to citrate.



Question: Sketch a mechanism of the reaction of the enolate of acetyl CoA with the ketone carbonyl of oxaloacetate to form citryl CoA.

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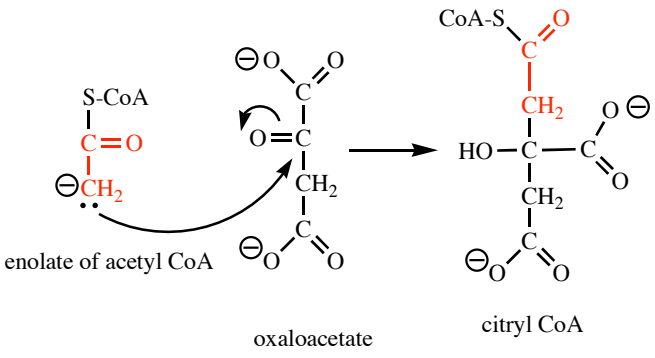
Answer:



Organic chemists form enolates by removing α-hydrogens of carbonyl compounds with strong bases. Enzymes provide these bases in biological systems, as you will see.

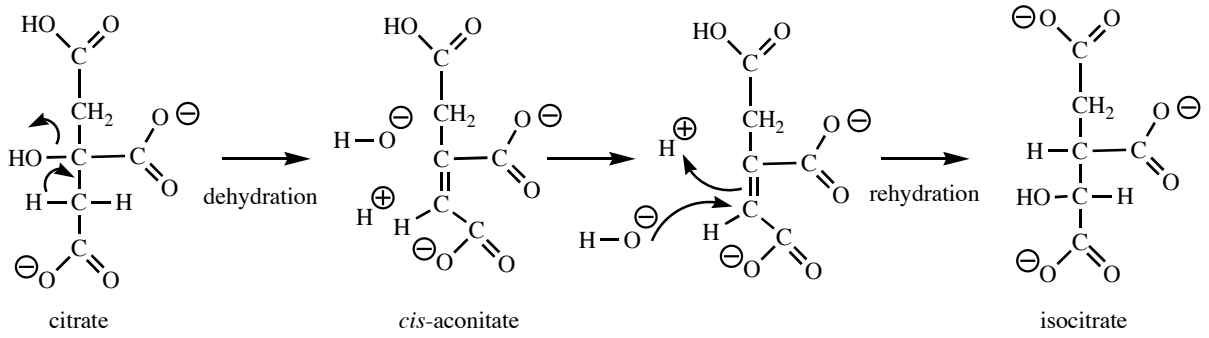
Reaction (2.2)

Earlier, I pointed out that isocitrate and citrate are structural isomers. The conversion of citrate to isocitrate is, in a formal sense, the movement of a hydroxyl group from carbon 3 of citrate to the adjacent carbon, and the movement of a hydrogen atom in the opposite direction. There are no common organic reactions in which atoms on adjacent atoms simply change places, and as you have seen so far, these biological reactions all look a lot like familiar ones from organic chemistry.

Question: Propose two common organic reactions that, in sequence, convert citrate to isocitrate.

———

Answer: One possibility is dehydration of citrate to form a 2,3-alkene (a tricarboxylate called aconitate), and then rehydration of the alkene with opposite orientation of the hydrogen and hydroxide ions of water:

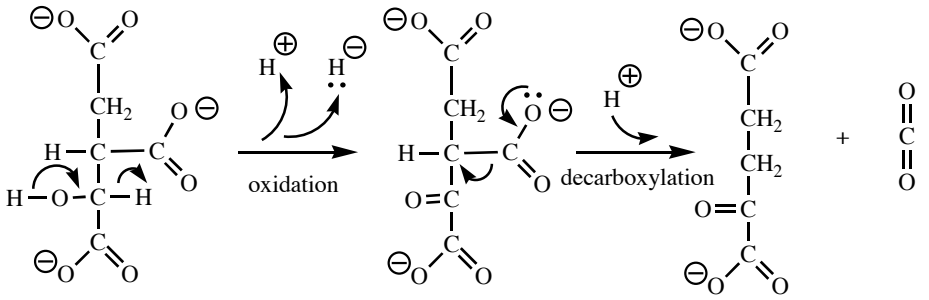


The enzyme that catalyzes this reaction is called *aconitase hydratase*, which implies that its function is to add water to aconitate, and there is evidence for the existence of aconitate as an intermediate in the reaction.

Notice the stereospecificity of this reaction. The first step could produce either a *cis*- or *trans*-alkene, but it produces only the *cis*. The second step produces two new chiral centers, and so could result in as many as four different products. As mentioned earlier, only one, 2(*R*),3(*S*)-isocitrate, is produced. And once again we see that these at-first-glance complex biochemical reactions have relatively simple analogies in common organic reactions.

Reaction (2.3)

Isocitrate is next converted to α-ketoglutarate, which entails oxidation of a secondary alcohol to a ketone, followed by decarboxylation:



Question:

Biological oxidants are usually flavins or nicotinamides. Which do you think is the hydride acceptor in the first step of this process?

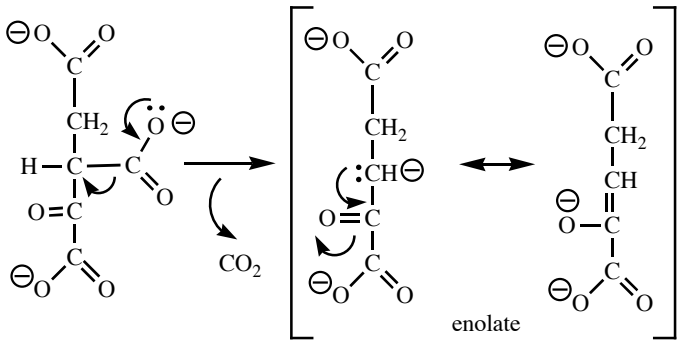
———

Answer: A nicotinamide. As stated earlier, oxidations at polar bonds, like the C–O bond of isocitrate, are usually promoted by nicotinamides. In this case, the oxidant is NAD^+.

Question: Why is it much more likely that oxidation is <u>followed</u> by decarboxylation, rather than preceded by it?

———

Answer: After oxidation of the secondary alcohol to a ketone, the carboxylate on C-3 becomes a β-keto carboxylate. In the lab, β-keto carboxylic acids, or β-carboxy ketones, decarboxylate spontaneously or with gentle heating. Decarboxylation, or loss of the C-3 carboxylate as CO_2, is assisted by the β-keto group, because the immediate product of the decarboxylation is an enolate ion. The β-hydroxy group of isocitrate offers no such assistance. The initial oxidation of alcohol to ketone thus prepares the molecule for easy decarboxylation.



Reactions (2.4) and (2.5)

If you find that that reaction (2.4) does not resemble any common reaction that you recall from organic chemistry, do not despair. This one is a complex, multistep process that we will tiptoe gingerly around for now. Later you will find that it is quite rich in processes you have seen before. Reaction (2.5) is hydrolysis of the thioester succinyl CoA, a process very similar to reaction (1.1) in Figure 1.

Reactions (2.6) Through (2.8)

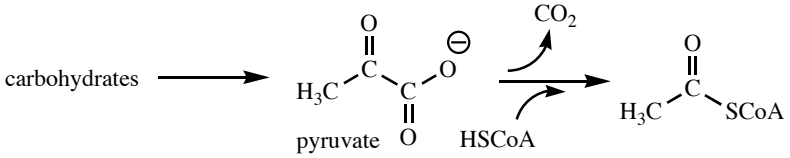
Question: This sequence of three reactions is chemically and mechanistically homologous to a three-reaction sequence in Figure 1. The substrates are different here, but the reactions and their mechanisms are identical. Identify the sequence.

———

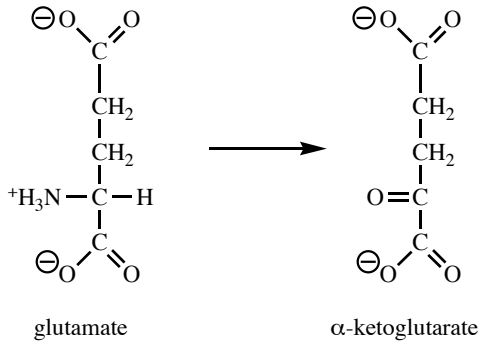
Answer: Reactions (1.3) through (1.5) entail the same reactions, dehydrogenation to convert a C–C single bond to a double bond; stereospecific addition of water across the double bond to produce a secondary alcohol; and oxidation of the alcohol to a ketone. As in the earlier sequence, the oxidizing agent for the initial dehydrogenation is a flavin (FAD), and for the alcohol-to-ketone oxidation is a nicotinamide (NAD^+). This set of reactions is a little theme that recurs in several places in cellular metabolism. Likewise, the complex reaction (2.4) occurs in two different central metabolic pathways. Metabolism looks quite complex at first, but many recurring themes make learning metabolism much easier.

Beyond Fatty Acids

As stated earlier, the carbons of many nutrients arrive at the final stages of oxidation as acetyl groups of acetyl CoA. Glucose and other carbohydrates are degraded to pyruvate, which is oxidatively decarboxylated to acetyl CoA in a process analogous to reaction (2.4).



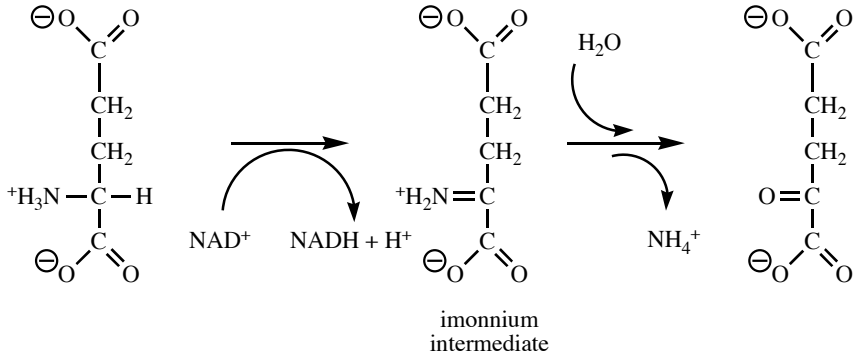
Some of the carbons from catabolism of amino acids also come into the central pathways as acetyl CoA, but some come directly into the TCA cycle as intermediates. For example, the amino acid glutamate loses its amine group to produce α-ketoglutarate, a TCA intermediate.



Glutamate is an α-amino acid (or 2-amino acid) as are all the amino acids that cells use to build proteins. At physiological pH, the amine group, a weak base, exists in its protonated or ammonium form; the carboxyl group, like those of the acids in the TCA cycle, are in carboxylate form. Nevertheless, biochemists invariably refer to them as

amino acids (they don't have an amino group, and they don't have a carboxyl group, but they are called amino acids—go figure).

The presence of nitrogen in amino acids calls for some special catabolic measures not needed for fats and carbohydrates, which are composed of carbon, hydrogen, and oxygen only. For many amino acids, the first step in their catabolism is loss of the amine group. Nitrogen loss occurs either by transfer to another α-keto acid (called transamination), or oxidation to an immonium ion, followed by hydrolysis, as follows:



This sequence, conversion of ammonium to immonium followed by hydrolysis with loss of ammonium, is widespread in the chemistry of biological amines.

Question: Sketch a mechanism for the hydrolysis of the immonium intermediate.

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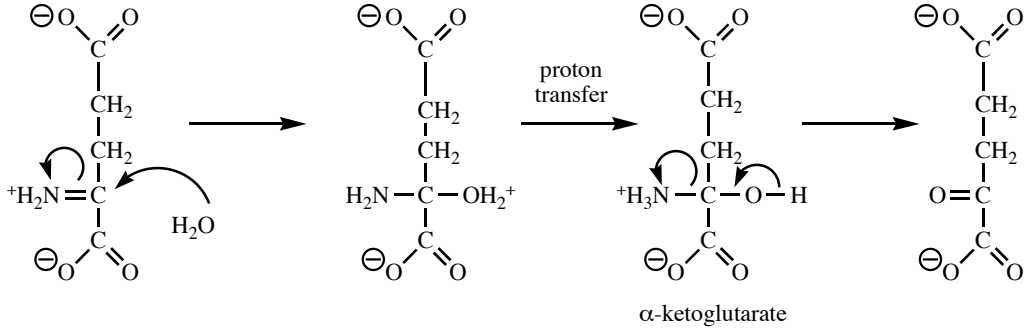
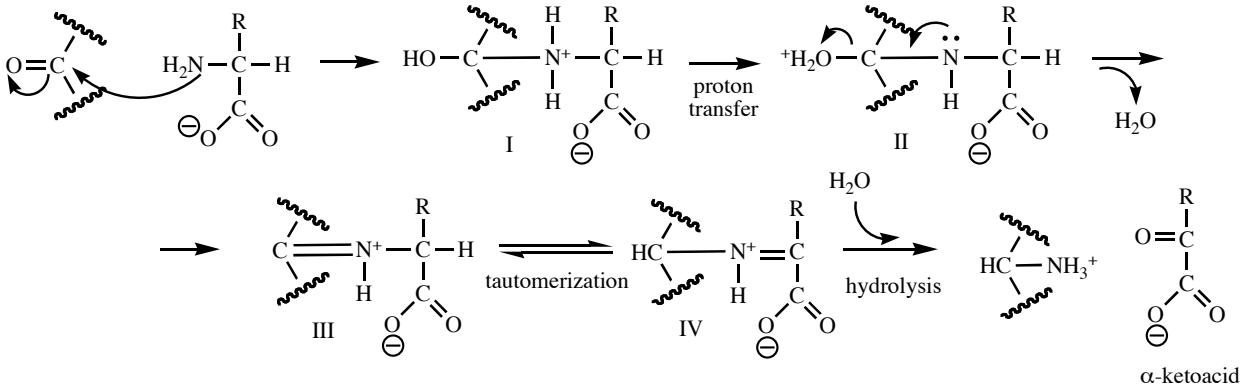
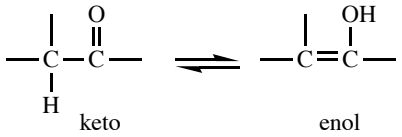
Answer:



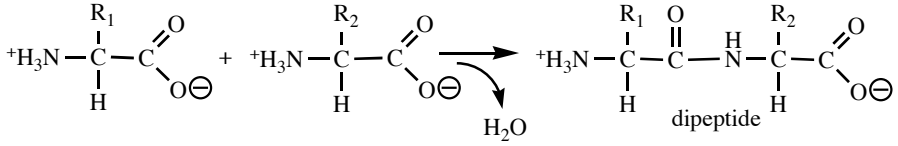
Transfer of an amine group to another carbonyl compound, such as another α-ketoacid, occurs more or less like this:



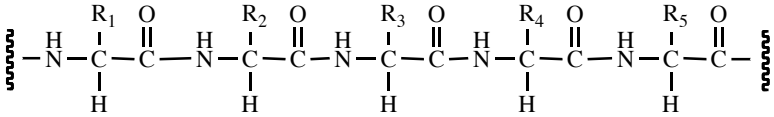
A key step in this transfer is the **tautomeric equilibrium** involving intermediates III and IV. Hydrolysis of III would give back the starting materials, leaving the nitrogen on the amino acid. But hydrolysis of IV results in removal of nitrogen from the amino acid, producing an α-ketoacid and a new amine. Recall that **tautomers**, unlike resonance contributors, are distinct substances (different compounds) in equilibrium with each other, and they differ by the position of a hydrogen and a double bond. You probably first encountered tautomers as the keto and enol forms of ketones or aldehydes having an α-hydrogen:



Another important reaction of amino acids is amide formation.



Biochemists call amide links between amino acids *peptide bonds*, and you can see that the compound made from two amino acids, called a dipeptide, has free amino and carboxyl groups for attachment of additional amino acids, allowing the production of long repeating structures. Proteins are **polymers**, long chains produced by joining amino acids with amide linkages:



From 20 different amino acids (differing in their R groups), nature makes an enormous number of different proteins. After all, with a supply of 20 different amino acids, you could make 20^2, or 400 different dipeptides; 20^3, or 8000 tripeptides; and so forth.

Question: Using 20 different amino acids, and using each one as many times as you like, how many polypeptides of 61 amino acids could you make? Express the number in scientific notation.

———

Answer: $20^{61} = 2.3 \times 10^{79}$. This number is approximately the same as the estimated number of atoms in the universe!

Most proteins are much larger than 61 amino acids. So although nature makes an unimaginably large number of proteins, it only makes a tiny fraction of what is possible. And making polymers from a modest number of building blocks is an efficient (there's that word again) way to produce great variety.

A final word about efficiency: Look back at Figures 1 and 2 and note that any saturated, even-carbon fatty acid can be oxidized completely to CO_2 by 14 enzymatic reactions. In addition, the acetyl CoA produced from degradation of carbohydrates and amino acids is also oxidized by the citric-acid cycle, so relatively few enzymes handle most of the carbon oxidation in metabolism. This is an example of the underlying economy in the evolved design of living organisms. Making an enzyme entails great cost in energy and carbon resources. Efficiency in the use of resources allows an organism to produce more offspring from the same resources than can a less efficient organism. If this efficiency is heritable, then the most efficient organisms become the most common ones. In metabolic pathways, we see the molecular results of evolution by natural selection.

LOOKING BACK

We have now examined the reactions that break down one type of nutrient—the fatty acids of triglycerides—into carbon dioxide and water. We have also gotten a glimpse of how the catabolic pathway for this nutrient intersects pathways for breaking down carbohydrates and proteins. I have presented these processes as pure organic chemistry, which is only one aspect of the story they can tell us about how living organisms work. As we continue, we will use this sequence of processes as a context in which to develop the central themes of biochemistry. As you proceed, try to ask how each new topic is connected to, and gives more insight into, the processes outlined in Figures 1 and 2. The following questions help to make these connections.

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PRELUDE TO BIOCHEMISTRY
25 Questions Leading to Further Study

The following questions lead you from the now-familiar territory of Figures 1 and 2 to the major topics of a typical biochemistry course. Read the first question now, and pursue its answer in the appropriate chapter of your biochemistry text. Each time you complete a chapter in your text, return here for a quick review of Figures 1 and 2, and then tackle the next question.

1. Thioesterification of fatty acids occurs in the cytoplasm of cells, while beta oxidation and the citrate cycle occur in mitochondria.

Question 1: What other metabolic processes occur in cells, and in what compartments do they occur?

The first chapter of most biochemistry texts features an introduction to the major compartments of the cell and the processes that occur in each. Such chapters usually also present the four main types of biological molecules: *lipids* (which include triglycerides and fatty acids), *carbohydrates*, *nucleic acids*, and *proteins*. Study your text's introductory chapter in order to see fatty-acid chemistry in the context of other metabolic processes and other types of biomolecules.

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2. The solvent of life is water. All of the reactions in Figures 1 and 2 occur in the aqueous medium of the cell. Even the ionization states of the intermediates in Figures 1 and 2 depend on the properties of water.

Question 2: What properties of water make it an excellent solvent for the chemical reactions of life? How do its properties affect the nature of biomolecules?

Almost all biochemistry texts have an early chapter entitled "Water". Consult that chapter in your text to review what you learned about water in general chemistry, and to see how water influences the properties and reactions of biomolecules.

_____

3. The fatty acids released by reaction (1.1) in Figure 1 are transported from the small intestine to cells of body tissues aboard a variety of proteins. Proteins are responsible for a fantastic variety of cellular processes in addition to transport. A living cell contains tens of thousands of different proteins. Yet proteins are composed of only 20 simple building blocks: the amino acids.

Question 3: What are proteins, and how can there be such a tremendous variety of proteins and protein functions?

Your biochemistry text contains one or two chapters on amino acids, the building blocks of proteins; the reactions that link amino acids to form proteins; and the evolutionary questions we can answer by comparing amino-acid sequences in proteins. Consult this material to begin learning about the most diverse family of biomolecules.

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4. Proteins are more than primary structure. Each protein chain folds into a highly ordered three-dimensional structure that can carry out specific functions, like transport, recognition of foreign molecules, and catalysis.

Question 4: The final electron acceptor in the oxidation of fats (Figures 1 and 2) is molecular oxygen, O_2. How does oxygen get from the atmosphere into mitochondria, in the hearts of cells?

Part of the answer lies with two oxygen carriers, myoglobin and hemoglobin. These two molecules often serve as first examples of how protein structure produces highly specific function. Your biochemistry text contains one or more chapters on the three-dimensional structure of proteins, helping you to understand how the one-dimensional sequence of proteins gives rise to three-dimensional molecular structures that can carry out highly specific molecular functions such as oxygen transport and distinguishing between native and foreign substances. Consult this material in your text to take your understanding of proteins into three dimensions.

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5. Ligand binding, as in oxygen transport by globins and inactivation of antigens by antibodies, are relatively simple protein functions. Catalysis by protein catalysts—enzymes—is much more complex. Each of the chemical reactions in Figures 1 and 2 is catalyzed by a different enzyme, which acts with great substrate specificity, regiospecificity, and stereospecificity. Chemists must use their most sophisticated tools to figure out how enzymes work. One of the most powerful tools is chemical kinetics.

Question 5. How does measurement of reaction rates help us to formulate mechanisms of enzymatic reactions?

From proposed models of catalysis, we can derive rate laws and compare them with rate laws derived from measuring the effects of concentration, pH, and temperature on reaction rates. Your biochemistry text contains a very important chapter on enzyme kinetics. Consult this chapter to begin to see how kinetics illuminates enzyme action at the molecular level.

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6. As pointed out earlier, the reactions in Figures 1 and 2 are rigidly regiospecific and stereospecific. In addition, these reactions occur much faster in the cell than they would occur in the organic chemist's reaction flask.

Question 6. What molecular tricks are up the sleeves of enzymes, allowing them such astonishing acceleration and exquisite control of complex reactions?

Consult the chapter on enzyme mechanisms in your text, and examine proposed mechanisms of catalysis of a few thoroughly studied enzymes. These examples serve as archetypes to help you understand other enzymes as you encounter them in your studies. From these examples, your text formulates a small number of central principles of enzyme action.

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7. Flavin and nicotinamide derivatives act as oxidants in several reactions of Figures 1 and 2. Such compounds bind to enzymes and act as chemical adjuncts, providing functional-group chemistry not available from the side chains of the 20 common amino acids. Depending on their roles and modes of association with enzymes, such compounds are called coenzymes, cofactors, cosubstrates, or prosthetic groups.

Question 7. What other adjunct molecules are involved in the reactions of Figures 1 and 2? Other than oxidation and reduction, what chemical functions do they provide?

Some texts have a separate chapter that introduces most of the adjunct molecules that broaden the range of protein functions. Such a chapter provides broad insight into the chemistry at many enzyme active sites. Many of these substances are not synthesized in human cells, but instead are derived from vitamins—essential trace nutrients in your diet. If your text contains a separate chapter on coenzymes and vitamins, study it to learn the mechanisms associated with these molecular accessory tools. Your study will be rewarded with greater chemical understanding when you encounter these molecular tools in their many roles in cell function.

Note: Some biochemistry texts introduce coenzymes and vitamins on the fly, as they are encountered in the study of cellular process, instead of in a separate chapter. Either way, pay special attention to each mechanism of coenzyme action as you encounter it. Each mechanism provides insight into many enzymatic processes.

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8. Fatty acids are only one of several nutrients from which the cell derives energy. Carbohydrates are another major energy source. Carbohydrates and their building blocks—monosaccharides—are more complex and varied than lipids.

Question 8: How can we find order in the wild variety of carbohydrates? What patterns of structure and chemistry can we discern? In addition to providing energy, which roles do carbohydrates play in cells.

The chapter on carbohydrate is more than a catalog of structures and functions. It introduces a relatively small number of structural themes and reactions that recur throughout carbohydrate chemistry. Study this chapter to become a discerning carbohydrate-watcher.

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9. Recall that thioesterification of fatty acids occurs in the cytoplasm, while β-oxidation and the TCA cycle occur in a distinct cellular compartment, the mitochondrion.

Question 9. How is the integrity of cellular compartments maintained? Specifically, what role do water-insoluble components, such as the long hydrocarbon chains of fatty acids, play in producing boundaries around the outside of cells and around cellular compartments like mitochondria, golgi apparatus, and lysosomes?

Fatty acids are important components of complex *lipids*, the only class of biomolecules that feature water-insoluble components. Among the many roles that lipids play, some are storage forms of energy, while others are the foundation of membranes, which form the cell's boundaries. Your text contains one or two chapters on lipids and membranes. Study these chapters to the cell confines molecular processes to particular locations, and how specific nutrients and signals cross the boundaries.

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10. We noted some recurring themes in Chapter Zero: the use of flavins as oxidizers of nonpolar substrates (C–C bonds to C=C), and of nicotinamides as oxidizers of polar substrates (C–O to C=O); the use of ATP as an activating agent for carboxylate condensations; and the cyclic use of a small number of enzymes to break down complex substances by repetition of simple steps.

Question 10. What aspects of fatty-acid oxidation point to principles that apply broadly across all of metabolism? What other principles bring order to the tangle of metabolic pathways?

A crucial chapter in a biochemistry text is its introduction to metabolism. Here you will find general principles of the cell's reaction sequences, deeper understanding of energy transfer in metabolism, and insight into metabolic regulation, with abundant examples. Study this chapter to develop the skills needed to see metabolism whole.

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11. Catabolism in convergent, with diverse nutrients producing only a small number of products from which energy is derived. We saw in Chapter Zero that fatty acids provide acetyl CoA for oxidation in the TCA cycle. Acetyl Co A is also an intermediate in the oxidation of other nutrients.

Question 11. How are carbohydrates broken down, and how does the cell conserve the energy released by their degradation? How does carbohydrate catabolism converge with fatty-acid catabolism?

In most biochemistry texts, the first metabolic pathway examined in detail is glycolysis—the conversion of glucose to pyruvate, a direct precursor of acetyl CoA. Study this chapter to get your first look at how nutrient degradation gives rise to ATP, as well as your first specific examples of the five notable aspects that make all pathways cohere: flow of carbon, flow of phosphoryl groups, flow of reducing power, regulation, and connection to other pathways.

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12. Fatty-acid breakdown, by way of β-oxidation and the TCA cycle, produces reduced cofactors such as $FADH_2$ and NADH. On the other hand, the synthesis of fatty acids, and most biosynthesis, consumes reduced cofactors, especially NADPH. Reduced cofactors are an energy source, in the same same sense as is ATP.

Question 12. How does the cell produce NADPH for biosynthesis of fatty acids and other compounds? How is NADPH production regulated to balance its production with that of ATP, the other major energy source for biosynthesis?

Most biochemistry texts contain a second chapter on carbohydrate metabolism, with three main topics: 1) the pentose pathway, an alternative catabolic pathway for glucose in which energy is conserved as reducing power of NADPH; 2) biosynthesis of glucose; and 3) the synthesis and breakdown of starches. Study this chapter to begin putting the catabolism of glucose and fatty acids into a broader cellular context.

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13. In oxidative catabolism, almost all the carbons end up as acetyl CoA, which is oxidized to CO_2 and H_2O by the TCA cycle. Chapter Zero introduced you to the organic chemistry of the cycle, but there is much more to it.

Question 13. How do the final oxidations of catabolism take place? How does the cell "choose" which of the many sources of acetyl CoA to consume?

Your text devotes a chapter to the TCA cycle, which is in many respects the heart of metabolism. Every organism that uses oxygen derives most of its energy from nutrients by way of the TCA cycle and the reoxidation of its coenzymes by oxygen (in mitochondrial electron transport and oxidative phosphorylation). Study this chapter to get to know the hub of energy production and the center of regulation in all aerobic cells.

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14. Catabolic processes produce $FADH_2$ and NADH. Most of the cell's production of ATP comes from reoxidizing these reduced coenzymes by oxygen.

Question 14. How do mitochondria transfer electrons from reduced cofactors to molecular oxygen and simultaneously conserve the abundant free energy available?

The next logical step to take in understanding energy metabolism is to look at electron transport, by which electrons are transferred from reduced coenzymes to oxygen, and oxidative phosphorylation, by which the energy of electron transport drives the synthesis of ATP from ADP and phosphate ion. As you study this chapter, you will get to know nature's fundamental energy source and the most important form of conserved metabolic energy. You thought it was ATP, but no: it is the transmembrane proton gradient.

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15. Nature's fundamental fundamental form of conserved energy, the transmembrane proton gradient, is just as important in the plant kingdom, where the sun's radiant energy is conserved first as a proton gradient. Some simple photosynthetic organisms run their whole metabolic business on a proton gradient produced directly from light by a single membrane protein. In plants, the machinery for harvesting light energy is far more complex.

Question 15. How does light produce ATP and reducing power in plants?

The sun takes center stage in the chapter on photosynthesis, which surveys the capture of light energy and its use to produce reducing power and ATP, both of which are then consumed in carbohydrate synthesis. Be prepared to invoke many some strange and esoteric ideas of chemistry and physics in order to understand these processes, the producers of energy on which all earthly life relies.

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16. The catabolism and the synthesis of glucose both occur in the cytoplasm and share many enzymes. Not so for fatty-acid catabolism and synthesis, which have some chemistry in common, but not a single shared enzyme. If fact, the two processes occur in separate cellular compartments.

Question 16. How are fatty acids broken down and synthesized? How are these two processes, which would be wasteful if they occurred simultaneously, coordinated despite occurring in distinct locations within the cell?

At last, we return to the central subject of Chapter Zero, fatty acids themselves, to learn how their consumption and production fit into the larger picture of the cell. Your text's chapter on this subject also includes pathways by which many other lipids, including membrane lipids, and steroids like cholesterol, or constructed.

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17. All cellular processes depend heavily on proteins, which are built from amino acids. When amino acids are plentiful, they also serve as energy-rich nutrients.

Question 17. How do the carbons of amino acids enter into the central, energy-conserving, metabolic pathways? How do catabolic processes for 20 or more different amino acids converge to a relatively small number of intermediates? How does the presence of nitrogen in amino acids pose special metabolic problems—and opportunities—for cells?

The chapter on amino acid metabolism introduces you to the biological chemistry of nitrogen, as well as to many roles that amino acids play in addition to serving as building blocks of proteins.

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18. Nucleotides are more important as coenzymes and as building blocks (of nucleic acids) than as sources of energy.

Question 18. How are nucleotides like ATP, GTP, NAD^+, FAD, and coenzyme A synthesized?

Many texts conclude the coverage of metabolism with an examination of pathways leading to nucleotides. Such chapters are usually sketchy because of the complexity of these pathways and the large number of specialized reactions in them. Study this chapter to complete your picture of metabolism of all classes of biomolecules.

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NOTE: Some recent texts contain a separate chapter on the integration of metabolism in humans, including discussions of the specialized roles of each major organ in the body. In other texts, this information is integrated into the chapters on carbohydrates, lipids, and amino acids. If your text contains such a chapter, it serves as an excellent review and overview of metabolism, especially its regulation and inter-organ coordination in mammals.

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19. Each cell in your body contains all the information required to make any type of human cell, including all the proteins and nucleotides of metabolism, regulation, transport, and other functions. All of these recipes are stored as a relatively simple form: sequences of nucleotides in DNA.

Question 19. What properties of nucleotides make them suited for information storage?

The typical chapter on nucleic acids begins a major section on the metabolism of information. It lays the groundwork for understanding how DNA and RNA are synthesized, and how information stored therein is expressed in the form of proteins and functional RNAs.

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20. As cell divide, both daughter cells receive a full complement of the information of the genome.

Question 20. What is the mechanism by which cells copy the DNA of entire genomes with amazing accuracy, and maintain the integrity of this information despite the fragility of polymeric nucleic acids?

All molecular processes are error-prone. The energy differences among pathways to possible products determine the proportions of each product produced. In replication, there is only a small difference in energy between putting the correct and an incorrect nucleotide into a growing chain; therefore, chemical principles predict a high error rate. But nature finds a way. Your chapter on replication and repair help you to understand how nature meets the challenge of using fragile molecules as stable repositories of information.

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21. The classic role of RNA is that of a messenger—transferring copies of DNA sequence information from nucleus to cytoplasm. Scientists are now learning that RNA is more versatile than previously thought.

Question 21. How are the protein-sequence instructions of nuclear DNA transcribed into RNA copies? How is this information selected and modified to fit the needs of a particular cell?

Recent chapters on transcription of RNA have expanded to include post-transcriptional processing of sequence, and more recently, the roles of small RNAs as regulatory agents. Study your text's chapter on translation lay the groundwork for following fast-moving research into the surprisingly varied life of RNA.

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22. The information of messenger RNA is translated into protein sequence with the assistance of many other forms of RNA, including transfer RNAs, translation factors, and the ribosome, which is an RNA enzyme containing some accessory proteins.

Question 22. How does the information of messenger RNA guide the high-fidelity synthesis of specific protein sequences?

Your text's chapter on protein synthesis completes the story of information flow from the linear information in nuclear DNA to the three-dimensional structure of proteins.

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23. Our knowledge of the molecular details of replication, transcription, and translation, along with our understanding of how these processes are regulated, has resulted in development of many tools for manipulating genetic information, and for altering the genes of organisms.

Question 23. How do biological scientists read, compare, and alter the genetic information in cells? How do they produce organisms with new properties?

We are in the midst of an information revolution. Our capacity to learn and compare genetic information, and to alter genomes in order to see how they work, continues to grow rapidly. Your text's chapter on recombinant DNA technology lays groundwork—usually already dated by the time your text comes off the presses—for you to catch up and stay abreast of this exciting and powerful technology.

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24. By this time, you probably take for granted the correctness of the many protein and nucleic-acid structures shown in your text, as if someone is reporting to you from actual observation of these molecules. In a molecular model, there is sometime more, and sometimes less, than meets the eye!

Question 24. How do scientists determine the structures of complex molecules? How reliable are the structural models that are available through the Protein Data Bank and other structural databases?

Some biochemistry courses include macromolecular structure determination as a special topic. Scientists derive models of protein structure by three methods: x-ray crystallography, NMR spectroscopy, and homology modeling. If your course includes a unit on this subject, study the readings provided to become a wise user of macromolecular models, and to avoid drawing unsupported or inappropriate conclusions from faulty models.

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25. Your text's chapters on metabolism built for you a general understanding of cell signaling. Exciting applications of many types of signaling are found in the five senses.

Question 25. What is a nerve impulse? How does sensory information, like like and sound, produce nerve impulse from sensory organs?

Some biochemistry courses include the five senses as a special topic. If yours does, study the readings provided to see how the same signaling themes you saw in metabolism help to you to understand the molecular basis of perception.