A rapid assay of acyl-coenzyme A:lysolecithin acyltransferase activity

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Summary A simple and rapid procedure for the assay of acyl-coenzyme A:1-acyl-*sn*-glycero-3-phosphocholine acyltransferase (lysolecithin acyltransferase, LLAT [EC 2.3.1.23]) activity in crude enzyme preparations is described. The incubation system utilizes lysolecithin and [1-^{14}C]-oleoyl-coenzyme A as substrates. Labeled fatty acid released due to accompanying acyl-coenzyme A hydrolase [EC 3.1.2.2] activity is first removed by di-isopropyl ether extraction. The labeled lecithin produced due to LLAT action is then quantitatively recovered by partition of the incubation medium with di-isopropyl ether–*n*-butanol 60:40 (v/v). Selective extraction of the labeled lecithin formed and avoidance of customary thin-layer chromatographic isolation procedures permits assay of LLAT activity with excellent accuracy at a substantial saving of time. The entire assay can be completed in less than 30 min as compared to 2–3 hrs when following conventional procedures.—**Hayase, K., S. Parthasarathy, C. M. Eppler, and W. J. Baumann.** A rapid assay of acyl-coenzyme A:lysolecithin acyltransferase activity. *J. Lipid Res.* 1980. **21:** 484–488.

Abbreviations: LLAT, lysolecithin acyltransferase; acyl-coenzyme A:1-acyl-*sn*-glycero-3-phosphocholine acyltransferase (EC 2.3.1.23); CoASH, coenzyme A; acyl-CoA, acyl-coenzyme A; TLC, thin-layer chromatography.

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Lysolecithin acyltransferase (LLAT; [EC 2.3.1.23]) catalyzes acyl transfer from acyl-coenzyme A to 1-acyl-*sn*-glycero-3-phosphocholine (lysolecithin) resulting in the formation of 1,2-diacyl-*sn*-glycero-3-phosphocholine (lecithin). The enzyme was originally discovered in rat liver microsomes (1), and has since been found in many other systems (2–6). LLAT plays an important role in choline-phospholipid synthesis and is involved in the regulation of cellular phospholipid metabolism through its participation in the deacylation-reacylation cycle (4, 7–9).

Essentially three different assay procedures have heretofore been used for measuring LLAT activity. The procedures are based i) on the appearance of radiolabeled lecithin from labeled acyl-CoA (1, 3, 4, 10, 11); ii) on the appearance of radiolabeled lecithin from labeled lysolecithin (1, 5, 6, 12); and iii) on the spectrophotometric quantitation of liberated CoASH from acyl-CoA (2, 13) upon lysolecithin acylation. The first two methods require rather time-consuming thin-layer chromatographic steps to separate lecithin from labeled fatty acid or lysolecithin, respectively, and to isolate the labeled lecithin formed. The latter method depends on continuous spectrophotometric monitoring of liberated CoASH, after being linked to 5,5′-dithio-*bis*-(2-nitrobenzoic acid), and requires adjustment of apparent LLAT activity for accompanying acyl-CoA hydrolase activity (2, 10, 11, 13).

The LLAT assay described in the present report is a time-saving modification of procedure i) and relies on selective solvent partition of the products of lysolecithin acylation. The assay takes into account the presence of long-chain acyl-CoA hydrolase [EC 3.1.2.2] in common acyltransferase systems.

MATERIALS AND METHODS

[1-^{14}C]Oleoyl-CoA (sp act, 44.4 mCi/mmol; radiopurity > 98%) was purchased from New England Nuclear Corp., Boston, MA, and oleoyl-CoA from Sigma Chemical Co., St. Louis, MO. Lysolecithin was prepared by phospholipase A_2 hydrolysis of egg yolk lecithin (14); phospholipase A_2 (*Ophiophagus hannah* venum) was obtained from Miami Serpentarium Laboratories, Miami, FL. Di-isopropyl ether was purchased from Aldrich Chemical Co., Milwaukee, WI.

Rat liver microsomes used as enzyme source were isolated by established procedures (11) and stored at −70°C prior to use. Protein was determined by a modification (15) of the method of Lowry et al. (16). Radioactivities were measured by scintillation counting in a Packard Tri-Carb Model 2405 scintillation spectrometer. The counting fluid (15 ml/sample) was prepared from 12 g Omnifluor (New England Nuclear), 1000 ml of Triton X-100, and 2000 ml of toluene (counting efficiency, 80–90%).

The recovery of lecithin and fatty acid by the present partition technique was determined in control experiments which showed that 92.9% of labeled lecithin added was recovered in the di-isopropyl ether-butanol phase and that 97.1% of labeled fatty acid was recoverable by di-isopropyl ether extraction.

Lysolecithin acyltransferase assay

The assay mixture containing 50 nmol of 1-acyl-*sn*-glycero-3-phosphocholine, 50 μmol of Tris-HCl buffer (pH 7.2), 10 μmol of $MgCl_2$, 0.01 μCi of [1-^{14}C]oleoyl-CoA, 20 nmol of oleoyl-CoA, and up to 100 μg of microsomal protein in a final volume of 0.4 ml per tube (10 × 100 mm) is incubated in a shaking water bath at 37°C for 10 min. Di-isopropyl ether (3 ml) is added, each tube is immediately vortexed for 15 sec and then centrifuged for 3 min using a table top centrifuge. The upper phase is carefully removed by means of a Pasteur pipette, and the lower phase is reextracted with 3 ml of di-isopropyl ether as described above. In order to determine acyl-CoA hydrolase activity, both upper phases are combined, transferred into a counting vial, evaporated to dryness under nitrogen, and the radioactivity associated with the extract due to oleic acid liberated from [1-^{14}C]oleoyl-CoA is measured.

The lower aqueous phase of the incubation mixture is extracted by vortexing with 2 ml of di-isopropyl ether–*n*-butanol, 60:40 (v/v), for 15 sec followed by centrifugation for 3 min. The lower phase is carefully removed by a Pasteur pipette, and the upper phase is washed with 3 ml of water, aided by vortexing and centrifugation as before, in order to remove residual amounts of precursor [1-^{14}C]oleoyl-CoA from the organic layer. An aliquot (conveniently 1 ml from a total of 2 ml) is transferred into a counting vial, the sample is evaporated to dryness under nitrogen, and radioactivity is measured by scintillation counting. Radioactivity of this sample is exclusively associated with lecithin and is representative of LLAT activity in the system.

RESULTS AND DISCUSSION

The inverse effect of lysolecithin on lysolecithin acyltransfer and on acyl-CoA hydrolysis as catalyzed by rat liver microsomes is illustrated in **Fig. 1.** In the presence of 0.23 nmol (0.01 μCi) of [1-^{14}C]oleoyl-

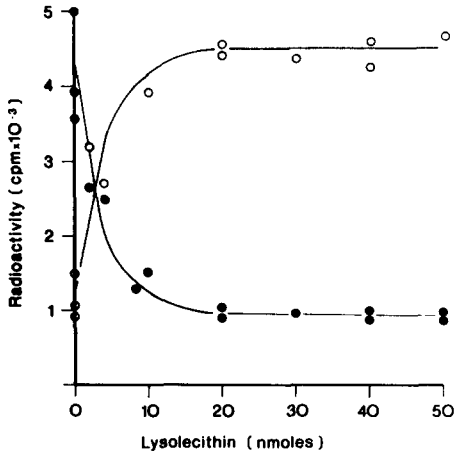

Fig. 1. Effect of exogenous lysolecithin on apparent lysolecithin acyltransferase and acyl-CoA hydrolase activities. Incubations were carried out using 98 μg of microsomal protein in the incubation system (see Materials and Methods); and oleoyl-CoA (0.01 μCi/0.23 nmol). Symbols: ○ —— ○, labeled lecithin formed due to LLAT; ● —— ●, labeled fatty acid due to acyl-CoA hydrolase. Each point represents the mean of at least one duplicate set of data.

CoA (sp act 44 mCi/mmol) and in the absence of exogenous lysolecithin, apparent acyl-CoA hydrolase activity reflected in labeled fatty acid formation was nearly four times higher than the LLAT activity as judged by synthesis of labeled lecithin. A trend towards such predominance of hydrolase activity in the absence of exogenous lysolecithin is well documented in the literature (2). However, upon addition of lysolecithin to the incubation mixture, hydrolase activity decreased sharply and at about the same rate as LLAT activity increased.

Because LLAT activity of commonly used crude enzyme preparations is generally accompanied by acyl-CoA hydrolase activity, a procedure was designed that would permit, in a rapid and convenient manner, the separation of labeled lecithin and of labeled fatty acid from residual [1-^{14}C]oleoyl-CoA precursor without the use of chromatography. This, indeed, can be readily accomplished by differential lipid partition. Fatty acid produced by hydrolase action on the acyl-CoA substrate can first be extracted selectively from the assay mixture by partition with di-isopropyl ether; then the lecithin formed by LLAT catalyzed acylation of lysolecithin can be recovered quantitatively by extraction of the aqueous phase with di-isopropyl ether–*n*-butanol 60:40 (v/v).

Table 1 illustrates the accuracy and reproducibility of the technique. Because the ratio of acyl-CoA hydrolase activity to LLAT activity is known to vary widely for different protein preparations (from 1:2 to 1:5; see references 1, 2, 11), it was considered essential to establish the suitability of the method under such drastically different conditions. The table gives a comparison of the amounts of fatty acid (cpm, hydrolase) and lecithin (cpm, LLAT) recovered from the incubation system by the present partition technique, by the present partition technique followed by

TABLE 1. Comparison of LLAT assays based on [1-^{14}C]oleoyl-CoA as substrate[a]

	Enzyme Activities Found in Experiments Utilizing Different Microsomal Preparations from Rat Liver											
	Experiment 1				Experiment 2				Experiment 3			
	Hydrolase		LLAT		Hydrolase		LLAT		Hydrolase		LLAT	
Assay procedure	cpm	(sp act)	cpm	(sp act)	cpm	(sp act)	cpm	(sp act)	cpm	(sp act)	cpm	(sp act)
Differential lipid partition[b]	259	(23.4)	2394	(216.4)	874	(49.2)	4236	(238.4)	941	(59.7)	1971	(124.9)
Differential lipid partition followed by TLC[c]	302	(27.3)	2185	(197.5)	802	(45.1)	4374	(246.1)	905	(57.4)	1729	(109.6)
Total lipid extraction followed by TLC[d]	273	(24.7)	2461	(222.5)	673	(37.9)	4221	(237.5)	865	(54.8)	1999	(126.7)

[a] Incubations were carried out at 37°C for 10 min as described in Materials and Methods. Results given are means of duplicates. Enzyme activities are listed as radioactivities (cpm) associated with fatty acid (for hydrolase) or lecithin (for LLAT) and as specific enzyme activities (sp act) in nmol of product formed per mg protein per hr.

[b] Fatty acids were recovered from the incubation mixture by di-isopropyl ether extraction, lecithin by partition with di-isopropyl ether–*n*-butanol 60:40 (v/v); see Materials and Methods. The reproducibility of the procedure of LLAT assay was established in separate quadruplicate sets of experiments for different rat liver microsomal preparations (52–98 μg protein), and the following typical mean ± standard deviations (and ranges) were measured for LLAT activities expressed in cpm lecithin formed: 5905 ± 87 (5793–6005); 4245 ± 114 (4161–4375); 2005 ± 25 (1971–2029). Corresponding control values in the absence of protein were 157 ± 24 (126–184).

[c] Each extract (see footnote *b*) was evaporated to dryness, taken up in 0.1 ml of $CHCl_3$, and the solution was applied to layers of silica gel H (Merck), 0.5 mm thick. After developing the plates in chloroform–methanol–water 65:35:8 (by vol), fractions were made visible by inserting the plates into an iodine chamber, areas corresponding to fatty acids (R_f 0.9) and lecithin (R_f 0.5) were scraped off, and the products were eluted with chloroform–methanol–water 50:40:10 (by vol). The fatty acid fraction was identified also by co-migration (R_f 0.7) with carrier using the TLC developing system, hexane–diethyl ether–acetic acid 60:12:1 (by vol).

[d] Total lipids were extracted from the incubation system according to Bligh and Dyer (17), the solvent was evaporated, and the lipids were taken up in 0.1 ml of chloroform and subjected to TLC as described above (see footnote *c*).

thin-layer chromatographic lipid isolation, and by total lipid extraction followed by thin-layer chromatographic lipid isolation. The data show that although the hydrolase/LLAT ratio varied from 1:10 (Experiment 1) to 1:2 (Experiment 3), the activities determined by the three procedures were essentially identical within the normal range of deviation expected. The radioactivity recovered by di-isopropyl ether extraction was associated with fatty acid only. The radioactivity of the di-isopropyl ether–*n*-butanol extract was exclusively due to labeled lecithin. Phospholipase A_2 treatment of the lecithin fraction showed that more than 90% of the label was located at position-2.

The mean ± standard deviations for LLAT activities determined by the present differential lipid partition method were generally in the range of ±2%. Small amounts of radioactivities recovered in the organic phases of control experiments, i.e., from the incubation system in the absence of microsomal protein, were attributed to contaminant [1-^{14}C]oleic acid present in [1-^{14}C]oleoyl-CoA that appeared in the di-isopropyl ether layer, and to traces of [1-^{14}C]oleoyl-CoA taken up by di-isopropyl ether–butanol (see Table 1, footnote b).

The LLAT assay based on selective extraction of labeled fatty acid and labeled lecithin with di-isopropyl ether and di-isopropyl ether–butanol, respectively, from the [1-^{14}C]oleoyl-CoA-containing incubation mixture utilizes a partition method similar to that described by Cham and Knowles (18) for the extraction of serum lipids. The procedure is rapid and reproducible and becomes particularly attractive and convenient when large numbers of samples are to be assayed. Under the extraction conditions chosen, in vitro produced fatty acid and lecithin are recovered quantitatively, although complete extraction of endogenous phospholipids may require more rigorous extraction conditions (18). The assay procedure can be simplified further in most instances when hydrolase activity would not be followed; the di-isopropyl ether fraction can then be removed by a Pasteur pipette connected to a vacuum trap. The present lysolecithin acyltransferase assay is, of course, not applicable if labeled lysolecithin is to be used as substrate.

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