Arachidonic Acid Formed by Peroxisomal β-Oxidation of 7,10,13,16-Docosatetraenoic Acid Is Esterified into 1-Acyl-sn-glycero-3-phosphocholine by Microsomes*

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Peroxisomal β-oxidation of linoleic acid and arachidonic acid was depressed when 1-palmitoyl-sn-glycero-3-phosphocholine and microsomes were included in incubations. This reduction was due to the esterification of the substrate into the acceptor by microsomal 1-acyl-sn-glycero-3-phosphocholine acyltransferase. The first cycle of the β-oxidation of 7,10,13,16-docosatetraenoic acid was independent of 1-acyl-sn-glycero-3-phosphocholine and microsomes. However, when arachidonate was produced it was esterified rather than serving as a substrate for continued β-oxidation. When arachidonate and linoleate were incubated with peroxisomes alone, 2-trans-4,7,10-hexadecatetraenoic acid and 2-trans-4-decadienoic acid were the respective end products of β-oxidation. 2-Oxo-8,11-heptadecadienone, a catabolite produced from linoleate, was most likely a nonenzymatic decarboxylation product of 3-oxo-9,12-octadecadienoic acid. In addition to the termination of β-oxidation by microsomal-peroxisomal communication, our results with linoleate and arachidonate suggest that the reaction catalyzed by 2-trans-4-cis-dienoyl-CoA reductase is the control step in double bond removal. In addition, the β-ketothiolase step may play a regulatory role in the peroxisomal β-oxidation of linoleate but not arachidonate or 7,10,13,16-docosatetraenoic acid.

Hepatic biosynthesis of PUFA,[1] from dietary precursors, is a microsomal process that proceeds via alternating position-specific acyl-CoA desaturases and malonyl-CoA-dependent chain elongation reactions (1). Catabolism of PUFA, via the β-oxidation spiral, may take place in both mitochondria and peroxisomes. A generally accepted role for peroxisomes is to chain-shorten long-chain fatty acids which may then be transported to mitochondria where the process is completed (2–4). Under normal physiological conditions, mitochondrial β-oxidation, once initiated, is generally thought to proceed to completion with little if any accumulation of chain-shortened products. Biosynthesis and catabolism of PUFA are thus two totally separate processes that are compartmentalized within the cell.

It has, however, long been recognized that in the intact animal long-chain PUFA are partially β-oxidized with the esterification of chain-shortened intermediates into membrane lipids. When 4,7,10,13,16-22:5 (5) and 7,10,13,16-22:4 (6) were fed to rats raised on a fat-free diet, there was a large increase of esterified arachidonate in hepatic lipids. When [3-¹⁴C]10,13,16-22:3 was administered to rats, it was possible to detect esterified arachidonate in both mitochondrial and microsomal phospholipids (7). The implication of this finding is that [3-¹⁴C]10,13,16-22:3 is β-oxidized to [1-¹⁴C]8,11,14-20:3 which then moves to the endoplasmic reticulum where it is desaturated to arachidonate. The physiological relevance of this apparent intracellular movement of fatty acids between the endoplasmic reticulum and a site for β-oxidation was not apparent in these early studies.

We recently refuted the commonly accepted, but untested, hypothesis that desaturation of 7,10,13,16,19-22:5 to 4,7,10,13,16,19-22:6 is catalyzed by a microsomal acyl-CoA-dependent 4-desaturase. In these studies, we showed that 7,10,13,16,19-22:5 was the precursor for 4,7,10,13,16,19-22:6, but the pathway proceeded as follows: 7,10,13,16,19-22:5 ⇒ 9,12,15,18,21-24:5 ⇒ 6,9,12,15,18,21-24:6 ⇒ 4,7,10,13,16,19-22:6. The first two reactions take place in the endoplasmic reticulum followed by the transfer of 6,9,12,15,18,21-24:6 to a site for partial β-oxidation where it is chain-shortened to 4,7,10,13,16,19-22:6 (8). Recent studies have shown that 7,10,13,16-22:4 is metabolized to 4,7,10,13,16-22:5 via an analogous pathway.[2]

Martinez (9) reported that liver phospholipids of a patient with Zellweger's disease contained low levels of esterified 4,7,10,13,16-22:5 and 4,7,10,13,16,19-22:6. These compositional findings suggest that peroxisomes are required for the synthesis of these two fatty acids from dietary precursors. When [3-¹⁴C]7,10,13,16-22:4 was incubated with rat hepatocytes or injected into the tail vein of rats, its primary anabolic fate was partial β-oxidation to arachidonate followed by its esterification into phospholipids (10). Fibroblasts from control patients metabolized [3-¹⁴C]7,10,13,16-22:4 to yield esterified arachidonate. Conversely, fibroblasts from a Zellweger's patient did not metabolize the substrate to yield any esterified arachidonate. Both cells, however, metabolized the substrate into radioactive water-soluble compounds (11). Collectively, the results suggest that the partial β-oxidation process is confined to peroxisomes.

In the study reported here, we compare the rates of β-oxidation of 1-¹⁴C- versus 3-¹⁴C-labeled 7,10,13,16-22:4 and show direct intracellular communication between peroxisomes and microsomes in that the primary metabolic fate of arachidonate is esterification into exogenously added 1-acyl-GPC rather than continued β-oxidation.

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[1] The abbreviations used are: PUFA, polyunsaturated fatty acids; 1-acyl-GPC, 1-palmitoyl-sn-glycero-3-phosphocholine; TES, N-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid; HPLC, high performance liquid chromatography; GC-MS, gas chromatography-mass spectrometry.

[2] B. S. Mohammed, S. Sankarappa, and H. Sprecher, manuscript in preparation.

EXPERIMENTAL PROCEDURES

Materials—ATP, NADH, CoASH, Hepes, dithiothreitol, TES, NADP⁺, NAD⁺, and essentially fatty acid-free bovine serum albumin were from Sigma. Antimycin A, lactate dehydrogenase, and Nycodenz (Accudenz) were obtained respectively from Fluka, Boehringer Mannheim, and Accurate Chemical and Scientific Corp. All phospholipids were purchased from Avanti while linoleic, arachidonic, and 7,10,13,16-docosatetraenoic acid were from Nu-Check Prep. [U-^{14}C]Linoleic acid and 5,6,8,9,11,12,14,15-[^{3}H]arachidonic acid were from DuPont NEN while [1-^{14}C]linoleic acid, [1-^{14}C]arachidonic acid, [1-^{14}C]- and [3-^{14}C] 7,10,13,16-docosatetraenoic acid, 4-decenoic acid, 5,8-tetradecadienoic acid, 7,10-hexadecadienoic acid, 4,7,10-hexadecatrienoic acid, 17,17,18,18-d_4-linoleic acid, and 19,19,20,20-d_4-arachidonic acid were made by total synthesis (12–14).

Isolation of Peroxisomes and Microsomes from Rat Liver—Male Sprague-Dawley rats were maintained on a chow diet containing 0.5% clofibrate (prepared by Dyets, Bethlehem, PA) for 8 days prior to being sacrificed. Peroxisomes were isolated in essence as described by Das *et al.* (15). Liver was homogenized in 0.25 M sucrose, 0.1 mM EDTA, 0.1% ethanol, 10 mM TES, pH 7.5. The nuclei and mitochondria obtained by centrifugation at 600 × *g* for 10 min and 3300 × *g* for 10 min were discarded. The light mitochondrial pellet that sedimented at 25,000 ×*g* for 10 min was washed with the homogenizing buffer and recentrifuged at 25,000 × *g* for 10 min. This light mitochondrial fraction was resuspended in the homogenizing medium in a volume corresponding to 1 ml/g liver. Two ml of this suspension were layered on 15 ml of 35% Nycodenz (w/v) containing 10 mM TES, 0.1% ethanol, and 0.1 mM EDTA, pH 7.5, and centrifuged at 56,000 ×*g* for 50 min. The peroxisomal pellet was suspended in the incubation medium which contained 130 mM KCl, 20 mM Hepes, pH 7.2, and the protein concentration was adjusted to 3 mg/ml using the Coomassie Blue reagent (Pierce Chemical Co.) to assay protein. The purity of the peroxisomes was determined by marker enzyme analysis using catalase for peroxisomes (16), glucose-6-phosphatase for microsomes (17), and succinate cytochrome *c*-reductase for mitochondria (18). The relative specific activities (average of six determinations ± S.D.) for glucose-6-phosphatase and succinate cytochrome *c*-reductase in peroxisomes relative to the total liver homogenate were, respectively, 1.45 ± 0.31 and 0.21 ± 0.08. The activity of catalase in peroxisomes was 1486 ± 372 units/mg of peroxisomal protein. In order to prepare microsomes, the 25,000 × *g* supernatant was centrifuged at 110,000 × *g* for 45 min. The microsomal pellet was resuspended in a medium containing 0.6 M KCl, 0.1 M phosphate, pH 7.4, and the microsomal pellet obtained by centrifugation at 110,000 ×*g* for 45 min was suspended in 130 mM KCl, 20 mM Hepes, pH 7.2, and stored at –80 °C (19).

Peroxisomal β-Oxidation—Peroxisomes (300 µg of protein/ml) were incubated at 37 °C in a shaking water bath in a medium that contained 130 mM KCl, 20 mM Hepes, 0.1 mM EGTA, 0.5 mM NAD⁺, 0.1 mM NADP⁺, 0.1 mM dithiothreitol, 0.2 mM CoASH, 10 mM Mg^{2+}ATP, 20 mM pyruvate, 10 µg of antimycin, and 2 units of lactate dehydrogenase (20). Reactions were initiated by the addition of the sodium salt of the fatty acid (2 Ci/mol) that was bound to bovine serum albumin in a 2:1 molar ratio. Aliquots of 200 µl were removed and added to an equal volume of 5% HClO₄. After 30 min at 4 °C, the samples were centrifuged and 200 µl was counted to measure acid-soluble radioactivity. Other incubations also contained 100 µM 1-acyl-GPC and 300 µg of microsomal protein. After 30 min, the reactions were terminated by addition of 1.7 ml of MeOH and 3.4 ml of CHCl₃. The tubes were centrifuged, the bottom organic layer was taken to dryness, and the neutral lipids were separated from phospholipids by sequential elution of Pasteur pipettes packed with Unisil (Clarkson Chemical Co., Williamsport, PA) with CHCl₃ and MeOH. The MeOH was removed under N₂, 1 ml of CHCl₃/MeOH was added, and an aliquot was counted. A second aliquot was applied to a Whatman LK5 plate which was developed with CHCl₃/MeOH/40% methylamine (65:25:5, v/v) (21). The zones corresponding to various phospholipids were scraped into scintillation vials for counting. The remainder of the total phospholipids was interesterified by stirring them overnight with anhydrous 5% HCl in MeOH. The methyl esters were separated by HPLC by eluting a 0.46 × 25 cm Zorbax ODS column with acetonitrile/water (90:10, v/v) at 1 ml per min. The effluent was mixed with ScintiVerse LC (Fisher) (3 ml/min), and radioactivity was detected with a Beckman 171 radioisotope detector.

Metabolite Isolation and Characterization—Peroxisomes were incubated with either 100 µM [U-^{14}C]linoleic acid (2 Ci/mol) or [5,6,8,9,11,12,14,15-^{3}H]arachidonic acid (10 Ci/mol). After 30 min, the incubations were terminated by addition of 0.25 ml of 4 N NaOH and 1.7 ml of MeOH. The contents were stirred overnight, acidified with 6 N
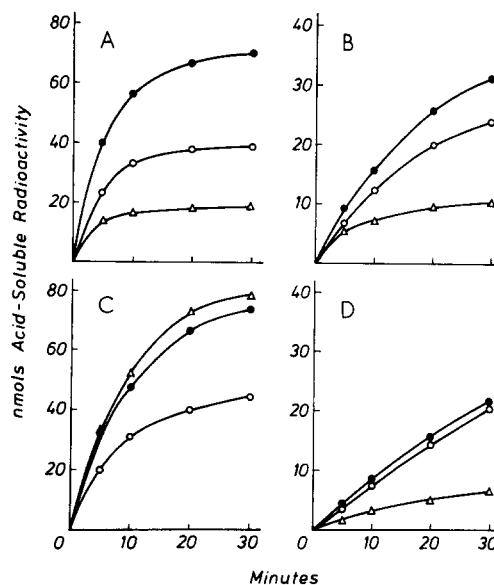

FIG. 1. **The time-dependent β-oxidation of [1-^{14}C]9,12-18:2 (*A*), [1-^{14}C]5,8,11,14-20:4 (*B*), [1-^{14}C]7,10,13,16-22:4 (*C*), and [3-^{14}C] 7,10,13,16-22:4 (*D*).** Peroxisomes (300 µg of protein/ml) were incubated alone with 50 (○) or 100 (●) µM concentrations of fatty acids as well as when incubations with 100 µM levels of fatty acids also contained 100 µM 1-acyl-GPC and 300 µg of microsomal protein (△). Incubation conditions are described under "Experimental Procedures." At the indicated times, 200 µl was removed and added to 200 µl of cold 5% HClO₄. After centrifugation, 200 µl of the supernatant was used to measure acid-soluble radioactivity.

HCl, and 3.4 ml of CHCl₃ was added. The bottom layer was taken to dryness, and the residue was reacted with 5% anhydrous HCl in MeOH. The radioactivity was then monitored by HPLC by eluting the column with acetonitrile/H₂O (85:15, v/v). Large-scale incubations were then carried out using 17,17,18,18-d_4-linoleic acid and 19,19,20,20-d_4-arachidonic acid. Appropriate fractions were collected from the column effluent, the acetonitrile was removed under N₂, and metabolites were recovered by extraction with 20% diethyl ether in petroleum ether. Trace amounts of water were removed by passing the combined extract through a Pasteur pipette packed with granular Na₂SO₄. The solvent was removed under N₂, and the compounds were dissolved in iso-octane and analyzed by mass spectrometry. Mass spectrometry was carried out with a Hewlett-Packard Model 5970A mass selective detector and a 5770 gas chromatograph containing a 30-m × 0.25-mm, inside diameter, DB-225 column (J and W Scientific). Injections were made in the splitless mode at 70 °C, and, after 3 min, the oven was programmed to 185 °C at 15 °C/min.

RESULTS

The results in Fig. 1 compare the time-dependent rates of β-oxidation when peroxisomes were incubated with either 50 µM (○) or 100 µM (●) [1-^{14}C]9,12-18:2 (*A*), [1-^{14}C]5,8,11,14-20:4 (*B*), [1-^{14}C]7,10,13,16-22:4 (*C*), or [3-^{14}C]7,10,13,16-22:4 (*D*). At 5 min, the rates of β-oxidation of all three 1-^{14}C-labeled substrates were greater when the higher level of substrate was used. In the latter case, the nanomoles of acid-soluble radioactivity produced at 5 min from 100 µM [1-^{14}C]9,12-18:2, [1-^{14}C]5,8,11,14-20:4, and [1-^{14}C]7,10,13,16-22:4 were thus, respectively, 40, 11, and 31. Arachidonic acid was a comparatively poor substrate for peroxisomal β-oxidation as was previously reported (22). The nonlinearity, in the apparent time-dependent rate of β-oxidation, was due both to substrate depletion and the generation of unlabeled chain-shortened products which continue to serve as substrates for the β-oxidation spiral. When the rate of two cycles of β-oxidation was assayed using [3-^{14}C]7,10,13,16-22:4, the amount of acid-soluble radioactivity produced was relatively independent of substrate concentration (Fig. 1*D*).

TABLE I
The percent of radioactivity in individual phospholipids

Phospholipids were separated by thin layer chromatography using Whatman LK5 plates which were developed with $CHCl_3/MeOH/40\%$ methylamine (65:25:5, v/v) (21). The zones corresponding to authentic phospholipids were scraped into scintillation vials and counted.

Phospholipid	Substrate		
	$[1\text{-}^{14}C]$ 9,12-18:2	$[1\text{-}^{14}C]$ 5,8,11,14-20:4	$[3\text{-}^{14}C]$ 7,10,13,16-22:4
Phosphatidylethanolamine	2.7	5.6	10.6
Phosphatidylcholine	92.5	88.5	83.0
Sphingomyelin	2.9	2.5	3.0
Phosphatidylserine	1.0	1.1	1.4
Phosphatidylinositol	0.8	2.3	2.0

When the four substrates (100 μM) were now incubated with 100 μM 1-acyl-GPC and 300 μg of microsomal protein (△), there was a decline in the amount of acid-soluble radioactivity produced from $[1\text{-}^{14}C]9,12\text{-}18:2$, $[1\text{-}^{14}C]5,8,11,14\text{-}20:4$, and $[3\text{-}^{14}C]7,10,13,16\text{-}22:4$. The rate of β-oxidation of $[1\text{-}^{14}C]$ 7,10,13,16-22:4 was not depressed by addition of 1-acyl-GPC and microsomes.

Incubations after 30 min with the four substrates and 1-acyl-GPC and microsomes were then extracted as per Folch *et al.* (23). When $[1\text{-}^{14}C]9,12\text{-}18:2$, $[1\text{-}^{14}C]5,8,11,14\text{-}20:4$, $[3\text{-}^{14}C]$ 7,10,13,16-22:4, and $[1\text{-}^{14}C]7,10,13,16\text{-}22:4$ were the substrates, the amount of radioactivity recovered in the bottom layer was, respectively, 68, 82, 62, and 15% of what was added to the incubations.

In essence, all of the radioactivity in the bottom organic layer was esterified into phospholipids. This was established either by sequentially eluting the lipids from a Unisil column with 10 ml of $CHCl_3$ and 10 ml of MeOH or by chromatographing an aliquot of total lipids on Whatman LK5 plates using diethyl ether/hexane/acetic acid (70:30:1, v/v). More than 95% of the radioactivity was in the MeOH eluate or remained at the origin of the thin layer plate.

The results in Table I tabulate the percent of radioactivity in individual phospholipids when they were separated by thin layer chromatography from those incubations containing $1\text{-}^{14}C\text{-}$ labeled linoleate or arachidonate and $[3\text{-}^{14}C]7,10,13,16\text{-}22:4$. As expected, the majority of the radioactivity co-migrated with choline phosphoglycerides, thus showing that the exogenously added acceptor was esterified. When $[1\text{-}^{14}C]5,8,11,14\text{-}20:4$ or $[3\text{-}^{14}C]7,10,13,16\text{-}22:4$ was the substrate, small amounts of radioactivity co-migrated with ethanolamine phosphoglycerides suggesting that microsomes may have contained trace amounts of 1-acyl-*sn*-glycero-3-phosphoethanolamine. The total phospholipids were now interesterified, and the methyl esters were analyzed by HPLC. When $[1\text{-}^{14}C]9,12\text{-}18:2$ was the substrate, over 97% of the esterified radioactivity co-migrated with methyl linoleate. In a similar way when $[1\text{-}^{14}C]5,8,11,14\text{-}20:4$ was the substrate, again over 97% of the radioactivity had a retention time identical with arachidonate. Conversely, when $[3\text{-}^{14}C]7,10,13,16\text{-}22:4$ was the substrate, 77% of the esterified radioactivity was arachidonate and 19% was 7,10,13,16-22:4. These results show that when $[1\text{-}^{14}C]9,12\text{-}18:2$ and $[1\text{-}^{14}C]5,8,11,14\text{-}20:4$ were the substrates the reduced rates of β-oxidation were due to esterification of the substrate into 1-acyl-GPC. The rate of the first cycle of 7,10,13,16-22:4 β-oxidation was in essence independent of exogenous 1-acyl-GPC. As soon as arachidonate was produced, its primary metabolic fate was esterification into the acceptor rather than continued β-oxidation.

When $[3\text{-}^{14}C]7,10,13,16\text{-}22:4$ was incubated with peroxisomes, the only radioactive metabolite that was detected was arachidonate. The results in Fig. 2 show the time-dependent β-oxidation of 7,10,13,16-22:4 and the accumulation of arachi-
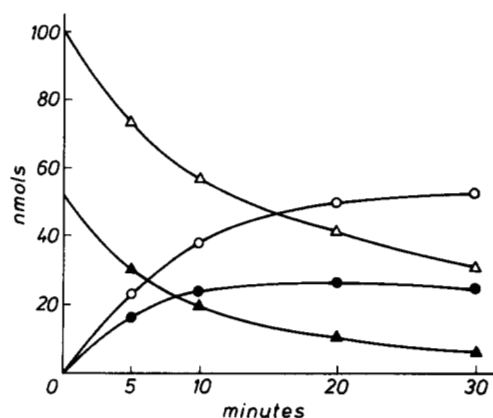

FIG. 2. **The time-dependent disappearance of 7,10,13,16-22:4 when peroxisomes were incubated with either 50 (▲) or 100 μM (△) $[1\text{-}^{14}C]7,10,13,16\text{-}22:4$ *versus* the accumulation of labeled arachidonate when peroxisomes were incubated with 50 (●) or 100 (○) μM $[3\text{-}^{14}C]7,10,13,16\text{-}22:4$**

donate. The results clearly show that arachidonate accumulates thus potentially making it available for movement out of the peroxisomes for use as as a substrate for membrane lipid biogenesis. However, the results in Fig. 1 also show that when $[1\text{-}^{14}C]5,8,11,14\text{-}20:4$ was incubated alone with peroxisomes it was β-oxidized although at a relatively slow rate. To determine what products were generated by β-oxidation of arachidonate, peroxisomes were incubated with 100 μM tritium-labeled arachidonic acid. When the methyl esters were analyzed by HPLC, it was not possible to detect any radioactive compound where we would expect to find 18-carbon compounds. Only two radioactive compounds were detected with the latter compound having a retention time identical with methyl 4,7,10-16:3. These two compounds were then isolated from incubations that were carried out with $19,19,20,20\text{-}d_4\text{-}5,8,11,14\text{-}20:4$. When the latter eluting compound, from HPLC, was analyzed by GC-MS, its retention time was identical with methyl 4,7,10-16:3 (16.5 min) and it could be identified, from its mass spectrum, as methyl $15,15,16,16\text{-}d_4\text{-}4,7,10\text{-}16:3$. The earlier eluting more polar compound had a retention time of 19.0 min when analyzed by GC-MS and it had small ions at $m/z = 266$ (1%) and at $m/z = 234$ (M – 32; 1%). It is thus most likely methyl $15,15,16,16\text{-}d_4\text{-}2\text{-}trans\text{-}4,7,10\text{-}16:4$.

As shown in Fig. 3, a more complex radiochromatogram was obtained when the products of an incubation with peroxisomes and $[U\text{-}^{14}C]9,12\text{-}18:2$ were separated by HPLC. Compounds 1, 2, 3, and 5 co-eluted, respectively, with the methyl esters of 4-10:1, 3,6-12:2, 5,8-14:2, and 7,10-16:2. These metabolites were then isolated from incubations carried out with $17,17,18,18\text{-}d_4\text{-}9,12\text{-}18:2$. When fraction 1 was analyzed by GC-MS, it was resolved into two compounds with the faster eluting component being methyl $9,9,10,10\text{-}d_4\text{-}4\text{-}10:1$. The mass spectrum of the second compound had an ion at $m/z = 186$ (20%) as well as at 155 (M – 31; 15%) and thus is most likely methyl $9,9,10,10\text{-}d_4\text{-}2\text{-}trans\text{-}4\text{-}10:2$. When metabolite 4 was analyzed by GC-MS, its spectrum had a small ion at $m/z = 254$ (3%) with a base peak at $m/z = 43$ and no other ions of apparent diagnostic value. When this compound was analyzed by thin layer chromatography using hexane/diethyl ether/acetic acid (70:30:1, v/v), it co-migrated with authentic 2-oxo-heptadecanone. It was then derivatized by reaction with methoxamine-HCl which, when analyzed by GC-MS, gave two closely eluting compounds as would be expected for *syn* and *anti* forms of a ketone. The mass spectrum of the latter compound, as shown in Fig. 4, had a molecular ion at $m/z = 283$ as well as ions at $m/z = 268$ (M – 15) and 252 (M – 31). By comparing this spectrum
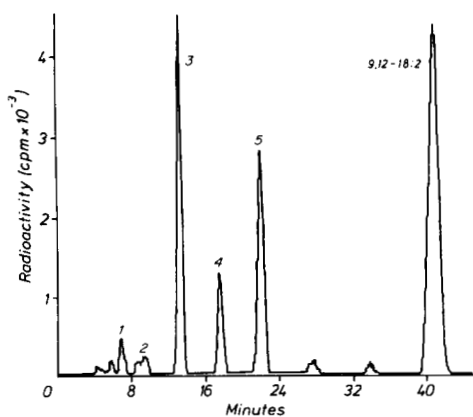
FIG. 3. **A radiochromatogram showing the products of an incubation when peroxisomes (300 µg of protein/ml) were incubated with 100 µm [U-^{14}C]9,12-18:2.** After 30 min, the incubation was terminated, and methyl esters were made and separated by HPLC as described under "Experimental Procedures."
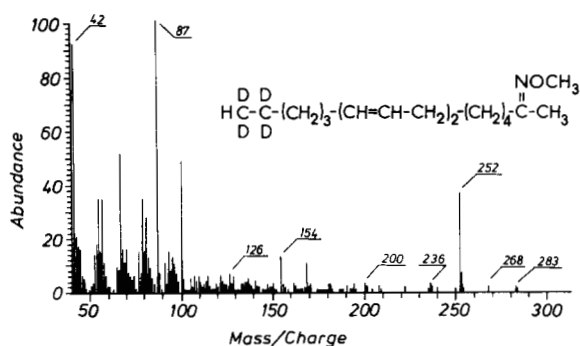

FIG. 4. **The mass spectrum of the methyloxime derivative of metabolite 4 after incubating peroxisomes with 17,17,18,18-d_4-9,12-18:2.**

with that of the methyloxime derivative of synthetic 2-oxo-7,10,13-nonadecatrienone, it was possible to assign its structure as 16,16,17,17-d_4-2-oxo-8,11-heptadecadienone.

DISCUSSION

It is generally accepted that peroxisomes function to chain-shorten fatty acids which then move to the mitochondria where the process is completed (2–4). The termination of peroxisomal fatty acid β-oxidation may be regulated more by competing reactions within the cell than by an absolute chain length-specific block in the β-oxidation spiral. When peroxisomes were incubated with [U-^{14}C]palmitic acid, the complete homologous series of chain-shortened intermediates, including hexanoyl-CoA, was detected (20). Hexanoyl-CoA is a substrate for fatty acid oxidase (24). Peroxisomes thus have the ability to totally β-oxidize palmitic acid. In the study reported here, we show that the depressed rate of β-oxidation of [1-^{14}C]9,12-18:2 and [1-^{14}C]5,8,11,14-20:4, when peroxisomes were incubated with 1-acyl-GPC and microsomes, is due to the esterification of the substrate into the acceptor. By comparing the metabolism of 1-^{14}C- *versus* 3-^{14}C-labeled 7,10,13,16-22:4, it was possible to show that the primary metabolic fate of arachidonate, when it was produced, was esterification into the acceptor rather than continued β-oxidation. Lands *et al.* (25) reported that the V_{max} values for acylation of arachidonate and 7,10,13,16-22:4 into 1-acyl-GPC were, respectively, 72 and 5 nmol/min/mg of rat liver microsomal protein. When 7,10,13,16-22:4 was incubated alone with peroxisomes, it was rapidly β-oxidized. When microsomes and 1-acyl-GPC were now added to the incubation, only small amounts of substrate were removed via esterifica-

tion, and, thus, the rate of β-oxidation was not depressed. However, when arachidonate was produced via β-oxidation, it was preferentially transported out of peroxisomes for esterification rather than for continued β-oxidation.

The types of products that accumulate when peroxisomes are incubated alone with a fatty acid is determined by the structural features of the substrate. When [3-^{14}C]7,10,13,16-22:4 was incubated alone with peroxisomes, it was not possible to detect the accumulation of any radioactive 20- or 22-carbon intermediates prior to the first two β-ketothiolase steps. Hiltunen *et al.* (22) reported that tritium-labeled arachidonic acid was metabolized by peroxisomes into an undefined product that they suggested was either 5,8-14:2 or 4,7,10-16:3. Our results are similar to theirs, but we were able to identify 4,7,10-16:3 and present evidence that it was metabolized by fatty acid oxidase to 2-*trans*-4,7,10-16:4.

[U-^{14}C]9,12-18:2 was chain-shortened via four cycles of β-oxidation to yield 4-10:1 which also was metabolized by fatty acid oxidase to yield 2-*trans*-4-10:2. The most noteworthy difference between the β-oxidation of linoleate *versus* arachidonate was the accumulation of a product identified as 2-oxo-8,11-heptadecadienone. Most likely, this compound was formed by spontaneous decarboxylation of 3-oxo-9,12-18:2 during saponification and subsequent esterification (26). The rates of β-oxidation of [1-^{14}C]7,10,13,16-22:4 and [1-^{14}C]9,12-18:2 as assayed by the production of acid-soluble radioactivity were similar. The possible accumulation of 3-oxo-9,12-18:2 but not 3-oxo-7,10,13,16-22:4 suggests that the reaction catalyzed by β-ketothiolase may be a rate-controlling reaction in the β-oxidation of linoleate as has previously been reported for palmitate, where 3-oxo-16:0 accumulated (20).

The end products detected from the β-oxidation of both linoleate and arachidonate are the first metabolites that would require the NADPH-dependent 2-*trans*-4-*cis*-dienoyl-CoA reductase for continued β-oxidation. When Wigren *et al.* (27) incubated 12-(*S*)-hydroxy-5,8,10,14-20:4 with liver peroxisomes, they detected 8-hydroxy-4,6,10-16:3 as the end catabolite which they suggested moved to mitochondria to complete the β-oxidation process. Neither theirs nor our incubations contained any NADPH. The accumulation of substrates for the 2,4-dienoyl-CoA reductase might thus be due to the absence of a required cofactor rather than a block in the β-oxidation spiral. In recent studies, we have observed that the amount of acid-soluble radioactivity produced from [1-^{14}C]4,7,10-16:3 and [1-^{14}C]4,7,10,13,16-22:5 was relatively independent of exogenous NADPH.[3] It thus appears that the reaction catalyzed by peroxisomal 2,4-dienoyl-CoA reductase may be a rate-limiting step but not an absolute metabolic block in removing double bonds from even-numbered carbon atoms in polyunsaturated fatty acids. Recently, it has been shown that liver mitochondria contain two isoforms of 2,4-dienoyl-CoA reductase while the peroxisomal enzyme is a different protein. Only about 10% of the total activity in liver is associated with peroxisomes when enzyme activity was assayed using 2,4-hexadienoyl-CoA as the substrate (28). It is thus possible when substrates for the peroxisomal 2,4-dienoyl-CoA are produced that they are in part transported to mitochondria to complete the β-oxidation process.

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