CHAPTER 5

Oxidation of fatty acids in eukaryotes

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1. The pathway of β-oxidation: a historical account

Fatty acids are a major source of energy in animals. The study of their biological degradation began in 1904 when Knoop [1] performed the classical experiments that led him to formulate the theory of β-oxidation. In his experiments, Knoop used fatty acids with phenyl residues in place of the terminal methyl groups. The phenyl residue served as a reporter group because it was not metabolized, but instead was excreted in the urine. When Knoop fed phenyl substituted fatty acids with an odd number of carbon atoms, like phenylpropionic acid (C₆H₅-CH₂-CH₂-COOH) or phenylvaleric acid (C₆H₅-CH₂-CH₂-CH₂-COOH), to dogs, he isolated from their urine hippuric acid (C₆H₅-CO-NH-CH₂-COOH), the conjugate of benzoic acid and glycine. In contrast, phenyl-substituted fatty acids with an even number of carbon atoms, such as phenylbutyric acid (C₆H₅-CH₂-CH₂-COOH), were degraded to phenylacetic acid (C₆H₅-CH₂-COOH) and excreted as phenylaceturic acid (C₆H₅-CH₂-CO-NH-CH₂-COOH). These observations led Knoop to propose that the oxidation of fatty acids begins at carbon atom 3, the β-carbon, and that the resulting β-keto acids are cleaved between the α-carbon and β-carbon to yield fatty acids shortened by two carbon atoms. Knoop’s experiments prompted the idea that fatty acids are degraded in a stepwise manner by successive β-oxidation. In the years following Knoop’s initial study, Dakin [2] performed similar experiments with phenylpropionic acid. Besides hippuric acid he isolated the glycine conjugates of the following β-oxidation intermediates: phenylacrylic acid (C₆H₅-CH=CH-COOH), β-phenyl-β-hydroxypropionic acid (C₆H₅-CHOH-CH₂-COOH), and benzoylacetic acid (C₆H₅-CO-CH₂-COOH). At the same time, Embden and coworkers demonstrated that in perfused livers unsubstituted fatty acids are degraded by β-oxidation and converted to ketone bodies. Thus, by 1910 the basic information necessary for formulating the pathway of β-oxidation was available.

After a 30-year period of little progress, Munoz and Leloir in 1943, and Lehninger in 1944, demonstrated the oxidation of fatty acids in cell-free preparations from liver. Their work set the stage for the complete elucidation of β-oxidation. Detailed investigations with cell-free systems, especially the studies of Lehninger, demonstrated the need for energy to ‘spark’ the oxidation of fatty acids. ATP was shown to meet this requirement and to be essential for the activation of fatty acids. Activated fatty acids were shown by Wakil and Mahler, as well as by Kornberg and Pricer, to be thioesters formed from fatty acids and coenzyme A. This advance was made possible by earlier studies of Lipmann and coworkers who isolated and characterized coenzyme A, and Lynen [3] and
coworkers who proved the structure of 'active acetate' to be acetyl-CoA. Acetyl-CoA was found to be identical with the two-carbon fragment removed from fatty acids during their degradation. The subcellular location of the β-oxidation system was finally established by Kennedy and Lehninger, who demonstrated that mitochondria were the cellular components most active in fatty acid oxidation. The mitochondrial location of this pathway agreed with the observed coupling of fatty acid oxidation to the citric acid cycle and to oxidative phosphorylation. The most direct evidence for the proposed β-oxidation cycle emerged from enzyme studies carried out in the fifties primarily in the laboratories of Green in Wisconsin, Lynen in Munich, and Ochoa in New York. Their studies were greatly facilitated by newly developed methods of protein purification and by the use of spectrophotometric enzyme assays with chemically synthesized intermediates of β-oxidation as substrates.

2. Uptake and activation of fatty acids in animal cells

Fatty acids are transported between organs either as unesterified fatty acids complexed to serum albumin or in the form of triacylglycerols associated with lipoproteins. Triacylglycerols are hydrolyzed outside of cells by lipoprotein lipase to yield free fatty acids. The mechanism by which free fatty acids enter cells remains poorly understood despite a number of studies performed with isolated cells from heart, liver, and adipose tissue [4]. Kinetic evidence has been obtained for both a saturable and non-saturable uptake of fatty acids. The saturable uptake, which predominates at nanomolar concentrations of free fatty acids, is presumed to be carrier-mediated, whereas the non-saturable uptake, which is significant only at higher concentrations of free fatty acids, has been attributed to non-specific diffusion of fatty acids across the membrane. Several suspected fatty acid transport proteins have been identified [5]. However, their specific function(s) in fatty acid uptake and their molecular mechanisms remain to be elucidated.

Once long-chain fatty acids have crossed the plasma membrane, they either diffuse or are transported to mitochondria, peroxisomes, and the endoplasmic reticulum where they are activated by conversion to their CoA thioesters. Whether this transfer of fatty acids between membranes is a facilitated process or occurs by simple diffusion is an unresolved issue. The identification of low-molecular-weight (14–15 kDa) fatty acid binding proteins (FABPs) in the cytosol of various animal tissues prompted the suggestion that these proteins may function as carriers of fatty acids in the cytosolic compartment [6]. FABPs may also be involved in the cellular uptake of fatty acids, their intracellular storage, or the delivery of fatty acids to sites of their utilization. The importance of FABPs in fatty acid metabolism is supported by the observation that the uptake and utilization of long-chain fatty acids are reduced in knock-out mice lacking heart FABP. These animals exhibit exercise intolerance and, at old age, develop cardiac hypertrophy. Nonetheless, the molecular mechanism of FABP function remains to be elucidated.

The metabolism of fatty acids requires their prior activation by conversion to fatty acyl-CoA thioesters. The activating enzymes are ATP-dependent acyl-CoA synthetases,
which catalyze the formation of acyl-CoA by the following two-step mechanism in which $E$ represents the enzyme:

$$
E + R-\text{COOH} + ATP \xrightleftharpoons{Mg^2+} (E : R-\text{CO-AMP}) + PP_i
\quad (E : R-\text{CO-AMP}) + \text{CoASH} \rightarrow R-\text{CO-SCoA} + \text{AMP} + E
$$

The evidence for this mechanism was primarily derived from a study of acetyl-CoA synthetase. Although the postulated intermediate, acetyl-AMP, does not accumulate in solution, and therefore only exists bound to the enzyme, the indirect evidence for this intermediate is very compelling. Other fatty acids are assumed to be activated by a similar mechanism, even though less evidence in support of this hypothesis has been obtained. The activation of fatty acids is catalyzed by a group of acyl-CoA synthetases that differ with respect to their subcellular locations and their specificities for fatty acids of different chain lengths [7]. Their chain-length specificities are the basis for classifying these enzymes as short-chain, medium-chain, long-chain and very-long-chain acyl-CoA synthetases.

A short-chain-specific acetyl-CoA synthetase that is present in mammalian mitochondria has been purified and its cDNA has been cloned. This 71-kDa enzyme, which is most active with acetate as a substrate but exhibits some activity towards propionate, has been detected in mitochondria of heart, skeletal muscle, kidney, adipose tissue and intestine, but not in those of liver. A cytosolic 78-kDa acetyl-CoA synthetase has been identified in liver, intestine, adipose tissue and mammary gland, all of which have high lipogenic activities. Expression studies support the hypothesis that the cytosolic enzyme synthesizes acetyl-CoA for lipogenesis, whereas the mitochondrial acetyl-CoA synthetase activates acetate headed for oxidation.

Medium-chain acyl-CoA synthetases are present in mitochondria of various mammalian tissues. The partially purified enzyme from beef heart mitochondria acts on fatty acids with 3–7 carbon atoms, but is most active with butyrate. In contrast, the 66-kDa enzyme purified from bovine liver mitochondria activates fatty acids with 3–12 carbon atoms with hexanoate being the best substrate. This enzyme also activates aromatic carboxylic acids like benzoic acid and its substituted derivatives. Overall, liver mitochondria, in contrast to heart mitochondria, contain medium-chain acyl-CoA synthetase activities with much broader substrate specificities toward fatty acids of varying chain lengths and structures.

Long-chain acyl-CoA synthetase is a membrane-bound enzyme that is associated with the endoplasmic reticulum, peroxisomes, and the outer mitochondrial membrane. The enzyme acts efficiently on saturated fatty acids with 10–20 carbon atoms and on common unsaturated fatty acids having 16–20 carbon atoms. Molecular cloning and expression studies of long-chain acyl-CoA synthetase have revealed the existence of five different isozymes (ACS 1–5) in the rat. A detailed investigation of the hepatic acyl-CoA synthetases ACS 1, 4 and 5 suggests that ACS 1 and 4, which are present in the endoplasmic reticulum and related subcellular structures, function in lipid biosynthesis while ACS 5 with a mitochondrial localization may catalyze the activation of fatty acids for $\beta$-oxidation. Such functional commitment of isozymes has previously been recognized. For example, long-chain acyl-CoA synthetases ACS I and ACS II of
Candida lipolytica are thought to activate long-chain fatty acids for complex lipid synthesis and peroxisomal β-oxidation, respectively.

Very long-chain acyl-CoA synthetase activates fatty acids with 22 or more carbon atoms and also acts on long-chain and branched-chain fatty acids. This membrane-bound enzyme is strongly expressed in liver where it is associated with the endoplasmic reticulum and peroxisomes but not with mitochondria. Purification of the 70-kDa very long-chain acyl-CoA synthetase enabled the cloning of the rat and human cDNAs coding for this enzyme. Sequence homologies with other cDNAs resulted in the identification of choloyl-CoA synthetase and in the demonstration that fatty acid transport protein 1 (FATP 1), a suggested transporter of fatty acids across the plasma membrane, exhibits very long-chain acyl-CoA synthetase activity.

In addition to ATP-dependent acyl-CoA synthetases, several GTP-dependent acyl-CoA synthetases have been described. The best known of these enzymes is succinyl-CoA synthetase, which cleaves GTP to GDP plus phosphate and functions in the tricarboxylic acid cycle. Although a mitochondrial GTP-dependent acyl-CoA synthetase activity was described, the existence of a distinct enzyme with such activity has been questioned.

3. Fatty acid oxidation in mitochondria

In animal cells, fatty acids are degraded in both mitochondria and peroxisomes, whereas in lower eukaryotes β-oxidation is confined to peroxisomes. Mitochondrial β-oxidation provides energy for oxidative phosphorylation and generates acetyl-CoA for ketogenesis in liver. The oxidation of fatty acids with odd numbers of carbon atoms proceeds by β-oxidation and yields, in addition to acetyl-CoA, 1 mole of propionyl-CoA per mole of fatty acid. Propionyl-CoA is further metabolized to succinate.

3.1. Mitochondrial uptake of fatty acids

Fatty acyl-CoA thioesters that are formed at the outer mitochondrial membrane cannot directly enter the mitochondrial matrix, where the enzymes of β-oxidation are located, because the inner mitochondrial membrane is impermeable to CoA and its derivatives. Instead, carnitine carries the acyl residues of acyl-CoA thioesters across the inner mitochondrial membrane. The carnitine-dependent translocation of fatty acids across the inner mitochondrial membrane is schematically shown in Fig. 1 [8]. The reversible transfer of fatty acyl residues from CoA to carnitine is catalyzed by carnitine palmitoyltransferase I (CPT I), which is an enzyme of the outer mitochondrial membrane. The resultant acylcarnitines cross the inner mitochondrial membrane via the carnitine:acylcarnitine translocase. This carrier protein catalyzes a rapid mole for mole exchange of acylcarnitine for carnitine, carnitine for carnitine, and acylcarnitine for acylcarnitine. This exchange, especially of acylcarnitine for carnitine, is essential for the translocation of long chain fatty acids from the cytosol into mitochondria. In addition, the translocase facilitates a slow unidirectional flux of carnitine across the inner mitochondrial membrane. This unidirectional flux of carnitine may be important
for mitochondria of organs other than liver to acquire carnitine, which is synthesized in the liver. The rat liver translocase, which has a subunit molecular mass of 32.5 kDa, has been purified and its cDNA has been cloned. The protein has also been functionally reconstituted into proteoliposomes. In the mitochondrial matrix, carnitine palmitoyltransferase II (CPT II), an enzyme that is associated with the inner mitochondrial membrane, catalyzes the transfer of acyl residues from carnitine to CoA to form acyl-CoA thioesters that then enter the β-oxidation spiral. CPT II has been purified from mitochondria of bovine heart and rat liver. The purified enzyme has a subunit molecular mass of approximately 70 kDa and catalyzes the reversible transfer of acyl residues with 10–18 carbon atoms between CoA and carnitine. The cDNAs of rat and human CPT II have been cloned and sequenced. The predicted amino acid sequences of the corresponding proteins show a greater than 80% homology with each other. CPT I, in contrast to CPT II, is reversibly inhibited by malonyl-CoA, its natural regulator, and is covalently modified and inactivated by CoA derivatives of certain alkyl glycidic acids [8]. The latter property was utilized to label this protein for generating sequence information that permitted the molecular cloning of CPT I. Human and rat cDNAs code for 88-kDa proteins that are highly homologous (88%) to each other and also are very similar (50%) to CPT II. An isoform of liver CPT I (L-CPT I) is present in skeletal muscle (M-CPT I) while both isoforms are expressed in heart mitochondria. L-CPT I and M-CPT I are products of different genes and have different kinetic properties. M-CPT I compared to L-CPT I is much more sensitive toward malonyl-CoA (K_i ≈ 20
nM vs. 2 μM) but has a lower affinity for carnitine ($K_m \approx 500 \mu M$ vs. 30 μM). CPT I is anchored in the outer mitochondrial membrane via two transmembrane segments so that the 46 N-terminal residues and the large C-terminal catalytic domain remain on the cytosolic side of the membrane. CPT I together with CPT II and acyl-CoA synthetase seem to be concentrated at contact sites between the inner and outer mitochondrial membrane. The effective expression of active CPT I in the yeast *Pichia pastoris*, which is devoid of this enzyme, made it possible to study the structure–function relationship of this enzyme. The general conclusion of these studies is that the cytosolic N-terminal region of CPT I harbors positive and negative regulatory elements that determine the sensitivity of L-CPT I toward malonyl-CoA and the affinity of M-CPT I for carnitine [9].

In addition to CPT I and CPT II, mitochondria contain a carnitine acetyltransferase, which has been purified and its cDNA has been cloned. The enzyme from bovine heart has an estimated molecular mass of 60 kDa and is composed of a single polypeptide chain. It catalyzes the transfer of acyl groups with 2–10 carbon atoms between CoA and carnitine. The function of this enzyme has not been established conclusively. Perhaps, the enzyme regenerates free CoA in the mitochondrial matrix by transferring acetyl groups and other short-chain or medium-chain acyl residues from CoA to carnitine. The resultant acylcarnitines can leave mitochondria via the carnitine:acylcarnitine translocase and can be metabolized by the same or other tissues, or can be excreted in urine. In addition, carnitine acetyltransferase together with CPT II may convert acylcarnitines that were formed by the partial β-oxidation of fatty acids in peroxisomes to acyl-CoAs for further oxidation in mitochondria.

Short-chain and medium-chain fatty acids with less than 10 carbon atoms can enter mitochondria as free acids independent of carnitine. They are activated by short-chain and medium-chain acyl-CoA synthetases that are present in the mitochondrial matrix.

### 3.2. Enzymes of β-oxidation in mitochondria

The enzymes of β-oxidation either are associated with the inner mitochondrial membrane or are located in the mitochondrial matrix. The reactions catalyzed by these enzymes are shown schematically in Fig. 2, which also provides a hypothetical view of the physical and functional organization of these enzymes.

In the first of four reactions that constitute one cycle of the β-oxidation spiral acyl-CoA is dehydrogenated to 2-trans-enoyl-CoA according to the following equation.

$$\text{R}\text{−CH}_2\text{−CH}_2\text{−CO−SCoA + FAD} \rightarrow \text{R}\text{−CH=CH−CO−SCoA + FADH}_2$$

Four acyl-CoA dehydrogenases with different but overlapping chain length specificities cooperate to assure the complete degradation of all fatty acids that can be metabolized by mitochondrial β-oxidation. The names of the four dehydrogenases, short-chain, medium-chain, long-chain, and very-long-chain acyl-CoA dehydrogenases, reflect their chain-length specificities. Purification of these enzymes has permitted detailed studies of their molecular and mechanistic properties [10,11]. The first three dehydrogenases are soluble matrix enzymes with similar molecular masses between 170 and 190 kDa. They are composed of four identical subunits, each of which carries a tightly, but non-covalently bound, flavin adenine dinucleotide (FAD). Their cDNAs
have been cloned and sequenced. High degrees of homology (close to 90%) have been observed for the same type of enzyme from man and rat and significant homologies (30–35%) are apparent when different enzymes from one source are compared. The crystal structure of medium-chain acyl-CoA dehydrogenases at 2.4 Å resolution confirmed the homotetrameric structure of the enzyme with one FAD bound per subunit in an extended conformation [12]. Very-long-chain acyl-CoA dehydrogenase, in contrast to the three other dehydrogenases, is a protein of the inner mitochondrial membrane. Purification of this enzyme and its molecular cloning established that it is a 133-kDa homodimer with one FAD bound per subunit. The four dehydrogenases differ with respect to their specificities for substrates of various chain lengths. Short-chain acyl-CoA dehydrogenase only acts on short-chain substrates like butyryl-CoA and hexanoyl-CoA. Medium-chain acyl-CoA dehydrogenase is most active with substrates from hexanoyl-CoA to dodecanoyl-CoA, whereas long-chain acyl-CoA dehydrogenase preferentially acts on octanoyl-CoA and longer-chain substrates. Very-long-chain acyl-CoA dehydrogenase extends the activity spectrum to longer-chain substrates, including those having acyl chains with 22 and 24 carbon atoms. However, long-chain acyl-CoA dehydrogenase may have a specific function in the β-oxidation of unsaturated fatty acids because this enzyme, in contrast to very long-chain acyl-CoA dehydrogenase, effectively dehydrogenates 4-enoyl-CoAs and 5-enoyl-CoAs, which are β-oxidation intermediates of unsaturated fatty acids. This hypothesis is supported by the observed accumulation of
intermediates of unsaturated but not of saturated fatty acids in knock-out mice lacking long-chain acyl-CoA dehydrogenase. The dehydrogenation of acyl-CoA thioesters involves the removal of a proton from the α-carbon of the substrate and hydride transfer from the β-carbon to the FAD cofactor of the enzyme to yield 2-trans-enoyl-CoA and enzyme-bound FADH₂ [12]. Studies based on X-ray crystallography, chemical modifications, and site-specific mutagenesis established that glutamate 376 is the base responsible for the α-proton abstraction in medium-chain acyl-CoA dehydrogenase. Other acyl-CoA dehydrogenases follow a similar mechanism. Reoxidation of FADH₂ occurs by two successive single-electron transfers from the dehydrogenase to the FAD prosthetic group of a second flavoprotein named electron-transferring flavoprotein (ETF), which donates electrons to an iron–sulfur flavoprotein named ETF:ubiquinone oxidoreductase. The latter enzyme, a component of the inner mitochondrial membrane, feeds electrons into the mitochondrial electron transport chain via ubiquinone. The flow of electrons from acyl-CoA to oxygen is schematically shown below.

\[
\begin{align*}
R-\text{CH}_2-\text{CH}-\text{CO}-\text{SCoA} & \rightarrow \text{FAD(acyl-CoA dehydrogenase)} \rightarrow \\
\text{FAD(ETF)} & \rightarrow \text{FAD}/[4\text{Fe}4\text{S}](\text{ETF:ubiquinone oxidoreductase}) \rightarrow \\
\text{ubiquinone} & \rightarrow \rightarrow \rightarrow \rightarrow \text{oxygen}
\end{align*}
\]

ETF is a soluble matrix protein with a molecular mass of close to 60 kDa. It is composed of two non-identical subunits of similar molecular masses with one FAD per protein dimer. The crystal structure of ETF revealed the location of FAD in a cleft between the two subunits. In addition to the four acyl-CoA dehydrogenases involved in fatty acid oxidation, two acyl-CoA dehydrogenases specific for metabolites of branched-chain amino acids have been isolated and purified. They are isovaleryl-CoA dehydrogenase and 2-methyl-branched chain acyl-CoA dehydrogenase.

In the second step of β-oxidation 2-trans-enoyl-CoA is reversibly hydrated by enoyl-CoA hydratase to L-3-hydroxyacyl-CoA as shown below.

\[
\begin{align*}
R-\text{CH}=\text{CH}-\text{CO}-\text{SCoA} + \text{H}_2\text{O} & \rightarrow R-\text{CH(OH)}-\text{CH}_2-\text{CO}-\text{SCoA}
\end{align*}
\]

Two enoyl-CoA hydratases have been identified in mitochondria [4]. The better characterized of the two enzymes is enoyl-CoA hydratase or crotonase, which is a 161-kDa homohexamer. The best substrate of crotonase is crotonyl-CoA (CH₃-CH=CH-CO-SCoA), which is hydrated to form L(S)-3-hydroxybutyryl-CoA. The activity of the enzyme decreases with increasing chain length of the substrate so that the activity with 2-trans-hexadecenoyl-CoA is only 1–2% of the activity achieved with crotonyl-CoA. Crotonase also hydrates 2-cis-enoyl-CoA to D-3-hydroxyacyl-CoA and exhibits very low Δ³,Δ²-enoyl-CoA isomerase activity. The crystal structure of crotonase revealed that it is a member of the hydratase/isomerase superfamily with two active site glutamate residues that function as general acid and general base in the syn addition of water to crotonyl-CoA. The second enoyl-CoA hydratase, referred to as long-chain enoyl-CoA hydratase, is virtually inactive with crotonyl-CoA, but effectively hydrates medium-chain and long-chain substrates. The activities of crotonase and long-chain enoyl-CoA hydratase complement each other thereby assuring high rates of hydration
of all enoyl-CoA intermediates. Long-chain enoyl-CoA hydratase is a component enzyme of the trifunctional \( \beta \)-oxidation complex, which additionally exhibits long-chain activities of L-3-hydroxyacyl-CoA dehydrogenase and 3-ketoacyl-CoA thiolase [13]. This \( \beta \)-oxidation complex is a protein of the inner mitochondrial membrane. It consists of equimolar amounts of a large \( \alpha \)-subunit with a molecular mass of close to 80 kDa and of a small \( \beta \)-subunit with a molecular mass of approximately 48 kDa. Cloning and sequencing of the cDNAs that code for this complex revealed significant homologies of the amino-terminal and central regions of the large subunit with enoyl-CoA hydratase and L-3-hydroxyacyl-CoA dehydrogenase, respectively, and of the small subunit with 3-ketoacyl-CoA thiolase. These homologies are indicative of the locations of the component enzymes on the complex.

The third reaction in the \( \beta \)-oxidation cycle is the reversible dehydrogenation of L-3-hydroxyacyl-CoA to 3-ketoacyl-CoA catalyzed by L-3-hydroxyacyl-CoA dehydrogenase as shown in the following equation.

\[
R-CH(OH)-CH_2-CO-SCoA + NAD^+ \rightarrow R-CO-CH_2-CO-SCoA + NADH + H^+
\]

Four L-3-hydroxyacyl-CoA dehydrogenases have been identified in mitochondria.

L-3-Hydroxyacyl-CoA dehydrogenase is a soluble matrix enzyme, which has a molecular mass of approximately 65 kDa and is composed of two identical subunits [4]. The enzyme and its cDNA have been sequenced. The crystal structure of the pig heart enzyme revealed a bilobal structure with the NAD\(^+\) binding site in the N-terminal region and the substrate binding site in the cleft between the C-terminal and N-terminal domains. The enzyme is specific for NAD\(^+\) as a coenzyme. It acts on L-3-hydroxyacyl-CoAs of various chain lengths but is most active with medium-chain and short-chain substrates. A second L-3-hydroxyacyl-CoA dehydrogenase was recently isolated from bovine liver. This enzyme is a homotetramer with a subunit molecular mass of 27 kDa. Its substrate specificity resembles that of the L-3-hydroxyacyl-CoA dehydrogenase described above. However, the enzyme’s primary function may be in androgen metabolism and not in \( \beta \)-oxidation because it exhibits significant 17\( \beta \)-hydroxysteroid dehydrogenase activity and is absent or almost absent from some tissues with high \( \beta \)-oxidation activity. Long-chain L-3-hydroxyacyl-CoA dehydrogenase is a component enzyme of the trifunctional \( \beta \)-oxidation complex or trifunctional protein [13]. This dehydrogenase is active with medium- and long-chain substrates, but not with 3-hydroxybutyryl-CoA, and hence complements the soluble dehydrogenase to assure high rates of dehydrogenation over the whole spectrum of \( \beta \)-oxidation intermediates. A soluble short-chain L-3-hydroxy-2-methylacyl-CoA dehydrogenase is also present in the mitochondrial matrix. This enzyme, which acts on short-chain substrates with or without 2-methyl substituents, is believed to function only in isoleucine metabolism.

In the last reaction of the \( \beta \)-oxidation cycle 3-ketoacyl-CoA is cleaved by thiolase as shown below.

\[
R-CO-CH_2-CO-SCoA + CoASH \rightarrow R-CO-SCoA + CH_3-CO-SCoA
\]

The products of the reaction are acetyl-CoA and an acyl-CoA shortened by two carbon atoms. The equilibrium of the reaction is far to the side of the thiolytic cleavage
products thereby driving β-oxidation to completion. All thiolases that have been studied in detail contain an essential sulphydryl group, which participates directly in the carbon–
carbon bond cleavage as outlined in the following equations where E–SH represents
thiolase.

\[
E-\text{SH} + R-\text{CO}-\text{CH}_2-\text{CO}-\text{SCoA} \rightarrow R-\text{CO}-S-E + \text{CH}_3-\text{CO}-\text{SCoA}
\]

\[
R-\text{CO}-S-E + \text{CoASH} \rightarrow R-\text{CO}-\text{SCoA} + E-\text{SH}
\]

According to this mechanism, 3-ketoacyl-CoA binds to the enzyme and is cleaved
between its α and β carbon atoms. An acyl residue, which is two carbons shorter than
the substrate, is transiently bound to the enzyme via a thioester bond, while acetyl-CoA
is released from the enzyme. Finally, the acyl residue is transferred from the sulphydryl
group of the enzyme to CoA to yield acetyl-CoA.

Several types of thiolases have been identified, some of which exist in multiple forms
[4]. Mitochondria contain three classes of thiolases: (1) acetoacetyl-CoA thiolase or
acetyl-CoA acetyltransferase, which is specific for acetoacetyl-CoA (C₄) as a substrate;
(2) 3-ketoacyl-CoA thiolase or acetyl-CoA acyltransferase, which acts on 3-ketoacyl-
CoA thioesters of various chain lengths (C₄–C₁₆) and (3) long-chain 3-ketoacyl-CoA
thiolase, which acts on medium-chain and long-chain 3-ketoacyl-CoA thioesters, but
not on acetoacetyl-CoA. The latter two enzymes are essential for fatty acid β-oxidation,
whereas acetoacetyl-CoA thiolase most likely functions only in ketone body and
isoleucine metabolism. Long-chain 3-ketoacyl-CoA thiolase is a component enzyme
of the membrane-bound trifunctional β-oxidation complex [13], whereas the other two
thiolases are soluble matrix enzymes. All mitochondrial thiolases have been purified
and their cDNAs have been cloned and sequenced. A comparison of amino acid
sequences proved all mitochondrial thiolases to be different, but homologous, enzymes.
3-Ketoacyl-CoA thiolase is composed of four identical subunits with a molecular mass
of close to 42 kDa. This enzyme acts equally well on all substrates tested except for
acetoacetyl-CoA, which is cleaved at half the maximal rate observed with longer chain
substrates.

The absence or near absence of intermediates of β-oxidation from mitochondria
prompted the idea of intermediate channeling due to the existence of a multienzyme
complex of β-oxidation enzymes in intact mitochondria. The identification and char-
acterization of at least two isozymes for each of the four reactions of the β-oxidation
spiral led to the presentation of a model for their physical and functional organization
as shown in Fig. 2 [14]. By this model, the membrane-bound, long-chain specific
β-oxidation system, consisting of very-long-chain acyl-CoA dehydrogenase and the tri-
functional β-oxidation complex, converts long-chain to medium-chain fatty acyl-CoAs,
which are completely degraded by the matrix system of soluble enzymes that have a
preference for medium-chain and short-chain substrates. An assumption underlying this
model is that all enzymes thought to function in fatty acid β-oxidation are essential for
this process. So far this assumption has proven to be correct. The characterization of
inherited disorders of fat metabolism in humans has revealed that each of the many
β-oxidation enzymes found to be deficient in a patient is essential for the normal
degradation of fatty acids (for more detail see Section 5).
3.3. β-Oxidation of unsaturated fatty acids

Unsaturated fatty acids, which usually contain cis double bonds, also are degraded by β-oxidation. However, additional (auxiliary) enzymes are required to act on the pre-existing double bonds once they are close to the thioester group as a result of chain-shortening [15]. All double bonds present in unsaturated and polyunsaturated fatty acids can be classified either as odd-numbered double bonds, like the 9-cis double bond of oleic acid and linoleic acid or as even-numbered double bonds like the 12-cis double bond of linoleic acid. Since both classes of double bonds are present in linoleic acid, its degradation illustrates the breakdown of all unsaturated fatty acids. A summary of the β-oxidation of linoleic acid is presented in Fig. 3.

Linoleic acid, after conversion to its CoA thioester(I), undergoes three cycles of β-oxidation to yield 3-cis,6-cis-dodecadienoyl-CoA(II) which is isomerized to 2-trans,6-cis-dodecadienoyl-CoA(III) by Δ³,Δ²-trans-enoyl-CoA isomerase, an auxiliary enzyme of β-oxidation. 2-trans,6-cis-Dodecadienoyl-CoA(III) is a substrate of β-oxidation and can complete one cycle to yield 4-cis-decenoyl-CoA(IV), which is dehydrogenated to 2-trans,4-cis-decadienoyl-CoA(V) by medium-chain acyl-CoA dehydrogenase. 2-trans,4-cis-Decadienoyl-CoA(V) cannot continue on its course through the β-oxidation spiral, but instead is reduced by NADPH in a reaction catalyzed by 2,4-dienoyl-CoA reductase. The product of this reduction, 3-trans-decenoyl-CoA(VI), is isomerized by Δ³,Δ²-trans-enoyl-CoA isomerase to 2-trans-decenoyl-CoA(VII), which can be completely degraded by completing four cycles of β-oxidation. Altogether, the degradation of unsaturated fatty acids in mitochondria involves at least Δ³,Δ²-enoyl-CoA isomerase and 2,4-dienoyl-CoA reductase as auxiliary enzymes in addition to the enzymes of the β-oxidation spiral.

More recent is the demonstration that odd-numbered double bonds can be reduced at the stage of 5-enoyl-CoA intermediates formed during the β-oxidation of unsaturated fatty acids. Shown in Fig. 4 is the sequence of reactions that explains the NADPH-dependent reduction of 5-cis-enoyl-CoA(II) [16]. After introduction of a 2-trans double bond by acyl-CoA dehydrogenase, the resultant 2,5-dienoyl-CoA(II) is converted to 3,5-dienoyl-CoA(III) by Δ³,Δ²-enoyl-CoA isomerase. A novel enzyme, Δ³,Δ²,Δ⁴-dienoyl-CoA isomerase, converts 3,5-dienoyl-CoA(III) to 2-trans,4-trans-dienoyl-CoA(IV) by a concerted shift of both double bonds. Finally, 2,4-dienoyl-CoA reductase catalyzes the NADPH-dependent reduction of one double bond to produce 3-trans-enoyl-CoA(V), which, after isomerization to 2-trans-enoyl-CoA(VI) by Δ³,Δ²-enoyl-CoA isomerase, can reenter the β-oxidation spiral. Although the significance of this new pathway has not been fully explored, it seems likely that it provides an avenue for the metabolism of 3,5-dienoyl-CoAs that may be formed fortuitously by Δ³,Δ²-enoyl-CoA isomerase acting on 2,5-dienoyl-CoA intermediates.

Two Δ³,Δ²-enoyl-CoA isomerases exist in rat mitochondria. One is mitochondrial Δ³,Δ²-enoyl-CoA isomerase that has been purified and its cDNA has been cloned [17]. This enzyme is a multimer of one type of subunit with a molecular mass of 30 kDa. In addition to converting the CoA derivatives of 3-cis-enolic acids and 3-trans-enolic acids with 6–16 carbon atoms to the corresponding 2-trans-enoyl-CoAs, the enzyme catalyzes the conversion of 2,5-dienoyl-CoA to 3,5-dienoyl-CoA and of
Fig. 3. \(\beta\)-Oxidation of linoleoyl-CoA.
3-ynoyl-CoA to 2,3-dienoyl-CoA. A second $\Delta^3,\Delta^2$-enoyl-CoA isomerase has been identified in mitochondria. This isomerase is identical with peroxisomal $\Delta^3,\Delta^2$-enoyl-CoA isomerase and therefore has a dual subcellular localization. It is more active with long-chain than medium-chain substrates and has a preference for 3-trans-enoyl-CoA as compared to 3-cis-enoyl-CoAs.

2,4-Dienoyl-CoA reductase has been purified and its cDNA has been cloned. This enzyme is a homotetramer with a native molecular mass of 124 kDa. The reductase has a specific requirement for NADPH. A second mitochondrial 2,4-dienoyl-CoA reductase has been observed but has not yet been characterized.

$\Delta^3,\Delta^5,\Delta^2,4$-Dienoyl-CoA isomerase is a member of the hydratase/isomerase superfamily with a 32-kDa subunit in a homohexameric arrangement. It is present in both mitochondria and peroxisomes because of mitochondrial and peroxisomal targeting signals at its N-terminus and C-terminus, respectively. The crystal structure of this isomerase revealed the presence of one active site glutamate and aspartate residue each, which catalyze simultaneous proton transfers that facilitate the $3,5 \rightarrow 2,4$ double bond isomerization with substrates having acyl chains with 8–20 carbon atoms. It also catalyzes the isomerization of 3,5,7-trienoyl-CoA to 2,4,6-trienoyl-CoA. This reaction is a likely step in the $\beta$-oxidation of conjugated linoleic acid like 9-cis,11-trans-octadecadienoic acid.

3.4. Regulation of fatty acid oxidation in mitochondria

The rate of fatty acid oxidation is a function of the plasma concentration of unesterified fatty acids. Unesterified or free fatty acids are generated by lipoprotein lipase or are released from adipose tissue into the circulatory system, which carries them to other tissues or organs. Hormones like glucagon and insulin regulate the lipolysis of triacylglycerols in adipose tissue (see Chapter 10). The utilization of fatty acids for either oxidation or lipid synthesis depends on the nutritional state of the animal,
more specifically on the availability of carbohydrates. Because of the close relationship
among lipid metabolism, carbohydrate metabolism, and ketogenesis, the regulation of
fatty acid oxidation in liver differs from that in tissues like heart and skeletal muscle,
which have an overwhelming catabolic function. For this reason, the regulation of fatty
acid oxidation in liver and heart will be discussed separately.

The direction of fatty acid metabolism in liver depends on the nutritional state of the
animal. In the fed animal, the liver converts carbohydrates to fatty acids, while in fasted
animals fatty acid oxidation, ketogenesis, and gluconeogenesis are the more active
processes. Clearly, there exists a reciprocal relationship between fatty acid synthesis
and fatty acid oxidation. Although it is well established that lipid and carbohydrate
metabolism are under hormonal control, it has been more difficult to identify the
mechanism that regulates fatty acid synthesis and oxidation. McGarry and Foster [18]
have proposed that the concentration of malonyl-CoA, the first committed intermediate
in fatty acid biosynthesis, determines the rate of fatty acid oxidation. The essential
features of their hypothesis are presented in Fig. 5. In the fed animal, where glucose is
actively converted to fatty acids, the concentration of malonyl-CoA is elevated. Malonyl-
CoA at micromolar concentrations inhibits hepatic CPT I thereby decreasing the transfer
of fatty acyl residues from CoA to carnitine and their translocation into mitochondria.
Consequently, β-oxidation is depressed. When the animal changes from the fed to the
fasted state, hepatic metabolism shifts from glucose breakdown to gluconeogenesis
with a resultant decrease in fatty acid synthesis. The concentration of malonyl-CoA
decreases, and the inhibition of CPT I is relieved. Furthermore, starvation causes an
increase in the total CPT I activity and a decrease in the sensitivity of CPT I toward
malonyl-CoA. Altogether, during starvation acylcarnitines are more rapidly formed and
translocated into mitochondria thereby stimulating β-oxidation and ketogenesis.

It appears that the cellular concentration of malonyl-CoA is directly related to the
activity of acetyl-CoA carboxylase, which is hormonally regulated. The short-term
regulation of acetyl-CoA carboxylase involves the phosphorylation and dephosphoryla-
tion of the enzyme (see Chapter 6). In the fasting animal, a high [glucagon]/[insulin]
ratio causes the phosphorylation and inactivation of acetyl-CoA carboxylase. As a
consequence, the concentration of malonyl-CoA and the rate of fatty acid synthesis
decrease, while the rate of β-oxidation increases. A decrease of the [glucagon]/[insulin]
ratio reverses these effects. Thus, both fatty acid synthesis and fatty acid oxidation are
regulated by the ratio of [glucagon]/[insulin].

It has been suggested that malonyl-CoA also regulates fatty acid oxidation in non-
hepatic tissues like heart and skeletal muscle [19,20]. The formation of malonyl-CoA in
these tissues is catalyzed by a 280-kDa isoform (ACC 2) of the 265-kDa acetyl-CoA
carboxylase (ACC 1) that is the predominant form of lipogenic tissues. The disposal
of malonyl-CoA is thought to be catalyzed by cytosolic malonyl-CoA decarboxylase.
If so, the tissue concentration of malonyl-CoA is determined by the activities of both
the carboxylase and decarboxylase. Both enzymes seem to be regulated. ACC 2 is
phosphorylated and inactivated by AMP-dependent kinase in response to stress caused
by ischemia/hypoxia and exercise and is activated allosterically by citrate. A concern
about this model for the regulation of fatty acid oxidation in heart and skeletal muscle
is the discrepancy between the micromolar tissue concentration of malonyl-CoA and
the nanomolar $K_i$ of muscle CPT I (M-CPT I) for malonyl-CoA. This enzyme should be completely inhibited at all times unless the effective malonyl-CoA concentration is lower due to binding to other proteins or due to intracellular compartmentation.

In heart, and possibly in other tissues, the rate of fatty acid oxidation is tuned to the cellular energy demand in addition to being dependent on the concentration of plasma free fatty acids [21]. At sufficiently high concentrations (>0.6 mM) of free fatty acids the rate of fatty acid oxidation is only a function of the cellular energy demand. Studies with perfused hearts and isolated heart mitochondria have shown that a decrease in the energy demand results in elevated concentrations of acetyl-CoA and NADH and in lower concentrations of CoA and NAD$^+$. The resultant increases in the ratios of [acetyl-CoA]/[CoA] and [NADH]/[NAD$^+$] in the mitochondrial matrix may be the cause for the reduced rate of $\beta$-oxidation. Experiments with isolated heart mitochondria have provided support for this view. Moreover, these experiments support the conclusion that the ratio of [acetyl-CoA]/[CoA], and not of [NADH]/[NAD$^+$],
controls the rate of β-oxidation. Although the site of this regulation has not been identified unequivocally, it is possible that the \([\text{acetyl-CoA}] / [\text{CoA}]\) ratio regulates the activity of 3-ketoacyl-CoA thiolase and thereby controls the flux of fatty acids through the β-oxidation spiral.

4. **Fatty acid oxidation in peroxisomes**

Peroxisomes and glyoxysomes, collectively referred to as microbodies, are subcellular organelles capable of respiration. They do not have an energy-coupled electron transport system like mitochondria, but instead contain flavin oxidases, which catalyze the substrate-dependent reduction of oxygen to \(H_2O_2\). Because catalase is present in these organelles, \(H_2O_2\) is rapidly reduced to water. Thus, peroxisomes and glyoxysomes are organelles with a primitive respiratory chain where energy released during the reduction of oxygen is lost as heat. Glyoxysomes are peroxisomes that contain the enzymes of the glyoxylate pathway in addition to flavin oxidases and catalase. Peroxisomes or glyoxysomes are found in all major groups of eukaryotic organisms including yeasts, fungi, protozoa, plants and animals.

An extramitochondrial system capable of oxidizing fatty acids was first detected in glyoxysomes of germinating seeds. When rat liver cells were shown to contain a β-oxidation system in peroxisomes [22], the interest in the peroxisomal pathway was greatly stimulated and one set of β-oxidation enzymes was soon identified and characterized [23,24]. It should be noted that peroxisomal β-oxidation occurs in all eukaryotic organisms, whereas mitochondrial β-oxidation seems to be restricted to animals. Studies of peroxisomal β-oxidation were aided by the use of certain drugs, e.g. clofibrate and di(-2-ethylhexyl)phthalate, which induce the expression of the enzymes of peroxisomal β-oxidation and in addition cause the proliferation of peroxisomes in rodents. The induction of peroxisomal β-oxidation by xenobiotic proliferators or fatty acids involves the peroxisomal proliferator-activated receptors (PPARs), which are members of the nuclear hormone receptor family and which recognize peroxisomal proliferator response elements upstream of the affected structural genes [4]. Although rat liver peroxisomes are capable of chain-shortening regular long-chain fatty acids, their main function seems to be the partial β-oxidation of very-long-chain fatty acids, methyl-branched carboxylic acids like pristanic acid, prostaglandins, dicarboxylic acids, xenobiotic compounds like phenyl fatty acids, and hydroxylated 5-β-cholestanolic acids, formed during the conversion of cholesterol to cholic acid.

4.1. **Fatty acid uptake by peroxisomes**

The mechanism of fatty acid uptake by peroxisomes is poorly understood. Although small molecules like substrates and cofactors can freely cross the membrane of isolated peroxisomes from animals, it seems that in vivo the peroxisomal membrane constitutes a permeability barrier that would require transporters to facilitate the uptake of substrates and cofactors [25]. In animal and yeast cells, long-chain fatty acids are activated outside of the peroxisomal membrane. Long-chain acyl-CoAs are thought to enter peroxisomes
in a facilitated process involving half ABC transporters like ALDP, ALDRP, PMP70, and PMP69 in animal cells and Pxa1p and Pxa2p in yeast. In contrast, very long-chain fatty acids in animal cells and medium-chain fatty acids in yeast can be activated in the peroxisomal matrix. The β-oxidation of medium-chain fatty acids in yeast requires two membrane proteins that are assumed to facilitate the uptake of medium-chain fatty acids and ATP, respectively.

4.2. Pathways and enzymology of peroxisomal α-oxidation and β-oxidation

The first step in peroxisomal β-oxidation (see Fig. 6) is the dehydrogenation of acyl-CoA to 2-trans-enoyl-CoA catalyzed by acyl-CoA oxidase. This enzyme, in contrast to the mitochondrial dehydrogenases, transfers two hydrogens from the substrate to its FAD cofactor and then to O₂, which is reduced to H₂O₂. Rat liver contains three acyl-CoA oxidases with different substrate specificities. Their names, palmitoyl-CoA oxidase, pristanoyl-CoA oxidase, and trihydroxycoprostanoyl-CoA oxidase are indicative of their preferred substrates [26]. Interestingly, human liver contains only one branched-chain acyl-CoA oxidase besides palmitoyl-CoA oxidase. Cloning and sequencing of the gene and cDNAs coding for rat acyl-CoA oxidase revealed two

![Diagram](image-url)
isoforms of this enzyme as the result of the alternative use of two exons [24]. The rat liver palmitoyl-CoA oxidase is a homodimer with a molecular mass of close to 150 kDa. Ligands of PPARs induce the expression of this enzyme but not of the other two acyl-CoA oxidases. Palmitoyl-CoA oxidase is inactive with butyryl-CoA and hexanoyl-CoA as substrates, but dehydrogenates all longer chain substrates with similar maximal velocities. Acyl-CoA oxidases from organisms other than mammals are either active with substrates of all chain lengths or the organisms express more than one acyl-CoA oxidase with complementing chain length specificities. Consequently, fatty acids can be completely degraded in yeasts, plants, and other lower eukaryotic organisms, but not in mammals.

The next two reactions of β-oxidation, the hydration of 2-enoyl-CoA to 3-hydroxyacyl-CoA and the NAD+-dependent dehydrogenation of 3-hydroxyacyl-CoA to 3-ketoacyl-CoA, are catalyzed in rat liver peroxisomes by multifunctional enzymes 1 (MFE 1) and 2 (MFE 2) [25,27]. Both multifunctional enzymes harbor enoyl-CoA hydratase and 3-hydroxyacyl-CoA dehydrogenase activities. MFE 1 additionally exhibits Δ\(^3\),Δ\(^2\)-enoyl-CoA isomerase activity. 3-Hydroxyacyl-CoA intermediates formed and acted on by MFE 1 have the L-configuration, whereas the hydroxy intermediates produced by MFE 2 have the D-configuration. Both MFE 1 and MFE 2 convert medium-chain and long-chain 2-trans-enoyl-CoAs to 3-ketoacyl-CoAs but show little or no activity with short-chain substrates. MFE 2 is slightly more active than MFE 1 with longer-chain substrates. However, MFE 2 alone acts on substrates with 2-methyl branches like those formed during the β-oxidation of pristanic acid and hydroxylated 5β-cholestanolic acid. MFE 1 consists of a single 80-kDa polypeptide while MFE 2 is a homodimer with a molecular mass of approximately 150 kDa. Yeast and fungi contain only one multifunctional enzyme each with D-specific enoyl-CoA hydratase and 3-hydroxyacyl-CoA dehydrogenase activities.

The last reaction of β-oxidation, the CoA-dependent cleavage of 3-ketoacyl-CoA, is catalyzed by 3-ketoacyl-CoA thiolase. Two rat 3-ketoacyl-CoA thiolases coded for by different genes have been detected. One is constitutively expressed, whereas the expression of the other is highly induced in response to peroxisomal proliferators [25,27]. Both enzymes are homodimers with molecular masses of close to 80 kDa. Both exhibit little activity toward acetoacetyl-CoA, but are active with all longer chain substrates except for 3-keto-2-methylacyl-CoA intermediates formed during the β-oxidation of pristanic acid and hydroxylated 5β-cholestanolic acids. However, these intermediates are acted upon by another 3-ketoacyl-CoA thiolase (SCP\(_t\)-thiolase) that was identified in peroxisomes during a study of the 58-kDa precursor of sterol carrier protein-2. The C-terminal segment of this 58-kDa protein is identical with sterol carrier protein-2, whereas the N-terminal domain harbors the thiolase, which is most active with medium-chain substrates. The crystal structure of the peroxisomal 3-ketoacyl-CoA thiolase from Saccharomyces cerevisiae at 2.8 Å resolution shows two cysteine residues in close proximity at the presumed active site.

Because peroxisomes contain at least two enzymes for each step of β-oxidation, specific functions for these enzymes were inferred from their substrate specificities. Most of the predictions were verified by analyzing fatty acids that accumulate in patients and/or knock-out mice deficient for individual enzymes. Together, these data
support the proposal that branched-chain acyl-CoA oxidase, MFE 2, and SCP, thiolase are essential for the degradation of pristanic acid and hydroxylated 5-β-cholestanolic acid. The β-oxidation of very-long-chain fatty acids requires the involvement of acyl-CoA oxidase and MFE 2. 3-Ketoacyl-CoA thiolase is believed, but has not been proven to function in the breakdown of straight-chain fatty acids. Surprisingly the knock-out mouse for MFE 1 does not exhibit an obvious phenotype, thus leaving the function of this enzyme in doubt.

Unsaturated fatty acids are degraded in peroxisomes by the pathways outlined in Figs. 3 and 4. Two Δ^3,Δ^2-enoyl-CoA isomerase are present in rat liver peroxisomes. One is the monofunctional Δ^3,Δ^2-enoyl-CoA isomerase that is also present in mitochondria, the other is the Δ^3,Δ^2-enoyl-CoA isomerase activity of multifunctional enzyme 1. The monofunctional Δ^3,Δ^2-enoyl-CoA isomerase has a preference for long-chain substrates and may play the major role in the partial β-oxidation of long-chain unsaturated fatty acids. So far this isomerase has only been obtained by cloning and heterologous expression based on its homology to the sole Δ^3,Δ^2-enoyl-CoA isomerase of yeast. The crystal structure of yeast Δ^3,Δ^2-enoyl-CoA isomerase revealed the presence of a single glutamate residue at the active site, which catalyzes a 1,3-proton transfer that results in the shift of the double bond. A 2,4-dienoyl-CoA reductase distinct from the mitochondrial reductase but homologous with the yeast 2,4-dienoyl-CoA reductase has been identified in mammalian peroxisomes by a cloning and expression approach. This enzyme acts on a wide spectrum of 2,4-dienoyl-CoAs but is most active with medium-chain substrates. Δ^3,5 Δ^2,4-Dienoyl-CoA isomerase of mammalian peroxisomes is the same enzyme that is also present in mitochondria (see Section 3.3).

The products of peroxisomal β-oxidation in animals are chain-shortened acyl-CoAs, acetyl-CoA, and NADH. The β-oxidation of chain-shortened acyl-CoAs is completed in mitochondria. For this purpose, acyl-CoAs, including acetyl-CoA, are thought to leave peroxisomes as acylcarnitines, which can be formed by peroxisomal carnitine octanoyltransferase and/or carnitine acetyltransferase [25]. These reactions, as well as the observed hydrolysis of acetyl-CoA to acetate, would regenerate CoA in peroxisomes. The transporters that facilitate the exit of the β-oxidation products from peroxisomes have not yet been identified.

Phytanic acid (3,7,11,15-tetramethylhexadecanoic acid), a component of the human diet that is derived from phytol, a constituent of chlorophyll, is not degraded by β-oxidation because its 3-methyl group interferes with this process. Instead it is chain shortened by α-oxidation in peroxisomes as outlined in Fig. 7 [25,27]. Activation of phytanic acid(I) to phytanoyl-CoA(II) by long-chain or very long-chain acyl-CoA synthetase converts it to a substrate of hydroxyphytanoyl-CoA hydroxylase. Cleavage of the resultant 2-hydroxyphytanoyl-CoA(III) by 2-hydroxyphytanoyl-CoA lyase yields pristanal(IV) and formyl-CoA that are oxidized to pristanic acid(V) and CO₂, respectively. Pristanic acid after activation to pristanoyl-CoA is a substrate of β-oxidation because the 2-methyl group does not interfere with the process as long as the 2-methyl group has the S configuration. A 2-methylacyl-CoA racemase that is present in both peroxisomes and mitochondria epimerizes (2R)-pristanoyl-CoA and (25R)-trihydroxycholestanoyl-CoA to their S isomers that are substrates of peroxisomal β-oxidation.
5. Inherited diseases of fatty acid oxidation

Disorders of fatty acid oxidation were first described in 1973 when deficiencies of carnitine and carnitine palmitoyltransferase (CPT II) were identified as causes of muscle weakness [28]. Patients with low levels (5–45% of normal) of CPT II have recurrent episodes of muscle weakness and myoglobinuria, often precipitated by prolonged exercise and/or fasting. Almost a decade later, a deficiency of medium-chain dehydrogenase was identified in patients with a disorder of fasting adaptation [28]. This
relatively common disorder is characterized by episodes of non-ketotic hypoglycemia provoked by fasting during the first 2 years of life. Between episodes, patients with medium-chain acyl-CoA dehydrogenase deficiency appear normal. Therapy is aimed at preventing fasting, if necessary by the intravenous administration of glucose, and includes carnitine supplementation. The molecular basis of medium-chain acyl-CoA dehydrogenase deficiency is an $A \rightarrow G$ base transition in 90% of the disease causing alleles. This mutation results in the replacement of lysine-329 by a glutamate residue, which impairs the assembly of subunits into the functional tetrameric enzyme. In the years following the identification of medium-chain acyl-CoA dehydrogenase deficiency, fatty acid oxidation disorders due to the following enzymes deficiencies have been described: short-chain acyl-CoA dehydrogenase, very-long-chain acyl-CoA dehydrogenase, electron-transferring flavoprotein (ETF), ETF:ubiquinone oxidoreductase, 3-hydroxyacyl-CoA dehydrogenase, long-chain 3-hydroxyacyl-CoA dehydrogenase, tri-functional $\beta$-oxidation complex, 2,4-dienoyl-CoA reductase, carnitine palmitoyltransferase I, and carnitine:acylcarnitine translocase [28,29]. A deficiency of mitochondrial acetoacetyl-CoA thiolase impairs isoleucine and ketone body metabolism, but not fatty acid oxidation.

Many of these disorders are associated with the urinary excretion of acylcarnitines, acyl conjugates of glycine, and dicarboxylic acids that are characteristic of the metabolic block. A general conclusion derived from studies of these disorders is that an impairment of $\beta$-oxidation makes fatty acids available for microsomal $\omega$-oxidation by which fatty acids are oxidized at their terminal ($\omega$) methyl group or at their penultimate ($\omega - 1$) carbon atom. Molecular oxygen is required for this oxidation and the hydroxylated fatty acids are further oxidized to dicarboxylic acids. Long-chain dicarboxylic acids can be chain-shortened by peroxisomal $\beta$-oxidation to medium-chain dicarboxylic acids, which are excreted in urine.

Several disorders associated with an impairment of peroxisomal $\beta$-oxidation have been described [30]. Of these, Zellweger syndrome and neonatal adrenoleukodystrophy are characterized by the absence, or low levels, of peroxisomes due to the defective biogenesis of this organelle. As a result of this deficiency, compounds that are normally metabolized in peroxisomes, for example very long-chain fatty acids, dicarboxylic acids, hydroxylated 5-$\beta$-cholestanolic acids, and also phytanic acid, accumulate in plasma [25,30]. Infants with Zellweger syndrome rarely survive longer than a few months due to hypotonia, seizures and frequently cardiac defects. In addition to disorders of peroxisome biogenesis, defects of each of the three enzymes of the peroxisomal $\beta$-oxidation spiral and of the peroxisomal very long-chain acyl-CoA synthetase (X-linked adrenoleukodystrophy) have been reported [25,30]. Most of these patients were hypotonic, developed seizures, failed to make psychomotor gains, and died in early childhood. The importance of $\alpha$-oxidation in humans has been established as a result of studying Refsum’s disease, a rare and inherited neurological disorder. Patients afflicted with this disease accumulate large amounts of phytanic acid due to a deficiency of phytanoyl-CoA hydroxylase [25,30].
6. Future directions

Fatty acid oxidation has been studied for almost a century with the result that a fairly detailed view of this process has emerged. The molecular characterization of most β-oxidation enzymes has yielded a wealth of structural information while the dynamics of this pathway remain less well understood. This is in part due to an absence of information about the organization of the β-oxidation enzymes and the impact such organization has on the control of the process. Also a number of questions about the regulation of this process remain unanswered, especially about its regulation in extrahepatic tissues. Even the extensively studied regulation of hepatic fatty acid oxidation by malonyl-CoA continues to be further investigated to provide an understanding of the regulatory mechanism at the molecular level. In spite of impressive progress in the area of peroxisomal β-oxidation, aspects of this process remain unresolved. For example, it is unclear how fatty acids enter peroxisomes and how products exit from this organelle. Also, the transcriptional regulation of this process has not been fully explored. Moreover, the cooperation between peroxisomes and mitochondria in fatty acid oxidation remains to be studied. Not all of the reactions of the β-oxidation spiral have been verified experimentally and hence some may not take place as envisioned. Finally, the complete characterization of known disorders of β-oxidation in humans and the identification of new disorders will raise questions about some accepted features of this process and will prompt re-investigations of issues thought to be resolved.

Abbreviations

ACC    acetyl-CoA carboxylase
ACS    acyl-CoA synthetase
CPT    carnitine palmitoyltransferase
ETF    electron-transferring flavoprotein
FABP   fatty acid binding protein
MFE    multifunctional enzyme
PPAR   peroxisomal proliferator-activated receptor
SCP    sterol carrier protein

References
