Formation of Vesicles by the Action of Acyl-CoA:1-Acyllysophosphatidylcholine Acyltransferase from Rat Liver Microsomes: Optimal Solubilization Conditions and Analysis of Lipid Composition and Enzyme Activity†

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ABSTRACT: The enzyme acyl coenzyme A:1-acyllysophosphatidylcholine acyltransferase (acyl-CoA:lysoPC acyltransferase) can be isolated in newly formed phosphatidylcholine (PC) vesicles by solubilization of rat liver microsomes with the two substrates lysoPC and acyl-CoA. In this study, we sought to optimize the conditions for the formation of PC vesicles and analyzed the lipid composition and enzyme activity of the newly formed vesicles. Analysis of PC vesicles formed by incubation of the microsomal preparation with 1-($C_{16:0}$)lysoPC and $C_{18:1}$CoA, $C_{18:2}$CoA, or $C_{20:4}$CoA showed that the optimal protein:lysoPC ratio was 1:5 (by weight) and the optimal lysoPC:acyl-CoA ratio was 1:1 (molar amounts). PC formation increased with incubation time; after 20 h of incubation at 37 °C, approximately 75% of the lysoPC was converted to PC in the incubation mixture. The phospholipid molecular species composition of the vesicles reflected almost exclusively the substrates used; the vesicles contained approximately 33% of the total acyl-CoA:lysoPC acyltransferase activity from the microsomes and demonstrated a single protein band with a molecular mass of 21 kDa by gel electrophoresis. The procedure selected for the enzyme specific for lysoPC acylation, as enzyme activity toward lysophosphatidylethanolamine (lysoPE), lysophosphatidylserine (lysoPS), and lysophosphatidylinositol (lysoPI), was very low. In addition, the utilization of different acyl-CoA substrates for acylation of lysoPC was different from that in microsomes. These results show that an enzyme specific for the formation of PC from lysoPC can be isolated in PC vesicles with a designed phospholipid molecular species composition and that the lipid environment plays an important role in the regulation of the enzyme's affinity for its substrates.

Acyl coenzyme A:lysophosphatidylcholine acyltransferase (acyl-CoA:lysoPC acyltransferase)[1] (EC 2.3.1.23) plays an important role in phospholipid renewal processes. First described in rat liver microsomes (Lands, 1960), this enzyme activity has been detected in mammals (Choy & Arthur, 1989) and other vertebrates (Holub *et al.*, 1976), as well as in plants (Devor & Mudd, 1971), insects (Heckman *et al.*, 1977), bacteria (Proulx & Van Deenen, 1966), protozoa (Okuyama *et al.*, 1977), and fungi (Das & Banerjee, 1977). In mammalian cells, most of the enzyme activity occurs in the microsomal fraction (Eibl *et al.*, 1969), but activity has been reported in the mitochondrial and plasma membrane fractions (Arthur *et al.*, 1987; Colard *et al.*, 1980). These studies indicate that a family of proteins is involved in the acylation of different lysophospholipids. Since the enzyme plays a critical role in the maintenance of the complex phospholipid molecular species composition of biological membranes, it seems crucial to define the interaction of these proteins with their lipid environment.

The characterization of the enzyme in the above studies was complicated by the use of intact biological membranes with a number of unknown factors, including a complex phospholipid molecular species composition and the presence of a number of enzymes. Attempts to purify and characterize the pure enzyme have been hampered by difficulties in solubilization of it from the membrane domain without inactivation of it. An enzyme from bovine brain and heart microsomes has been isolated (Deka *et al.*, 1986; Sanjanwala *et al.*, 1988) but was very unstable under the conditions used. In rat liver microsomes, the enzyme can be solubilized with detergents (Mukherjee *et al.*, 1992), but greater success has been obtained with 1-acyllysoPC (Hasagawa-Sasaki & Ohno, 1980). When deoxycholate (DOC)-washed rat liver microsomes were solubilized with equal molar amounts of 1-acyllysoPC and $C_{18:1}$CoA and incubated at room temperature, PC vesicles were formed. These vesicles, containing an active enzyme with a specific activity of 9−10 μmol min^{-1} (mg of protein)$^{-1}$, could then be isolated in a glycerol density gradient (Deamer & Boatman, 1980; Gavino & Deamer, 1982). These studies however did not define the lipid composition of the vesicles or the effect of such an environment on the activity of the enzyme.

In the present study, we sought to optimize the conditions for solubilization of DOC-washed rat liver microsomes with 1-($C_{16:0}$)lysoPC and different acyl-CoAs to form PC vesicles of defined phospholipid molecular species composition by the action of acyl-CoA:lysoPC acyltransferase. We analyzed

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[1] Abbreviations: acyl-CoA:lysoPC acyltransferase, acyl coenzyme A:1-acyllysophosphatidylcholine acyltransferase; PL, phospholipid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol; FA, fatty acid; $C_{16:0}$CoA, palmitoyl-CoA; $C_{18:1}$CoA, oleoyl-CoA; $C_{18:2}$CoA, linoleyl-CoA; $C_{20:4}$CoA, arachidonoyl-CoA; DOC, sodium deoxycholate; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); SDS−PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis.

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the lipid composition, purity, and activity of acyl-CoA:lysoPC acyltransferase in the PC vesicles and evaluated the effects of differences in the PC molecular species environment on enzyme activity.

EXPERIMENTAL PROCEDURES

Materials. [1-^{14}C]$C_{16:0}$CoA (specific activity of 57.0 mCi/mmol) and [1-^{14}C]$C_{20:4}$CoA (specific activity of 46.3 mCi/mmol) were purchased from DuPont-New England Nuclear Corp., Boston, MA. [1-^{14}C]$C_{18:1}$CoA (specific activity of 55.0 mCi/mmol) and 1-[1-^{14}C]($C_{16:0}$)lysoPC (specific activity of 56.0 mCi/mmol) were obtained from Amersham Corp., Arlington Heights, IL. Econo-Safe scintillation fluid was obtained from Research Products International Corp., Mount Prospect, IL. Silica plates for thin-layer chromatography were purchased from Analtech, Inc., Newark, DE. $C_{16:0}$CoA, $C_{18:1}$CoA, $C_{18:2}$CoA, $C_{20:4}$CoA, 1-acyllysoPI (from soybean with mixed fatty acyl composition), and sodium deoxycolate (DOC) were products of Sigma Chemical Co., St. Louis, MO. 1-Acyl-lysoPC ($C_{16:0}$), 1-acyllysoPE ($C_{16:0}$), and 1-acyllysoPS ($C_{16:0}$) were obtained from Avanti Polar Lipids, Inc. Alabaster, AL. All other compounds used were reagent grade.

Preparation of Microsomes. Rat livers were a generous gift from Dr. Faddy Wanna and were used fresh. DOC-washed rat liver microsomes were produced as described by Hasegawa-Sasaki and Ohno (1980). The microsomal suspension was centrifuged at 225000*g*, and the pellet was washed with 0.33% DOC, resuspended to 6 mg/mL in 20 mM Tris-HCl (pH 7.4) containing 0.2 mM ethylenediaminetetraacetic acid (EDTA), divided into 200 μL aliquots containing 200 μg of protein, and stored at −70 °C. Acyl-CoA:lysoPL acyltransferase activity in the sample was stable for several months. The protein concentration was determined as described by Bradford (1976).

Formation and Isolation of PC Vesicles. DOC-washed rat liver microsomes containing 200 μg of protein were solubilized with various amounts of 1-($C_{16:0}$)lysoPC and $C_{18:0}$CoA, $C_{18:2}$CoA, or $C_{20:4}$CoA. Microsomes were solubilized by addition of a solution of 20 mM 1-($C_{16:0}$)lysoPC in 20 mM Tris-HCl (pH 7.4). The suspension was vortexed for 1 min, diluted to 1:1 with 20 mM Tris-HCl, and placed in a bath sonicator for 30 s. To this was added a solution of 4 mM acyl-CoA in 20 mM Tris-HCl, and the suspension was again vortexed for 1 min, followed by sonication for 30 s. The volume was increased to 1 mL with 20 mM Tris-HCl and made 1 mM with dithiothreitol (DTT), 1 mM with EDTA, and 0.02% with NaN_3.

The reaction mixtures were incubated at 37 °C for various time periods, and PC vesicles were isolated in a glycerol gradient by centrifugation (Gavino & Deamer, 1982). Briefly, preparations that turned turbid, as indicated by increased absorbance at 500 nm, were sonicated for 30 s in a bath sonicator, placed in a centrifuge tube, and diluted to 50% with glycerol. Then, 30, 15, and 0% glycerol solutions were layered on the reaction mixtures, and the gradient preparation was centrifuged at 30 000 rpm for 12 h. Vesicle PC bands were examined and photographed against a dark background.

Characterization of Incubation Mixtures and PC Vesicles. In one set of experiments, the incubation mixtures and PC vesicles formed from different substrates with or without radioactive label were analyzed to determine their composition. Lipids from PC vesicles or microsomes were extracted (Bligh & Dyer, 1959), dried under N_2, resuspended in 60 μL of $CHCl_3/CH_3OH$ (2:1 by volume), and applied to a silica TLC plate. The plates were developed in $CHCl_3/CH_3OH/CH_3COOH$/0.9% NaCl (100:50:16:5 by volume). PL classes were quantitated by measuring inorganic phosphate (Rouser, 1970). The FA composition of PC was determined by gas chromatography after transesterification in MeOH/HCl (Kuypers *et al.*, 1988). PC molecular species were determined according to Kuypers *et al.* (1991). Acyl-CoA was measured as described by Hornberger and Patscheke (1990). Radiolabeled compounds were counted for radioactivity in a liquid scintillation counter.

Measurement of Acyl-CoA:LysoPL Acyltransferase Activity. Acyl-CoA:lysoPC acyltransferase activity in PC vesicles and microsomes was assayed by measuring the formation of [1-^{14}C]PC from 1-[1-^{14}C]($C_{16:0}$)lysoPC and acyl-CoA. To determine the activity of acyl-CoA:lysoPL acyltransferases toward different PL classes, [1-^{14}C]$C_{20:4}$CoA and 1-($C_{16:0}$)-lysoPC, 1-($C_{16:0}$)lysoPE, 1-($C_{16:0}$)lysoPS, or 1-acyllysoPI were used as substrates. The enzyme reaction generating radioactive PC, PS, or PE was stopped by addition of 0.5 mL of incubation mixture to 2 mL of $CHCl_3/CH_3OH$ (2:1 by volume). The enzyme reaction generating radioactive PI was stopped by addition of 0.5 mL of incubation mixture to 15 mL of $CHCl_3/CH_3OH$/6 N HCl (25:50:1 by volume); the mixture was incubated for 30 min with occasional vortexing, and 5 mL of H_2O and 5 mL of $CHCl_3$ were added to obtain two phases. Lipids were extracted and separated by TLC as described above. PC, PS, PI, or PE bands were scraped off and counted for radioactivity in a liquid scintillation counter.

Gel Electrophoresis. PC vesicles were dialyzed for 3 days with several changes of 5 mM Tris buffer (pH 7.4) to remove salts and glycerol and extracted twice with $CHCl_3/CH_3OH$ (2:1 by volume) to remove lipids. The water and interface containing the proteins were dried in a SpeedVac and resuspended in SDS sample buffer containing 4.5 M urea. The same sample buffer was used for the microsomes. SDS−PAGE was carried out as described by Laemmli (1970). The proteins were resolved on a 13% separation gel with a 4% stacking gel. The gel was run at 50 V until the dye front reached the separation gel and was then run at 150 V. After it was stained (Bio-Rad Silver Stain kit), the gel was scanned in a densitometer to estimate the amount of protein associated with the PC vesicles.

RESULTS

Optimal Conditions for PC Vesicle Formation. The optimal conditions for the formation of PC vesicles were found when DOC-washed microsomes containing 200 μg of protein were solubilized with 2000 nmol each of 1-($C_{16:0}$)lysoPC and $C_{18:2}$CoA (Table 1a). PC formation increased with incubation time; after 20 h, approximately one-half of the substrate was found as PC in the newly formed vesicles (Table 1b). The optimal protein:lysoPC ratio was 1:5 (by weight), and the optimal lysoPC:acyl-CoA ratio was 1:1 (molar amounts).

The increase in absorbance at 500 nm did not correlate with the formation of PC vesicles. In fact, absorbance increased the most in the sample with 4000 nmol of

Table 1: Amount of PC in the Upper Vesicle Band after Solubilization of DOC-Washed Microsomes with Different Amounts of 1-($C_{16:0}$)lysoPC and $C_{18:2}$CoA at 37 °C for 8 h (a) or with 2000 nmol of Each Substrate for 3, 8, or 20 h (b)

a

lysoPC:acyl-CoA (nmol:nmol)	2000:2000 (heat-inactivated control)	1000:1000	2000:2000	3000:3000	4000:2000	2000:4000
ΔA_{500}[a]	0	0.048	0.190	0.157	0.302	0
PC in upper band (nmol)	−	30	600	350	20	−

b

incubation time (h)	3	8	20
PC in upper band (nmol)	400	600	1050

[a] (ΔA_{500} is the change in absorbance at 500 nm).

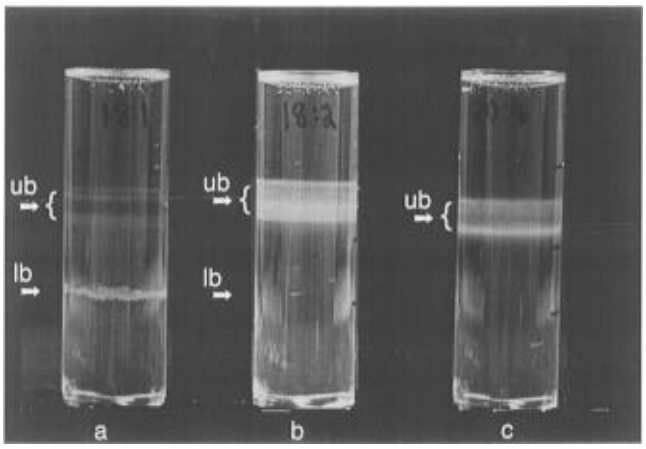

FIGURE 1: PC vesicles formed from 200 μg of DOC-washed rat liver microsomal protein solubilized with 2000 nmol of 1-[1-^{14}C]lysoPC and 2000 nmol of [1-^{14}C]$C_{18:1}$CoA (a), $C_{18:2}$CoA (b), or [1-^{14}C]$C_{20:4}$CoA (c) and isolated in a glycerol step gradient. After 20 h of incubation at 37 °C, two bands of low-density PC vesicles were found in the 15% glycerol solution; these two bands were collected as the upper band (ub). A band of high-density PC vesicles was found in the 30% glycerol solution when [1-^{14}C]$C_{18:1}$CoA or $C_{18:2}$CoA was used as the acyl substrate.

lysoPC, in which minimal amounts of PC were formed (Table 1a). A 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) assay showed formation of free CoA and increased absorbance regardless of whether PC formed (data not shown).

PC vesicles formed by incubation of the microsomal preparation with 2000 nmol each of lysoPC and different acyl donors for 20 h are shown in Figure 1. Glycerol step gradient analysis demonstrated two turbid bands in the 15% glycerol solution, which were collected together and will be referred to as the upper band. A lower band was found in the 30% glycerol solution when $C_{18:1}$CoA or $C_{18:2}$CoA was used as the acyl donor, but not when $C_{20:4}$CoA was used.

Characterization of Incubation Mixtures and PC Vesicles. Table 2a shows the composition of PC, lysoPC, acyl-CoA, and FA in the incubation mixtures before centrifugation and in the upper and lower PC vesicle bands in the experiments shown in Figure 1.

When $C_{18:2}$CoA was used as the acyl donor, the incubation mixture contained approximately 1400 nmol of PC. Approximately 77 nmol of the PC originated from the microsomes (Table 3); therefore, more than 1300 nmol of PC was formed from 1-($C_{16:0}$)lysoPC and $C_{18:2}$CoA by acyl-CoA:lysoPC acyltransferase activity. In addition to a fair amount of FA, the incubation mixture contained more lysoPC than acyl-CoA. The upper band contained approximately 71% of the total amount of PC, whereas the lower band contained less than 10%. The upper band also contained 50% of the remaining lysoPC substrate and 14% of the remaining acyl-CoA.

When $C_{18:1}$CoA was used as the acyl donor, the location of the bands was similar to that obtained with $C_{18:2}$CoA; however, the upper band was less intense, and the lower band was more intense. The incubation mixture contained approximately 1300 nmol of PC. The upper and lower bands each contained approximately 500 nmol of PC, as well as nearly all of the remaining lysoPC but less than 35% of the remaining acyl-CoA.

When $C_{20:4}$CoA was used as the acyl donor, approximately 1400 nmol of PC formed. The upper band contained 78% of the total amount of PC. The amounts of lysoPC and acyl-CoA associated with the band were similar to those obtained with $C_{18:2}$CoA. In all three experiments, most of the remaining acyl-CoA was found in the 50% glycerol solution (data not shown).

Table 2b shows the PC:lysoPC, PC:acyl-CoA, and PC:FA ratios in the three experiments shown in Figure 1. When $C_{18:1}$CoA was used as the acyl donor, the PC:lysoPC ratio was lower in the upper band than in the incubation mixture. When $C_{18:2}$CoA or $C_{20:4}$CoA was used, the PC:lysoPC ratio was higher in the upper band than in the incubation mixture. In all three experiments, the PC:acyl-CoA ratio was higher in the upper band than in the incubation mixture.

Taken together, these findings show that using different acyl-CoA substrates with 1-($C_{16:0}$)lysoPC to solubilize DOC-washed rat liver microsomes markedly alters the composition and density of the newly formed PC vesicles.

PL Composition of PC Vesicles Formed from 1-($C_{16:0}$)-LysoPC and $C_{18:2}$CoA. Table 3 shows the PL composition of the microsomal preparation and the PC vesicles formed after 10 h of incubation with 2000 nmol each of 1-($C_{16:0}$)lysoPC and $C_{18:2}$CoA. The solvent system used for TLC did not separate PS and PI. The amounts of PE and PI/PS relative to PC were 39.5 and 32.4%, respectively, in microsomes but were less than 1% in the vesicles.

FA Composition of PC in the Vesicles. In a similar experiment, $C_{18:1}$CoA, $C_{18:2}$CoA, and $C_{20:4}$CoA were used as acyl donors and the FA composition of vesicle PC in the upper band was analyzed by gas chromatography (Table 4). All of the FA detected in vesicle PC originated from the substrates. Moreover, a single molecular species of PC was detected in the vesicles (Figure 4A,C).

Acyl-CoA:LysoPL Acyltransferase Activity in Microsomes and PC Vesicles Formed from 1-($C_{16:0}$)LysoPC and $C_{20:4}$CoA. Acyl-CoA:lysoPL acyltransferase activity in microsomes and in the upper band was studied using $C_{20:4}$CoA and different lysoPL substrates (Figure 2). In microsomes, the rates of PS, PE, and PI formation relative to PC formation were 99,

Table 2: Characterization of Incubation Mixture and PC Vesicles Formed from 1-($C_{16:0}$)lysoPC and Different Acyl-CoA Substrates after 20 h of Incubation[a]

a

	$C_{18:1}$CoA			$C_{18:2}$CoA			$C_{20:4}$CoA	
compound	mixture (nmol)	upper band (nmol)	lower band (nmol)	mixture (nmol)	upper band (nmol)	lower band (nmol)	mixture (nmol)	upper band (nmol)
PC	1311	505	543	1477	1046	129	1460	1145
lysoPC	592	332	251	452	224	75	429	271
acyl-CoA	426	82	64	340	46	32	358	51
FA	297	88	74	252	98	41	336	119

b

	$C_{18:1}$CoA			$C_{18:2}$CoA			$C_{20:4}$CoA	
ratio	mixture	upper band	lower band	mixture	upper band	lower band	mixture	upper band
PC:lysoPC	2.21	1.52	2.16	3.26	4.67	1.72	3.40	4.23
PC:acyl-CoA	3.08	6.15	8.48	4.34	22.74	4.03	4.08	22.45
PC:FA	4.41	5.74	7.34	5.86	10.67	3.15	4.35	9.62

[a] Each result is the mean of three independent experiments.

Table 3: Phospholipid Composition of DOC-Washed Microsomes and Upper PC Vesicles Formed by Incubation of the Microsomal Preparation with 1-($C_{16:0}$)lysoPC and $C_{18:2}$CoA for 10 h[a]

	DOC-washed microsomes		upper band	
	nanomoles	relative amount (%)	nanomoles	relative amount (%)
PC	76.5 ± 2.2	100.0 ± 2.9	784.7 ± 19.7	100.0 ± 2.5
PE	30.2 ± 0.2	39.5 ± 0.1	7.4 ± 0.3	0.6 ± 0.2
PI/PS	24.8 ± 1.9	32.4 ± 0.6	5.1 ± 0.3	0.6 ± 0.2

[a] Each result is the mean ± the standard deviation of four independent experiments.

Table 4: Fatty Acid Composition of PC from DOC-Washed Microsomes and Upper PC Vesicles Formed from 2000 nmol Each of 1-($C_{16:0}$)lysoPC and Different Acyl-CoA Substrates after 10 h of Incubation[a]

FA found in PC	DOC-washed microsomes	($C_{16:0}$)lysoPC and $C_{18:1}$CoA	($C_{16:0}$)lysoPC and $C_{18:2}$CoA	($C_{16:0}$)lysoPC and $C_{20:4}$CoA
16:0	1.00	1.00	1.00	1.00
18:0	0.81 ± .02	nd	nd	nd
18:1	0.27 ± .01	0.96 ± 0.07	nd	nd
18:2	0.66 ± .01	nd	0.86 ± 0.04	nd
20:4	0.68 ± .06	nd	nd	0.90 ± 0.07
22:6	0.14 ± .01	nd	nd	nd

[a] Results, expressed as a fraction relative to the peak area of $C_{16:0}$FA, are the mean ± the standard deviation of four independent experiments. nd is not detectable.

57, and 36%, respectively (Figure 2A). Figure 2B shows acyl-CoA:lysoPL acyltransferase activity in PC vesicles formed from 1-($C_{16:0}$)lysoPC and $C_{20:4}$CoA. In contrast to microsomes, the PC vesicles showed very low acyl-CoA: lysoPL acyltransferase activity when lysoPS, lysoPE, or lysoPI was used as a substrate. The rates of PS, PE, and PI formation relative to the formation of PC were 22, 6, and 8%, respectively (Figure 2B).

In experiments in which lysoPS, lysoPE, and lysoPI were used as substrates for the enzyme reaction, lysoPC in the the newly formed vesicles (Table 2a) must be taken into consideration as a competing substrate. In each PC vesicle assay, 10 nmol of PC, containing 2.4 nmol of lysoPC as calculated from the PC:lysoPC ratio (Table 2b), and 25 nmol of lysoPL substrate were used. Therefore, 8−9% of the lysoPL substrate was lysoPC from the PC vesicles. However, when lysoPS, lysoPE, or lysoPI was used, less than 2% of the PL formed was PC (data not shown).

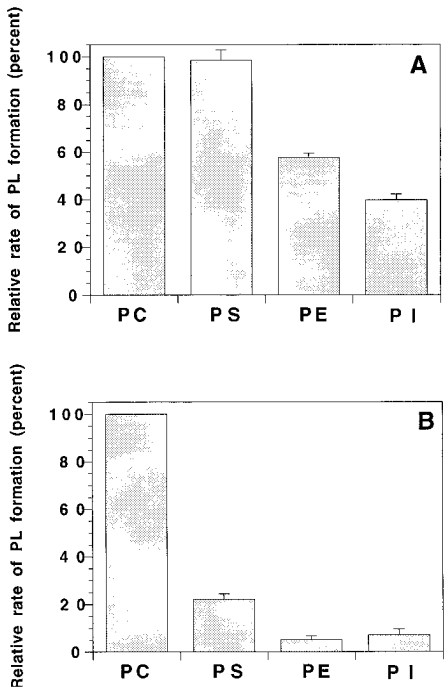

FIGURE 2: Activity of acyl-CoA:lysoPL acyltransferases toward different lysoPL substrates. A 600 μL reaction mixture of 20 mM Tris-HCl (pH 7.4) contained 10 nmol of [1-^{14}C]$C_{20:4}$CoA, 25 nmol of different lysoPL substrates, and 10 μg of DOC-washed rat liver microsomal protein (A) or an amount of PC vesicles corresponding to 10 nmol of PC (B). Microsomes and PC vesicles were incubated at 37 °C for 10 and 30 min, respectively. The results are the mean ± the standard deviation of four independent experiments.

Acyl-CoA:LysoPC Acyltransferase Activity in Microsomes and PC Vesicles Incubated with Different Acyl-CoA Substrates. Figure 3A shows acyl-CoA:lysoPC acyltransferase activity in microsomes incubated with different acyl-CoA substrates. The rate of PC formation was highest when $C_{18:2}$CoA was the acyl donor. When $C_{20:4}$-CoA, $C_{18:1}$CoA, and $C_{16:0}$CoA were used, PC formation was 68, 52, and 22%, respectively, of that obtained with $C_{18:2}$CoA.

Figure 3B shows acyl-CoA:lysoPC acyltransferase activity in PC vesicles formed from 1-($C_{16:0}$)lysoPC and $C_{18:2}$CoA and incubated with different acyl-CoA substrates. The rate of PC formation was highest when $C_{20:4}$CoA was used as the acyl donor. When $C_{20:4}$CoA, $C_{18:1}$CoA, and $C_{16:0}$ CoA

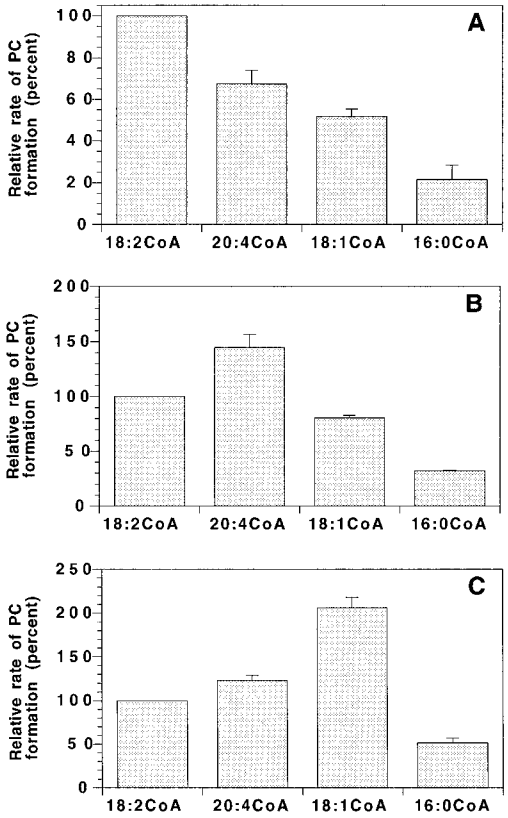

FIGURE 3: Acyl-CoA:lysoPC acyltransferase activity toward different acyl-CoA substrates. A 600 μL reaction mixture of 20 mM Tris-HCl (pH 7.4) contained 25 nmol of 1-[1-^{14}C]lysoPC, 10 nmol of different acyl-CoA substrates, and 10 μg of DOC-washed rat liver microsomal protein (A) or an amount of PC vesicles corresponding to 10 nmol of ($C_{16:0-18:2}$)PC (B) or ($C_{16:0-20:4}$)-PC (C). Microsomes and PC vesicles were incubated at 37 °C for 10 and 30 min, respectively. The results are the mean ± the standard deviation of four independent experiments.

were used, PC formation was 145, 81, and 32%, respectively, of that obtained with $C_{18:2}$CoA.

Figure 3C shows acyl-CoA:lysoPC acyltransferase activity in PC vesicles formed from 1-($C_{16:0}$)lysoPC and $C_{20:4}$CoA and incubated with different acyl-CoA substrates. The rate of PC formation was highest when $C_{18:1}$CoA was used as the acyl donor. When $C_{18:1}$CoA, $C_{20:4}$CoA, and $C_{16:0}$ CoA were used, PC formation was 206, 123, and 52%, respectively, of that obtained with $C_{18:2}$CoA.

Again, leftover substrate in the PC vesicles, in this case respectively $C_{18:2}$CoA and $C_{20:4}$CoA, must be considered when evaluating the enzyme activity. In each PC vesicle assay, 10 nmol of PC, containing 0.4 nmol of leftover $C_{18:2}$CoA and 0.4 nmol of leftover $C_{20:4}$CoA substrate as calculated from the PC:acyl-CoA ratio (Table 2b), was used. Because 10 nmol of acyl-CoA substrate was added in each assay, the contamination from acyl-CoA in the PC vesicles was only 4%.

Taken together, these results indicate that acyl-CoA:lysoPC acyltransferase activity is different in either its microsomal environment or vesicles of different PC molecular species design and show that the activity of the enzyme is affected by the phospholipid molecular species environment.

Modulation of PC Molecular Species in PC Vesicles. Incubation of the PC vesicles with different acyl-CoA substrates led to the formation of new PC molecular species (Figure 4). Vesicles generated from 1-($C_{16:0}$)lysoPC and $C_{18:2}$CoA contained only ($C_{16:0-18:2}$)PC (Figure 4A). When these vesicles were incubated with 1-($C_{16:0}$)lysoPC, $C_{18:1}$CoA, and $C_{20:4}$CoA, ($C_{16:0-18:1}$)PC and ($C_{16:0-20:4}$)PC were formed (Figure 4B). Similarly, vesicles generated from 1-($C_{16:0}$)lysoPC and $C_{20:4}$CoA contained only ($C_{16:0-20:4}$)PC (Figure 4C) and incorporated ($C_{16:0-18:2}$)PC after incubation with 1-($C_{16:0}$)lysoPC and $C_{18:2}$CoA (Figure 4D). The relative amounts of these new PC molecular species depended on the incubation time (data not shown).

Purification of an Acyl-CoA:LysoPC Acyltransferase from PC Vesicles. In experiments to characterize the protein in the vesicles, 400 μg of microsomes was solubilized with 4000 nmol each of 1-($C_{16:0}$)lysoPC and $C_{18:2}$CoA and incubated for 8 h at 37 °C. The PC vesicles were isolated and analyzed by SDS−PAGE as described in Experimental Procedures. The vesicles contained one major protein band with a molecular mass of approximately 21 kDa (Figure 5). The protein content of the vesicles, estimated by densitometry, was 1.3 μg. The estimated specific activity of acyl-CoA:lysoPC acyltransferase in the PC vesicles formed with 1-($C_{16:0}$)lysoPC and $C_{18:2}$CoA as substrates was 10 μmol min^{-1} (mg of protein)$^{-1}$, which was 200-fold higher than in rat liver microsomes [51.11 ± 3.95 nmol min^{-1} (mg of protein)$^{-1}$].

DISCUSSION

Although acyl-CoA:lysoPC acyltransferase activity has been characterized in subcellular fractions of many tissues and organisms, the majority of these studies has been performed on intact membrane systems, thereby involving several unknown factors. Reacylation of phospholipids plays a very importent role in the maintenance of the membrane phospholipid molecular species composition. It is logical to expect that protein lipid interactions will modulate the activity of acyl-CoA:lysoPL acyltransferase. Hence, reconstitution of a pure protein in a well-defined phospholipid environment seems important for understanding the effect of the bilayer on the enzyme activity. The importance of the lipid environment is further indicated by the fact that very few attempts to purify these enzymes have been successful due to the difficulties in separation of the enzyme from its lipid environment without loss of enzyme activity. In studies where the enzyme was purified from bovine brain and heart microsomes, the incipient solubilization of the microsomes with DOC resulted in a purified enzyme that was highly unstable (Deka *et al.*, 1986; Sanjanwala *et al.*, 1988). To study the characteristics of the purified enzyme, it seems crucial to maintain it in a phospholipid environment. This approach was first used by the laboratory of Deamer (Gavino & Deamer, 1982). These studies however did not attempt to define or alter the phospholipid molecular species environment in which the enzyme was reconstituted or measure the effect on its activity. In this study, we describe the optimalization of the conditions for solubilization of DOC-washed rat liver microsomes with 1-($C_{16:0}$)lysoPC and different acyl-CoAs to form PC vesicles of defined phospholipid molecular species composition by the action of acyl-CoA:lysoPC acyltransferase. We analyzed the lipid composition, purity, and activity of acyl-CoA:lysoPC acyltransferase in the PC vesicles.

Since the enzyme's activity is retained in these vesicles for several days, they form an ideal system for evaluation of the lipid protein interactions that modulate the activity of this protein.

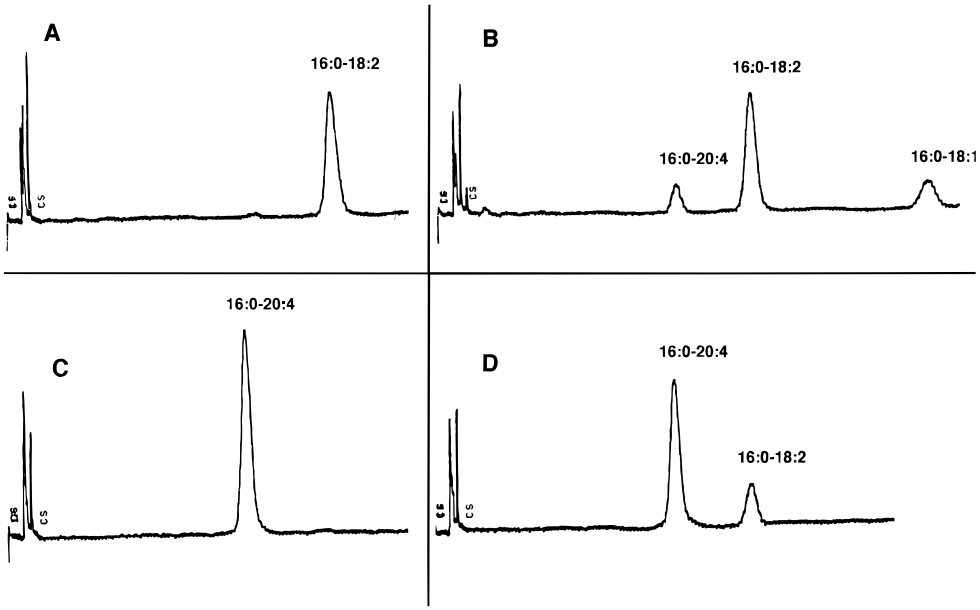

FIGURE 4: HPLC analysis of PC molecular species. PC vesicles were generated from microsomes by incubation with 1-($C_{16:0}$)lysoPC and $C_{18:2}$CoA (A) or 1-($C_{16:0}$)lysoPC and $C_{20:4}$CoA (C). The ($C_{16:0-18:2}$)PC vesicles were subsequently incubated with 1-($C_{16:0}$)lysoPC, $C_{18:1}$-CoA, and $C_{20:4}$CoA (B), while the ($C_{16:0-20:4}$)PC vesicles were incubated with ($C_{16:0}$)lysoPC and $C_{18:2}$CoA (D) at 37 °C for 30 min.

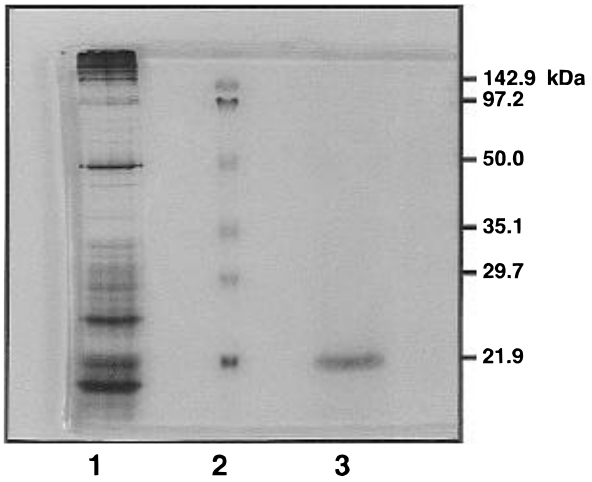

FIGURE 5: SDS−PAGE analysis of DOC-washed rat liver microsomal protein (7 μg, lane 1), molecular size markers (lane 2), and low-density PC vesicles formed from 400 μg of microsomes incubated with 4000 nmol each of lysoPC and $C_{18:2}$CoA (lane 3).

PC Vesicle Formation. In this study, PC vesicles formed when 1-($C_{16:0}$)lysoPC and $C_{18:1}$CoA, $C_{18:2}$CoA, or $C_{20:4}$CoA were used to solubilize DOC-washed rat liver microsomes. The optimal protein:lysoPC ratio was 1:5 (by weight), and the optimal lysoPC:acyl-CoA ratio was 1:1 (molar amounts) (Table 1a). When we used a protein:lysoPC ratio of 1:10 (by weight) as suggested by Gavino and Deamer (1982), no PC vesicles formed (data not shown). Gavino and Deamer (1982) also suggested that the formation of PC vesicles could be followed by measuring the increase in absorbance at 500 nm. In our study, however, the increase in absorbance did not correlate with the formation of PC vesicles. Very small amounts of PC formed under conditions that greatly increased absorbance (Table 1a). We found a substantial amount of FA in the reaction mixture (Table 2a), and a DTNB assay showed liberation of free CoA regardless of whether PC vesicles formed. These findings suggest that the increase in absorbance is caused by free FA and free CoA from hydrolysis of acyl-CoA as well as free CoA resulting from acylation of lysoPC.

Enzyme Activity. It was reported that acyl-CoA:lysoPC acyltransferase activity was not affected by large amounts of substrates used in the reaction mixture and that the substrates were depleted after 1 h of incubation (Gavino & Deamer, 1982). In our study, 1200−1400 nmol of PC formed after 20 h of incubation at 37 °C under optimal solubilization conditions with 1-($C_{16:0}$)lysoPC and $C_{18:1}$CoA, $C_{18:2}$CoA, or $C_{20:4}$CoA (Table 2a). This corresponded to an enzyme activity that was less than 5% of the activity found when small amounts of substrates were used. These results suggest that acyl-CoA substrates with a high rate of incorporation into PC should be used to overcome the low enzyme activity in the presence of large amounts of substrate. The rate of PC formation in DOC-washed rat liver microsomes is lower when $C_{16:0}$CoA is used as the acyl donor than when $C_{18:1}$CoA, $C_{18:2}$CoA, or $C_{20:4}$CoA is used (Figure 3A). When $C_{16:0}$CoA was used, no PC formed (data not shown). When $C_{18:2}$CoA or $C_{20:4}$CoA was used, 70−80% of the newly formed PC was found in vesicles in the upper band (Table 2a). Using different acyl-CoA substrates affected the lipid composition and density of the vesicles. When any of the three acyl-CoA substrates was used, two bands of low-density PC vesicles were found in the 15% glycerol solution, and when $C_{18:1}$CoA or $C_{18:2}$CoA was used, a band of high-density PC vesicles was found in the 30% glycerol solution (Figure 1). Using $C_{18:2}$CoA or $C_{20:4}$CoA as the substrate resulted in vesicles consisting of approximately 75% newly formed PC. The PL originating from the microsomes was less than 1% as compared to the newly formed PC (Table 3). Nearly all the non-PC lipid consisted of lysoPC and FA (Table 2a). A substantial amount of high-density PC vesicles formed from 1-($C_{16:0}$)-lysoPC and $C_{18:1}$CoA (Figure 1A). Both the low- and high-density PC vesicles formed from 1-($C_{16:0}$)lysoPC and $C_{18:1}$CoA contained substantial amounts of leftover substrates (Table 2b).

All of the isolated low-density PC vesicle bands contained acyl-CoA:lysoPC acyltransferase activity. Enzyme activity was stable in the PC vesicles for at least 14 days. Because of the leftover substrates in the PC vesicles formed from 1-($C_{16:0}$)lysoPC and $C_{18:1}$CoA, enzyme activity was characterized in vesicles formed from 1-($C_{16:0}$)lysoPC and $C_{18:2}$-CoA or $C_{20:4}$CoA. Compared with microsomes, PC vesicles formed from 1-($C_{16:0}$)lysoPC and $C_{20:4}$CoA had very low activity toward lysoPS, lysoPE, and lysoPI (Figure 2). In addition, compared with microsomes, PC vesicles formed from 1-($C_{16:0}$)lysoPC and $C_{18:2}$CoA or $C_{20:4}$CoA showed different rates of PC formation, depending on the acyl-CoA substrate used (Figure 3). Acyl-CoA:lysoPL acyltransferases specific for the head group of PL have been identified in microsomes from bovine brain and heart (Deka *et al.*, 1986; Sanjanwala *et al.*, 1988; Sanjanwala *et al.*, 1989). The results shown in Figure 2 suggest that an enzyme specific for the choline head group is isloated in the formed PC vesicles. Additional experiments using lysoPS, lysoPE, or lysoPI to solubilize DOC-washed rat liver microsomes could clarify if the procedure requires specific enzymes. The results shown in Figure 3 suggest that changes in the lipid environment affect the enzyme's affinity for its acyl-CoA substrates. Although the pattern seems complicated, the enzyme's affinity for its acyl-CoA substrates is clearly different in ($C_{16:0-18:2}$)PC vesicles or ($C_{16:0-20:4}$)PC vesicles and altered when compared to rat liver microsomes. Incubating PC vesicles with 1-($C_{16:0}$)lysoPC and different acyl-CoA substrates led to the incorporation of PC molecular species of choice (Figure 4B and 4D). By sequential addition of small amounts of substrates for the enzyme, the amount of PC in the vesicles would increase and the final PC molecular species composition would depend on the substrates added. Analyzing the specific activity of the enzyme in relation to the PC composition of the vesicles could help answer important questions concerning the effects of the lipid environment on enzyme activity.

Protein Analysis. Gel electrophoresis of the proteins in the low-density PC vesicles formed from 1-($C_{16:0}$)lysoPC and $C_{18:2}$CoA showed one major protein band with a molecular mass of 21 kDa (Figure 5). The protein was purified to approximately 95% homogeneity. Approximately 33% of the total acyl-CoA:lysoPC acyltransferase activity from microsomes was found in the PC vesicles, and the specific activity of the enzyme was 10 μmol min^{-1} (mg of protein)$^{-1}$. Since the amount of acyl-CoA:lysoPC acyltransferase has to be higher in the vesicles loaded in lane 3 than in the microsomes loaded in lane 1, our data strongly suggest that the 21 kDa protein is an acyl-CoA:lysoPC acyltransferase. We are currently analyzing its amino acid composition.

Conclusions. When DOC-washed rat liver microsomes were solubilized with lysoPC and either $C_{18:1}$CoA, $C_{18:2}$CoA, or $C_{20:4}$CoA and incubated at 37 °C, PC vesicles were formed by the action of acyl-CoA:lysoPC acyltransferase. The optimal protein:lysoPC ratio was 1:5 (by weight), and the optimal lysoPC:acylCoA ratio was 1:1 (molar amounts). Analysis of the vesicles showed small amounts of leftover PL from the microsomes and leftover substrates from the incubation mixture. The isolated PC vesicles contained acyl-CoA:lysoPC acyltransferase activity, and SDS−PAGE analysis showed one major protein band with a molecular mass of 21 kDa. These results indicate that an acyl-CoA:lysoPC acyltransferase forms its own environment by generating vesicles. Since enzyme activity is stable in these vesicles for several days, they provide a good system for the study of enzyme characteristics in a bilayer of well-defined phospholipid molecular species composition. The lipid environment can be regulated in two ways. Depending on the structure of the lysoPC and the acyl-CoA used to solubilize the microsomes, the enzyme can be isolated in PC vesicles consisting of the PC molecular species composition of choice. Moreover, after isolation of the PC vesicles, the active enzyme can change the lipid surroundings by making PC from added substrates with different acyl chains.

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