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Effects of Chain Length and Sulphur Position of Thia Fatty Acids on Their Incorporation into Phospholipids in 7800 C1 Hepatoma Cells and Isolated Rat Hepatocytes, and Their Effects on Fatty Acid Composition of Phospholipids

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ABSTRACT. Incorporation of thia fatty acids and their effects on the fatty acid composition in phospholipids has been investigated in 7800 C1 hepatoma cells and cultured hepatocytes. 3-Thia fatty acids of chain lengths from dodecyl- to hexadecyl-thioacetic acid were incorporated into phospholipids during a 3-day incubation. Longer and shorter 3-thia fatty acids were barely detectable. Tetradecylthioacetic acid, 3-thia stearate, and their Δ^9-desaturated derivatives were maximally incorporated into whole-cell phospholipids. The amount of tetradecylthioacetic acid incorporated into phospholipids of hepatoma cells remained almost identical in cells cultured for 3 days or adapted over a period of 1 year. Δ^9-desaturated metabolites of long chain thia fatty acids (C_{13}- to C_{16}-S-acetic acid) were identified by GC-MS in phospholipids. 3-Thia stearate appeared to be the best substrate for Δ^9 desaturase. Incubation of hepatoma cells with thia fatty acids led to alterations in the amount of normal fatty acids in total phospholipids. The amounts of 16:0 and 18:1 decreased and 18:2(n-6) and 20:5 (n-3) increased. Changes in the normal fatty acid composition of phospholipids were seen both with thia acids incorporated into phospholipids and those not incorporated. These effects, therefore, may be only partially dependent on displacement of normal fatty acids by thia fatty acids. Morris 7800 C1 hepatoma cell acyl-CoA synthetase (ACS) and peroxisomal acyl-CoA oxidase (ACO) were induced by thia fatty acids of all chain lengths, and with the sulphur atom(s) in different positions. Control experiments with hepatocytes revealed a similar incorporation of thia fatty acids in these physiologically more normal cells. BIOCHEM PHARMACOL 51;6:751–758, 1996.

KEY WORDS. thia fatty acids; phospholipids; gas chromatography; hepatoma cells; hepatocytes; desaturase

The 3-thia fatty acid tetradecylthioacetic acid has a pronounced hypolipidemic effect in rats [1], and the 4-thia fatty acid tetradecylthiopropionic acid (4-thia stearate) induces fatty liver [2] in these animals. We have, therefore, studied the metabolic fates of these and related thia fatty acids.

Tetradecylthioacetic acid, which cannot be β-oxidized, stimulates fatty acid oxidation in hepatocytes [3], probably through release of carnitine acyl transferase inhibition following lowered malonyl-CoA levels [4]. Furthermore, a series of lipid-metabolizing enzymes is induced by tetradecylthioacetic acid [5]. 4-Thia stearate, which can be β-oxidized [6], inhibits fatty acid oxidation [2, 7].

TTAcr‡, the primary metabolite of 4-thia stearate, is an inhibitor of carnitine palmitoyltransferase II and fatty acid oxidation in hepatocytes [8]. Thus, thia fatty acids evidently influence the balance between oxidation of fatty acids and their esterification into complex lipids. Thia fatty acids are, themselves, incorporated into phospholipids.

The 3-thia fatty acids dodecylthioacetic acid [9] and tetradecylthioacetic acid [10], and a 4-thia fatty acid, tetradecylthiopropionic acid (4-thia stearate) [11], are incorporated into phospholipids *in vitro*. Tetradecylthioacetic acid alters the fatty acid composition of total phospholipids in Morris 7800 C1 hepatoma cells [10]. An unknown metabolite of tetradecylthioacetic acid was found in phospholipids of the hepatoma cells in that study and was presumed to be its desaturated derivative.

In the present work, we have systematically investigated the effect of thia fatty acid chain length on the induction of two lipid metabolizing enzymes (ACS and ACO) previously stud-

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‡ *Abbreviations:* ACO, acyl-CoA oxidase; ACS, acyl-CoA synthetase; TTAcr, tetradecylthioacrylic acid; C_n-S-acetate, alkythioacetic acid; C_{15}-S-acetate and C_{14}-S-propionate equal 3- and 4-thia stearate, respectively.

ied in our laboratory. We have also investigated the incorporation of thia fatty acids with different chain lengths and with different sulphur substitution positions into whole-cell phospholipids in Morris 7800 C1 hepatoma cells. The effects of thia fatty acids on the normal fatty acid composition of phospholipids in these cells were also studied. Some experiments using isolated rat hepatocytes in cell culture were also carried out to compare hepatoma cells with a system approaching conditions in normal liver.

MATERIALS AND METHODS

Materials

Ham's F10 medium, horse serum, and calf serum were from Flow Laboratories (Irvine, U.K.). Dulbecco's Modified Eagle (DME) medium, Ultroser G (a serum substitute), gentamicin, anti-pleuropneumonia-like organism agent (anti-PPLO), fungizone, and penicillin-streptomycin were from Gibco (Grand Island, NY).

3- And 4-thia fatty acids were synthesized as previously described [12, 13].

BCl_3-methanol (borontrichloride-methanol) was purchased from Supelco, Inc. (U.S.A.). Chromatography- or GR-grade solvents and other reagents used in sample preparation for gas chromatographic analysis were from Merck (Darmstadt, Germany).

Preparation of 9-Thia Stearate and 3,10-Dithia Stearate

9-Thia stearate was prepared by reacting 2.5 g 8-bromooctanoic acid (Fluka) (11.2 mmol) with 2.3 mL nonanethiol (Fluka) (12.2 mmol) in the presence of NaOH (2 g) in 200 ml ethanol. The mixture was stirred and heated to approximately 40°C until a clear solution was obtained. A massive precipitate was formed upon cooling to room temperature. This was isolated by filtration and dissolved in 150 mL 80% warm methanol and acidified with 4 mL 6N HCl. A clear solution was obtained. Slow cooling to 4°C gave crystalline-pure 9-thia stearate that was isolated by filtration. The yield was nearly 100%. Thin layer chromatography on silica plates with hexane:ether:formic acid (50:50:2) showed only one spot with iodine and, after methylation with borotrichloride:methanol, only one dominating peak was seen in gas chromatography.

3,10-Dithia stearate was prepared in a two-step reaction. First, a mixture of 6-bromooctylthioacetic acid and 1,6-dibromohexane was prepared. One mL of thioglycolic acid (15 mmol) in 10 mL ethanol was added dropwise with stirring to 8 g of 1,6-dibromohexane (Fluka) (33 mmol) in 500 mL ethanol containing 10 g of NaOH. After 30 min. of continued stirring (under nitrogen) 10 mL octanethiol (Fluka) (60 mmol) was added. A mixture of 3,10-dithiastearate and 1,6-bis(octylthio)hexane was formed. After stirring overnight (a precipitate was formed) 100 mL of water was added and a nearly clear solution was obtained upon heating. This alkaline mixture was extracted twice with 150 and 100 mL hexane to remove the bis(octylthio)-hexane. The clear ethanol/water phase was held at −20°C overnight and a precipitate of sodium dithia-stearate was formed. This was isolated by filtration and redissolved in 180 mL 80% ethanol and acidified with 4 mL 6N HCl. After cooling to 4°C, approximately 3 g of pure 3,10-dithia steric acid was isolated by filtration. Thin layer chromatography and gas chromatography showed a single spot and one gas chromatographic peak, respectively.

The compounds were also recrystallized from warm hexane.

Cell Culture of 7800 C1 Morris Hepatoma Cells

7800 C1 Morris hepatoma cells [14] were cultivated as monolayer in 145/20 mm cell culture dishes (Costar) with F10 medium as described by Wu *et al.* [15]. The serum added to the F10 medium gave approximately 500 μM fatty acids (36% linoleic acid, 16% oleic acid, 18% stearic acid, 17% palmitic acid, and 0.2% arachidonic acid) in the medium [10]. Various thia fatty acids were added to the medium when the cells had reached the plateau phase. The medium containing thia fatty acids was renewed on the second day. After 3 days, the medium was removed, the cells were washed twice with 5 mL 0.1 M PBS buffer (pH 7.4) and then scraped into 2 mL of the same buffer. The cell suspension was sonicated for 10 sec with an ultrasonicator cell disruptor and then kept on ice until enzyme measurements and lipid extraction were completed.

Preparation of Rat Hepatocytes

Parenchymal hepatocytes were prepared from male Wistar rats (approx. 250 grams) according to the method of Berry and Friend [16] as modified by Seglen [17]. Isolated rat hepatocytes were centrifuged (600 g), resuspended, and plated in DME medium containing 20 mM HEPES (pH 7.4), Ultroser G (2%, v/v, containing approx. 0.6 mM fatty acids, leading to 12 μM fatty acids in the final medium (20% linoleic acid), gentamicin (50 μg/mL) and 0.37% (w/v) $NaCO_3$ at a cell density of approximately 2 million cells/dish (i.d. 60 mm). The cells were incubated overnight at 37°C in air/CO_2 (19:1). This medium was replaced the next morning with the one containing thia fatty acid. The medium was changed again after 8 hr. After 24 hr, the cells were harvested using the procedure described above. In experiments with hepatocytes, the thia acids were added in F10 medium so that these experiments could be compared with the hepatoma cell studies.

Assays of ACO and ACS

ACO activity was determined spectrophotometrically at 502 nm as described by Small *et al.* [18], with minor modifications. Homogenized hepatoma cells (5 μg protein) in Triton X-100 (0.004%) were incubated with 0.1 M Tris buffer (pH 8.5), 50 μM 2′,7′-dichlorofluorescin diacetate ($\Delta\epsilon_{502}$ = 91000 $M^{-1} \cdot cm^{-1}$), 16 μM FAD^+, 60 U peroxidase, and bovine serum albumin (0.06%, w/v). Reactions were carried out at room temperature (*ca.* 23°C) and were started by the addition of 125 nmol acyl-CoA esters into 1 mL incubation mixture.

Activity of ACS was determined with a radioisotope method [15] and a spectrophotometric method [13].

Protein was measured by the method of Lowry *et al.* [19].

Extraction of Lipids from Cells and Preparation of Samples for Gas Chromatography

n-Butanol extraction of lipids and separation of lipid classes was done by the procedure of Hvattum *et al.* [10]. The phospholipid and triacylglycerol fractions obtained were transesterified according to Metcalfe and Schmitz [20] with a minor modification. The column eluate was evaporated to dryness under nitrogen (when not methylated immediately it was redissolved in 1 mL chloroform). One mL BCl_3-methanol was added. The tubes were flushed with nitrogen and heated in boiling water or in a sandbath at 100°C for 20 min. After cooling, 1 mL of water was added and the methyl esters were extracted with 2 mL of hexane. The hexane phase was transferred to another tube after centrifugation (1100 *g*, 5 min), and a small amount of Na_2SO_4 was added to remove remaining water. After mixing and standing at room temperature for 1 hr, the hexane phase was transferred to another tube. The extract was evaporated under nitrogen and the residue redissolved in 250 µL hexane.

In most experiments, this method led to a variable extra peak that was eluted after the 3-thia fatty acids. This was identified as the sulphoxide of 3-thia fatty acids by comparison with sulphoxide standards. Its formation proved unpredictable. Both 3-thia fatty acids and their Δ^9-desaturated metabolites were converted to their sulphoxides to the same extent (20–70%). These derivatives were evidently formed during sample preparation, because it has been shown that the sulphoxide of 3-thia fatty acid cannot be activated by ACS [21]. The sulphoxides, therefore, cannot be incorporated into the phospholipids although they can be formed biologically in rat liver [22]. Transmethylation at different temperatures or with methanol-HCl had no influence on sulphoxide formation. Repeated tests showed that sulphur oxidation occurred during the isolation of the phospholipids. Contents of the thia fatty acids and their corresponding sulphoxides in phospholipids were quantified separately because of the difference between their response factors. The levels of incorporation of 3-thia fatty acids into phospholipids in Table 1 represent the sum of unchanged thia acids and their sulphoxides.

Gas Chromatography Analysis

The methylated fatty acids of phospholipids and triacylglycerol were analyzed using a 50 m non-polar capillary column (0.25 mm internal diam., SGE-50QC2/BP1, Ringwood, Vic., Australia) on a Shimadzu GC-14A gas chromatograph (Shimadzu Europe, Duisburg, Germany) fitted with a flame ionization detector (FID). One µL samples were injected in the split mode (closed split 40 sec; injector temperature: 290°C). Column temperature was held at 140°C for 5 min, then increased at a rate of 4°C/min to 250°C, which was maintained for 30 min.

Fatty acids were quantified using an internal standard method [23] relative to the heptadecanoic acid released from the added internal standard lipid. Results were calibrated with the response factor of individual fatty acids relative to heptadecanoic acid (its response factor was set to 1.0). All response factors were determined in triplicate at different concentrations. The response factor of the Δ^9-desaturated metabolite was assumed to be the same as corresponding thia fatty acid, because standards are not available.

GC-MS

Phospholipid fractions obtained from n-butanol extracts were hydrolyzed and the resulting free fatty acids we converted to picolinyl esters as reported by Hvattum *et al.* [10]. The identities of thia fatty acyl components were verified by subjecting these esters [24] to GC-MS using a Shimadzu GCMS QP2000 instrument equipped with a non-polar 40 m DB1 quartz capillary column (0.18 mm internal diameter, J&W Scientific, Folsom, CA).

Statistical Treatment of Data

Data were first evaluated statistically with the one-way analysis of variance (ANOVA), and then were analyzed with the Turkey-Kramer multiple comparisons test (GraphPad Software Inc. 1993). Differences between groups were considered significant when $P < 0.05$.

RESULTS

Induction of ACS and ACO by Different Thia Fatty Acids in 7800 C1 Hepatoma Cells

A three-day incubation of 7800 C1 hepatoma cells with 3-thia fatty acids increased both ACS and peroxisomal ACO, two of several enzymes induced by 3-thia fatty acids [5] (Fig. 1). No significant correlation between chain length and enzyme induction was observed. C_{14}-S-acetic acid induced ACS [15] and ACO in these cells as well as in hepatocytes [25].

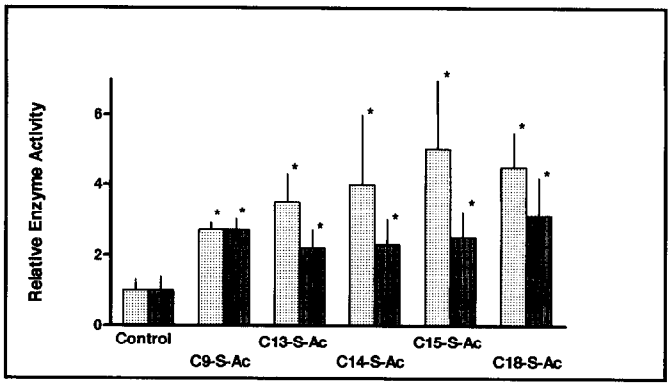

FIG. 1. The effects of thia fatty acids (80 µM) on acyl-CoA synthetase (ACS) and acyl-CoA oxidase (ACO) in Morris 7800 C1 hepatoma cells and rat liver hepatocytes in culture. Enzyme activity is expressed relative to control activity (control = 1). The mean values ± SD are presented from 4 experiments with 7800 C1 hepatoma. The asterisks indicate $P < 0.05$. ▒ ACO; ▓ ACS.

Incorporation of This Fatty Acids into Phospholipids in 7800 C1 Hepatoma Cells and Isolated Hepatocytes

Incorporation of tetradecylthioacetic acid (C_{14}-S-acetic acid) into phospholipids of hepatoma cells reached a maximum after 3 days when cells were cultured in medium containing 80 μM thia fatty acid. Hepatoma cells adapted to a medium with 80 μM C_{14}-S-acetic acid for 1 year contained approx. the same amount of C_{14}-S-acetic acid in total phospholipids as that observed after a 3-day incubation (Table 1). Therefore, a 3-day culture period was utilized for the experiments reported in this communication.

Table 1 shows the effect of chain length on incorporation of 3-thia fatty acids into total phospholipids of 7800 C1 hepatoma cells. C_{12}- to C_{16}-S-acetic acids were well incorporated into phospholipids and incorporation of shorter and longer thia fatty acids was either slight or undetectable. Maximal incorporation was observed with C_{15}-S-acetic acid and C_{14}-S-acetic acid when their unsaturated metabolites were taken into account. C_9-S and C_{10}-S acetic acids were hardly detectable in phospholipids, although they induced ACS and ACO (Fig. 1).

Isolated hepatocytes have a similar pattern of thia fatty acid incorporation into phospholipids as that seen in 7800 C1 hepatoma cells (Table 1). The only difference was a slight shift towards shorter chain lengths. C_{12}-S-acetic acid was incorporated to a larger degree than observed in hepatoma cells and C_{18}-S-acetic acid incorporation was not detectable.

Table 2 shows that the 4-thia acid C_{14}-S-propionate and, especially 9-thia stearate, were poorly incorporated into phospholipids in hepatoma cells and in hepatocytes when compared with the non-β-oxidizable C_{15}-S-acetic acid. 13-thia stearate was also poorly incorporated (not shown). All of these acids have the same chain length. This difference may be at least partially explained by mitochondrial β-oxidation of the 4-thia and 13-thia acids. When aminocarnitine, an inhibitor of mitochondrial β-oxidation [26], was added to the medium (2 experiments), the incorporation of 9-thia and 13-thia stearate into phospholipids increased 5–10-fold in hepatoma cells (not shown).

Identification of Δ^9-Desaturated Metabolites

Analysis of cell extracts from experiments with long-chain thia fatty acids revealed an extra peak that was eluted before the main peak of the thia fatty acid. The differences in retention times suggested the introduction of a double bond [10]. To characterize this material, we incubated Morris 7800 C1 hepatoma cells with C_{14}-S-acetic acid. The presence of a Δ^9-desaturated thia fatty acyl moiety in phospholipids was confirmed by gas chromatography and mass spectrometry of the corresponding picolinyl esters. When run on nonpolar columns, picolinyl ester gives gas chromatographic elution patterns similar to those of methyl esters [27]. The former facilitates assignment of alkyl chain double bonds in gas mass spectrometry. Interpretation of the gas mass spectrum (Fig. 2) according to Harvey [28] and Grav *et al.* [24] suggested the structure 1-(carboxymethylthio)-tetradeca-6-ene, picolinyl ester (Fig. 2).

Table 1 shows that, in hepatoma cells, the most extensive Δ^9-desaturation occurred with C_{15}-S-acetic acid (3-thia stearate). Forty percent of the total C_{15}-S-acetic acid incorporated into phospholipids was found as the desaturated metabolite 3-thia oleic acid. The highest incorporation into phospholipids (sum of original thia acid and desaturated thia acid) was found with 3-thia stearate in these cells.

Δ^9-desaturated metabolites were also found in the isolated hepatocytes, although in relatively smaller amounts than in hepatoma cells. However, it must be kept in mind that the hepatocytes were analyzed after 1 day, while the hepatoma cells were analyzed after 3 days. As in the hepatoma cells, the highest extent of desaturation was found with 3-thia stearate.

TABLE 1. Incorporation of thia fatty acids into total phospholipids of C7800 C1 Morris hepatoma cells and isolated rat hepatocytes

	Incorporation of thia fatty acids into phospholipids (% of total fatty acids)					
	7800 C1 Morris hepatoma cells			Isolated hepatocytes		
Thia fatty acid	Nonmetabolized	Δ°-desaturated	Total	Nonmetabolized	Δ°-desaturated	Total
C_9-S-acetic acid	ND*	ND	ND	–	–	–
C_{10}-S-acetic acid	ND	ND	ND	ND	ND	ND
C_{12}-S-acetic acid	10.4 ± 3.5	ND	10.4	23.4 ± 21.1	ND	23.4, 21.1
C_{13}-S-acetic acid	26.6 ± 3.1	0.9 ± 0.3	27.5	26.5 ± 2.1	0.6 ± 0.06	27.1
C_{14}-S-acetic acid	24.6 ± 4.8	6.8 ± 3.4	31.4	28.0 ± 3.5	1.5 ± 0.2	29.5
C_{14}-S-acetic acid[b]	21.0 ± 3.0	4.9 ± 3.6	25.9	–	–	–
C_{15}-S-acetic acid	19.0 ± 2.1	13.2 ± 4.2	32.2	20.9 ± 1.9	3.1 ± 0.3	24.0
C_{16}-S-acetic acid	18.1 ± 4.3	4.2 ± 0.5	22.3	15.8, 16.4	0.5, 0.5	16.3, 16.3
C_{18}-S-acetic acid	6.2, 3.0	ND	6.2, 3.0	ND	ND	ND

* ND, Not detectable.

7800 C1 Morris hepatoma cells were grown to the plateau phase and then cultured with 80 μM of thia fatty acids for 3 days. Isolated hepatocytes were cultured with DME medium overnight and then exposed to 80 μM of thia fatty acids for 24 hr. Incorporation of thia fatty acids into phospholipids is presented as the mean ± SD from 3–5 separate experiments or as separate values from 2 experiments.

TABLE 2. Incorporation of C_{14}-S-propionic acid and C_{14}-S-acrylic acid into total phospholipids in 7800 C1 Morris hepatoma cells and isolated rat hepatocytes

	Incorporation into phospholipids (% of total fatty acids)							
	7800 Cl hepatoma cells				Isolated hepatocytes			
Added thia fatty acid	C_{14}-S-acrylate	Δ^2 saturated (4-thia-stearate)	Δ^9 desaturated (4-thia-oleate)	Total	C_{14}-S-acrylic acid	Δ^2 saturated (4-thia stearate)	Δ^9 desaturated (4-thia oleate)	Total
80 μM C_{14}-S-propionic acid	0	11.0 ± 0.9	5.9 ± 1.5	16.9	0	4.8 ± 0.7	1.5 ± 0.4	6.3
25 μM C_{14}-S-acrylate*	4.7, 4.1	1.3, 2.3	1.5, 1.5	2.8, 3.8	0	2.2 ± 0.8	1.3 ± 0.4	3.5

* A lower concentration of C_{14}-S-acrylate was used because of its toxicity.

Reduction of TTAcr to 4-thia Stearate Before Incorporation

We have previously shown that TTAcr is activated to its CoA ester and that this is a poor substrate for glycerophosphate acyl transferase [8]. In the present study, we have shown that C_{14}-S-acrylate is incorporated into phospholipids in hepatoma cells but not hepatocytes. It is striking that C_{14}-S-propionate is found in cells incubated with the thia acrylate. No C_{14}-thia acrylate was found in phospholipids from cells incubated with C_{14}-S-propionate (Table 2). These observations indicate that C_{14}-S-acrylyl-CoA can be reduced by a 2-enoyl-CoA reductase to 4-thia stearyl-CoA, which then is a substrate for acyl-CoA Δ^9-desaturase.

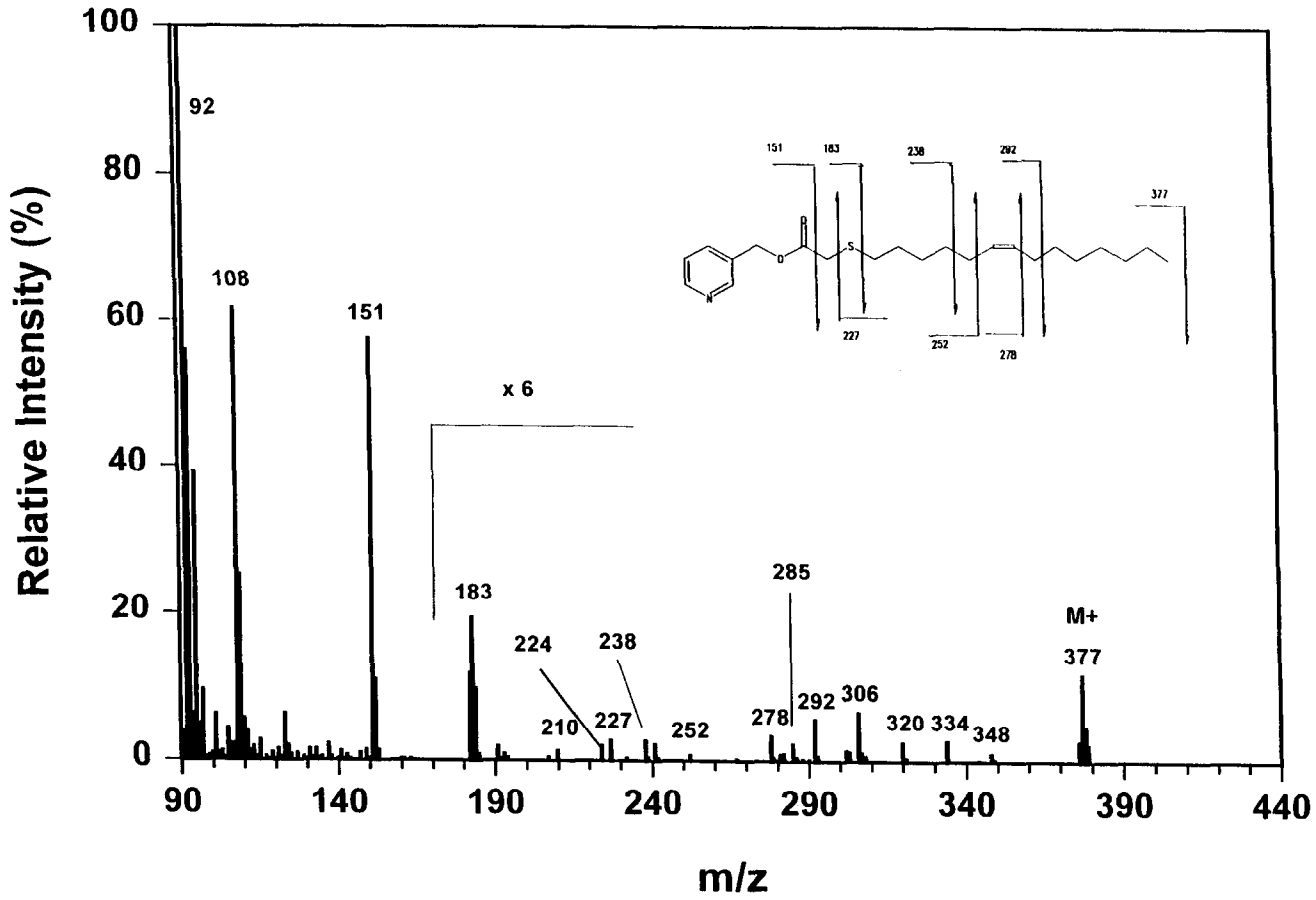

FIG. 2. Mass specturm of the picolinyl ester of desaturated metabolite of tetradecylthisacetic acid. Morris 7800 C1 hepatoma cells were incubated with C_{14}-S-acetic acid and the phospholipids we isolated. The latter were then analysed with gas chromatography and mass spectrography. The insert indicates the presumed origin of major diagnostic ions.

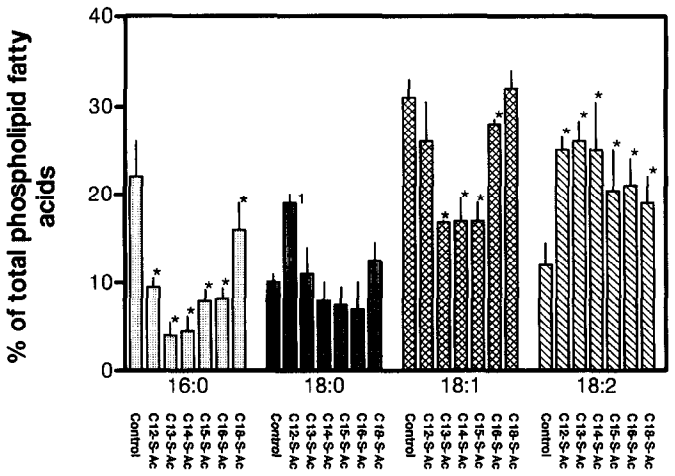

FIG. 3. Effect of chain-length variation of 3-thia fatty acids on the fatty acid composition of phospholipids in Morris 7800 C1 hepatoma cells. Morris 7800 C1 hepatoma cells were grown to the plateau phase and then cultured further with various 3-thia fatty acids. (80 μM) for 3 days. Fatty acids in phospholipids were quantitated by gas chromatography. The fatty acid composition of phospholipids is presented as % of total fatty acids. The bars represent means ± SD from 3 experiments. Asterisks indicate $P < .05$ or less compared to controls. 1, The apparent increase in 18:0 with C_{12}-S-acetate is an artifact caused by the concurent elution of the sulfoxide of the thia acid and stearate.

Effect of Chain Length of 3-Thia Fatty Acids on the Normal Fatty Acid Composition of Phospholipids

The data in Fig. 3 show that, in hepatoma cells, thia fatty acids with chain lengths of from C_{12}-S-acetate to C_{18}-S-acetate reduced phospholipid palmitate. 3-Thia palmitate (C_{13}-S-acetic acid) and C_{14}-S-acetic acid exerted the strongest effects.

No significant alteration in phospholipid stearate levels were noted, with the apparent exception of C_{12}-S-acetate. This apparent increase in phospholipid stearate seen following treatment of hepatoma cells with C_{12}-S-acetic acid is an artifact caused by elution of the sulfoxide of the thia acid concurrently with stearate. In the case of oleate, C_{13}-S-acetate, C_{14}-S-acetate, and C_{15}-S-acetic acid reduced the content to similar degrees. Shorter and longer thia acids were either less effective or had no effect. All 3-thia fatty acids tested increased linoleic acid (18:2) in the phospholipids of hepatoma cells.

Effect of the Position of Sulphur Atom in the Chain of Thia Stearic Acids on the Fatty Acid Composition of Phospholipids

Table 3 shows that thia stearate with a sulphur atom(s) at differing positions have divergent effects on the phospholipid content of normal fatty acids in hepatoma cells. Two experiments with hepatocytes gave similar but less pronounced results (not shown).

All of the thia stearates decreased palmitate (16:0) in phospholipids both in hepatoma cells (Table 3) and hepatocytes, 3-thia stearate showing the strongest effect. 3-Thia stearate decreased palmitate approx. 70% in hepatoma cells (45% in hepatocytes), which correlates with 3-thia stearate's high incorporation level in phospholipids. However, 3,10-dithia stearate also markedly reduced phospholipid palmitate without being incorporated into phospholipids to a significant extent. Thus, alteration of the normal fatty acid content in phospholipids is not entirely dependent upon thia fatty acid incorporation. 9-Thia stearate and 3,10-dithia stearate, which are poorly incorporated into phospholipids, increased stearate approx. 1.5–2-fold and decreased 18:1 (mainly oleate, n-9) approx. 20–30% in the phospholipids of hepatoma cells (Table 3). This suggests that the Δ^9 and Δ^{10} sulphur atoms in these acids inhibit Δ^9-desaturation of stearate. All of the thia stearates tested increased linoleic acid in the phospholipids of hepatoma cells. In hepatocytes (2 experiments), the content of linoleic acid in phospholipids was twice that in hepatoma cells. When cultured with F10 medium, a further marginal increase was observed in 4-, 9-thia and 3,10-dithia stearate treated cells (not shown).

In hepatoma cells, eicosapentanoic acid (EPA, 20:5, n-3) was increased 2–3-fold in phospholipids when treated with thia stearates (with the exception of 4-thia stearate). EPA was not detectable in the phospholipids of the hepatocytes. In hepatocytes, arachidonic acid (20:4,n-6) in total phospholip-

TABLE 3. Effect of thia stearic acids on the fatty acid composition of phospholipids in 7800 C1 Morris hepatoma cells

Fatty acid in total phospholipids	Fatty acid composition of phospholipids, % of total					
	None	Stearate	3-Thia stearate	4-Thia stearate	9-Thia stearate	3, 10-Dithia stearate
16:0	23.9 ± 1.9	21.7 ± 1.6	7.5 ± 1.3^{a,b}	9.7 ± 1.0^{a,b}	18.6 ± 2.2^{a,b,c}	11.3 ± 0.8^{a,b,c,e}
18:0	9.4 ± 1.1	12.2 ± 1.5	6.7 ± 2.3^{b}	8.0 ± 0.3	13.7 ± 1.0^{a,c,d}	18.9 ± 2.9^{a,b,c,d,e}
18:1	31.6 ± 1.0	34.9 ± 1.6^{a}	17.6 ± 0.9^{a,b}	24.1 ± 0.9^{a,b,c}	24.3 ± 2.0^{a,b,c}	20.3 ± 0.8^{a,b,c,d,e}
18:2	11.3 ± 0.3	10.3 ± 1.2	19.6 ± 1.1^{a,b}	24.5 ± 4.5^{a,b}	18.3 ± 1.4^{a,b}	25.3 ± 5.2^{a,b,c,e}
20:4	16.6 ± 4.1	14.8 ± 1.8	12.6 ± 2.2	11.5 ± 2.1	13.9 ± 2.8	12.7 ± 3.5
20:5	1.8 ± 0.3	2.5 ± 0.3	3.8 ± 0.6^{a}	2.2 ± 0.8	5.4 ± 1.4^{a,b,d}	6.2 ± 1.4^{a,b,c,d}
22:6	1.3 ± 0.2	1.4 ± 0.3	1.2 ± 0.2	1.3 ± 0.6	1.2 ± 0.3	1.2 ± 0.3
Incorporation of added fatty acid	–	2.8 ± 0.4	32.2 ± 4.2^{b}	16.9 ± 1.5^{b,c}	1.3 ± 0.6^{c,d}	1.7 ± 0.5^{c,d}

Hepatoma cells were cultured with 80 μM stearate or thia stearates for 3 days. The fatty acid composition of the phospholipids was then analyzed. The values represent the means of 3 experiments ± SD: a, b, c, d, e indicate significant differences ($P < 0.05$) from control, stearate, 3-thia stearate, 4-thia stearate, and 9-thia stearate, respectively.

ids increased approx. 30% with 9-thia stearate or 3,10-dithia stearate.

Stearate had none of these effects in hepatoma cells or hepatocytes (Table 3).

DISCUSSION

The Effect of Chain Length of Thia Fatty Acids in their Enzyme Induction, Δ^9-Desaturation and Incorporation into Phospholipids

Peroxisomal ACO and ACS activities are increased both *in vitro* [12, 15, 25, 29] and *in vivo* [9, 30] with 3-thia fatty acid treatment. Our present work shows that, in the 7800 C1 hepatoma cells, other medium and long-chain 3-thia fatty acids also induce these enzymes with little chain-length specificity.

Previous studies showed that 4-thia stearate does not induce peroxisomes *in vivo* [1, 5, 31, 32] but, in 7800 C1 hepatoma cells, 4-thia stearate does induce peroxisomal acyl-CoA oxidase activity [12]. One possible explanation for the lack of effect of 4-thia fatty acids seen *in vivo* is the toxic effects of these thia fatty acids. 4-Thia stearate and especially its metabolite TTAcr inhibit growth of 7800 C1 hepatoma cells (expressed as a reduction in protein per dish). Separate experiments showed that reduction of the concentration of TTAcr to levels below 80 μM resulted in a marked induction of ACO activity without decreased cell growth. The apparent lack of induction of peroxisomes seen *in vivo* may be due to the toxic effects of 4-thia fatty acids and their acrylic acid metabolite.

The present study has shown that there is only a partial correlation between chain-length specificity for incorporation of thia fatty acids into total cell phospholipids and the effects of thia fatty acids upon the fatty acid composition of these phospholipids. In particular, the increase in linolic acid was independent of thia fatty acid incorporation. No correlation was found between the chain-length specificity for induction of peroxisomal ACO and ACS and for incorporation into total cell phospholipids. Enzyme induction and effects on the normal fatty acid composition of phospholipids seem to be essentially independent of thia fatty acid incorporation into phospholipids.

Morris 7800 C1 hepatoma cells and isolated hepatocytes produce Δ^9-desaturated metabolites of long-chain thia fatty acids. It appears that Δ^9-desaturase has a high chain-length specificity for thia stearates. Only thia fatty acids between C_{14}-S-acetate and C_{16}-S-acetate were Δ^9-desaturated in 7800 C1 hepatoma cells. Among normal saturated fatty acids, stearate is the preferred substrate [33]. Recently Diczfalusy *et al.* [34] used C_{14}-S-acetate as substrate in assay of the Δ^9-desaturase.

Effects of Thia Fatty Acids on the Fatty Acid Composition of Phospholipids

Saturated fatty acids tend to esterify at the *sn*-1 position in glycerophospholipids during *de novo* synthesis [35]. In the present work, only the incorporation into total phospholipids was measured. We have previously shown that dodecylthioacetic acid, tetradecylthioacetic acid and tetradecylthiopropionic acid [9–11] are incorporated mainly into phosphatidylcholine and, to some degree, into phosphatidylethanolamine. We have also shown that 4-thia stearate [11] is incorporated mainly in the *sn*-1 position of phosphotidylcholine. It is likely, therefore, that all the saturated thia fatty acids are incorporated mainly in the *sn*-1 position of phospholipid.

Increases of linoleic acid in the phospholipids in hepatoma cells have previously been observed in the presence of tetradecylthioacetic acid [10]. The medium that we routinely used to cultured 7800 C1 hepatoma cells is relatively rich (approx. 180 μM) in linoleic acid [10]. Therefore, the increase may be a result of increased uptake of this fatty acid from the medium in the presence of tetradecylthia-acetate. This may be caused, at least partially, through induction of ACS (Fig. 1).

Long-chain thia fatty acids increased the content of EPA (20:5, n-3) in hepatoma cells (Table 3) and arachidonic acid (20:4, n-6) in the hepatocytes (not shown). Increases in (n-6) polyunsaturated fatty acids in rat liver after administration of clofibric acid (a peroxisome proliferator) *in vivo* has been reported [36]. It was suggested that peroxisome proliferators may act directly on the desaturation and elongation processes in the synthesis of polyunsaturated fatty acids. However, in our experiments, no increased conversion of linolic acid to arachidonic acid was observed in the hepatoma cells, and the content of arachidonic acid increased in hepatocytes.

Conclusions

3-Thia fatty acids seem to resemble normal saturated fatty acids in phospholipid synthesis processes. Moving the sulphur atom towards the middle of the chain almost abolished incorporation into whole cell phospholipids. However, when β-oxidation (chain-shortening) was inhibited with aminocarnitine, these acids were also incorporated. All thia fatty acids in low concentrations have similar induction effects on long-chain ACS and ACO. They also stimulate the incorporation of polyunsaturated fatty acids into phospholipids. Thia fatty acids appear to have similar effects on the composition of phospholipids in hepatoma cells and isolated hepatocytes, but the effects are more pronounced in hepatoma cells.

The behavior of thia fatty acids in phospholipid synthesis and remodeling processes suggests that thia fatty acids can be used as experimental tools in investigations of the interconversion and incorporation of fatty acids into phospholipids of cell membranes.

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