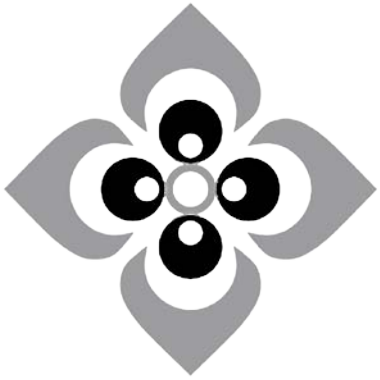

Consortium for Educational Communication

Module
on
Biosynthesis Of Fatty Acids

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TEXT

Introduction

Fatty acids are a class of compounds containing a long hydrophobic hydrocarbon chain and a terminal carboxylic group. Fatty acid synthesis in animals occurs in cytosol and in plants cells in chloroplast stroma. Fatty acid synthesis is the creation of fatty acids from acetyl CoA and NADPH through the action of enzymes called fatty acid synthases. Most of the acetyl CoA which is converted into fatty acids is derived from carbohydrates via gycolytic pathway. This pathway also provides the glycerol with which free fatty acids can combine (by ester bond) to form triglycerides. The NADPH is generated in the cytosol by pentose phosphate pathway and by the malic enzyme which oxidises malate into pyruvate and carbon dioxide generating NADPH.

Biosynthesis of fatty acids involves stepwise addition of two carbon units and these carbon atoms are supplied by acetyl CoA. This acetyl CoA is formed in mitochondrial matrix and cannot cross inner mitochondrial membrane. An acetyl CoA shuttle system is required to transport it into the cytosol. Within mitochondria acetyl CoA reacts with oxoloacetic acid to form citrate catalysed by citrate synthetase. The citrate leaves the mitocondiria and in cytosol reacts with CoA and ATP to form acetyl CoA and Oxoloacetic acid catalysed by enzyme citrate lyase.

Fatty acid

Fatty acids are the simplest form of lipids and serve as constituents in a large number complex form of lipids fatty acids are long chain hydrocarbons (4 to 36 carbons long) with one carboxylic group. Fatty acids in biological systems usually contain an even number of carbon atoms. The 16- and 18- carbon fatty acids are most common the alkyl chain may be saturated and unsaturated. Unsaturated fatty acids may contain one or more double bonds. Fatty acids are amphipathic by nature; that is, they have both non-polar and polar ends.

A fatty acid contains a long hydrocarbon chain and a terminal carboxylate group. Fatty acids have four major physiological roles.

i. Fatty acids are building blocks of phospholipids and glycolipids. These amphipa-

thic molecules are important components of biological membranes

ii. Many proteins are modified by the covalent attachment of fatty acids, which targets them to membrane locations.

iii. Fatty acids are fuel molecules. They are stored as triacylglycerols (also called neutral fats or triglycerides), which are uncharged esters of fatty acids with glycerol. Fatty acids mobilized from triacylglycerols are oxidized to meet the energy needs of a cell or organism.

iv. Fatty acid derivatives serve as hormones and intracellular messengers.

Predominant fatty acids found in mammals

Common name	Systematic name	No. of C atoms	No. of double bonds
Lauric	Dodecanoic	12	0
Myristic	Tetradecanoic	14	0
Palmitic	Hexadecanoic	16	0
Stearic	Octadecanoic	18	0
Palmitoleic	Cis-Δ^9-hexadecenoic	16	1
Oleic	Cis-Δ^9-Octadecenoic	18	1
Linoleic	All Cis-Δ^9,Δ^{12}-Octadecadienoic	18	2
Linonic	All Cis-Δ^9,Δ^{12}, Δ^{15}-Octadecatrienoic	18	3
Arachidonic	All Cis-Δ^5,Δ^8,Δ^{11},Δ^{14}-eisosatetraenoic	20	4

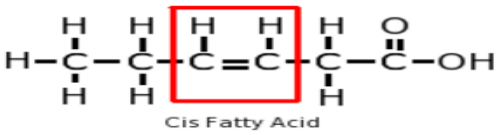
By an older system, in a fatty acid second carbon is referred to as alpha carbon, third carbon as beta carbon and the end methyl carbon as omega carbon.

Saturated and unsaturated fatty acids

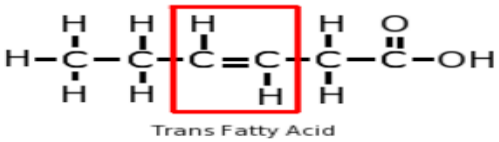
Saturatyed fatty acids have no double bonds in the chain. Their general formula is CH_3-$(CH_2)_n$ – COOH where n specifies the number of methylene groups between the methyl and carboxylic carbons. Examples of predominant saturated fatty acids are lauric, myristic, palmitic and others.

Unsaturated fatty acids have one or more double bonds, and called monounsaturated

or poly unsaturated respectively. The double bonds in naturally occurring fatty acids are generally in a cis as opposed to a trans configuration. The double bonds of polyunsaturated fatty acids are almost never conjugated (alternating single and double bonds)



Cis Fatty Acid



Trans Fatty Acid

Unsaturated fatty acids have lower melting point that the saturated fatty acids. Most plant lipids have unsaturated fatty acids while most animal lipids have saturated fatty acids aquatic animals of cold waters, however, posses unsaturated fatty acids. Carboxylic group of a fatty acid is hydrophilic, polar or water soluble while the rest of the hydrocarbon chain is non-polar, lipophillic, hydrophobic or water insoluble. Polar end sticks to water so that fatty acids form a monomolecular layer over it. The water soluble end of fatty acid also reduces surface tension and increases the wetting or cleaning power of water. Soap action is actually the action of fatty acids contained in it. The long carbon chains of fatty acids are assembled in a repeating four-step sequence. A saturated acyl group produced by this set of reactions becomes the substrate for subsequent condensation with an activated malonyl group. With each passage through the cycle, the fatty acyl chain is extended by two carbons. When the chain length reaches 16 carbons, the product (palmitate, 16:0) leaves the cycle. Carbons C-16 and C-15 of the palmitate are derived from the methyl and carboxyl carbon atoms, respectively, of an acetyl-CoA used directly to prime the system at the outset; the rest of the carbon atoms in the chain are derived from acetyl-CoA via malonyl-CoA. Both the electron-carrying cofactor and the activating groups in the reductive anabolic sequence differ from those in the oxidative catabolic process. Recall that in oxidation, NAD^+ and FAD serve as electron acceptors and the activating group is the thiol (OSH) group of coenzyme A. By contrast, the reducing agent in the synthetic sequence is NADPH and the activating groups are two different enzyme-bound OSH groups, as described below. All the reactions in the synthetic process are catalyzed by a multienzyme complex, fatty acid synthase. Although the details of

enzyme structure differ in prokaryotes such as Escherichia coli and in eukaryotes, the four-step process of fatty acid synthesis is the same in all organisms.

Essential and non-essential fatty acids

Based on whether the body can produce fatty acids, fatty acids can be classified into essential and non-essential fatty acids. The main difference between the two is that essential fatty acids cannot be produced by the body and have to be consumed through food or dietary supplements. Non-essential fatty acids can be produced by the body although they can still be ingested from some of the food that we eat.

- Essential Fatty Acids

Basically, for humans, there are only two kinds of essential fatty acids. These are the alpha-linoleic acid, which is a kind of omega-3 fatty acid, and linoleic acid, which is a type of omega-6 fatty acid. There is a third category of essential fatty acids, which are actually known as conditional essential fatty acids because they become essential only on certain developmental or illness conditions. Examples of conditional essential fatty acids are the gamma-linolenic acid, which a kind of omega-6 fatty acid, and docosahexaenoic acid, which is an omega-3 fatty acid. The omega-9 fatty acids are considered to be non-essential fatty acids because they can be produced using other fatty acids and carbohydrates.

The balance and ratio between the three kinds of omega fatty acids is important. Many factors influence the need for the omega acids and affect the ratio in which they should be consumed. Generally, the amount of omega 3 should be higher than that of omega 6. Age and gender are the most important factors. The main functions of the essential fatty acids are to maintain healthy cell membranes, assist in the development of the brain and nervous system, and help in the production of hormone-like substances. They also play a vital role in the prevention and breakdown of bad cholesterol in arteries.

Omega 6 is found in vegetable oil and it is believed that this particular type of omega fatty acid helps with the regulation of blood flow and levels of blood cholesterol. Omega 3 has an anti-inflammatory effect, especially beneficial to the heart when inflammation of the vessel walls cause atherosclerosis. It is mainly found in fish oils such as tuna and mackerel. Certain nuts contain fatty acids, especially almonds and walnuts, but it

is important to eat them in moderation. Dark, leafy vegetables, like spinach and kale and flaxseed also contain omega 3 fatty acids.

- Non Essential Fatty Acids

Some non-essential fatty acids are actually important for the body. Of course, the saturated fats are considered to be non-essential fatty acids because they are not required by the body and instead may cause harm. Trans fatty acids, although these are a kind of unsaturated fat, are also non-esential fatty acids and should be avoided in the same manner that we should avoid saturated fats because of findings that increased consumption of trans fats is proportional to an increase in the risk of coronary heart disease. It is also used in supplements when used as development enhancements. Meanwhile, there are non-essential fatty acids which are simply classified as such because the human body can synthesize them from other nutrients like carbohydrates and other unsaturated fatty acids. These are the omega-9 fatty acids, which can lower blood cholesterol and control blood sugar. However, since the human body can create omega-9 fatty acids, there is no need to include them in one's diet.

Fatty acid synthesis

The dietary carbohydrates and amino acids, when consumed in excess can be converted to fatty acids and stored as triacylglycerols. De nova synthesis of fatty acids occurs predominantly in liver, kidney, adipose tissue and lactating mammary glands. The enzyme machinery for fatty acid production is located in the cytosomal fraction of the cell. Fatty acid biosynthesis in the cytosol requires a sufficient concentration of NADPH and acetyl-CoA. NADPH is generated in the cytosol by the pentose phosphate pathway, and by the malic enzyme which oxidizes malate into pyruvate and CO_2, generating NADPH. The acetyl-CoA formed by amino acid degradation is insufficient for fatty acid biosynthesis. Acetyl CoA molecules are the building blocks of fatty acid synthesis. There are 3 principle ways of producing acetyl-CoA in the cytosol of the cell:

1. Amino acid degradation produces acetyl-CoA.
2. Fatty acid oxidation in the matrix of the mitochondria produces acetyl-CoA which is converted into citrate which is transported into the cytosol by the tricarboxylate

transporter. ATP-citrate lyase convertes citrate in the cytosol into acetyl-CoA.

3. Glycolysis generates pyruvate which can be carboxylated in the mitochondria into oxaloacetate and then converted into citrate which is transported into the cytosol by the translocase. ATP-citrate lyase converts citrate in the cytosol into acetyl-CoA.

The acetyl CoA molecules need to be activated for fatty acid biosynthesis. The pathway for the biosynthesis of saturated fatty acids is identical in all organisms. A sequence of seven enzymes catalysed reactions converts two carbon units to four carbon units.

Formation of malonyl CoA

Acetyl CoA is carboxylated to form **malonyl CoA** by acetyl CoA carboxylase which is a biotin containing enzyme. The carboxylation reaction is irreversible and is the first committed step of fatty acid biosynthesis. The mechanism of this carboxylase is the same as pyruvate carboxylase and propionyl CoA carboxylase. ATP is used to activate bicarbonate in the form of carboxyphosphate which leads to the carboxylation of biotin. The activated CO_2 group is transferred to acetyl CoA to form malonyl CoA.

$$\mathbf{HCO_3^- + ATP + acetyl\text{-}CoA \rightarrow ADP + P_i + malonyl\text{-}CoA}$$

Acetyl CoA carboxylase has three domains:

a. A biotin carboxyl group carrier protein.

b. Biotin carboxylase which adds CO_2 to biotin.

c. A transcarboxylase which transfers the CO_2 group from biotin to acetyl CoA to form malonyl CoA.

In animals, acetyl CoA carboxylase (ACC) is a filamentous polymer composed of 230 kD protomers. Each protomer contains the biotin carboxyl carrier protein, the carboxylase and the transcarboxylase domains as well as allosteric regulatory sites. The polymeric form of this enzyme is active, the individual protomers are not. The activity of ACC is dependent of the equilibrium between the two forms of this enzyme.

The remaining reactions of fatty acid synthesis are catalyzed by a multifunctional

enzyme complex called **fatty acid synthase complex.** Fatty acid synthase is a polypeptide chain with multiple domains, each with distinct enzyme activities required for fatty acid biosynthesis. it is not a single enzyme but a whole enzymatic system composed of two identical 272 kDa multifunctional polypeptides, in which substrates are handed from one functional domain to next. Its main function is to catalyse the synthesis of palmate from acetyl coA and Malanoyl-coA, in presence of NADPH. There are two types of fatty acid synthases:

Type I systems utilize a single large, multifunctional polypeptide and are common to both mammals and fungi (although structural arrangement of fungal and mammalian synthases differ). Type 1 fatty acid synthase system is also found in corynebacteria, mycobacteria and nocardia). In these bacteria, FSA 1system produces palmititic acid and cooperates with the FAS11 system to produce a greater diversity of lipid products.

Type II is found in bacteria and archaea, and is characterised by the use of discreate, monofunctional enzymes for fatty acid synthesis. Inhibitors of this pathway (FAS11) are being investigated as possible antibiotics.

In eukaryotic cells, including humans, the fatty acid synthase exists as a dimer with two identical units. Each monomer possesses the activities of seven different enzymes and an acyl carrier protein (ACP) bound to 4′- phosphopantethine. Fatty acid synthase functions as a single unit catalysing all the seven reactions. Dissociation of the synthase complex results in loss of the enzyme activities. In prokaryotes, the fatty acid syntheses is carried is carried out by a multi enzyme complex in association with a separate acyl carrier protein. This is in contrast to eukaryotes where ACP is a part of fatty acid synthase.

Formation of acetyl ACP and malonyl ACP

- The two carbon fragments of acetyle CoA is transferred to ACP of fatty acid synthase, catalyzed by the enzyme, acetyl CoA-ACP transacylase. The acetyl unit is then transferred from ACP to cystine residue of the enzyme. Thus ACP cite falls vacant

- The enzyme malonyl CoA-ACP transacylase transfers malonate from malonyl CoA to bind to ACP.

$$\text{Acetyl CoA} + \text{ACP} \rightleftharpoons \text{acetyl ACP} + \text{CoA}$$
$$\text{Malonyl CoA} + \text{ACP} \rightleftharpoons \text{malonyl ACP} + \text{CoA}$$

Other reactions in the formation of fatty acids

Step 1 (condensation): The first reaction in the formation of a fatty acid chain is condensation of the activated acetyl and malonyl groups to form acetoacetyl-ACP, an acetoacetyl group bound to ACP through the phosphopantetheine OSH group; simultaneously, a molecule of CO2 is produced. In this reaction, catalyzed by β-ketoacyl-ACP synthase (KS), the acetyl group is transferred from the Cys OSH group of the enzyme to the malonyl group on the OSH of ACP, becoming the methyl-terminal two-carbon unit of the new acetoacetyl group. The carbon atom of the CO2 formed in this reaction is the same carbon originally introduced into malonyl CoA from $HCO3^-$ by the acetyl-CoA carboxylase reaction. Thus CO2 is only transiently in covalent linkage during fatty acid biosynthesis; it is removed as each two-carbon unit is added.

$$\text{Acetyl ACP} + \text{malonyl ACP} \longrightarrow \text{acetoacetyl ACP} + \text{ACP} + CO_2$$

The β-oxidation of fatty acids, cleavage of the bond between two acyl groups (cleavage of an acetyl unit from the acyl chain) is highly exergonic, so the simple condensation of two acyl groups (two acetyl-CoA molecules, for example) is highly endergonic. The use of activated malonyl groups rather than acetyl groups is what makes the condensation reactions thermodynamically favorable. The methylene carbon (C-2) of the malonyl group, sandwiched between carbonyl and carboxyl carbons, is chemically situated to act as a good nucleophile. In the condensation, decarboxylation of the malonyl group facilitates the nucleophilic attack of the methylene carbon on the thioester linking the acetyl group to β-ketoacyl-ACP synthase, displacing the enzyme's OSH group. Coupling the condensation to the decarboxylation of the malonyl group renders the overall process highly exergonic. A similar carboxylation-decarboxylation sequence facilitates the formation of phosphoenolpyruvate from pyruvate in gluconeogenesis. By using activated malonyl groups in the synthesis of fatty acids and activated acetate in their degradation, the cell makes both processes energetically favorable, although one is effectively the reversal of the other. The extra energy required to make fatty acid synthesis favorable is provided by the ATP used to synthesize malonyl-CoA from

acetyl-CoA and $HCO3^-$.

Step 2 Reduction of the Carbonyl Group, the acetoacetyl-ACP formed in the condensation step now undergoes reduction of the carbonyl group at C-3 to form D-hydroxybutyryl-ACP. This reaction is catalyzed by ketoacyl-ACP reductase(KR) and the electron donor is NADPH. Notice that the D--hydroxybutyryl group does not have the same stereoisomeric form as the L-hydroxyacyl intermediate in fatty acid oxidation.

Step 3 Dehydration The elements of water are now removed from C-2 and C-3 of D--hydroxybutyryl-ACP to yield a double bond in the product, trans-2- butenoylACP. The enzyme that catalyzes this dehydration is hydroxyacyl-ACP dehydratase (HD).

Step 4 Reduction of the Double Bond Finally, the double bond of trans-2-butenoyl-ACP is reduced (saturated) to form butyryl-ACP by the action of enoyl-ACP reductase (ER); again, NADPH is the electron donor.

Synthesis of palmitic acid

The carbon chain attached to ACP is transferred to cysteine residue and the reactions cited above are repeated 6 more times. Each time, the fatty acid chain is lengthened by a two carbon unit. At the end of 7 cycles, fatty acid synthesis is complete and a 16-carbon fully saturated fatty acid-palmitate bound to ACP is produced. The enzyme palmitile tioesterase separates palmitate from fatty acid synthase by a hydrolytic activity in the complex. This completes the synthesis of palmitate. Small amounts of longer fatty acids such as stearate (18:0) are also formed. In certain plants (coconut and palm, for example) chain termination occurs earlier; up to 90% of the fatty acids in the oils of these plants are between 8 and 14 carbons long.

7 Acetyl-CoA + 7CO2 + 7ATP → 7 malonyl-CoA + 7ADP + 7Pi

Acetyl-CoA + 7malonyl-CoA + 14NADPH + $14H^+$ → palmitate + 7CO2 + 8CoA + 14NADP + 6H2O

The over all process is:

8 Acetyl-CoA + 7ATP + 14NADPH + $14H^+$ → palmitate + 8 CoA + 7ADP + 7Pi + $14NADP^+$ + 6H2O

The biosynthesis of fatty acids such as palmitate thus requires acetyl-CoA and the input of chemical energy in two forms: the group transfer potential of ATP and the reducing power of NADPH. The ATP is required to attach CO2 to acetyl-CoA to make malonyl-CoA; the NADPH is required to reduce the double bonds.

Unsaturation of fatty acids

Both prokaryotes and eukaryotes are capable of introducing double bond(s) in a newly synthesized fatty acid. In eukaryotes, a microsomal system that contains NADH-cytochrome b5 reductase, Cyt b5, and an oxygen-dependent desaturase(a mixed-function oxidase or mixed-function oxygenase) containing a single non-heme iron atom cause desaturation. All components of the desaturase system are integral membrane proteins. Mammals are unable to introduce double bonds beyond Δ^9 in the fatty acid chain. Hence they cannot synthesize either linoleic acid or linolenic acid.

Regulation of fatty acid biosynthesis

Acetyl-CoA is formed into malonyl-CoA by acetyl-CoA Acetyl-CoA is formed into malonyl-CoA by acetyl-CoA-carboxylase, at which point malonyl-CoA is destined to feed into the fatty acid synthesis pathway. Acetyl-CoA carboxylase is the point of regulation in saturated straight-chain fatty acid synthesis, and is subject to both phosphorylation and allosteric regulation. Regulation by phosphorylation occurs mostly in mammals, while allosteric regulation occurs in most organisms. Allosteric control occurs as feedback inhibition by palmitoyl-CoA and activation by citrate. When there are high levels of palmitoyl-CoA, the final product of saturated fatty acid synthesis, it allosterically inactivates acetyl-CoA carboxylase to prevent a build-up of fatty acids in cells. Citrate acts to activate acetyl-CoA carboxylase under high levels, because high levels indicate that there is enough acetyl-CoA to feed into the krebs cycle and produce energy. High plasma levels of insulin in the blood plasma (e.g. after meals) cause the dephosphorylation of acetyl-CoA carboxylase, thus promoting the formation of malonyl-CoA from acetyl-CoA, and consequently the conversion of carbohydrates into fatty acids, while epinephrine and glycagon (released into the blood during starvation and exercise) cause the phosphorylation of this enzyme, inhibiting lipogenesis in favor of fatty acid oxidation via beta-oxidation.