Leaky $\beta$-Oxidation of a trans-Fatty Acid

INCOMPLETE $\beta$-OXIDATION OF ELAIDIC ACID IS DUE TO THE ACCUMULATION OF 5-TRANS-TETRADECENOYL-CoA AND ITS HYDROLYSIS AND CONVERSION TO 5-TRANS-TETRADECENOYL-CARNITINE IN THE MATRIX OF RAT MITOCHONDRIA

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The degradation of elaidic acid (9-trans-octadecenoic acid), oleic acid, and stearic acid by rat mitochondria was studied to determine whether the presence of a trans double bond in place of a cis double bond or no double bond affects $\beta$-oxidation. Rat mitochondria from liver or heart effectively degraded the coenzyme A derivatives of all three fatty acids. However, with elaaidoyl-CoA as a substrate, a major metabolite accumulated in the mitochondrial matrix. This metabolite was isolated and identified as 5-trans-tetradecenoyl-CoA. In contrast, little or none of the corresponding metabolites were detected with oleoyl-CoA or stearoyl-CoA as substrates. A kinetic study of long-chain acyl-CoA dehydrogenase (LCAD) and very long-chain acyl-CoA dehydrogenase revealed that 5-trans-tetradecenoyl-CoA is a poorer substrate of LCAD than is 5-cis-tetradecenoyl-CoA, while both unsaturated acyl-CoAs are poor substrates of very long-chain acyl-CoA dehydrogenase when compared with myristoyl-CoA. Tetradecenoic acid and tetradecenoylcarnitine were detected by gas chromatography/mass spectrometry and tandem mass spectrometry, respectively, when rat liver mitochondria were incubated with elaaidoyl-CoA but not when oleoyl-CoA was the substrate. These observations support the conclusion that 5-trans-tetradecenoyl-CoA accumulates in the mitochondrial matrix, because it is less efficiently dehydrogenated by LCAD than is its cis isomer and that the accumulation of this $\beta$-oxidation intermediate facilitates its hydrolysis and conversion to 5-trans-tetradecenoylcarnitine thereby permitting a partially degraded fatty acid to escape from mitochondria. Analysis of this compromised but functional process provides insight into the operation of $\beta$-oxidation in intact mitochondria.

Unsaturated fatty acids with trans double bonds, also referred to as trans fatty acids, are part of the human diet, because they are present in dairy products, meat of ruminants, and partially hydrogenated vegetable oils (1). Clinical studies

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§ The abbreviations used are: CHAPS, 3-(3-cholamidopropyl)dimethylammonio-1-propanesulfonate; crotonase, enoyl-CoA hydratase; dienoyl-CoA isomerase, $\Delta^\text{12},\Delta^\text{14}$-dienoyl-CoA isomerase; enoyl-CoA isomerase, $\Delta^\text{12}$-enoyl-CoA isomerase; HPLC, high performance liquid chromatography; GC, gas chromatography; MS, mass spectrometry; LCAD, long-chain acyl-CoA dehydrogenase; VLCAD, very long-chain acyl-CoA dehydrogenase; Mops, 4-morpholinepropanesulfonic acid.

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β-Oxidation of Elaidic Acid in Rat Mitochondria

liver dienoyl-CoA isomerase (9) were purified by published procedures. Synthesis of Substrates—2-trans-Tetradecenoic acid was synthesized by reacting malonic acid with n-dodecane as described in principle by Linsead et al. (10). 5-trans-Tetradecenoyl-CoA, 5-trans-tetradecenoyl-CoA, and 2-trans-tetradecenoyl-CoA were synthesized from 5-cis-tetradecenoic acid, 5-trans-tetradecenoic acid, and 2-trans-tetradecenoic acid, respectively, by the mixed anhydride method as described by Fong and Schulz (11). 2-trans-Tetradecenoyl-CoA was partially converted to 1,3-hydroxytetradecenoyl-CoA by hydration in the presence of crotonase in 0.1 M KP, (pH 8.0). 3-Ketohexadecanoyl-CoA was synthesized as described previously (12). All products were purified by HPLC. The pH values of the acyl-CoA solutions were adjusted to ~3.4, and the concentrations of thioester solutions were determined by the method of Ellman (13) after cleaving the thioester bond with hydroxylamine at pH 7.11 (11).

Isolation of Mitochondria from Rat Liver and Respiration Measurements—Rat liver mitochondria were isolated as described by Neergaard and Cannon (14) from male Sprague-Dawley rats (240–260 g) kept on a standard chow and then fasted for 24 h before the isolation of mitochondria. For respiration measurements, 1.5 mg of rat liver mitochondria were incubated in 1.9 ml of incubation buffer containing 20 mM Tris-HCl (pH 7.4), 4 mM KF, 0.1 M KCl, 4 mM MgCl₂, 0.1 M EGTA, bovine serum albumin (0.5 mg/ml), and 0.5 mM L-malate. l-Carnitine (1 mM) was added to the incubation mixture, and acyl-CoA dehydrogenase reaction was initiated by the addition of 15 μl of the indicated fatty acyl-CoA and 1 mM ADP to achieve state 3 respiration. Rates of respiration were measured polarographically with a Clark oxygen electrode attached to a YS-oxygraph.

Analysis of Acyl-CoAs Present in the Mitochondrial Matrix—When fatty acyl-CoAs were analyzed that are present in the mitochondrial matrix, a 1:4:1:1 mixture used for preparation of thioester solution was added to the sample (15). The mixture was heated at 60 °C for 10 min. The supernatant was diluted 5-fold before it was passed slowly through a C₁₅ Sep-Pak column. The bound CoA derivatives were eluted with 2.5 ml of methanol. The extraction process was repeated and 1 ml of 500 mM ammonium phosphate (pH 5.5) was added to the combined methanolic extracts. After removal of methanol under N₂, samples were applied to a μBondapak C₁₈ reverse-phase column (30 cm × 3.9 mm) attached to a Waters gradient HPLC system. The absorbance of the eluent was monitored at 254 nm. Separation was achieved by linearly increasing the acetonitrile/H₂O (91:9, v/v) content of the 50 mM ammonium phosphate elution buffer (pH 5.5) from 50% to 80% in 30 min at a flow rate of 2 ml/min. The concentrations of thioester solutions were determined by the method of Rashed et al. (15). Deuterium-labeled acylcarnitines (0.2 pmol of C4-C14 and 0.4 pmol of C16-C18) were added as internal standards to 15 μl of neutralized extract and centrifuged. The supernatant was dried under a stream of nitrogen then butyrylated by reacting it with 0.1 ml of 3 N HCl in butanol at 55 °C for 15 min. The sample was dried under a stream of nitrogen and dissolved in 0.15 ml of acetonitrile/ethyl acetate (1:1 v/v) and 0.1 ml of 3 mM FAD (buffer A). The suspension was sonicated 10 times for 20 s each at 4 °C and then centrifuged at 100,000 × g for 1 h. The supernatant was loaded onto a DEAЕ-Sepharose column (2.5 × 40 cm) that had been equilibrated overnight with buffer A. After washing the column with buffer A, the column was eluted with a linear gradient from 0 to 300 mM NaCl in buffer A. Fractions were collected from the bottom after slowly inserting a thin decafluorobutane stream. Active fractions were pooled, concentrated, and dialyzed against a YM-10 membrane, and then dialyzed against 50 mM KPi (pH 7.6) containing 10% glycerol, 0.3 mM EDTA, and 1 mM FAD (buffer B). The sample was applied to a hydroxyapatite column (2.5 × 25 cm), equilibrated with buffer B, and eluted with a linear gradient from 50 to 400 mM KPi (pH 7.6) in buffer B. Active fractions were collected, concentrated, and dialyzed overnight against 10 mM KP (pH 8.0), 0.3 mM EDTA, 15% glycerol (buffer C), the sample was applied to a Blue Sepharose CL-6B column (1.5 × 10 cm) equilibrated with buffer C. The column was developed with a linear gradient from 0 to 0.6 M KCl in buffer C. Active fractions were combined, concentrated, and dialyzed against 10 mM KPi (pH 7.5), 0.3 mM EDTA, and 20% glycerol (buffer D). The sample was loaded onto a Q-Sepharose column (0.5 × 5 cm) previously equilibrated with buffer D. The column was developed with a linear gradient from 0 to 0.5 M KPi, in buffer D. Active fractions were pooled, concentrated to 0.6 M, diluted with glycerol to ~1 ml, and stored at ~80 °C.

Partial Purification of Mitochondrial Thioesterase and CPT II from Rat Liver—For the partial purification of thioesterase, rat liver mitochondria were stirred at 50,000 × g for 10 min at 4 °C. The supernatant was purified by chromatography on a self-generated gradient of iodixanol (OptiPrep). Equal volumes of iodixanol (50%, v/v) containing 0.25% sucrose, 1 mM EDTA, and 10 mM Mops-NaOH (pH 7.4) and a suspension of heavy mitochondria were mixed (final iodixanol concentration = 25%; p = 1.150 g/ml) and then transferred to 10-ml tubes and centrifuged at 180,000 g, for 3 h in a TLS-55 fixed angle rotor at 4 °C using the slow acceleration and braking modes. Fractions were collected from the bottom after slowly inserting a thin
glass tube through the bottom of the tube. Catalase and malate dehydrogenase were assayed as marker enzymes for mitochondria and peroxisomes, respectively. Fractions containing most mitochondria were combined and diluted 2-fold with MST isolation buffer (210 mM mannitol, 70 mM sucrose, 10 mM Tris-HCl, pH 7.4, and 0.1 mM EDTA) before they were harvested by centrifugation at 17,500 × g for 20 min. The mitochondrial pellet was suspended, dialyzed against MST isolation buffer for at least 4–6 h, and then sonicated 6 times for 20 s each at intervals of 30 s to keep the temperature of the suspension at 4 °C. The suspension of sonicated mitochondria was centrifuged for 1 h at 100,000 × g, and the resultant supernatant was brought to 70% saturation with ammonium sulfate. Precipitated proteins were collected by centrifugation at 15,000 × g for 10 min, dissolved in 3 ml of 20 mM KP, (pH 7.0) containing 0.5 mM benzamidine, 0.5 mM dithiothreitol, 10% glycerol, 30 mM NaCl, and applied to a Sepharch 5-200HR column (2.5 × 40 cm). After elution with the same buffer, active fractions were identified, pooled, concentrated to 0.6 ml, and stored at −80 °C. For the purification of CPT II, mitochondria (1.3 mg/ml) in 20 mM KPi (pH 7.4) containing 1 mM KCl (buffer A) were treated with 0.2% CHAPS for 10 min at 4 °C. The resultant suspension was centrifuged at 1,500 × g for 10 min, and the supernatant was applied to an Octyl-Sepharose CL-4B column (1 × 4 cm) equilibrated with buffer A containing 0.2% CHAPS. CPT II was eluted with buffer A containing 1% CHAPS, and active fractions were pooled, concentrated, and lyophilized at −80 °C.

Enzyme and Protein Assays—Thioesterase and CPT II activities were determined spectrophotometrically by measuring the release of coenzyme A from acyl-CoAs with Ellman’s reagent (13) at 412 nm. A typical plot program. One unit of enzyme activity is defined as the amount of substrate to product per minute. Protein concentrations were determined by the dye-binding assay as described by Bradford (19) with bovine serum albumin as standard.

RESULTS

Respiration Rates of Rat Mitochondria with cis and trans Monounsaturated Fatty Acyl-CoAs as Substrates—The perfused rat heart has been reported to produce 5-trans-tetradecenoic acid from elaidic acid, which appears to be a sufficient energy source for sustaining the heartbeat (4). In an effort to compare the effectiveness of elaidic acid with oleic acid and stearic acid as substrates of mitochondrial β-oxidation, we measured respiration rates of coupled rat liver and heart mitochondria with several fatty acyl-CoAs as substrates. As shown in Fig. 1, stearoyl-CoA and elaidoyl-CoA supported equal rates of respiration in rat liver mitochondria, whereas oleoyl-CoA sustained a rate that was 50% higher than the rates observed with the other two substrates. Rates obtained with myristoyl-CoA, 5-cis-tetradecenoyl-CoA, and 5-trans-tetradecenoyl-CoA were higher than the rate supported by any of the three longer chain acyl-CoAs. In agreement with a previous report (20), elaidoyl-CoA was degraded in rat heart mitochondria at a rate that was 50 and 33% lower than rates observed with oleoyl-CoA and stearoyl-CoA, respectively (results not shown). These data indicate that elaidic acid is an adequate substrate of β-oxidation in both rat liver and heart mitochondria.

Detection and Identification of 5-trans-Tetradecenoyl-CoA in Rat Mitochondria Incubated with Elaidoyl-CoA—In an effort to detect possible differences between the β-oxidation of elaidoyl-CoA and oleoyl-CoA, coupled rat liver mitochondria were incubated with either elaidoyl-CoA, oleoyl-CoA, or stearoyl-CoA at state three respiration and fatty acyl-CoAs were analyzed by HPLC. Because no CoA was added to the incubation mixture, fatty acyl-CoAs other than the substrate were overwhelmingly, if not completely, present in the mitochondrial matrix. Shown in Fig. 2 are HPLC chromatograms of fatty acyl-CoAs that were isolated from rat liver mitochondria after incubation with stearoyl-CoA (Fig. 2A), oleoyl-CoA (Fig. 2B), or elaidoyl-CoA (Fig. 2C) for 5 min. One major peak in each chromatogram corresponds to the fatty acyl-CoA that served as substrate. A second peak marked “IS” is due to pentadecanoyl-CoA that was added as an internal standard after the incubation was completed to facilitate the quantification of metabolites. However, in Fig. 2C, a third prominent peak is visible, which, based on its elution time, was tentatively identified as 5-trans-tetradecenoyl-CoA (ΔΔC14). After further purification by HPLC, this material was hydrolyzed and converted to its methyl ester. Analysis of the methyl ester by gas chromatography/mass spectrometry proved this material to be a homogeneous compound with a molecular ion at m/z of 258 as expected of methyl tetradecenolate upon chemical ionization in the presence of ammonia (data not shown). The material presumed to be 5-trans-tetradecenoyl-CoA had a UV spectrum with an absorbance maximum around 260 nm that is typical of an acyl-CoA (see spectrum 1 in Fig. 3). Upon treatment with acyl-CoA oxidase, spectrum 1 changed to spectrum 2. The observed increase in the absorbance around 260 nm agrees with the expected conversion of 5-trans-tetradecenoyl-CoA to 2,5-trans-tetradecadienoyl-CoA. Treatment of the latter compound with enoyl-CoA isomerase yielded spectrum 3 that is characteristic of 3,5-dienoyl-CoAs. Finally, when the compound, presumed to be 3,5-tetradecadienoyl-CoA, was incubated with dienoyl-CoA isomerase, spectrum 3 changed to spectrum 4 with absorbance maxima at 260 and 300 nm, which is characteristic of 2,4-dienoyl-CoA. Taken together, the data demonstrate that 5-tetradecenoyl-CoA accumulated in the matrix of mitochondria that oxidized elaidoyl-CoA. Because the intermediate most likely retained the original trans double bond, it was assumed to be 5-trans-tetradecenoyl-CoA. The amount of this metabolite after 1 to 2 min of incubation was 0.88 ± 0.22 nmol/mg mitochondrial protein. When oleoyl-CoA served as a substrate, a metabolite with chromatographic behavior identical to that of
authentic 5-cis-tetradecenoyl-CoA was observed (see Fig. 2B). However, the putative cis metabolite was present at a level 10-times lower than the trans compound (Fig. 2, compare B and C), and therefore no attempt was made to further identify it. Altogether, it is very likely that the metabolite of oleoyl-CoA was 5-cis-tetradecenoyl-CoA. Tetradecanoyl-CoA (myristoyl-CoA), the corresponding metabolite of stearoyl-CoA, was not detected and therefore did not accumulate at a level that was higher than the background noise.

Kinetics of the Dehydrogenation of 5-trans- and 5-cis-Tetradecenoyl-CoA and Myristoyl-CoA by LCAD and VLCAD—The observed accumulation of 5-trans-tetradecenoyl-CoA in the matrix of rat liver mitochondria raised the question as to the cause for the build-up of this metabolite. We hypothesized that 5-trans-tetradecenoyl-CoA may be a relatively poor substrate of LCAD and/or VLCAD, both of which dehydrogenate long-chain acyl-CoAs. This prediction is supported by the data shown in Table I. The catalytic efficiency ($k_{cat}/K_m$) of LCAD was 4-times lower with 5-trans-tetradecenoyl-CoA than with 5-cis-tetradecenoyl-CoA or tetradecanoyl-CoA (myristoyl-CoA). This lower catalytic efficiency is due to a 4-fold higher $K_m$ for 5-trans-tetradecenoyl-CoA compared with the $K_m$ values for the other two substrates. In contrast VLCAD acted equally well on the cis and trans isomers of 5-tetradecenoyl-CoA, which, however, were poorer substrates of this enzyme than was tetradecenoyl-CoA. The latter observation agrees with a previous report showing that saturated acyl-CoAs are better substrates of VLCAD than the corresponding unsaturated substrates with 4,5- or 5,6-double bonds (21).

Identification of 5-trans-Tetradecenoic Acid as a Product of Elaidate β-Oxidation in Rat Liver Mitochondria—The reported formation of 5-trans-tetradecenoic acid in rat hearts perfused with elaidic acid (4) suggested that 5-trans-tetradecenoyl-CoA, a metabolite of elaidate β-oxidation, may be hydrolyzed in mitochondria and the resultant free fatty acid may exit from cells. To test this idea, isolated rat liver mitochondria were incubated with elaidoyl-CoA, oleoyl-CoA, or stearoyl-CoA for 5 min, and the resultant acidic products were extracted and analyzed by gas chromatography/mass spectrometry (GC/MS). Shown in Fig. 4 are the gas chromatograms of the acids extracted after incubating mitochondria with elaidoyl-CoA or oleoyl-CoA. Only the incubation of mitochondria with elaidoyl-CoA yielded a compound, marked C14:1 acid, with an elution time identical to that of 5-trans-tetradecenoic acid (see Fig. 4, A and B). The mass spectrum of this compound was virtually identical with that of 5-trans-tetradecenoic acid (Fig. 4, compare C and D). Hence 5-trans-tetradecenoyl-CoA, which is formed by β-oxidation of elaidoyl-CoA in mitochondria and accumulates in the matrix, was hydrolyzed. It was estimated that 1–2% of the elaidoyl-CoA present in the incubation mixture was converted to 5-trans-tetradecenoic acid. The hydrolysis of 5-trans-tetradecenoyl-CoA in the mitochondrial matrix requires an acyl-CoA thioesterase that was detected in the soluble extract from rat liver mitochondria. Shown in Fig. 5 is the substrate profile of a partially purified preparation of one or more of these thioesterases. The enzyme was most active with substrates having acyl chains with 12 and 14 carbon atoms and hence is best classified as a long-chain acyl-CoA thioesterase. The enzyme was highly active with 5-trans-tetradecenoyl-CoA, although slightly more so with the cis isomer. Intermediates of β-oxidation, e.g. 2-trans-tetradecenoyl-CoA, 3-hydroxytetradecenoyl-CoA, and 3-ketohexadecanoyl-CoA, were poorer substrates than regular fatty acyl-CoAs of equal chain length. $K_m$ and $V_{max}$ values for the thioesterase-catalyzed hydrolysis of tetradecanoyl-CoA (myristoyl-CoA) were determined to be 12.6 ± 0.8 μM and 22.8 ± 0.6 μmol/min/mg protein.
milliunits/mg, respectively, and for the hydrolysis of 5-trans-tetradecenoyl-CoA 7 ± 1 μM and 12.6 ± 0.6 milliunits/mg, respectively. Thus, the catalytic efficiency of this enzyme is unaffected by the presence of the 5-trans double bond.

Formation of 5-trans-Tetradecenoylcarnitine during the β-Oxidation of Elaidic Acid in Rat Liver Mitochondria—The observed hydrolysis of 5-trans-tetradecenoyl-CoA in mitochondria raised the question: Is the 5-trans-tetradecenoyl residue also transferred to carnitine? To answer this question, rat liver mitochondria were incubated with elaidoyl-CoA or oleoyl-CoA. B, expanded region of the gas chromatogram where 5-trans-tetradecenoic acid was eluted. C, mass spectrum of authentic 5-trans-tetradecenoic acid. Abbreviations: C14:1 acid, 5-trans-tetradecenoic acid; IS, internal standard (pentadecanoic acid).

Table I

| Kinetic constant | LCAD a | VLCA a |
|------------------|--------|--------|
|                  | C14:0-CoA | 5c-C14:1-CoA | 5t-C14:1-CoA | C14:0-CoA | 5c-C14:1-CoA | 5t-C14:1-CoA |
| V_max (units/mg) | 3.3 ± 0.3 | 3.0 ± 0.13 | 2.9 ± 0.2 | 1.4 ± 0.13 | 0.32 ± 0.02 | 0.88 ± 0.07 |
| K_m (μM)         | 0.41 ± 0.14 | 0.4 ± 0.06 | 1.6 ± 0.31 | 0.57 ± 0.19 | 0.44 ± 0.09 | 0.97 ± 0.28 |
| k_cat (s⁻¹)      | 9.9 ± 0.8 | 8.9 ± 0.4 | 8.5 ± 0.6 | 2.0 ± 0.2 | 0.42 ± 0.02 | 1.12 ± 0.09 |
| k_cat/k_m (s⁻¹ μM⁻¹) | 24 | 22 | 5 | 4 | 1 | 1 |

a Fluorescence-based assay with electron transferring flavoprotein as the second substrate. For details see "Experimental Procedures."
5-trans-tetradece-16:0, 5t-C16:1CoA, 9c-C16:1CoA, oleoyl-CoA.

tetradecenoyl-CoA than with tetradecanoyl-CoA as substrate.

An attempt was made to estimate the relative rates at which 5-trans-tetradece-16:0-CoA was hydrolyzed and converted to the carnitine derivative in the mitochondrial matrix. For this purpose, activities of CPT II and thioesterase were measured in extracts of rat liver mitochondria at one concentration of 5-trans-tetradece-16:0-CoA. The values thus obtained together with the kinetic parameters determined for the partially purified enzymes with the same substrate were used to calculate activities of the two enzymes as a function of the concentration of 5-trans-tetradece-16:0-CoA (Fig. 7). The data show that the conversion of 5-trans-tetradece-16:0-CoA to 5-trans-tetradece-16:0-CoA is favored by a factor of 1.5 over the hydrolysis of 5-trans-tetradece-16:0-CoA to 5-trans-tetradece-16:0-CoA (see Fig. 7). This conclusion agrees with the observed greater accumulation of 5-trans-tetradece-16:0-CoA than 5-trans-tetradece-16:0-CoA.

**DISCUSSION**

This study was initiated with the aim of analyzing the mitochondrial β-oxidation of trans fatty acids at the molecular level. The reported formation of 5-trans-tetradece-16:0-acid from elaidic acid (9-trans-octadecenoic acid) in perfused rat hearts (4) prompted the idea that β-oxidation of trans fatty acids may be an atypical process, because the substrate or part of the substrate was incompletely degraded. In contrast, oleic acid did not give rise to a partially degraded substrate. Despite its incomplete degradation, elaidic acid seemed to be a sufficient energy source to support high rates of respiration in isolated rat heart and liver mitochondria (see Ref. 20 and this study). Hence, the energy support high rates of respiration in isolated rat heart and liver mitochondria (see Ref. 20 and this study). Hence, the energy support high rates of respiration in isolated rat heart and liver mitochondria (see Ref. 20 and this study). Hence, the energy support high rates of respiration in isolated rat heart and liver mitochondria (see Ref. 20 and this study). Hence, the energy support high rates of respiration in isolated rat heart and liver mitochondria (see Ref. 20 and this study).

Another consequence of the accumulation of 5-trans-tetradece-16:0-CoA in the mitochondrial matrix is expected to cause the rate of its degradation to increase to a level that is achieved with a lower concentration of 5-cis-tetradece-16:0-CoA. This conclusion is supported by the observation that 5-trans-tetradece-16:0-CoA supports a rate of respiration that is only 20% lower than rates obtained with 5-cis-tetradece-16:0-CoA or myristoyl-CoA as substrates. Any remaining difference between the rates of elaidate and oleate β-oxidation may be due to the inhibitory effects of accumulated 5-trans-tetradece-16:0-CoA or to the adverse effect of the trans double bond on other reactions of β-oxidation.

The partial degradation of elaidic acid to 5-trans-tetradece-16:0-acid and 5-trans-tetradece-16:y carnitine raises the ques-
tion as to the fate of these metabolites and their possible impact on cellular metabolism and/or physiology. 5-trans-Tetradecenoylcarnitine can be converted back to 5-trans-tetradecenoyl-CoA by CPT II and degraded by H9252-oxidation either immediately or after leaving and re-entering mitochondria in the same or other tissues. 5-trans-Tetradecenoic acid, however, must be reactivated by conversion to its CoA derivative in the cytosol before it can be utilized as substrate of H9252-oxidation, lipid synthesis, and possibly protein myristoylation. Its possible substitution for myristoyl-CoA in the modification of proteins, especially of G proteins, could affect the functions of such proteins in cell signaling, because the presence of double bonds in the acyl chain has been shown to reduce the affinity of myristoylated proteins for lipid rafts (24, 25). In the retina, 5-cis-tetradecenoyl and 5-cis,8-cis-tetradecadienoyl residues partially replace the myristoyl group in transducin and some other G proteins (reviewed in Ref. 26). This heterogeneous acylation of transducin and other retinal proteins is assumed to be physiologically significant, although this idea remains to be proven.

The incomplete degradation of elaidic acid raises the specter of other monounsaturated and polyunsaturated trans fatty acids yielding novel fatty acids by partial H9252-oxidation. Because of continuing health concerns about the consumption of trans fatty acids, it seems prudent to study the H9252-oxidation of major trans fatty acids and to assess the biological effects of products that are formed by partial H9252-oxidation. Such evaluation should include conjugated linoleic acids, because they contain trans double bonds and are important constituents of the human diet due to their presence in dairy products, meat of ruminants, and partially hydrogenated vegetable oils.

The observed incomplete degradation of a fraction of elaidic acid contradicts the general conclusion that under normal conditions mitochondrial H9252-oxidation facilitates the complete breakdown of fatty acids and proceeds without the accumulation of extensive amounts of intermediates (reviewed in Ref. 5). The accumulation of substantial quantities of partially degraded fatty acids has only been observed in cases of enzyme deficiencies (27, 28) or when inhibitors of H9252-oxidation enzymes or respiration were added to mitochondria (29). However, under such conditions H9252-oxidation usually is severely or completely inhibited. The prevailing view of mitochondrial H9252-oxidation...


\textbf{Scheme 1. Degradation of elaidoyl-CoA in rat liver mitochondria.} Compound I, elaidoyl-CoA; compound II, 5-trans-tetradecenoyl-CoA; compound III, 5-trans-tetradecenoic acid; compound IV, 5-trans-tetradecenoylcarboxinone; compound V, 2-trans,5-trans-tetradecadienoyl-CoA. TE, thioesterase; CPT II, carnitine palmitoyltransferase.

\textbf{References}

1. Craig-Schmidt, M. C. (1998) in \textit{Trans Fatty Acids in Human Nutrition} (Sébeédio, J. L., and Christie, W. W., eds) pp. 59–113, The Oily Press, Dundee, Scotland.

2. Aro, A. (1998) in \textit{Trans Fatty Acids in Human Nutrition} (Sébeédio, J. L., and Christie, W. W., eds) pp. 235–260, The Oily Press, Dundee, Scotland.

3. Elman, G. L. (1959) \textit{J. Biol. Chem.} 234, 155–162.

4. Davidson, B. (1994) \textit{J. Biol. Chem.} 269, 1027–1033.

5. Thorpe, C. (1986) \textit{J. Biol. Chem.} 261, 1129–1141.

6. Ellman, G. L. (1959) \textit{Arch. Biochem. Biophys.} 82, 70–77.

7. Nadergaard, M. M., and Cannon, B. (1979) \textit{Methods Enzymol.} 58, 390–398.

8. Nadergaard, M. M., and Cannon, B. (1979) \textit{Methods Enzymol.} 58, 390–398.

9. Nadergaard, M. M., and Cannon, B. (1979) \textit{Methods Enzymol.} 58, 390–398.

10. Nadergaard, M. M., and Cannon, B. (1979) \textit{Methods Enzymol.} 58, 390–398.

11. Nadergaard, M. M., and Cannon, B. (1979) \textit{Methods Enzymol.} 58, 390–398.

12. Nadergaard, M. M., and Cannon, B. (1979) \textit{Methods Enzymol.} 58, 390–398.

13. Nadergaard, M. M., and Cannon, B. (1979) \textit{Methods Enzymol.} 58, 390–398.

14. Nadergaard, M. M., and Cannon, B. (1979) \textit{Methods Enzymol.} 58, 390–398.

15. Nadergaard, M. M., and Cannon, B. (1979) \textit{Methods Enzymol.} 58, 390–398.

16. Nadergaard, M. M., and Cannon, B. (1979) \textit{Methods Enzymol.} 58, 390–398.

17. Nadergaard, M. M., and Cannon, B. (1979) \textit{Methods Enzymol.} 58, 390–398.

18. Nadergaard, M. M., and Cannon, B. (1979) \textit{Methods Enzymol.} 58, 390–398.

19. Nadergaard, M. M., and Cannon, B. (1979) \textit{Methods Enzymol.} 58, 390–398.

20. Nadergaard, M. M., and Cannon, B. (1979) \textit{Methods Enzymol.} 58, 390–398.

21. Nadergaard, M. M., and Cannon, B. (1979) \textit{Methods Enzymol.} 58, 390–398.

22. Nadergaard, M. M., and Cannon, B. (1979) \textit{Methods Enzymol.} 58, 390–398.

23. Nadergaard, M. M., and Cannon, B. (1979) \textit{Methods Enzymol.} 58, 390–398.

24. Nadergaard, M. M., and Cannon, B. (1979) \textit{Methods Enzymol.} 58, 390–398.

25. Nadergaard, M. M., and Cannon, B. (1979) \textit{Methods Enzymol.} 58, 390–398.

26. Nadergaard, M. M., and Cannon, B. (1979) \textit{Methods Enzymol.} 58, 390–398.

27. Nadergaard, M. M., and Cannon, B. (1979) \textit{Methods Enzymol.} 58, 390–398.

28. Nadergaard, M. M., and Cannon, B. (1979) \textit{Methods Enzymol.} 58, 390–398.

29. Nadergaard, M. M., and Cannon, B. (1979) \textit{Methods Enzymol.} 58, 390–398.

30. Nadergaard, M. M., and Cannon, B. (1979) \textit{Methods Enzymol.} 58, 390–398.
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