Lysophospholipase–transacylase from rat lung: isolation and partial purification

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Abstract Incubation of rat lung supernatant with 1-[1-^{14}C]palmitoyl-*sn*-glycero-3-phosphocholine in the absence of any cofactors resulted in the release of radioactive fatty acid and the formation of phosphatidylcholine. The production of fatty acids (lysophospholipase activity) exceeded phosphatidylcholine formation (transacylase activity) about threefold, although the relative extent of phosphatidylcholine formation was considerably greater than previously reported by Abe et al. (Biochim. Biophys. Acta, **369:** 361–370, 1974). In agreement with these authors, evidence is presented suggesting that a single enzyme is responsible for both catalytic activities. The enzyme, provisionally denoted lysophospholipase–transacylase, was found primarily in the soluble fraction of rat lung and was purified approximately 250-fold. The enzyme had an estimated mol wt of 50,000. The ratio of lysophospholipase to transacylase activity in the purified enzyme could be varied depending upon the concentration and character of the lysophosphatidylcholine and the ratio of substrate to products. The degree of esterification of 1-acyl lysophosphatidylcholine was altered with mixtures of different molecular species of substrate, indicating acyl chain selectivity in the transfer process. This enzyme was capable of synthesizing disaturated phosphatidylcholine, an important component of the pulmonary surfactant. Three lysophospholipases purified from other sources did not possess this transacylase activity.

Supplementary key words disaturated phosphatidylcholine · pulmonary surfactant · phosphatidylcholine fatty acid rearrangement · substrate specificity.

Recent work (1–4) has indicated that the de novo synthesis of phosphatidylcholine in the lung is insufficient to account for the rapid turnover of disaturated phosphatidylcholine, an integral component of the pulmonary surfactant (5–8). This has prompted the search for rearrangement pathways that exchange the *sn*-2 unsaturated fatty acid, characteristic of phosphatidylcholines synthesized by the de novo pathway (1–4), for saturated fatty acids. Such pathways involve the phospholipase A_2 hydrolysis of the *sn*-2 fatty acid (9, 10) and coenzyme A dependent acyltransferase (11–13) or independent (4, 14–16) transacylase to esterify the lysophosphatidylcholine. The partial purification of a lysophospholipase-non-CoA-dependent transacylase from rat lung soluble fraction with a lysophospholipase–transacylase ratio of 30:1 has recently been reported (15). Here we report the purification of this enzyme under different conditions with a lysophospholipase–transacylase activity ratio of 3:1 and the response of the enzyme to pure lysophosphatidylcholine substrates in micellar and liposomal form.

The following reactions are compatible with our findings. Reaction 1 represents the lysophospholipase activity and Reaction 2 the non-CoA-dependent transacylase activity.

$$\text{LPC} + \text{E} \rightarrow \text{LPC:E} \rightarrow \text{GPC} + \text{E-FA}$$

$$\text{E-FA} + \text{H}_2\text{O} \rightarrow \text{E} + \text{FA} \qquad \text{Reaction 1}$$

$$\text{E-FA} + \text{LPC} \rightarrow \text{E} + \text{PC} \qquad \text{Reaction 2}$$

METHODS AND MATERIALS

Tissue preparation

Fifty young adult Wistar rats were killed by decapitation and the lungs were removed and immediately chilled in cold homogenization buffer (0.25 M sucrose, 50 mM phosphate buffer, pH 6.8, 1 mM EDTA, and 10% glycerol). All subsequent procedures, other than incubations and chromatography, were carried out at 0–4°C. The tissue was trimmed to remove the larger bronchi, weighed, and minced with scissors. Homogenization was carried out in a Potter tube with sufficient buffer to prepare a 20–25% homogenate. Approximately 20 strokes were needed to adequately homogenize the tissue. The homogenate was sub-

Abbreviations: LPC, lysophosphatidylcholine; GPC, glycerophosphorylcholine; FA, fatty acid; E, enzyme; PC, phosphatidylcholine.

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jected to differential centrifugation as previously described to derive nuclear, mitochondrial, lysosomal, microsomal, and supernatant fractions (17). Samples were assayed immediately or stored at −20°C. The supernatant fraction contained the majority of the lysophospholipase–transacylase and was prepared by centrifuging the homogenate at 105,000 *g* for 60 min in a Beckman L5-65 ultracentrifuge using a 30 rotor.

Assay system and synthesis of substrates

Lysophospholipase was assayed using 200 nmol of synthetic 1-[1-^{14}C]palmitoyl-*sn*-glycero-3-phosphocholine (sp act 100 dpm/nmol) in a total volume of 0.5 ml of 100 mM phosphate buffer, pH 6.5. After incubation for the indicated time periods at 37°C in a shaking water bath, the reaction was stopped by the addition of 2.5 ml of an extraction mixture consisting of *iso*propanol–heptane–1 N H_2SO_4 400:100:10 (18). After the addition of 100 mg of silicic acid (Merck, Keiselgel H, type 60) to bind the remaining lysophosphatidylcholine, the released fatty acids were extracted by the addition of 1.5 ml of heptane and 1.5 ml of water. After 15 sec of agitation, the phases were allowed to separate and 1.0 ml of the upper heptane phase was removed and counted to quantify the released fatty acids. Lysophospholipase–transacylase activities were assayed using the same incubation mixture. The lipids were extracted by the method of Bligh and Dyer (19) and separated by thin-layer chromatography (20) using lysophosphatidylcholine, phosphatidylcholine, and fatty acid standards. The chromatographed lipids were identified after exposure of the plate to I_2 and were eluted (methanol–chloroform 2:1) or scraped into counting vials. Samples for scintillation spectrometry were thoroughly mixed (Vortex mixer) with 10 ml of a solution of 2 parts toluene (0.5% PPO, 0.03% POPOP), 1 part Triton X-100, and 0.2 parts H_2O. Radioactivity was quantitated using a Packard Tri-carb or Amersham-Searle scintillation spectrometer and the necessary reference standards. Specific quenched standards were prepared for experiments using dual labels.

Radioactive and nonradioactive substrates were synthesized from the $CdCl_2$ adducts of *sn*-glycero-3-phosphocholine and [1-^{14}C]palmitoyl chloride, [1-^{14}C]stearoyl chloride, stearoyl chloride, and oleoyl chloride as previously described (21). The lyso compounds were prepared by treatment of the synthesized PC with phospholipase A_2 (*Crotalus adamanteus*) solution containing 0.05 M borate (pH 7.4), 0.005 M calcium acetate, and ether. Phosphatidylcholine synthesized by the lung enzyme was chromatographed, eluted from the silica, dried in vacuo, and degraded as outlined above, but using purified phospholipase A_2 (*Crotalus adamanteus*), which was a generous gift from Dr. Zwaal. The reaction products were separated by thin-layer chromatography and the radioactivity in the fatty acid and phosphatidylcholine was determined. Palmitoyl-propanediol-(1,3)-phosphocholine (deoxylysophosphatidylcholine) was synthesized by the method of Eibl and Westphal (22) and was a generous gift from A. J. Aarsman.

Purification of lysophospholipase–transacylase

From several pilot projects the following procedure was adopted. The supernatant from lung homogenate was brought to 65% saturation with solid ammonium sulfate, stirred for 30 min, and centrifuged for 20 min at 12,000 *g* (Sorvall RC2-B). The precipitate was dissolved in 50 ml of "buffer" (50 mM phosphate, pH 6.8, containing 10 mM β-mercaptoethanol and 10% glycerol). The protein solution was dialysed against 2 l of "buffer" for 16 hr and the precipitate that formed was removed by centrifugation (Sorvall RC2-B). Analysis of both the ammonium sulfate supernatant and the redissolved protein precipitate present after dialysis demonstrated no significant enzymic activity. Conductivity was comparable to the starting "buffer" for the DEAE–cellulose chromatography. The solution (210 ml) was pumped on a DEAE–cellulose (Whatman DE 52) column (2.5 × 28 cm) and washed with "buffer" until the eluate was free of protein (absorbance at 280 nm). This was followed by a 1-liter NaCl gradient (0–350 mM) in buffer at a flow rate of 45 ml/hr. The active fractions eluted at approximately 150 mM NaCl. The pooled active fractions (84 ml) were dialyzed against "buffer" for 2–4 hr and pumped on a hydroxylapatite (Bio-Gel HTP-BioRad Laboratories) column (3.3 × 10 cm) and washed with "buffer" until the eluate was free of protein. This column was eluted using a 400-ml phosphate gradient (50–200 mM), pH 6.8, containing 10 mM β-mercaptoethanol and 10% glycerol at a flow rate of 40 ml/hr. The active fractions eluted at approximately 100 mM phosphate and were concentrated from 50 to 5 ml using an ultrafiltration device (Amicon, UM-10 filter). This sample was pumped on an AcA-44 (LKB, 4% acrylamide, 4% agarose) column (1.5 × 92 cm), which had been equilibrated with "buffer" containing 0.5 M NaCl. The active fractions were pooled and could be stored for up to one month at −40°C with less than 10% loss of activity.

The gel filtration column was used to estimate the molecular weight of the enzyme by calibration with commercially available proteins of known molecular

TABLE 1. Subcellular distribution of rat lung lysophospholipase (LPL) and transacylase (TA)

Fraction	Protein	(%)	LPL	(%)[b]	TA	(%)[b]
	mg		*sp act*[a]		*sp act*[a]	
Nuclear	145.1	40.1	33.7	19.9	1.9	4.0
Mitochondrial	18.4	5.1	77.2	5.8	7.4	2.0
Lysosomal	3.7	1.0	95.2	1.4	5.8	0.3
Mitochondrial	23.8	6.6	48.0	4.6	12.1	4.3
Supernatant	170.8	47.2	98.0	68.3	35.4	89.4

[a] nmol/hr/mg protein.
[b] Percent distribution of total activity.

weight (bovine serum albumin, ovalbumin, and sperm whale myoglobin). The void volume was determined using Blue Dextran 2000.

Disc gel electrophoresis was conducted using 7.5% polyacrylamide, Tris–glycine buffer, pH 8.3, and 5 mA per tube for 2 hr according to the method of Davis (23). Protein determinations were performed by the method of Ross and Schatz (24) in order to carboxymethylate the β-mercaptoethanol present in the "buffer".

Inorganic phosphorus was determined using the micromethod of Chen, Toribarra, and Warren (25) following ashing by the method of Ames and Dubin (26). Liposomes were prepared as described by van den Besselaar, Verheyen, and van den Bosch (27).

Materials

All organic solvents were reagent grade and were redistilled before use. [1-^{14}C]- and [9,10-^{3}H]palmitic acid were obtained from the Radiochemical Center, Amersham, England and sp act were 56 mCi/mmol and 500 mCi/mmol, respectively; they were diluted with unlabeled palmitic acid as required. Beef pancreatic lysophospholipase and beef liver lysophospholipase I and II were purified by the methods of van den Bosch et al. (28) and de Jong et al. (29), respectively. Egg phosphatidylcholine was isolated by the method of Papahadjopoulos and Miller (30) and was a gift from A.M.H.P. van den Besselaar. Phospholipase A_2 (*Crotalus adamanteus*) was obtained from Sigma Chemical Company.

RESULTS

Table 1 indicates the subcellular distribution of protein and the specific activity of the lung enzymes, lysophospholipase and transacylase. As indicated, both enzymes were located predominantly in the soluble fraction.

Table 2 presents the results obtained in the purification of the lysophospholipase–transacylase from the soluble fraction of rat lung. Studies early in the purification indicated that glycerol had a protective effect on enzymatic activity and that the ratio of transacylase to lysophospholipase was higher than previously reported (15). Furthermore, it was determined that ammonium sulfate precipitation and DEAE–cellulose chromatography used in conjunction with glycerol permitted significant purification. Glycerol (10%) was used, thereafter, in all buffers and for storage of the enzyme. In addition, the purification was conducted as rapidly as feasible (approximately 4 days) to minimize the effects of proteolytic enzymes and to obviate freezing and thawing. A number of early attempts at purification using gel filtration (Sephadex G-150) after the hydroxylapatite step were unsuccessful, with total protein and enzyme activity appearing in the void volume. With the consideration that the proteins might be aggregating, gel filtration chromatography was conducted in the presence of 0.5 M NaCl. This permitted further separation of the proteins and produced a fourfold purification. Samples were retained at each purification step to determine the ratio of lysophospholipase and transacylase activity as reflected by the radioactivity in the released fatty acid and in phosphatidylcholine, respectively.

TABLE 2. Purification of lysophospholipase–transacylase from rat lung supernatant

Purification Step	Protein	Activity	Specific Activity	Recovery	Purification	Ratio FA/PC[a]
	mg	*nmol FA/min*	*nmol FA/min/mg*	*%*	*–fold*	
Supernatant	1782	15500	8.7	100		3.8
0–65% $(NH_4)_2SO_4$	731	16000	22	100	2.5	3.1
DEAE–cellulose	40	5480	137	35	16	2.3
Hydroxylapatite	6.5	3731	574	24	66	2.2
Ultrogel AcA-44	1.0	2150	2150	14	247	2.4

[a] Ratio of radioactivity in fatty acids and phosphatidylcholine.
The assay system consisted of 100 mM potassium phosphate buffer (pH 6.5) and 200 μM 1-[1-^{14}C]palmitoyl-*sn*-glycero-3-phosphocholine (93 dpm/nmol) in a final volume of 0.5 ml. Incubations were conducted for 10 min at 37°C.

Procedures that resulted in significant loss of enzymatic activity were CM-cellulose chromatography, acidification below pH 5.0, delipidation with butanol, and lyophilization. Proteins purified through the hydroxylapatite chromatographic step were further chromatographed using isoelectric focusing columns (ampholine gradients of pH 3–10 and 3.5–6.0). In each instance, a single protein precipitation band was observed at pH 4.0–4.5. Virtually all of the protein loaded on the column was associated with the precipitation band and, although it could be solubilized in "buffer," the enzymatic activity was markedly reduced. No changes in lysophospholipase and transacylase activity ratio were observed.

The lipid phosphorus content of three different enzyme preparations following hydroxylapatite chromatography and prolonged dialysis was determined and ranged between 23 and 56 nmol of lipid phosphorus per mg protein. Since less than 10 μg of purified protein were used for an assay, contamination with endogenous phospholipids associated with the enzymes was less than 0.5 nmol per incubation. In addition, the purified enzyme was incubated for 1 hr with

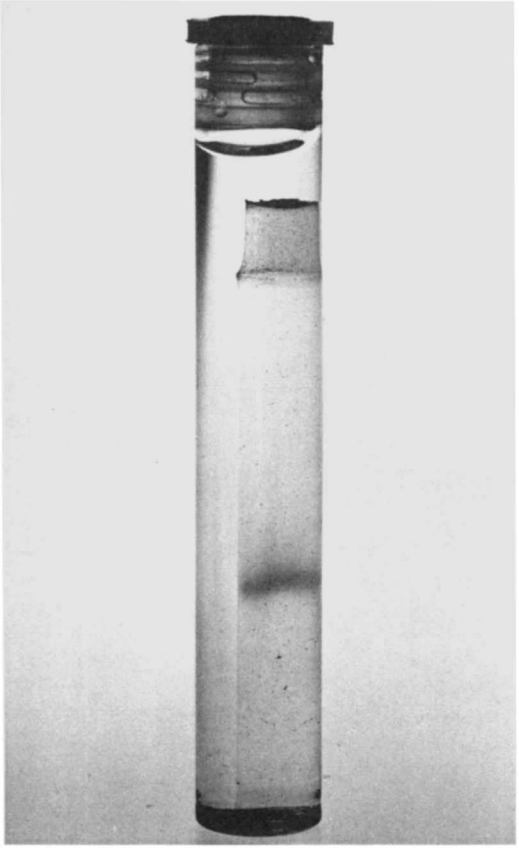

Fig. 1. Polyacrylamide dis gel electrophoresis of rat lung lysophospholipase–transacylase. The gel was loaded with 50 μg of the purified protein.

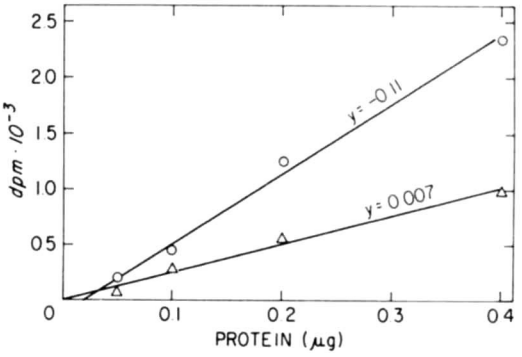

Fig. 2. Lysophospholipase–transacylase activity with increasing enzyme concentration. Assay system as in Table 2 with purified enzyme protein altered as noted. FA, ○ —— ○; PC, △ —— △.

1-stearoyl-2-[1-^{14}C]stearoyl-*sn*-glycero-3-phosphocholine and there was no evidence of phospholipase A_2 activity. The pH optimum for the purified lysophospholipase–transacylase was the same for both enzymatic activities and was found to be between pH 6.3 and 6.8.

Estimates of molecular weight using the calibrated gel filtration column (AcA-44) were approximately 50,000. Disc gel electrophoresis utilizing 50 μg of protein revealed a single band when stained with 1% Amido black (**Fig. 1**).

The activities of both the lysophospholipase and the transacylase were linear with increasing protein concentration up to 0.4 μg per 0.5 ml incubation volume (**Fig. 2**). Similar incubations for increasing periods of time up to 1 hr also demonstrated linearity with a constant ratio for the two enzymatic activities (**Fig. 3**). The linear regressions of the velocities of the lysophospholipase reactions calculated by the least squares method in Fig. 2 and Fig. 3 do not pass through the origin. **Fig. 4** presents the velocities of lysophospholipase and transacylase with increasing substrate concentration. The lysophospholipase activity follows the pattern of saturation kinetics, reaching maximum velocity at a lysophosphatidylcholine concentration of approximately 150 μM. The transacylase activity follows a somewhat different pattern, being at first linear and rapid and subsequently slower.

Table 3 illustrates the lack of effect of ATP and CoA on phosphatidylcholine synthesis. Added free fatty acids appear not to be incorporated into phosphatidylcholine synthesized by the lysophospholipase–transacylase. Similar results were presented recently by Abe, Akino, and Ohno (31).

Table 4 depicts the activity of the purified lysophospholipase on palmitoyl deoxylysophosphatidylcholine, indicating hydrolysis rates comparable to that observed using lysophosphatidylcholine substrates.

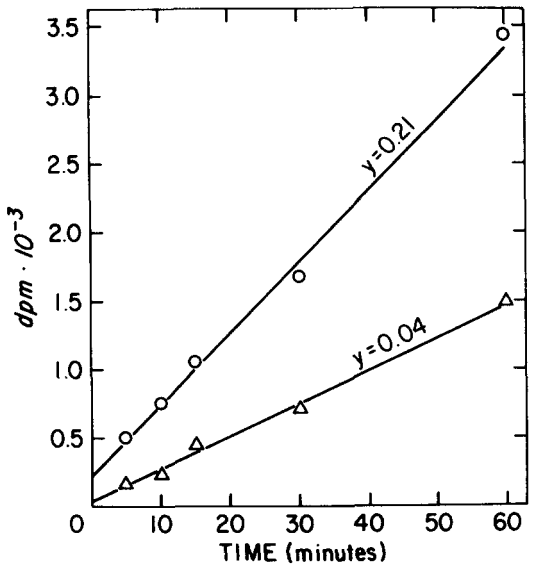

Fig. 3. Lysophospholipase–transacylase activity vs. time. Assay system as in Table 2 using 0.4 μg of purified protein. FA, ○ —— ○; PC, △ —— △.

The deoxylysophosphatidylcholine lacks a *sn*-2 hydroxyl group and, therefore, does not participate in the transacylase reaction. However, the addition of unlabeled 1-palmitoyl-*sn*-glycero-3-phosphocholine (acceptor substrate) does permit the formation of phosphatidylcholine using labeled palmitic acid from the deoxylysophatidylcholine substrate (donor substrate).

Table 5 shows a comparison of the lung lysophospholipase–transacylase with purified lysophospholipases from beef pancreas and liver and indicates that only the lung enzyme synthesizes phosphatidylcholine.

Table 6 is representative of the results of three experiments in which the lung enzyme was incubated

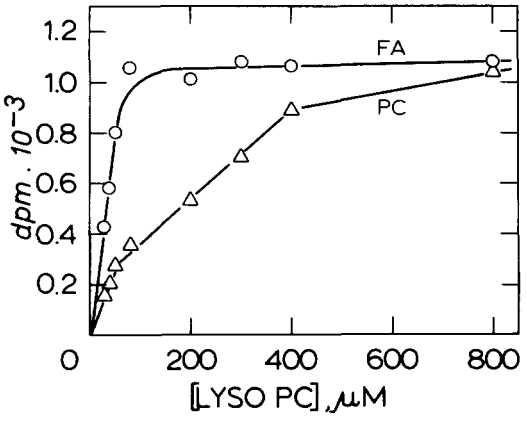

Fig. 4. Lysophospholipase–transacylase activity vs. increasing substrate concentration. Assay system as noted in Table 2 using 0.2 μg of purified protein and indicated substrate concentrations. 1-[1-^{14}C]Palmitoyl-*sn*-glycero-3-phosphocholine (sp act 400 dpm/nmol).

TABLE 3. Lack of effect of ATP, CoA, or fatty acid on phosphatidylcholine synthesis

Addition	Fatty Acid Hydrolyzed	Fatty Acid Incorporated into Phosphatidylcholine	
		[^{14}C]Palmitate	[^{3}H]Palmitate
	nmol	*nmol*	
None	24.8	10.6	
ATP	25.6	11.9	
CoA	25.3	11.0	
ATP; CoA	23.8	9.2	
[^{3}H]Palmitate	21.2	11.1	0.2
[^{3}H]Palmitate; ATP; CoA	25.0	11.8	0.0

Assay system as noted in Table 2 using 2.5 μg of purified protein. The samples containing [9,10-^{3}H]palmitate (sp act 400 dpm/nmol) were sonicated for 1 min at 50 MHZ prior to adding the enzyme. Concentrations used: ATP, 10 mM; CoA, 0.1 mM; and [^{3}H]palmitate, 0.05 mM.

with 1-[1-^{14}C]palmitoyl-*sn*-glycero-3-phosphocholine and equimolar amounts of 1-stearoyl- or 1-oleoyl-*sn*-glycero-3-phosphocholine. Incubations containing the labeled substrate diluted with an equal amount of unlabeled 1-stearoyl-*sn*-glycero-3-phosphocholine showed a more than twofold reduction in hydrolysis and phosphatidylcholine synthesis as evidenced by the reduced radioactivity in fatty acids and phosphatidylcholine. Addition of unlabeled 1-oleoyl-*sn*-glycero-3-phosphocholine caused much less reduction in the hydrolysis of 1-[1-^{14}C]palmitoyl-*sn*-glycero-3-phosphocholine and its conversion into phosphatidylcholine.

Table 7 depicts the results of incubations in which the lysophosphatidylcholine substrate was presented in micelles, in liposomes, or in mixed micelles with fatty acid. Hydrolysis occurred under all conditions, though it was greatest on micelles and least on liposomal lysophosphatidylcholine. Some comparative reduction in hydrolysis was apparent also in the incubation that contained mixed micelles consisting of 10 mol% of lysophosphatidylcholine and 90 mol% of palmitic acid. More remarkable was the near absence

TABLE 4. Acyltransfer from donor to acceptor substrate

Substrate	[^{14}C]Palmitate (nmol) in	
	Fatty Acid	Phosphatidylcholine
[1-^{14}C]Palmitoyldeoxylyso PC	63.4	0
[1-^{14}C]Palmitoyldeoxylyso PC *plus* 1-palmitoyllyso PC	28.0	4.1

The incubation mixture contained 100 mM potassium phosphate buffer (pH 6.5), 1.65 μg of purified protein, and 80 nmol of [1-^{14}C]palmitoyldeoxylysophosphatidylcholine (120 dpm/mmol) or 40 nmol of this substrate and 40 nmol of 1-palmitoyl-*sn*-glycero-3-phosphocholine in a final volume of 0.5 ml. Incubations were carried out for 10 min at 37°C.

TABLE 5. Transacylase activities of purified lysophospholipases

Enzyme	Fatty Acid Hydrolyzed	Fatty Acid Incorporated into PC
	nmol	*nmol*
Rat lung lysophospholipase–transacylase	24.8	10.5
Beef pancreas lysophospholipase	52.5	0
Beef liver lysophospholipase I	40.5	0
Beef liver lysophospholipase II	32.9	0

Assay system as noted in Table 2. Enzyme quantities used: lung lysophospholipase–transacylase, 2.5 μg; beef pancreas lysophospholipase, 2.0 μg; beef liver lysophospholipase I, 18.5 μg; and beef liver lysophospholipase II, 61.5 μg.

of phosphatidylcholine synthesis when the substrate was presented in liposomes made of 90 mol% of egg phosphatidylcholine.

DISCUSSION

The subcellular distribution of the lung lysophospholipase–transacylase isolated in our studies is similar to that reported by van den Bosch and de Jong (17) for beef liver lysophospholipase I, where this enzyme was found predominantly in the soluble fraction although some activity in the mitochondrial fraction also was demonstrated. The purified beef liver lysophospholipase I as well as two other purified lysophospholipases, however, were incapable of carrying out the transacylase reaction (Table 5).

The purification of the lysophospholipase–transacylase appeared to be facilitated by the use of 10% glycerol in that the loss of enzymatic activity was reduced and purification methods (ammonium sulfate preciptation and DEAE–cellulose chromatography) could be used that had previously been reported as unsatisfactory (15). The assay procedure used and the purification methods employed also permitted the preservation of more of the transacylase activity relative to the lysophospholipase. The specific activity of the lysophospholipase reported here is comparable to that previously reported (15), while the transacylase is approximately 10-fold higher. The ratio of lysophospholipase to transacylase activity decreased during purification, suggesting an apparent increase in transacylase activity. However, the impure enzyme may have included endogenous lysophosphatides which our assay system would not have detected. The final chromatography step in the purification of the lung lysophospholipase–transacylase was conducted using gel filtration (AcA-44) in the presence of 0.5 M NaCl. This gel sieve has an exlusion molecular weight of 130,000 and prior attempts to use it in the presence of 0.15 M NaCl resulted in no further purification, with both total protein and enzymatic activity appearing in the void volume. The fourfold purification experienced with the use of 0.5 M NaCl suggests a strong tendency for this protein to aggregate at low ionic strength. This seems further supported by the aggregation and precipitation in one inseparable band of all of the partially purified lysophospholipase–transacylase preparations upon chromatography on isoelectrofocusing columns.

As observed by Abe, Ohno, and Sato (15) and substantiated by our findings, this enzyme does not require coenzyme A activation of the fatty acid for the function of the transacylase (Table 2). We have interpreted these findings to indicate that the released fatty acid remains complexed to the enzyme until it is hydrolyzed (Reaction 1) or until it is esterified (Reaction 2) to another molecule of lysophosphatidylcholine.

Three different lines of evidence suggest that the hydrolysis of lysophosphatidylcholine and its con-

TABLE 6. Effect of lysolecithin species on palmitate transfer

Substrate	[1-^{14}C]Palmitate in		Positional Distribution of [1-^{14}C]Palmitate	
	Fatty Acid	Phosphatidylcholine	1-pos.	2-pos.
	nmol		%	
1-[1-^{14}C] 16:0 lyso PC[a]	77.6	80.8	49	51
1-[1-^{14}C] 16:0 lyso PC *plus* 1-stearoyl lyso PC	18.4	12.5	38	62
1-[1-^{14}C] 16:0 lyso PC *plus* 1-oleoyl lyso PC	49.4	33.7	36	64

[a] 1-Acyl lyso PC is 1-acyl-*sn*-glycero-3-phosphocholine.

The assay system consisted of 2.2 μg of purified protein and either 200 nmol of 1-[1-^{14}C]palmitoyl-*sn*-glycero-3-phosphocholine (93 dpm/nmol) or 100 nmol of this substrate and 100 nmol of the lysolecithin species as listed in a total volume of 0.5 ml of 100 mM potassium phosphate buffer (pH 6.5). Incubations were carried out for 20 min at 37°C.

TABLE 7. Influence of reaction products on lysophospholipase and transacylase activity ratio

Substrate	Amounts	Fatty Acid Hydrolyzed	Fatty Acid Incorporated into PC
	nmol	*nmol*	*nmol*
Lyso PC	80	48.0	21.7
Lyso PC + PC	80 + 720	17.8	0.8
Lyso PC + FA	80 + 720	11.6	9.3

Micellar 1-[1-^{14}C]palmitoyl-*sn*-glycero-3-phosphocholine (80 nmol, sp act 100 dpm/nmol), liposomal 1-[1-^{14}C]palmitoyl-*sn*-glycero-3-phosphocholine (80 nmol) plus egg phosphatidylcholine (720 nmol), and mixed micellar 1-[1-^{14}C]palmitoyl-*sn*-glycero-3-phosphocholine (80 nmol) plus palmitic acid (720 nmol) substrates were prepared by sonication (30 min at 60 MHZ under nitrogen in an ice bath) and centrifugation (20 min at 105,000 *g* in a Beckman L5-65, 50 rotor). These combinations were incubated with 2.5 μg of purified protein for 30 min at 37°C in a volume of 1.0 ml of 100 mM phosphate buffer (pH 6.5) and analyzed as described in Methods.

version into phosphatidylcholine were catalyzed by a single bifunctional protein. First, the ratio of lysophospholipase and transacylase activity remained virtually constant over a 250-fold purification procedure. At this stage, a single band in polyacrylamide disc gels was observed. Second, both reactions depended in a similar linear fashion upon variation in amount of protein and time in the kinetic experiments depicted in Figs. 2 and 3. Furthermore, the transfer of [1-^{14}C]-palmitate from 1-[1-^{14}C]palmitoyl-deoxylysophosphatidylcholine to unlabeled 1-palmitoyl-*sn*-glycero-3-phosphocholine (Table 4) supports the concept of one enzyme being responsible for the catalysis of both acyl hydrolysis and acyl transfer. This is illustrated further by the reactions in which deoxylysophosphatidylcholine was rapidly hydrolyzed by the lysophospholipase, indicating that lysophosphatidylcholine can be replaced by deoxylysophosphatidylcholine in Reaction 1. As expected, Reaction 2 does not take place with deoxylysophosphatidylcholine since the substrate analog lacks the free hydroxyl group at *sn*-2 to be esterified. When the incubation was repeated with an equimolar mixture of 1[1-^{14}C]palmitoyl-deoxylysophosphatidylcholine and unlabeled 1-palmitoyl-*sn*-glycero-3-phosphocholine, the production of ^{14}C-labeled fatty acid was reduced about half due to dilution of the radioactive substrate. If phosphatidylcholine formation by Reaction 2 was catalyzed by an enzyme other than the lysophospholipase–transacylase, one would expect unlabeled phosphatidylcholine to be formed exclusively from the unlabeled lysophosphatidylcholine. The appearance of radioactive phosphatidylcholine in this incubation strongly supports the concept that the ^{14}C-labeled fatty acid released from 1-[1-^{14}C]deoxylysophosphatidylcholine (donor substrate) is transferred by the same enzyme to the *sn*-2-hydroxyl of unlabeled lysophosphatidylcholine (acceptor substrate).

In the calculation of transacylase activity we have used 50% of the total counts in phosphatidylcholine synthesized by the lung enzyme. We had previously determined that the purified enzyme and synthetic 1-[1-^{14}C]palmitoyl-*sn*-glycero-3-phosphocholine produced phosphatidylcholine with an equal distribution of [^{14}C]palmitate between the *sn*-1 and *sn*-2 positions (Table 6). For such calculations, Abe et al. (15) used 70% since phospholipase A_2 degradation of phosphatidylcholine synthesized by their enzyme preparation esterified approximately 70% of [^{3}H]palmitate at the *sn*-2 position. These authors used biosynthetically prepared 1-[^{3}H]palmitoyl-*sn*-glycero-3-phosphocholine as a substrate. Inasmuch as this substrate was derived from rat liver and hence consisted of a mixture of radioactive palmitoyl and other unlabled lysophosphatidylcholine species, their findings can be explained by our results obtained with mixtures of synthetic lysophosphatidylcholines. Due to the not yet completely understood specificity of the enzyme, we also found about 65% of the [^{14}C]palmitate to be esterified at the *sn*-2 position in these instances (Table 6), thus mimicking the results obtained with biosynthetically prepared lysophosphatidylcholine. The deviation from the expected twofold reduction in both lysophosphatidylcholine hydrolysis and phosphatidylcholine synthesis upon dilution with equimolar amounts of other lysophosphatidylcholine species indicates acyl chain selectivity of the enzyme. It is apparent that in incubations containing only a single lysophosphatidylcholine species the phosphatidylcholine synthesized according to Reaction 2 described above should have a 50/50 distribution of the fatty acid in the *sn*-1 and *sn*-2 positions. Using phospholipase A_2, the predicted ratio was confirmed for the phosphatidylcholine synthesized from 1-[1-^{14}C]palmitoyl-*sn*-glycero-3-phosphocholine (Table 6). However, any preference of the enzyme for acyl chains, either at the donor or at the acceptor site, can give rise to deviation from such a 50/50 distribution. This indeed was observed in the phosphatidylcholines synthesized from the mixtures of lysophosphatidylcholine species. The higher amount of [^{14}C]palmitate in the *sn*-2 position of the phosphatidylcholine must reflect a preference of the enzyme for palmitate transfer.

Abe et al. (15) calculated a lysophospholipase–transacylase ratio of 30:1 in contrast to that reported here of approximately 3:1. The reason for this difference is not apparent, although it can be ascribed, at least in part, to the use of pure 1-palmitoyl-*sn*-glycero-3-phosphocholine, which gave the highest

ratio of phosphatidylcholine to fatty acid formation (Table 6).

In addition, our assays usually were performed using lysophosphatidylcholine concentrations of 200 μM as compared to 30 μM used by Abe et al. (15). As can be seen in Fig. 4 at low substrate concentrations, hydrolysis predominates (ratio 6:1) while at concentrations above saturation of the hydrolysis reaction (150 μM) esterification is further stimulated, e.g., at 800 μM substrate the lysophospholipase–transacylase ratio is 2:1. These results clearly indicate that the ratio of lysophospholipase to transacylase activity is variable depending on the substrate concentration. The relative importance of the transacylase reaction increases with increasing lysophosphatidylcholine concentration. Fig. 4 also illustrates the variable kinetics of the transacylase reaction in which the rate is initially rapid and then increases less rapidly before it appears to reach saturation. However, since we could not measure the concentration of the fatty acid–enzyme complex, we do not know the true substrate concentration relative to the product phosphatidylcholine. Perhaps the increasing concentration of phosphatidylcholine alters the micellar character of the lysophosphatidylcholine substrate, rendering it less available for esterification. Information presented in Table 7 shows that phosphatidylcholine in liposomes containing the lysophosphatidylcholine substrate nearly completely inhibits the formation of new phosphatidylcholine, suggesting product inhibition.

The functional role of lysophospholipases previously has been ascribed to deacylating lysophosphoglycerides in the catabolism of membrane phosphoglycerides (33). Although transfer reactions by hydrolytic enzymes are quite common in biochemistry (33–37), the capacity of phosphatidylcholine synthesis by lysophospholipases so far could be demonstrated only for the lung enzyme (Table 5). In this unique capacity the enzyme could contribute to the synthesis of the pulmonary surfactant, which is composed largely of disaturated phosphatidylcholine as the functional constituent. The mammalian lung is unique with the highest concentration of highly saturated phosphatidylcholine of any organ in the body (38, 39). Evidence has been presented indicating that the de novo synthesis of phosphatidylcholine in lung produces primarily unsaturated species (2, 3, 40). For the conversion of these unsaturated species into 1,2-dipalmitoyl-*sn*-glycerol-3-phosphocholine, two different redistribution mechanisms, both involving lysophosphatidylcholine, have been postulated. Several investigators have suggested the importance of acyl-CoA-dependent acyl-transferase in the formation of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (2, 11, 41–43). Although, in comparison to liver, relatively high activities of palmitoyl-CoA: 1-acyl-*sn*-glycero-3-phosphocholine acyltransferase have been found in lung (2, 11), the acyltransferase activities for unsaturated acyl-CoA's were even higher (2, 11, 13, 42, 43). Such data led Hasegawa-Sasaki and Ohno (13) to conclude that palmitoyl-CoA: 1-acyl lysophosphatidylcholine acyltransferase did not participate in the main pathway of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine formation in lung. However, in cells presumably representing type-II alveolar epithelium, the acyltransferase activity was slightly higher for palmitoyl CoA than for oleyl CoA (43).

A second fatty acid redistribution mechanism that has been suggested (1, 16, 31, 44) as contributing to 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine formation involves the non-CoA-dependent transacylation between two molecules of 1-acyl-*sn*-glycero-3-phosphocholine as originally described by Erbland and Marinetti (45). It is interesting to note that the activity of this enzyme increased by a factor of five in the developing mouse lung, reaching maximal activities one day before term, i.e, one day after cholinephosphotransferase activity reached its maximum value (4). In contrast, acyl-CoA:1-acyl lysophosphatidylcholine acyltransferase showed hardly any change in activity during development (see refs. 4 and 46 for discussion).

Current studies and opinion indicate that disaturated phosphatidylcholine in the lung is synthesized in the type-II alveolar cell, stored in the cytoplasm of this cell in lamellar bodies and finally secreted onto the alveolar surface, where it forms a surface film that inhibits alveolar collapse at low transpulmonary pressures (47). The pulmonary surfactant, in addition to disaturated phosphatidylcholine, is likely composed of protein (48) and lipopolysaccharides (49) and, in the rat, has been estimated to have a half-life of approximately 14 hr (50). Chevalier and Collet (51) injected mice with labeled choline, leucine, and galactose and studied the maturation of the lamellar body in the type-II alveolar cell using radioautography. They concluded that the surfactant synthesis begins in the endoplasmic reticulum, proceeds to the Golgi apparatus, subsequently forming a series of small particles or multivesiculated bodies that coalesce to form the mature lamellar body composed of lipid bilayers of saturated phospholipid.

It is interesting to speculate concerning the role of lysophosphatidylcholine and the soluble lysophospholipase–transacylase in the maturation of the lamellar body. Lucy (52) has hypothesized that lysophosphatidylcholine may be a factor that contributes to mem-

brane fusion. Such a role may be relevant during coalescence of the multivesiculated bodies. Furthermore, the coalesced particles demonstrate the first evidence of lipid bilayers characteristic of the mature lamellar body. It seems plausible that the soluble lysophospholipase–transacylase may act upon micelles of lysophosphatidylcholine, converting them to lipid bilayers at the same time fusion occurs. It is apparent, however, that caution is required in the interpretation of the physiological meaning of the in vitro experiments reported in this paper. As noted above, considerable variation in the quantity and character of the synthesized phosphatidylcholine may occur with changes in the substrate concentration, the acyl chain length and unsaturation, and the character of the lipid membrane containing the lysophosphatidylcholine substrate.

Furthermore, it will be important to determine the ratio of lysophospholipase to transacylase activity on lysophosphatidylcholine embedded in microsomal membranes. Such experiments should provide further insights as to whether the enzyme lysophospholipase–transacylase, which can synthesize dipalmitoylphosphatidylcholine in vitro, indeed contributes to the in vivo synthesis of this important component of the pulmonary surfactant.

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