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Properties of Phospholipase A_1/Transacylase in the White Muscle of Bonito *Euthynnus pelamis* (Linnaeus)

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The properties of phospholipase A_1 (PLA_1) obtained from the white muscle of bonito, *Euthynnus pelamis* (Linnaeus), were examined. The PLA_1 activity had a pH optimum from 6.5 to 7.0 for phosphatidylcholine (PC), and calcium ion was not required. The optimum temperature was from 20 to 30°C. When a fatty alcohol was used as an acceptor, a wax ester was produced by transferring a fatty acid at the *sn*-1 position of the donor's PC. The maximum production of lysophosphatidylcholine was shifted by 0.5 pH units to the acidic side and the pH optimum of wax ester synthesis was from 6.0 to 6.5. The synthesis was independent of calcium ion and Coenzyme A. The transacylation was also observed when 1-lyso-2-acyl-*sn*-glycero-3-phosphocholine was used as an acceptor. Fatty acid at the *sn*-1 position of the donor PC was transferred to the unoccupied hydroxy group of the acceptor at the *sn*-1 position. When 2,3-dipalmitoyl-*sn*-glycero-1-phosphocholine was used as the acyl donor, a similar amount of palmitic acid was transferred as in the case of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine. However, 1-acyl-2-lyso-*sn*-glycero-3-phosphocholine, a positional isomer, was a poor acceptor. These results indicate that the transacylation by the PLA_1 from bonito muscle is not stereospecific, but is position-specific both for the acyl donor and acceptor.

Key words: 2-acyl LPC, bonito muscle, fatty acid remodeling, phospholipase A_1, transacylase.

It is known that some phospholipase As have transacylase activities as well as their hydrolytic activities. A well known example is lecithin:cholesterol acyltransferase, which is responsible for the cholesterol transport in plasma (*1*). It catalyzes the esterification of free cholesterol in two steps. The first step involves phospholipase A_2 (PLA_2) activity, which hydrolyzes the fatty acyl group at the *sn*-2 position of phosphatidylcholine (PC), and the other step is transacylation, which transfers the fatty acid from the acyl-enzyme complex to a hydroxy group of cholesterol. The specific activities of PLA_2 and cholesterol esterification are similar in human and rat lecithin:cholesterol acyltransferases (*2*). The physiological acceptor is cholesterol, though, lysophosphatidylcholine (LPC) is also a good acceptor of fatty acid from the acyl intermediate, generating PC; this is called the idling reaction (*3*). Phospholipase A_1 (PLA_1) from rat liver plasma membranes, which has a high activity towards triglyceride, also catalyzes the transacylation in addition to hydrolysis (*4*). In this case, monoglyceride and long chain alcohol could be utilized as acyl acceptors, but lysophosphatidylethanolamine, diglyceride, and cholesterol did not accept the fatty acid from the donor's phosphatidylethanolamine (PE) (*5*).

In a previous study (*6*), a fairly high proportion of LPC, nearly 10% of the total phospholipid, was detected in the fresh muscle of bonito (*Euthynnus pelamis*) and LPC had further been accumulated, to almost twice the initial content, during frozen storage. LPC was found to be abundant in highly unsaturated fatty acids such as docosahexaenoic acid (DHA) and eicosapentaenoic acid, and was mainly composed of 1-lyso-2-acyl-*sn*-glycero-3-phosphocholine (2-acyl LPC). In connection with the occurrence of 2-acyl LPC, we detected PLA_1 activity in the white muscle (*6*). The PLA_1 activity may be the cause of the accumulation of 2-acyl LPC during frozen storage, though its role in bonito muscle is still unclear.

In the present study, we characterized the properties of PLA_1 activity in bonito muscle and examined its transacylase activity.

MATERIALS AND METHODS

Materials—1,2-Dioleoyl, 1,2-didocosahexaenoyl, 1-stearoyl-2-palmitoyl, 1-palmitoyl-2-stearoyl, and 1-stearoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholines were obtained from Avanti Polar Lipids (Birmingham, AL). 1,2-Dipalmitoyl, 1,2-distearoyl-*sn*-glycero-3-phosphocholine, and 2,3-dipalmitoyl-*sn*-glycero-1-phosphocholine were the products of Serdary Research Laboratories (London, Ontario, Canada). Palmityl and oleyl alcohols, and docosahexaenoic acid were from Serdary Research Laboratories. Oleyloleate was the product of Larodan Fine Chemicals

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Abbreviations: 1-acyl LPC, 1-acyl-2-lyso-*sn*-glycero-3-phosphocholine; DHA, docosahexaenoic acid; FFA, free fatty acid; 2-acyl LPC, 1-lyso-2-acyl-*sn*-glycero-3-phosphocholine; LPC, lysophosphatidylcholine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PLA_1, phospholipase A_1; PLA_2, phospholipase A_2.

(Westbury, NY). 1,2-Dilauroyl-*sn*-glycero-3-phosphocholine was synthesized by mixing lauric anhydride and glycerophosphorylcholine (*6*). The heat-treated venom from *Trimeresurus flavoviridis* was used as phospholipase A_2. Phospholipase C from *Bacillus cereus* and Coenzyme A were from Sigma Chemicals (St. Louis, MO). 1-Palmitoyl-2-[1-^{14}C]linoleoyl, 1,2-di[1-^{14}C]palmitoyl, and 1,2-di[1-^{14}C]oleoyl-*sn*-glycero-3-phosphocholines (1.85 GBq/mmol, 200 nmol, respectively) were the products of Amersham, Tokyo. 1,2-Di[1-^{14}C]palmitoyl PC was diluted with 100 μmol of cold 1,2-dipalmitoyl PC and the others were diluted with 100 μmol of egg yolk phosphatidylcholine (PC). Methanolic-HCl was purchased from Tokyo Kasei (Tokyo). The pre-coated thin-layer plates (Silica gel 60) were the product of Merck (Darmstadt, Germany). Silic AR (100-200 mesh) was from Mallinckrodt (Paris, KY). Toyopearl HW-55 S was the product of Tosoh, and DEAE-cellulofine was from Seikagaku Kogyo, both of Tokyo. 3,5-Dinitrobenzoyl chloride was from Dojindo Laboratories (Kumamoto). SepPak C18 cartridges were obtained from Waters Associates (Milford, MA). Acetonitrile and 2-propanol were of HPLC grade. All other chemicals were of reagent grade.

Preparation of Lysophosphatidylcholine—1-Stearoyl or 1-docosahexaenoyl LPC (1-DHA LPC) was prepared by the hydrolysis of 1,2-distearoyl or 1,2-didocosahexaenoyl PC with phospholipase A_2 (*6*). 2-Stearoyl or 2-docosahexaenoyl LPC (2-DHA LPC) was synthesized using beef heart choline plasmalogen as the starting material (*6*). Beef heart choline phosphoglyceride was first subjected to mild alkaline hydrolysis, and the resulting 1-radyl LPC was purified by preparative thin layer chromatography (TLC). Stearic acid or DHA was esterified with the 1-radyl LPC using the respective anhydride. The 1-alk-1′-enyl-2-stearoyl or -DHA PC was further subjected to mild acid hydrolysis, and then the formed 2-stearoyl or 2-DHA LPC was purified by preparative TLC. The yields of 2-acyl LPCs were about 20% as calculated from the starting ether-type LPC.

Phospholipase A_1 Assay—The deacylation of 1-palmitoyl-2-[1-^{14}C]linoleoyl-*sn*-glycero-3-phosphocholine (500 nmol, 50 nCi) by bonito muscle PLA_1 was evaluated using the following assay conditions. The reaction mixture consisted of 200 μmol of Tris-HCl buffer (pH 6.5), 2.5 μmol of sodium deoxycholate, and PLA_1 in a total volume of 1.1 ml. The mixture was vigorously stirred with a Taitec TC-8 concentrator at maximum speed at 25°C for 1 h, and lipids were extracted using the two-phase system of Bligh and Dyer (*7*). The extract was applied to a thin layer plate and developed with chloroform : methanol : H_2O (65 : 35 : 6, v/v). The regions corresponding to LPC and free fatty acid (FFA) as well as PC were detected in a chamber saturated with iodine vapor, and they were then scraped off. A scintillator was directly added to the powder and the radioactivity of each spot was counted. The specific activity of PLA_1 is expressed as nmol of 2-acyl LPC released per mg of protein per h.

Wax Ester Synthesis Assay—The wax ester synthesis by bonito muscle PLA_1 was evaluated using 1,2-di[1-^{14}C]-oleoyl-*sn*-glycero-3-phosphocholine (500 nmol, 50 nCi) as an acyl donor and oleyl alcohol (11 μmol) as an acceptor molecule (*8*). The reaction time was 2.5 h and conditions were the same as those used for the PLA_1 assay with the exception of the use of sodium acetate buffer (pH 6.0). The extracted lipids were applied to a thin layer plate and first developed with chloroform : methanol : H_2O (65 : 35 : 6, v/v) until the solvent reached half the height of the plate. The plate was then left under a hood to remove the solvent and was developed again to the top of the plate with petroleum ether : diethyl ether : 28% NH_4OH (30 : 80 : 1, v/v) to separate FFA and wax. The regions corresponding to the authentic standards, wax, FFA, PC, and LPC, were detected, scraped off and counted. The wax ester synthesis was also evaluated by gas chromatography using 1-stearoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine as an acyl donor. The wax was extracted with known amounts of heptadecanoic acid and palmityl alcohol. The extract was subjected to transmethylation with 5% methanolic HCl to prepare the fatty acid methyl ester and fatty alcohol, which were analyzed using a Shimadzu GC-14A gas chromatograph containing a capillary column coated with CBP 20 (0.22 mm diameter, 30 m length; Shimadzu, Kyoto). The conditions were the same as previously described (*6*). The methyl esters and fatty alcohol were identified by comparing their retention times with those of authentic standards. Similar amounts of fatty acid and alcohol (1 : 1.06 in molar ratio) were detected in the wax fraction.

Assay for Acylation of Lysophosphatidylcholine—The acylation of LPC (1.6 μmol) by bonito muscle PLA_1 was evaluated using diacyl PC (500 nmol) as an acyl donor and 1,2-dilauroyl PC (80 nmol) as an internal standard, which was added during the extraction step. The reaction conditions were the same as those for the wax ester synthesis. The newly generated PC as well as the substrate and internal standard were purified by preparative TLC. They were hydrolyzed with phospholipase C, and the resulting diglycerides were converted to their dinitrobenzoyl derivatives (*9*). They were separated using reversed-phase, high-performance liquid chromatography (HPLC) (Tosoh CCPM, dual pump system, Tokyo) with an ODS 120-T column using a mixture of acetonitrile : 2-propanol (90 : 10, v/v) as the eluting solution. Peaks were identified by comparison with those of molecular species from standard PC and bonito muscle PC (*10*). The specific activity of the transacylation is expressed as nmol of PC newly formed per mg of protein per 2.5 h.

Preparation of Bonito Muscle Phospholipase A_1—Bonitos *Euthynnus pelamis* (Linnaeus) were obtained from a local market in November, 1995, and the muscle was stored in a freezer. The following procedures were carried out at 4°C and centrifugation was performed at 10,000 × *g* for 30 min in a refrigerated centrifuge. The muscle was homogenized with 10 mM Tris-HCl buffer (pH 7.4) containing 1 mM EDTA. After filtration through gauze, the filtrate was centrifuged. To the supernatant, solid ammonium sulfate was added to produce 60% saturation. The precipitate was collected by centrifugation and dissolved in a small volume of 10 mM Tris-HCl buffer (pH 7.4) containing 1 mM EDTA, 20% glycerol and 1 mM *N*-acetyl-L-cysteine (A buffer). The solution was dialyzed overnight, and the precipitate formed during dialysis was removed by centrifugation. The solution was placed on the top of a column of TSKgel HW-55 S, which had been previously equilibrated with buffer A. Elution was carried out with the same buffer, and 5 ml fractions were collected. One peak with PLA_1 activity appeared near the void volume. The active fractions were pooled and applied to a DEAE-cellulo-

fine column. After the unadsorbed materials were washed out, the PLA_1 was eluted from the column with a linear gradient of KCl from 0 to 0.5 M. A symmetrical peak of PLA_1 activity was eluted with about 0.15 M KCl. Although about 20-fold purification was achieved from the supernatant by these chromatographies, attempts at further purification were unsuccessful. Accordingly, the general properties of PLA_1 were assessed using this fraction. The specific activities ranged from 200 to 285 nmol/mg/h among the preparations.

Determinations of Protein and Phosphorus—The protein concentration was determined by the method of Lowry *et al.* (*11*), and the phosphorus concentration was determined according to the procedure of Bartlett (*12*).

All data are expressed as the mean ± SD of three separate experiments.

RESULTS

Properties of Phospholipase A_1 and Wax Ester Synthesis—Incubation of the DEAE-cellulofine fraction with 1-palmitoyl-2-[1-^{14}C]linoleoyl PC resulted in a time-dependent accumulation of 2-[1-^{14}C]linoleoyl LPC. The generation of [1-^{14}C]linoleic acid was negligible, indicating that the preparation was devoid of PLA_2 activity and catalyzed the hydrolysis of the ester linkage at the *sn*-1 position of the glycerol moiety (Fig. 1). The PLA_1 activity was maximal between pH 6.5 and 7.0. The optimum temperature was 20-30°C, and about half of the activity remained even at 4°C. The PLA_1 activity was independent of calcium ion concentration and the addition of EDTA at 5 mM did not affect the activity (Fig. 2, A-C).

The addition of fatty alcohol to the reaction mixture resulted in the formation of a wax ester utilizing the alcohol as a fatty acid acceptor (Fig. 3). Radioactivity was detected time-dependently in the wax ester fraction when 1,2-di-[1-^{14}C]oleoyl PC was used as a donor. However, when 1-palmitoyl-2-[1-^{14}C]linoleoyl PC was used, the radioactivity detected was only around the background level (data not shown), indicating positional specificity for the *sn*-1 position of glycerol in the wax ester synthesis. In the

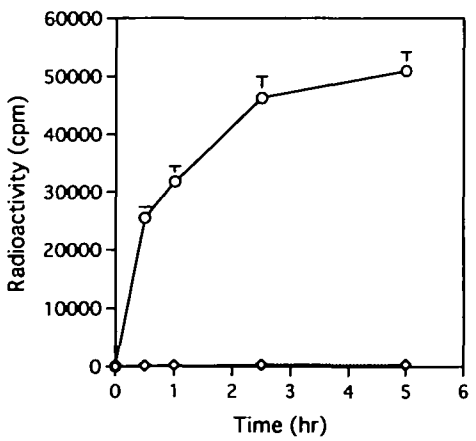

Fig. 1. **Time-course of products generation by phospholipase A_1.** Hydrolysis was determined with 500 μg of the PLA_1 preparation as described under "MATERIALS AND METHODS," and the reaction was terminated at the indicated times. Radioactivities of 2-[1-^{14}C]linoleoyl LPC (○) and [1-^{14}C]linoleic acid (◇) were counted.

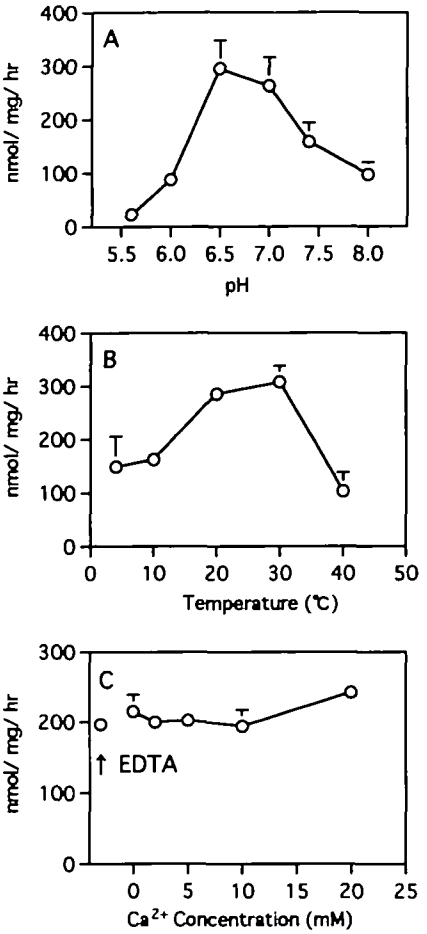

Fig. 2. **Effect of pH, temperature, and calcium concentration on the activity of phospholipase A_1.** (A) pH. The buffers used were sodium acetate (pH 5.6-6.0) and Tris-HCl (pH 6.5-8.0). (B) Temperature. (C) Calcium ion. The final concentration of EDTA was 5 mM.

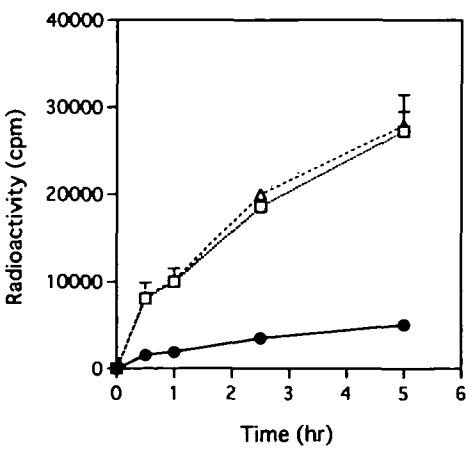

Fig. 3. **Time-course of products generation by transacylation.** Wax ester synthesis was determined with 1 mg of the PLA_1 preparation as described under "MATERIALS AND METHODS" using 1,2-di[1-^{14}C]oleoyl PC as an acyl donor, and the reaction was terminated at the indicated times. Radioactivities of [1-^{14}C]oleoyl LPC (△), [1-^{14}C]oleic acid (□), and oleyl [1-^{14}C]oleate (●) were counted.

presence of the fatty alcohol, the maximum formation of LPC was shifted to more acidic pH, and the wax ester synthesis was maximal around pH 6.0 and 6.5 (Fig. 4). The absence of calcium ion or the addition of Coenzyme A did not affect the wax ester synthesis (Table I).

Since the preparation used here could not be purified to homogeneity, it is not known whether the activities of PLA_1 and wax ester synthesis are present in the same protein.

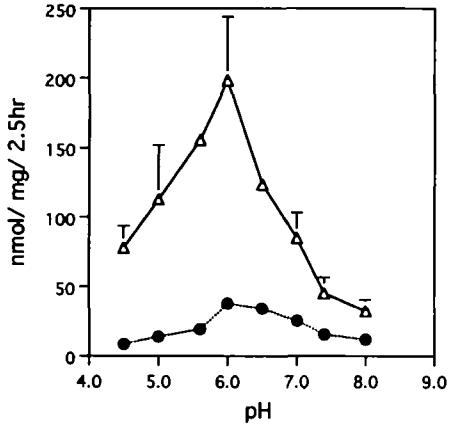

Fig. 4. **Effect of pH on the activity of wax ester synthesis.** The buffers used were the same as in Fig. 2. Radioactivities in [1-^{14}C]-oleoyl LPC (△) and oleyl[1-^{14}C]oleate (●) were counted.

The preparation was further subjected to DEAE-cellulofine column chromatography. As shown in Fig. 5, the elution positions of PLA_1 activities and wax ester synthesis were the same, and the relative radioactivity of 2-[1-^{14}C]linoleoyl LPC to oleyl[1-^{14}C]oleate was similar in each fraction. Treatment with phenylmethylsulfonyl fluoride dose-dependently inactivated the activities of PLA_1 and the wax ester synthesis (Fig. 6A). Heat-treatment also similarly decreased the two activities (Fig. 6B), suggesting that a single protein may possess both PLA_1 and transacylase activities. Accordingly, the following characterizations of the transacylase activity were performed using the DEAE-cellulofine fraction.

Formation of Phosphatidylcholine by Transacylation Using Lysophosphatidylcholine as an Acceptor—When 2-DHA LPC was used as an acceptor and 1,2-dipalmitoyl

TABLE I. **Effect of calcium ion and Coenzyme A on transacylation activity.** Wax ester synthesis was determined by calculating fatty acids derived from the wax fraction. *Oleyl alcohol or calcium was omitted from the complete system, and **440 nmol CoA and 8.25 μmol ATP were added to the complete system.

	Wax ester formed (nmol/mg/h)
Complete	37.1± 4.6
− alcohol*	1.8± 0.3
− Ca^{2+}*	40.0± 7.0
+ CoA, ATP**	34.1±11.0

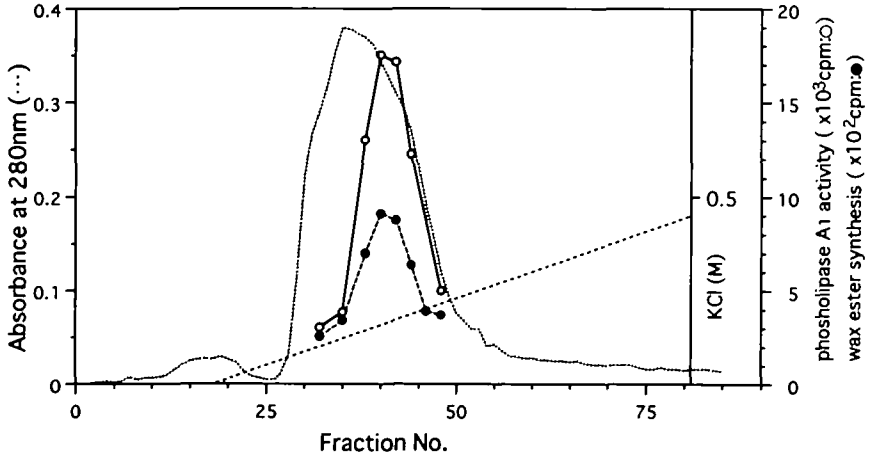

Fig. 5. **Column chromatography of the phospholipase A_1 preparation by DEAE-cellulofine.** The pooled fraction obtained by the first DEAE-cellulofine column chromatography was applied to the same column and eluted with KCl. The PLA_1 and wax ester synthesis activities of a 400 μl aliquot of each fraction were determined. Absorbance at 280 nm (·····); 2-[1-^{14}C]linoleoyl LPC (○); oleyl-[1-^{14}C]oleate (●).

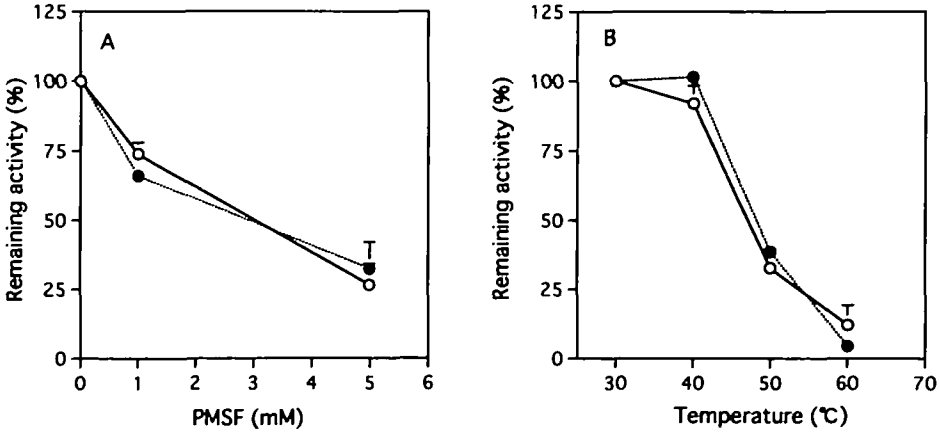

Fig. 6. **Effect of PMSF and heat-treatment on the activities of phospholipase A_1 and wax ester synthesis.** The PLA_1 preparation was preincubated with phenylmethylsulfonyl fluoride at 30°C for 2 h with constant shaking (A) or heated for 10 min at the appropriate temperature (B), the respective radiolabeled substrates were added, and radioactivities of 2-[1-^{14}C]linoleoyl LPC (○) and oleoyl[1-^{14}C]oleate (●) were counted.

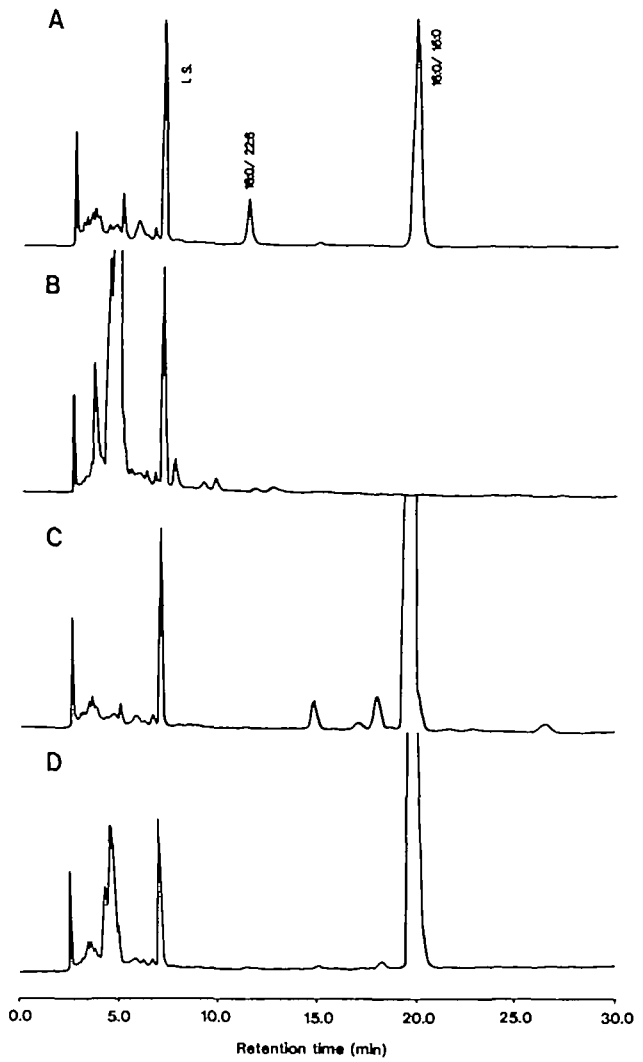

Fig. 7. **HPLC chromatograms of transacylation products.** The transacylation was performed with 880 μg of the PLA_1 preparation as described under "MATERIALS AND METHODS." 1,2-Dipalmitoyl PC was used as a donor and 2-DHA LPC as an acceptor. The HPLC chromatograms are (A) complete system, (B) minus donor, (C) minus acceptor, and (D) minus PLA_1 preparation.

PC was used as a donor, a peak with the same retention time as the standard 1-palmitoyl-2-DHA PC was formed (Fig. 7A). No 1-palmitoyl-2-DHA PC was formed when the donor or the acceptor or the PLA_1 preparation was omitted from the complete system (Figs. 7, B, C, and D). The amount of 1-palmitoyl-2-DHA PC formed was 37.6±7.2 nmol. It was calculated that about 7.5% of the donor's palmitic acid was transferred to the unoccupied hydroxyl group. In the absence of 2-DHA LPC, the peak area of 1,2-dipalmitoyl PC in Fig. 7C was comparable with that in Fig. 7D, indicating that 1,2-dipalmitoyl PC was not hydrolyzed at pH 6.0. This is in stark contrast to the complete system (Fig. 7A), in which a substantial amount of the donor was hydrolyzed. This was therefore examined using 1,2-di[1-^{14}C]palmitoyl PC (500 nmol, 50 nCi) as a substrate and 280 μg of the PLA_1 preparation. In the absence of 2-DHA LPC, radioactivity detected in the LPC fraction was around the background level, whereas 10,765±964 cpm in LPC was detected in the presence of 2-DHA LPC. Thus, a shift in the optimum pH for PLA_1 activity to the more acidic side also occurred in the presence of LPC, as was the case with the fatty alcohol, though the reason is not clear (Fig. 4).

TABLE II. **Effect of the donor phosphatidylcholine on transacylation activity.** 2-DHA LPC was used as an acceptor. *L-type (*sn*-3) and D-type (*sn*-1) Dipalmitoyl PC were subjected to hydrolysis with phospholipase A_2, and D-type dipalmitoyl PC was not hydrolyzed, whereas the L-type was completely hydrolyzed.

	PC molecular species formed		
	16:0/22:6	18:0/22:6	18:1/22:6
	(nmol/mg/2.5 h)		
Donor PC			
18:0/16:0 PC	2.5± 0.8	16.6±1.6	
16:0/18:0 PC	34.3±13.2	6.5±2.3	
16:0/16:0 PC (*sn*-3)*	37.6± 7.2		
16:0/16:0 PC (*sn*-1)*	40.2± 5.7		
18:1/18:1 PC			98.9±16.3

TABLE III. **Effect of the acceptor lysophosphatidylcholine on transacylation activity.** 1,2-Dioleoyl PC was used as a donor. *The assignment of this species was based on the relationship of retention times of the reversed isomers described by Kito *et al.* (*9*).

	PC molecular species formed	
	18:1/22:6 or 22:6/18:1*	18:1/18:0 or 18:0/18:1
	(nmol/mg/2.5 h)	
Acceptor LPC		
2-22:6 LPC	98.9±16.3	
1-22:6 LPC	6.8± 1.4	
2-18:0 LPC		82.9±10.0
1-18:0 LPC		2.0± 3.5

In order to demonstrate the specificity of the transacylation, the effect of donor PC was first examined using mixed acyl PC. As shown in Table II, when 1-stearoyl-2-palmitoyl PC was used as a donor, 1-stearoyl-2-DHA PC was formed, whereas 1-palmitoyl-2-DHA PC was mainly formed when 1-palmitoyl-2-stearoyl PC was used.

2,3-Dipalmitoyl PC (*sn*-1 PC) was then used as an acyl donor, and the rate of transacylation was compared with the case of 1,2-dipalmitoyl PC (*sn*-3 PC). As shown in Table II, similar amounts of 1-palmitoyl-2-DHA PC were formed irrespective of the stereospecificity of the donor PC at the *sn*-2 position.

Compared with palmitic, stearic and oleic acids at the *sn*-1 position of the donor PC, oleic acid was the most favorable for transacylation (Table II).

Next, the positional specificity of the transacylation for an acceptor was examined using 1,2-dioleoyl PC as the acyl donor. When 2-DHA LPC was used as the acceptor, 98.9± 16.3 nmol of 1-oleoyl-2-DHA PC was formed; however, in 1-DHA LPC, only one-fifteenth the amount of 1-DHA-2-oleoyl PC was formed. When the fatty acid bonded to LPC was changed from DHA to stearic acid, the amount of oleic acid transacylated from the donor to 2-stearoyl LPC did not change. However, the positional isomer, 1-stearoyl LPC, was scarcely acylated (Table III).

DISCUSSION

Substantial amounts of LPC have been detected even in fresh bonito muscle (*6*), as was originally reported by

Ohshima *et al.* (*13*). Fatty acid esterified in LPC was found to be rich in highly unsaturated fatty acids (*6, 14*). Unsaturated fatty acids are commonly found at the *sn*-2 position of glycerophospholipids. It is generally believed that fatty acid at the *sn*-2 position readily migrates when the *sn*-1 or *sn*-3 position is not occupied. However, the majority of LPC in bonito muscle was found to be 2-acyl LPC by gas chromatography/mass spectrometric analysis (*6*). Recently, 2-arachidonoyl glycerol, an endogenous cannabinoid receptor ligand, was also found in rat brain (*15*).

As shown in Fig. 1, the bonito muscle had PLA_1 activity. Unlike PLA_1s from rat liver (*5*), bovine brain (*16*), and rat platelet (*17*), which prefer PE (*5, 16*) and phosphatidylserine (*17*) as substrates, the present preparation hydrolyzed PC in addition to PE. The optimum temperature of PLA_1 was from 20 to 30°C, and about half of the activity remained at 4°C. Accordingly, PLA_1 in muscle might be responsible for LPC accumulation during the cold storage (*6*). However, the physiological significance of PLA_1 in the muscle is still unclear. Thus, we examined the transacylation of the PLA_1 preparation, because it is generally known that PLA_1 also has a transacylase activity (*4*), and evidence has been accumulating showing that transacylases as well as acyltransferases play an important role in the fatty acid remodeling in phospholipid (*18*).

When 1-stearoyl-2-arachidonoyl PC was used as a donor and oleyl alcohol as an acceptor molecule, 92.4% of the fatty acid detected in the wax fraction was stearic acid. As the present mixed acyl PC contained about 10% of the reversed isomer, it is suggested that the PLA_1 preparation has the ability to generate a wax ester by transferring the *sn*-1 fatty acid to the alcohol. The presence of calcium ion was not necessary for wax ester synthesis (Table I).

The PLA_1 preparation also has the ability to form PC from 2-DHA LPC, which is abundant in bonito muscle (Fig. 7). The specific activity of the transacylase was one-fifth of that of PLA_1. Fatty acid at the *sn*-1 position of the donor's PC was utilized for the transacylation to LPC (Table II). Thus, the transacylation was expected not to be stereospecific for the *sn*-2 position, and in fact, an unnatural *sn*-1 PC (D-type PC) was equally well utilized as an acyl donor. On the other hand, 1-acyl LPC did not serve as an acceptor (Table III). This was not due to hydrolysis of 1-acyl LPC by the PLA_1 preparation. The PLA_1 did hydrolyze 1-palmitoyl LPC in the absence of sodium deoxycholate, but the lysophospholipase activity was less than one-tenth of the PLA_1 activity, and in the presence of the detergent, the activity was inhibited (data not shown). Considering that a fatty acyl group is transferred from a putative acyl-enzyme intermediate to an acceptor molecule and that PLA_1 has a strict specificity for the *sn*-1 position of PC, it is conceivable that 2-acyl LPC accepts a fatty acyl group, but 1-acyl LPC does not. However, this idea requires confirmation, because the PLA_1 preparation was not purified to homogeneity.

It is well known that temperature is one of the most important environmental factors which affects the physiological activity of poikilotherms, such as fish. The first target of a temperature change is the biomembrane. Accumulations of highly unsaturated fatty acid, especially in the winter season, have often been reported (*19, 20*). In bonito muscle, 1,2-diDHA PC accounts for almost 10% of PC molecular species (data not shown). Furthermore, it has recently been demonstrated that modification of the fatty acid at the *sn*-1 position is important in determining the physiological properties of the biomembrane in relation to temperature adaptation (*21*). In carp liver PC and PE, 1-oleoyl-2-DHA species increased with the compensatory decreases in 1-palmitoyl-2-DHA and 1-stearoyl-2-DHA species upon cooling from 25 to 5°C (*21, 22*). These molecular species are formed by fatty acid remodeling of phospholipid through a deacylation-reacylation pathway (*18*). The PLA_1 coupled with acyltransferase and/or the transacylase detected in the bonito muscle might be responsible for the remodeling of phospholipid molecular species.

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