Purification and Properties of Lysophospholipase Produced by *Corticium centrifugum*[†]

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Lysophospholipase (EC 3. 1. 1. 5) from the culture broth of *Corticium centrifugum* was purified 92-fold in specific activity by DEAE-Sephadex and hydroxylapatite column chromatography. The isoelectric point was at about pH 3.9, and the molecular weight was about 130,000. The optimal pH was about 3.5~5.0. The stable pH range was from 7.0 to 8.0. Lysophospholipase activity was inhibited by Fe^{3+} , Hg^{2+} and Al^{3+} , but stimulated by various organic solvents. Diazobenzene *p*-sulfonic acid, N-bromosuccinimide and diisopropyl-fluorophosphate also inhibited the activity. This enzyme did not hydrolyze mono-, di- or tripalmitin or phosphatidylcholine. Apparent Michaelis constants of lysophospholipase activity for 1-acyl-LPC, 1-palmitoyl-LPC and 1-oleoyl-LPC were 0.35, 0.16 and 0.09 mM, respectively. The effect of detergents on the enzyme activity was observed to differ with the fatty acid composition of substrate.

It has previously been demonstrated that phospholipids acyl-hydrolases are present in the cell wall lytic enzyme preparation from *Corticium centrifugum*.¹⁾ To make clear the properties of and relations among various phospholipids acyl-hydrolases, i.e., phospholipases A and B and lysophospholipase, we attempted to purify the lysophospholipase of this sample at first. In this paper, purification of this enzyme and its properties are described.

MATERIALS AND METHODS

Enzyme. A lyophilized crude enzyme preparation was prepared from the culture broth of *C. centrifugum*

as follows. The mold was cultured in a medium, consisting of 4% of heat-treated brewer's yeast, 0.1% of KH_2PO_4 , 0.05% of KCl, 0.05% of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and 0.001% of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, at 26~28°C for 67~93 hr. The culture broth was filtered through two layers of cheese cloth and pulp layer, followed by ultrafiltration. The precipitate from the concentrated culture filtrate obtained by saturation with ammonium sulfate was collected and dialyzed against water for only 4 hr (because of the damage of the cellulose tubing by a cell wall lytic activity in the culture filtrate), followed by lyophilization. The purification of this crude enzyme preparation was done twice. The respective purified enzyme preparations were numbered I and II, of which specific activities were 68.0 and 50.9 units/mg, respectively. One unit of activity was defined as the quantity of enzyme which liberated 1 μmole of fatty acid per min.

Substrate. PC was prepared from egg yolk by the method of Pangborn,²⁾ and was purified by silicic acid column chromatography. LPC was prepared by the action of *Vipera russelli* phospholipase A (Sigma Chem. Co.) or *Crotalus adamanteus* phospholipase A (Worthington Biochem. Co.) on PC by the procedure of Hanahan,³⁾ and purified by silicic acid column chromatography. (u)-[¹⁴C]-1-Acyl-LPC was prepared by the action of *Vipera russelli* phospholipase A on (u)-[¹⁴C]-PC (New England Nuclear Co.) which was diluted with unlabeled PC from egg yolk previously. 1-[¹⁴C]-Palmitoyl-LPC was prepared by the action of *Crotalus adamanteus* (Sigma Chem. Co.) phospholipase A on

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Abbreviations: PC, phosphatidylcholine; LPC, lysophosphatidylcholine; FFA, free fatty acid; PCase, phosphatidylcholine acyl-hydrolase; LPCase, lysophospholipase; TLC, thin-layer chromatography; PPO, 2, 5-diphenyloxazole; POPOP, 1, 4-bis [2-(5-phenyloxazolyl)]-benzene.

L- α -dipalmitoyl-1-[14 C]-phosphatidylcholine (Applied Science Laboratories Inc.) which was diluted with unlabeled dipalmitoylphosphatidylcholine (Serdary Research Laboratories) previously. 1-[14 C]-Oleoyl-LPC was prepared by the same method as described above from L- α -dioleoyl-1-[14 C]-phosphatidylcholine (Applied Science Laboratories Inc.) which was diluted with unlabeled dioleoylphosphatidylcholine (Serdary Research Laboratories). These LPCs were purified by silicic acid column chromatography. The specific radioactivities of (u)-[14 C]-1-acyl-LPC, 1-[14 C]-palmitoyl-LPC and 1-[14 C]-oleoyl-LPC were 30, 45 and 95 cpm/m μ mole LPC, respectively.

Enzyme assay. LPCase activity, when unlabeled LPC was used as substrate, was assayed as follows. The reaction mixture contained 0.5 ml of aqueous solution of LPC (2 μ moles), 1.2 ml of 0.1 M acetate buffer, pH 4.0, and 0.3 ml of enzyme solution in a total volume of 2 ml. Incubation was carried out at 30°C for 8 min with shaking at a rate of 120 times per min. The reaction was stopped by addition of 2.5 volumes of methanol and by heating at 80°C for 1 min. The products were partitioned between chloroform and aqueous methanol by the procedure of Bligh and Dyer.⁴⁾ Enzyme activity was assayed by measuring FFA liberated in the chloroform phase by the method of Duncombe,⁶⁾ Satouchi *et al.*⁹⁾ and Itaya *et al.*,⁷⁾ with palmitic acid as standard. In some cases in which enzyme preparation I was used, incubation was carried out in 0.1 M acetate buffer, pH 3.6, at 45°C.

For assay of PCase, 0.5 ml of ultrasonic dispersion of PC (2 μ moles), which was sonicated with an ultrasonic oscillator (Kaijo Denki Model 4251S), 10 KHz, for 1 min, was used as substrate in place of aqueous solution of LPC. Other conditions and procedures were the same as above.

When labeled LPC was used as substrate, the reaction mixture contained LPC (1 μ mole) which was pipetted in the reaction tube as methanol solution and evaporated, 0.9 ml of 0.1 M acetate buffer, pH 4.0, and 0.1 ml of enzyme solution in a total volume of 1 ml. Enzyme reaction was carried out at 30°C for 8 min. The reaction was stopped by addition of 2.5 volumes of methanol and by heating at 80°C for 1 min. After the reaction, the products were partitioned between chloroform and aqueous methanol by the procedure of Bligh and Dyer,⁴⁾ and 2 ml of the chloroform phase was concentrated and spotted on Silica gel G plates (Merck). After TLC, each fraction of FFA, PC and LPC was scraped off, and its radioactivity was counted in a Packard 2425 liquid scintillation spectrometer with 10 ml of scintillation mixture containing 0.1 g of POPOP and 4 g of PPO per liter of toluene.

Thin-layer chromatography. The reaction products in the chloroform phase were analyzed by thin-layer chromatography, using silica gel plates (Merck) with

chloroform-methanol-water (80:35:5, v/v) as solvent. Spots were visualized by iodine vapor.

Isoelectric focusing. Isoelectric focusing was carried out in a 30 ml apparatus devised by Doi *et al.*,⁸⁾ in accordance with the method of Vesterberg.⁹⁾ Electrophoresis was performed with a carrier of pH 2.5~6.0 at 500~800 V (0.5~1.0 mA) for 50 hr at 0~4°C. After the electrophoretic run, the ampholine solution was fractionated to 1 ml, followed by measurement of pH and activity.

Determination of protein concentration. Protein concentration was determined by the method of Lowry *et al.*,¹⁰⁾ with bovine serum albumin as standard.

Disc electrophoresis. Disc electrophoresis was conducted in 7.5% gel for 90 min with a current of 2 mA/tube at 4°C in accordance with the method of Ornstein *et al.* (pH 9.4).¹¹⁾ Protein band was stained with Amide Black 10 B.

Estimation of molecular weight by gel filtration. The molecular weight of the enzyme was determined by gel filtration as described by Andrews.¹²⁾

RESULTS

Purification of LPCase

Step 1. Desalting. The crude enzyme was desalted by gel filtration with Bio-gel P-10.

Step 2. First DEAE-Sephadex Column Chromatography. The desalted solution was applied to a DEAE-Sephadex A-25 column (Fig. 1). LPCase activity was separated into three fractions. The most active fractions (No. 73~86) were combined, and concentrated with collodion-bag. The concentrated solution was dialyzed against 0.05 M Tris-HCl buffer, pH 7.3.

Step 3. Second DEAE-Sephadex Column Chromatography. The dialyzed solution was rechromatographed on a DEAE-Sephadex A-25 column. The active fractions were combined, and concentrated with collodion-bag. The concentrated solution was dialyzed against 10 mM phosphate buffer, pH 7.1.

Step 4. Hydroxylapatite Column Chromatography. The dialyzed solution was applied to a hydroxylapatite column (Fig. 2). The activity was eluted at 50 mM and 100 mM

FIG. 1. DEAE-Sephadex Column Chromatography of LPCase from *C. centrifugum*.

Desalted enzyme solution (320 ml, 6.72 g protein) was applied to a DEAE-Sephadex A-25 column (2.6×36 cm), which was equilibrated with 0.05 M Tris-HCl buffer, pH 7.3. The elution, at a rate of 30 ml/hr, was carried out with a linear gradient of NaCl concentration from zero to 0.8 M in the same buffer, and 10 ml fractions were collected.

-----, absorbance at 280 nm; ○—○, LPCase activity; □—□, PCCase activity; —, NaCl concentration.

buffer. The active fractions were concentrated and named 50 mM Frn. and 100 mM Frn., respectively. After concentration with collodion-bag, these fractions were preserved at -20°C .

The results of purification by above procedures are summarized in Table I. The 50 mM Frn. and 100 mM Frn. showed 37- and 92-fold increases in specific activity, respectively. The 100 mM Frn. was used for the investigation of various properties described below except for the estimation of molecular weight. The hydrolysis reaction showed a linear rate up to at least 10 min incubation by the standard assay method.

FIG. 2. Hydroxylapatite Column Chromatography of LPCase.

The dialyzed solution (8 ml, 132 mg protein) from the second DEAE-Sephadex (No. 51~57) was applied to a hydroxylapatite column (2.6×10 cm), which was consisted of hydroxylapatite and cellulose (1:1, v/v), and was equilibrated with 10 mM phosphate buffer, pH 7.1. The elution, at a rate of 35 ml/hr, was carried out with a stepwise increase in buffer concentration (10, 50 and 100 mM) and 5 ml fractions were collected. -----, absorbance at 280 nm; ○—○, LPCase activity; □—□, PCCase activity.

Homogeneity of purified enzyme

Homogeneities of both fractions were examined by disc electrophoresis (pH 9.4). The 50 mM Frn. had two LPCase components, and 100 mM Frn. had one (Fig. 3-(a) and (b)). The LPCase component of 50 mM Frn. (active fraction B in Fig. 3-(a)) was found a little contaminated with PCCase activity.

Estimation of molecular weight

The molecular weight of LPCase (50 mM Frn.: active fraction A in Fig. 3-(a)), determined by gel filtration with Sephadex G-200 (Fig. 4), was estimated to be about 130,000.

TABLE I. SUMMARY OF THE PURIFICATION OF LYSOPHOSPHOLIPASE PRODUCED BY *C. centrifugum*

Procedure	Protein (mg)	Activity (units)		Specific activity (units/mg)		Recovery (%)	
		LPCase	PCCase	LPCase	PCCase	LPCase	PCCase
Desalted solution of lyophilized protein of $(\text{NH}_4)_2\text{SO}_4$ ppt. (100% satn.)	6720	4964.4	5325.6	0.74	0.79	100	100
1st DEAE-Sephadex	875	1632.8	430.5	1.86	0.49	32.9	8.1
2nd DEAE-Sephadex	131.6	781.3	109.6	5.55	0.83	14.7	2.1
Hydroxylapatite							
50 mM Frn.	11.6	313.9	39.4	27.18	3.41	6.35	0.74
100 mM Frn.	1.3	91.1	0.0	68.00	0.00	1.83	0.00

FIG. 3. Disc Electrophoresis of LPCase.

About 30~50 μg of the purified enzyme was subjected to electrophoresis at pH 9.4. See the text for experimental details. (a) and (b) are the patterns of disc electrophoresis of 50 mm Frn. and 100 mm Frn., respectively.

FIG. 4. Estimation of Molecular Weight of LPCase by Gel Filtration.

Chromatography was carried out on a Sephadex G-200 column (1.5 $\times$ 95 cm) with 0.05 M Tris-HCl buffer, pH 7.4, containing 0.1 M KCl after the column had been equilibrated with the same buffer. V_e , the elution volume; V_0 , the void volume.

Isoelectric point

50 mm Frn. (active fraction A in Fig. 3-(a)) and 100 mm Frn. had the same isoelectric point at about pH 3.9.

Optimal pH of LPCase

As shown in Fig. 5, the pH optimum of LPCase activity was found to be 3.5~5.0.

FIG. 5. Effect of pH on LPCase Activity.

The enzyme activity was measured by the standard assay method with enzyme preparation I. Buffers used were: 0.1 M glycine-HCl buffer containing 0.1 M NaCl (pH 2.0~2.9), 0.1 M acetate buffer (pH 3.4~5.8), 0.03 M phosphate buffer (pH 6.8~7.9) and 0.1 M glycine-NaOH buffer containing 0.1 M NaCl (pH 8.8~10.6).

pH stability

The stability of LPCase in solutions of various pH values is shown in Fig. 6. The

FIG. 6. Effect of pH on the Stability of LPCase.

The enzyme solution was incubated at each pH value from 2.0 to 9.0 for 47 and 100 hr at 4°C, and the remaining activities were assayed. Buffers used were: 0.1 M sodium acetate-HCl buffer (pH 2.0~3.0), 0.1 M acetate buffer (pH 3.5~5.0), 0.05 M phosphate buffer (pH 5.5~8.0) and 0.05 M Tris-HCl buffer (pH 8.5~9.0).

○—○, incubation for 47 hr; ○---○, incubation for 100 hr.

enzyme was most stable in the pH range of 7.0 to 8.0, and more stable on the acidic than on the alkaline side.

Thermal stability

The enzyme was stable up to 35°C at pH 7.0, but it rapidly lost its activity at 45°C. Up to 40°C, the enzyme was more stable at pH 7.0 than at pH 4.0, but from 40 to 60°C, it was more stable at pH 4.0 (Fig. 7).

FIG. 7. Thermal Stability of LPCase.

The enzyme solution was incubated at various temperatures for 15 min at pH 4.0 and 7.0, and the remaining activity was assayed.

○—○, pH 7.0; ○---○, pH 4.0.

Effect of metal ions

The effect of various metal ions on LPCase activity is shown in Table II. None of the metal ions stimulated LPCase activity. Fe^{3+} , Hg^{2+} and Al^{3+} inhibited 100%, 46% and 32% of LPCase activity, respectively.

Effect of organic solvents

Enzyme activity was measured by incubation with various organic solvents (Table III). Ethanol, methanol, 1-propanol, 2-propanol and glycerol at a final concentration of 10% stimulated LPCase activity, but ethanol at 30% inhibited the activity. When the enzyme solution was incubated with 5~20% ethyl ether, enzyme activity was stimulated by 20~30%.

Effect of reagents

The effect of various reagents on LPCase

TABLE II. EFFECT OF METAL IONS ON LPCASE ACTIVITY

The enzyme activity was determined by the standard assay method with enzyme preparation I, except that 10 mM of one or another of the indicated reagents was added before the reaction. The activity is expressed in percentage of the activity level in the absence of metal salts. Ag^+ was used as the nitrate form and the other metal ions were used as the chloride form.

Metal ions (10 mM)	Activity (%)
None	100
Mg^{2+}	93
Ca^{2+}	93
Ba^{2+}	98
Al^{3+}	68
Mn^{2+}	102
Fe^{2+} ^{a)}	101
Fe^{3+}	3
Co^{2+}	98
Ni^{2+}	94
Cu^{2+}	94
Zn^{2+}	92
Ag^+	101
Hg^{2+}	54

^{a)} Fe^{2+} was prepared from $\text{FeCl}_2 \cdot n\text{H}_2\text{O}$ ($n=6$).

TABLE III. EFFECT OF ORGANIC SOLVENTS ON LPCASE ACTIVITY

The enzyme activities were determined by the standard assay method with enzyme preparation I, except that one or another of the listed organic solvents at the indicated concentration was added before the reaction. When the volume of organic solvent had to be increased, the equivalent volume of acetate buffer was decreased.

Organic solvents	Conc. (%)	Activity (%)
None	—	100
Ethanol	10	161
"	20	153
"	30	41
Methanol	10	200
1-Propanol	10	142
2-Propanol	10	196
Glycerol	10	124

activity is shown in Table IV. Despite the inhibition of enzymatic activity by Hg^{2+} , LPCase was not inhibited by specific SH reagents. Among imidazole group reagents, diethylpyrocarbonate did not inhibit LPCase activity, but diazobenzen *p*-sulfonic acid did inhibit 89% of it at 10 mM. N-Bromosuccinimide exhibited an inhibitory effect of 100%

TABLE IV. EFFECT OF VARIOUS REAGENTS ON LPCASE ACTIVITY

The enzyme was incubated with each reagent in 0.05 M phosphate buffer, pH 7.0, at 30°C for 30 min, and the residual activity was assayed. When diethylpyrocarbonate was used, the enzyme solution was incubated in 0.05 M phosphate buffer, pH 7.0, containing ethanol at 0°C for 30 min. When diisopropylfluorophosphate and phenylmethylfluoride were used, the enzyme solution was incubated in 0.1 M acetate buffer, pH 4.0, containing isopropanol at 0°C for 60 min.

Addition	Concentration (mM)	Inhibition (%)
N-Ethylmaleimide	10	3
Dithionitrobenzoic acid	1	4
<i>p</i> -Chloromercuribenzoate	1	0
Iodoacetamide	10	5
Monoiodoacetic acid	10	0
N-Acetylimidazole	10	0
1, 2-Cyclohexanedione	10	0
Diethylpyrocarbonate	10	0
Diazobenzene <i>p</i> -sulfonic acid	10	89
N-Bromosuccinimide	1	100
Diisopropyl-fluorophosphate	1	19
Phenylmethyl-sulfonylfluoride	1	7

at 1 mM. Among serine-OH group reagents, diisopropylfluorophosphate inhibited LPCase by 19% at 1 mM concentration.

Substrate specificity

Apparent Michaelis constants (K_m) of LPCase activity for (u)-[¹⁴C]-1-acyl-LPC, 1-[¹⁴C]-palmitoyl-LPC and 1-[¹⁴C]-oleoyl-LPC were calculated from Lineweaver-Burk plots to be 0.35, 0.16 and 0.09 mM, and V_{max} were 79.1, 58.7 and 52.1 μ moles/min per mg of protein, respectively, as shown in Fig. 8. The Lineweaver-Burk plots for 1-[¹⁴C]-palmitoyl-LPC and 1-[¹⁴C]-oleoyl-LPC were broken at 0.25 mM and 0.4 mM, respectively. Under the influence of 10% ethanol, their Michaelis constants were 0.36, 0.07 and 0.07 mM, and V_{max} were 131.6, 106.4 and 96.2 μ moles/min per mg of protein, respectively. In this case, the Lineweaver-Burk plot for (u)-[¹⁴C]-1-acyl-LPC was also broken at 0.34 mM, and the break points for 1-[¹⁴C]-palmitoyl-LPC and

FIG. 8. Effect of LPC Concentration on LPCase Activity.

The enzyme activities were determined by standard methods. (a) (u)-[¹⁴C]-1-acyl-LPC, (b) 1-[¹⁴C]-palmitoyl-LPC, (c) 1-[¹⁴C]-oleoyl-LPC. ○—○, without ethanol; ●—●, with 10% ethanol.

1-[¹⁴C]-oleoyl-LPC rose.

Mono-, di- and tripalmitin were used as substrates for lipase activity. These substrates were used as a dispersed solution by an ultrasonication or with 0.1% Triton X-100 or 10% ethyl ether. The enzyme solution was incubated with 2 mM substrates in acetate buffer, pH 4.0, at 30°C for 30 min, but these substrates were not hydrolyzed.

PC was used as substrate for PCase activity. The enzyme was incubated with an ultrasonic dispersion of PC (1 mM) in the acetate buffer, pH 4.0, at 30°C for 30 min, but PC was not hydrolyzed.

Effect of bovine serum albumin

The effect of bovine serum albumin on LPCase activity was investigated, with (u)-[¹⁴C]-1-acyl-LPC, 1-[¹⁴C]-palmitoyl-LPC and 1-[¹⁴C]-oleoyl-LPC used as substrates. LPCase

activity was not affected by bovine serum albumin below 500 $\mu\text{g/ml}$, regardless of the kind of substrate.

Effect of phosphatidylcholine

The enzyme solution was incubated with 1 mM labeled LPC and various concentrations of PC. As shown in Fig. 9, regardless of the

FIG. 9. Effect of Phosphatidylcholine on LPCase Activity.

The enzyme activities were determined by standard methods except that varying amounts of ultrasonic dispersion of PC were added before the reaction.

Substrates: ○—○, (u)-[¹⁴C]-1-acyl-LPC; ●—●, 1-[¹⁴C]-palmitoyl-LPC; ■—■, 1-[¹⁴C]-oleoyl-LPC.

FIG. 10. Effect of Detergents on LPCase Activity. The enzyme activities were determined by standard methods except that varying concentrations of detergents were added before the reaction.

Substrates: ○—○, (u)-[¹⁴C]-1-acyl-LPC; ●—●, 1-[¹⁴C]-palmitoyl-LPC; ■—■, 1-[¹⁴C]-oleoyl-LPC. Detergents: -----, Tween 80; —, Triton X-100.

kind of substrate, LPCase activity was little affected by PC.

Effect of detergents

The effect of detergents was investigated with various substrates as shown in Fig. 10. When (u)-[¹⁴C]-1-acyl-LPC was used as substrate, LPCase activity was stimulated by Triton X-100 at a concentration of 0.1% (w/v) and by Tween 80 at 0.1 and 0.4%. However, when 1-[¹⁴C]-palmitoyl-LPC and 1-[¹⁴C]-oleoyl-LPC were used as substrates, LPCase activity was inhibited by both detergents at all concentrations tested.

DISCUSSION

In the hydroxylapatite column chromatography, the active fraction was divided into 50 mM Frn. and 100 mM Frn. However, the properties of these fractions with respect to the pattern of disc electrophoresis, optimal pH, isoelectric point, effects of stimulators and inhibitors and K_m for LPC were similar, and we could not find any difference. The molecular weight of LPCase was determined with 50 mM Frn.

LPCase produced by *C. centrifugum* is similar to that of a mold, *Penicillium notatum*,¹³⁾ in acidic pH optimum (3.5~5.0), isoelectric point (pH 3.9), molecular weight (130,000) and thermal instability.

Inhibition by histidine reagents was observed with LPCase from cod muscle,¹⁴⁾ and diisopropylfluorophosphate inhibition was observed with bovine pancreas,¹⁵⁾ but no inhibition by N-bromosuccinimide has been reported.

This enzyme differed from those of the bovine pancreas¹⁵⁾ and *E. coli*¹⁶⁾ enzymes which show the lipase activity. Nor did it exhibit phospholipase B activity which was observed with *P. notatum*.¹³⁾

LPCase activity of *C. centrifugum* was not affected by bovine serum albumin. This result is similar to that observed with the LPCase of *Culex pipiens fatigans*.¹⁷⁾

The inhibition of LPCase by PC, which was observed with bovine pancreas¹⁵⁾ and mos-

quito,¹⁷⁾ was not observed with *C. centrifugum*.

The sensitivity of LPCase from *C. centrifugum* to stimulation by various organic solvents and detergents characteristically distinguishes this enzyme from other general LPCases.

The appearance of a break point and the difference of its values with each three substrates which is different in its fatty acid composition, in Lineweaver-Burk plots mean that the micellar form gives a better substrate for LPCase than the monomolecular form, and that each LPC has its own micellar structure determined by its fatty acid composition. Under the influence of 10% ethanol, though *K_m* did not change, *V_{max}* was greater than in the absence of ethanol. This result suggests that ethanol mainly affects the enzyme rather than the substrate in the hydrolysis of LPC. (u)-[¹⁴C]-1-Acyl-LPC was hydrolysed more rapidly than 1-[¹⁴C]-palmitoyl-LPC and 1-[¹⁴C]-oleoyl-LPC. The fatty acid composition of a carrier LPC of (u)-[¹⁴C]-1-acyl-LPC is shown in Table V. This indicates that the mixed composition of fatty acids of a carrier LPC gives a good micellar structure for this enzyme to (u)-[¹⁴C]-1-acyl-LPC.

TABLE V. MOLECULAR SPECIES OF LPC

Carbon number	Fatty acid		wt (%)
	C-1	C-2	
16	16: 0 (Palmitic acid)		79.3
18	18: 0 (Stearic acid)		16.6
	18: 1 (Oleic acid)		4.1

In the experiment concerning the effect of detergents on LPCase activity, the change of substrate from (u)-[¹⁴C]-1-acyl-LPC to 1-[¹⁴C]-palmitoyl-LPC or 1-[¹⁴C]-oleoyl-LPC was observed to reverse the effect of detergents. This result indicates that the interaction of LPC and

detergents is affected by the fatty acid composition of LPC, and (u)-[¹⁴C]-1-acyl-LPC that has a mixed composition of fatty acids can be more favorable to LPCase.

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