Purification and Properties of a Phospholipase A$_2$/Lipase Preferring Phosphatidic Acid, Bis(monoacylglycerol) Phosphate, and Monoacylglycerol from Rat Testis* 

Received for publication, March 22, 2002, and in revised form, September 5, 2002

Published, JBC Papers in Press, September 9, 2002 DOI 10.1074/jbc.M202817200

Masafumi Ito, Urban Tchoua, Mitsuhiro Okamoto, and Hiromasa Tojo‡

From the Department of Molecular Physiological Chemistry (H-1), Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan

Phospholipase A$_2$ (PLA$_2$) was purified to homogeneity from the supernatant fraction of rat testis homogenate. The purified 63-kDa enzyme did not require Ca$^{2+}$ ions for activity and exhibited both phosphatidic acid-prefering PLA$_2$ and monoacylglycerol lipase activities with a modest specificity toward unsaturated acyl chains. Anionic detergents enhanced these activities. Serine-modifying irreversible inhibitors, (p-aminodinophenyl) methanesulfonyl fluoride and methylarachidonyl fluorophosphonate, inhibited both activities to a similar extent, indicating that a single active site is involved in PLA$_2$ and lipase activities. The sequence of NH$_2$-terminal 12 amino acids of purified enzyme was identical to that of a carboxylesterase from rat liver. The optimal pH for PLA$_2$ activity (around 5.5) differed from that for lipase activity (around 8.0). At pH 5.5 the enzyme also hydrolyzed bis(monoacylglycerol) phosphate, or lysobisphosphatidic acid (LBPA), that has been hitherto known as a secretory PLA$_2$-resistant phospholipid and a late endosome marker. LBPA-enriched fractions were prepared from liver lysosome fractions of chloroquine-treated rats, treated with excess of pancreatic PLA$_2$, and then used for assaying LBPA-hydrolyzing activity. LBPA and the reaction products were identified by microbore normal phase high performance liquid chromatography/electrospray ionization ion-trap mass spectrometry. These enzymatic properties suggest that the enzyme can metabolize phosphatidic and lysobisphosphatidic acids in cellular acidic compartments.

Lysophosphatidic acid (LPA$^1$) is a key intermediate for de novo synthesis of phospholipids and triacylglycerol. In addition, recent studies have established that LPA serves as an intercellular signaling molecule that mediates diverse cellular functions, such as cell growth and cytoskeletal remodeling (1), via G protein-coupled receptors (2). These receptors comprise several isozymes (2), which fulfill distinct functions through different signaling pathways depending on isozymes. On the other hand, there is little information on the metabolic pathways and enzymes responsible for LPA synthesis; several pathways have been proposed depending on tissue and cell types (3). Phospholipase A$_2$ (PLA$_2$) or phospholipase A$_1$ (PLA$_1$) directly produces LPA from phosphatidic acid (PA) generated by combined action of phospholipase C and diacylglycerol kinase or by direct action of phospholipase D (PLD). Diacylglycerol lipase deacylates diacylglycerol produced in response to stimulation and then the product 2-monocacylglycerol can be phosphorylated by monoacylglycerol kinase, generating LPA. Finally, plasma lysophospholipase D can hydrolyze lysophosphatidylcholine (LPC), yielding LPA.

Of these enzymes PLA$_2$s are ubiquitous and have been studied most intensively. A variety of isozymes have been known, and some isoforms exhibit specificity for PA, including group II A PLA$_2$ (4), rat brain 58-kDa PLA$_2$ (5), and intracellular Ca$^{2+}$-independent PLA$_2$ (6). They might be candidates for LPA-synthesizing enzymes, but relevance to LPA synthesis in vivo has not yet been established, although it was suggested that group II A PLA$_2$ attacks PA-containing microvesicles, shed from damaged cells in inflammation, to produce LPA (7). In the course of a study on tissue distribution of PLA$_2$ activity toward mixed micelles of acidic phospholipid and cholate, we found that rat testis contained appreciable amounts of Ca$^{2+}$-independent PLA$_1$ and PLA$_2$ (8). Recently, PA-prefering PLA$_2$ was purified and cloned from bovine testis (9, 10). In this study, we purified to homogeneity a PLA$_2$ with a substantial specificity for PA, and monoacylglycerol lipase activities as a single 63-kDa molecule but did not exhibit PLA$_2$ and lysophospholipase activities.

The PLA$_2$ activity of the enzyme toward PA showed acidic pH optimum of 5.5, suggesting that the enzyme might work in cellular acidic compartments including endosome/lysosyme system. The late endosomes specifically contain another acidic phospholipid, bis(monoacylglycerol) phosphate (lysobisphos-
phosphatidic acid, LBPA), a structural isomer of PG, that takes part in a following acyl donor and acceptor combinations: mixtures of 1 mM LPC and various concentrations of taurocholate, cholate, deoxycholate (DOC), or Triton X-100. The pH of the assay mixtures was adjusted to 8.5. A Biogel A-0.5m column (25 cm; Bio-Rad) was used for the separation of PLA2 activity. The column was pre-equilibrated with 20 mM Tris-HCl (pH 7.5) containing 0.1 M NaCl, 1 mM EDTA, and 1 mM DTT. Then the solutions were applied to a phenyl-Sepharose column (10 m; Pharmacia) pre-equilibrated with Buffer A. The PLA2 activity was eluted with a concentration gradient of NaCl in Buffer A from 0 to 0.2 M for 60 min and then from 0.2 to 1 M for 80 min. The pooled PLA2 fractions were rechromatographed on the same column; the pH of the eluent was decreased to 8.0, and a smaller gradient of NaCl from 0 to 0.1 M for 90 min was used. The flow rate was 0.5 ml/min, and 1-ml fractions were collected. The active PLA2 pools were purified further by HPLC on a Cosmosil 250-5C4 column (7.5 × 75 mm; Nacalai Tesque) pre-equilibrated with Buffer A. The PLA2 activity was eluted with a concentration gradient of NaCl in Buffer A from 0 to 0.2 M for 60 min and then from 0.2 to 1 M for 80 min. The collected PLA2 activity was eluted at the retention time of 29 min, coinciding with a peak at 280 nm.

**Synthesis of PAs**—1-Palmityl-2-arachidonoyl-sn-glycero-3-phosphate (PAPA), 1-O-hexadecyl-2-oleoyl-sn-glycero-3-phosphate (1-O-hexadecyl-OPA), and 1,2-di-palmityl-sn-glycero-3-phosphate (1,2-DPPA) and its enantiomer (2,3-DPPA) were produced from the corresponding phosphatidylcholines by the action of PLD as described previously (17). Briefly, substrates (25 mg each) were resuspended in 2 ml of diethyl ether and then 100 units of Streptomyces sp.PLD (Sigma) were added in 1 ml of 0.2 M sodium acetate (pH 5.6) and 40 mM CaCl2 at room temperature. After a 12-h incubation with vigorous shaking, the diethyl ether was evaporated and then lipids were extracted by the acidic Bligh and Dyer method (16). The extracted lipids were resuspended in 0.2 ml of hexane/2-propanol (3:2) (v/v). Aliquots were purified by HPLC on a Phenomenex Ultrasphere ODS column (C18, 4.6 × 25 cm; Rainin Instrument Company, Inc.). The column was pre-equilibrated with Solvent A, hexane/2-propanol (4:6) (v/v) and developed with a linear gradient of Solvent B over 30 min (water (pH 7.5)/water (pH 10)/BuOH (1 × 1 cm); Tosoh Corporation) pre-equilibrated with 20 mM Tris-HCl (pH 7.0) containing 300 mM NaCl, 1 mM EDTA, and 1 M NaCl and 1 mM EDTA at 0.1 ml/min. The enzyme activity was eluted at the retention time of 30 min, coinciding with a peak at 280 nm.

**Preparations of LBPA-rich Lipid Fractions**—Male albino rats of the Sprague-Dawley strain were given an aqueous solution of chloroquine at 100 mg per kg of body weight per day through a stomach tube for 1 week. The rats were anesthetized with pentobarbital sodium by drawing blood from the abdominal aorta. The liver was removed and homogenized with Buffer B (0.25 M sucrose containing 5 mM Tris-HCl, 1 mM MgCl2, and 2 mM EDTA (pH 7.4)). The homogenate was filtered through a 1,000 × g for 10 min and then the supernatant was centrifuged at 105,000 × g for 30 min.
Si-100 (Merck) was slurry-packed into a column (Packings) as a mixer greatly improved mixing efficiency. Lichrosphere 100 was not used, but the column was connected directly to a mass spectrometer. For a column of 1-mm diameter, the use of Accurate (LC Packings) was not detectable after a SuperQ Toyopearl chromatography. Rat testis contains a PLA2 activity toward PA (9), which was not detectable after a SuperQ Toyopearl chromatography. Based on the specific activity of PLA2, presumably because of its nonspecific adsorption onto the column. Among several chromatographic steps involved in this purification strategy, Cosmolg QA HPLC at pH 8.0 was the most effective. At the final step of purification gel chromatography on a Super SW 3000 column allowed us to remove minor contaminants reproducibly. To ensure its high resolution, the HPLC was operated at a low flow rate of 0.1 ml/min, leading to the overall purification of 538-fold (see Table I and Fig. 1B). Inclusion of DTT and a non-ionic detergent in the eluent of chromatographies was essential for improving the recovery.

During purification we found that monoacylglycerol lipase activity was co-purified with PLA2 activity. Almost all of PLA2 activity toward PA bound to a TEAE-cellulose column, whereas a significant fraction of monoacylglycerol lipase activity flew through. The ratios of lipase to PLA2 activities after Biogel A-0.5m gel chromatography were rather constant (Table I). Silver-stained SDS-PAGE analysis showed a single band of 63-kDa protein (Fig. 1C). This molecular mass was similar to that estimated by gel chromatography on a Super SW 3000 column (72 kDa). To check whether this 63-kDa protein represented PA-PLA2/MGL, aliquots of purified enzyme were separated by polyacrylamide gel electrophoresis on two adjacent lanes of a native gel. A gel strip of one lane was stained with Coomassie Brilliant Blue, and the other lane was sliced into pieces of 3-mm length. Monoacylglycerol lipase and PLA2 activities were assayed with materials extracted from these gel slices, and both activities were recovered in the same strip containing a protein band. The omission of DTT from the eluent on gel chromatography at the final step of purification caused almost complete losses of both PLA2 and monoacylglycerol lipase activities. Similarly, the respective specific activities of purified enzyme for POPA and monooleoacylglycerol decreased significantly in a similar extent, from 0.22 and 4.0 nmol/min/mg (the specific activity ratio of lipase/PLA2 of 18.2) in the presence of 50 or 10 mM EDTA in the assay mixtures did not affect the enzyme activities toward POPA and monooleoacylglycerol lipase activities greater (taurocholate > cholate > DOC) than non-ionic Triton X-100; activity was measured at pH 8.5 to ensure solubility of cholate and DOC. The pH dependence of the enzyme action toward POPA and monooleoacylglycerol lipase activities greatly (taurocholate > cholate > DOC) than non-ionic Triton X-100; activity was measured at pH 8.5 to ensure solubility of cholate and DOC. The pH dependence of the enzyme action toward POPA and monooleoacylglycerol lipase activities was determined as described under “Experimental Procedures,” using 1 mM POPA/6 mM taurocholate and 1 mM monooleoacylglycerol/6 mM taurocholate mixed micelles as substrates.

### Experimental Procedures

**Purification of Testis Phospholipase A2/Lipase**

The PLA2 and monoacylglycerol lipase activities were determined as described under “Experimental Procedures” using 1 mM POPA/6 mM taurocholate and 1 mM monooleoacylglycerol/6 mM taurocholate mixed micelles as substrates.

#### Table I

| Steps         | PLA2 Specific activity | Monoacylglycerol lipase specific activity | Monoacylglycerol lipase/PLA2 | Purification |
|---------------|------------------------|------------------------------------------|------------------------------|-------------|
| Supernatant   | 1.6                    | 18.7                                     | 11.7                         | Fold        |
| TEAE-cellulose| 0.8                    | 8.6                                      | 10.8                         | 1.0         |
| Phenyl-Sepharose | 0.9                  | 7.5                                      | 8.3                          | 1.1         |
| SuperQ-Toyopearl | 0.9                  | 24.6                                     | 27.3                         | 1.1         |
| Biogel A-0.5m | 12.0                   | 210                                      | 17.5                         | 15.0        |
| Cosmolg QA (pH 8.5) | 20.0                 | 459                                      | 23.0                         | 25.0        |
| Cosmolg QA (pH 8.0) | 220                  | 4,000                                    | 18.2                         | 275         |
| Super SW 3000 | 430                    | 8,210                                    | 19.1                         | 538         |

*a* The ratios of monoacylglycerol lipase to PLA2 activities.  
*b* Based on the specific activity of PLA2.

### Protein Sequencing

**Purification of PA-PLA2/MGL from rat testis**

The PLA2 and monoacylglycerol lipase activities were determined as described under “Experimental Procedures,” using 1 mM POPA/6 mM taurocholate and 1 mM monooleoacylglycerol/6 mM taurocholate mixed micelles as substrates. The PLA2 and monoacylglycerol lipase activities were determined as described under “Experimental Procedures,” using 1 mM POPA/6 mM taurocholate and 1 mM monooleoacylglycerol/6 mM taurocholate mixed micelles as substrates.
8.5 for monoolein (Fig. 2B), but both activities extended broadly over a physiologically relevant pH range from pH 5.5 to 7.5. Fig. 3 shows the substrate specificity of the purified enzyme. The enzyme preferred anionic phospholipids POPA and POPG in this order but hardly hydrolyzed zweiterionic POPE and POPC. It released oleic acid from the sn-2 position of POPG, but not palmitic acid esterified at its sn-1 position, and can hydrolyze 1-O-hexadecyl-OPA as efficiently as POPA, confirming the A2 regiospecificity for these phospholipids (see Fig. 2A and Fig. 3A). As to the specificity for the sn-2 acyl groups, PA-PLA2/MGL much preferred unsaturated than saturated acyl chains (Fig. 3A). The enzyme also hydrolyzed bisphosphatidic acid, an anionic and more bulky substrate, to the extent similar to POPA, but we did not address the regiospecificity for this substrate. PA-PLA2/MGL hardly exhibited lysophospholipase activities toward 1-acyl lysophospholipids including LPA, LPC, LPI, and LPS at pH 5.5. PA-PLA2/MGL exhibited monoacylglycerol lipase activity with modest preference for polyunsaturated acyl groups at pH 8.5, with the specificity order linolenoyl > arachidonoyl > linoleoyl > oleoyl > stearoyl, whereas its di- and tri-acylgi-
tors, APMSF and MAFP, inhibited PLAs and lipase activities to a similar extent (Table II). PA-PLA2/MGL also exhibited esterase activity toward p-nitrophenyl esters of short and long chain fatty acids. The specific activity for the valerate ester (580 μmol/min/mg) was significantly greater than the myristate ester (12 μmol/min/mg).

**LBPA Hydrolysis by PA-PLA2/MGL**—The major molecular species of phospholipids in LBPA-rich lipids extracted from the lysosomal fractions of chloroquine-treated rat liver were as follows: phosphatidylcholine, 18:0–20:4, 16:0–18:2, 16:0–20:4, and 18:0–18:2 (1, 0.89, 0.70, and 0.49 of the relative intensities, respectively); phosphatidylethanolamine, 18:0–20:4, 18:0–18:2, 16:0–22:6, 16:0–20:4, and 18:0–22:6 (1, 0.5, 0.45, 0.43, and 0.23, respectively); phosphatidylinosine, 18:0–20:4 and 18:0–22:6 (1 and 0.3, respectively); and phosphatidylinositol, 18:0–20:4, where the numbers in parentheses indicate intensity ratios of each species to the most intense one, and species with the relative intensity of >0.1 were listed. Signals of PA were very weak but detectable at the retention times similar to those of phosphatidylinosine and contained predominant peaks of 18:0–20:4 species. The lipids were hydrolyzed thoroughly by excess of pancreatic PLA2. After this treatment the amounts of diacylphospholipids were less than 0.5% of those in untreated lipids except for 1-stearoyl-2-arachidonoyl-phosphatidylinositol and LBPA as revealed by mass spectrometry. Signals of PA were not detectable after the treatment. LBPA is a structural isomer of PG, but tandem mass spectrometry can discriminate both isomers; negative ion MS/MS spectrum of PG contains a peak (m/z = 791) for di-docosahexaenoyl (C22:6)-PG arisen from neutral loss of a glycerol moiety but not that of LBPA (Fig. 4B). The major LBPA molecular species were found to be di-C22:6- and C22:6-linoleoyl (C18:2)-LBPPAs as revealed in the presence of the respective fatty acid anions in negative ion MS/MS spectra (Fig. 4, A and B). Other phospholipids with such fatty acyl groups were not detectable after treatment with pancreatic PLA2; major lysocephospholipid molecular species found were either palmitoyl- and stearoyl-LPC or LPE and stearoyl-LPS, further supporting the assignment of unsaturated fatty acids into sn-2 position as described above. These unique fatty acyl compositions of LBPA and the substrate specificity of PA-PLA2/MGL allowed us to assay LBPA hydrolyzing activity by following the C22:6 and C18:2 release on incubation of purified PA-PLA2/MGL with the pancreatic PLA2-treated LBPA-rich lipid fractions. Fig. 5A shows the time course of C22:6 and C18:2 release. The specific activity for the total of C18:2 and C22:6 release (0.33 μmol/min/mg) was comparable with that toward PA, but C18:1 and C20:4 release was very low. Release of lysophosphatidylglycerol (LPG), another product of LBPA hydrolysis by PA-PLA2/MGL, was examined by negative ion HPLC/ESI-MS spectrometry (Fig. 5, B-D). The major species was found to be C22:6-LPG (m/z = 555). For comparison, we used endogenous N-palmitoyl-sphingomyelin (m/z = 747 of its formate adduct) as an internal standard (Fig. 5B). On MS/MS analysis the presence of a peak (m/z = 463) derived from the neutral loss of glycerol–H2O and that of C22:6 anion (m/z = 327) confirmed its structure.

**Sequence Analysis**—The sequence of the NH2-terminal 12 amino acids of the purified enzyme was YPSPPVVNTVK and is identical to those of rat liver serine esterases CES1A including microsomal serine carboxylesterase ES-10 (19) and cholesteryl esterase (20).

**DISCUSSION**

We purified to homogeneity a phospholipase A2 from rat testis that preferred both PA and monoacylglycerol. Purified enzyme showed a single band on a silver-stained gel (Fig. 1), ratios of the specific activity of PLA2 to lipase were constant at the final purification steps (Table I), and the two activities co-migrated on native gels required the presence of DTT in the assay mixtures and were similarly inhibited by MAFP and APMSF (Table II), suggesting that a single enzyme catalyzes PLA2 and monoacylglycerol lipase activity. Although mammalian PLA2s are rather specific for diradyl phospholipids, phos-

---

**Table II**

| Inhibitors | Residual activity | Monoooleoylglycerol substrate | POPA substrate |
|------------|------------------|------------------------------|---------------|
| APMSF (500 μM) | 1.3% | 4.8% |
| MAFP (50 μM) | 0.4% | 6.4% |

---

2 Intensity ratios do not necessarily represent concentration ratios, because ionization efficiencies of phospholipids of a given class depend significantly on acyl chain length.
pholipase B/lipase expressed in rat testis and intestine exhibits high PLA₂, lysophospholipase, and triacylglycerol lipase activities (15). PA-PLA₂/MGL is the second mammalian enzyme with PLA₂ and lipase activities. Interestingly, both enzymes hardly show PLA₁ activity, which is often associated with lipases (21).

PA-PLA₂/MGL displayed broad pH profiles with acidic pH optimum (5.5) toward PA, an acidic substrate, and with alkaline one (8.5) toward monoaoylglycerol, a neutral substrate (Fig. 2B). MAFF, a potent irreversible inhibitor for lipases and PLA₂, accepts a proton from the Ser O⁻ in the carboxylic group, and behaves as trimers on gel chromatography, a characteristic of the ES-10 esterase. There exists the ES-10 species with only a few amino acid substitutions, and this microheterogeneity in sequence caused it to switch substrate specificity. Rat lung CES1A esterase and liver cholesterol esterase differ in sequence by only four residues, three of which were important to confer cholesterol esterase activity on the lung enzyme as revealed by site-directed mutagenesis (28). Recent x-ray crystallography of rabbit CES2 esterase suggested not only the importance of the equivalent residues in maintaining substrate specificity but also the participation of a sugar chain in the binding site of the acyl intermediate (29). Moreover, rat liver ES-10 esterase functions as a triacylglycerol lipase that mobilizes cytoplasmic triacylglycerol stores on lipoprotein assembly (30), whereas PA-PLA₂/MGL hardly reacted with triacylglycerol (Fig. 3B). Hence, intensive protein structural analysis by mass spectrometry will be required to detect a few amino acid substitutions and post-translational modifications including glycosylation in PA-PLA₂/MGL, compared with the ES-10 enzyme, helping us clarify its mechanisms of controlling substrate specificity.

Recently, several PLA₂s that are optimally active at acidic pH have been characterized (5, 25, 31, 32). The lung 26-kDa PLA₂ with the peroxidase activity at alkaline pH as mentioned above can hydrolyze dipalmitoyl phosphatidylcholine, a lung surfactant, and showed a more acidic pH optimum (4.2–4.5), although its selectivity for acidic phospholipids has not been
tested (25). A 45-kDa \( \text{PLA}_2 \) partially purified from macrophage-like RAW 264.7 cells preferred PG, but not PA, and has a weak but significant \( \text{PLA}_1 \) activity (31). These two \( \text{PLA}_2 \)s differed considerably from PA-\( \text{PLA}\_2/\text{MGL} \) in substrate specificity and molecular mass (see Fig. 1 and Fig. 3). An ~58-kDa PA-prefering \( \text{PLA}_2 \) purified from rat brain (5) exhibited pH 6.0 optimum for hydrolyzing PA and is rather similar to PA-\( \text{PLA}\_2/\text{MGL} \), but significant differences should be noted; unlike PA-\( \text{PLA}\_2/\text{MGL} \), the enzyme did not prefer unsaturated acyl groups at \( \text{sn}-2 \) position at all and strongly binds to cation-exchange and heparin-ligated gels, suggesting its more basic nature (5). It was not tested whether the enzyme hydrolyzed monoacylglycerol. An ~40-kDa \( \text{PLA}_2 \) with an optimum pH of 4.5 purified from calf brain catalyzed transacylation of the acyl group at \( \text{sn}-2 \) position of phosphatidylcholine or phosphatidylethanolamine to 1-hydroxy group of \( N\)-acetyl ceramide, as well as \( \text{PLA}_1 \) reaction. This enzyme is different from PA-\( \text{PLA}\_2/\text{MGL} \) in substrate specificity, reactivity with serine-modifying irreversible inhibitors, net charge on the protein, and molecular mass (32).

The testis expresses the enzymes that have been purified and cloned and display the activities related to those of PA-\( \text{PLA}\_2/\text{MGL} \), i.e. 97.6-kDa PA-prefering \( \text{PLA}_1 \) (9, 10) and 33.2-kDa monoacylglycerol lipase (33). Apparent molecular mass (63 kDa) of PA-\( \text{PLA}\_2/\text{MGL} \) is quite different from those of the latter two enzymes. These three enzymes do not require \( \text{Ca}^{2+} \) ions for activity but show substrate specificities different from one another. PA-\( \text{PLA}\_2/\text{MGL} \) exhibited high monoacylglycerol lipase and \( \text{PLA}_2 \) activities with a specific activity toward the mixed micelles of taurocholate and either monooleoylglycerol (8.21 \( \mu \text{mol} / \text{min} / \text{mg} \)) or POPA (0.43 \( \mu \text{mol} / \text{min} / \text{mg} \)). In contrast, PA-prefering \( \text{PLA}_1 \) with a specific activity of 2.95 \( \mu \text{mol} / \text{min} / \text{mg} \) toward PA did not exhibit \( \text{PLA}_1 \) activity (9), and monoacylglycerol lipase with a specific activity of 350 \( \mu \text{mol} / \text{min} / \text{mg} \) toward monooleoylglycerol did not exhibit appreciable \( \text{PLA}_2 \), tri- and diacylglycerol lipase and lysophospholipase activities (34).

There are several biosynthetic pathways of LPA as mentioned in the Introduction, and the major pathway varies from tissue to tissue. One of the major sources of LPA in the serum was reportedly platelet in which the combined action of phospholipase C and kinase, a \( \text{PLA}_2 \)-independent pathway, mainly contributes to LPA synthesis (35), but a study with a \( \text{PLA}_2 \) inhibitor suggested for involvement of a \( \text{PLA}_2 \)-dependent pathway in LPA synthesis, depending on molecular species of LPA (36). We found that human platelets contained PA-\( \text{PLA}\_2/\text{MGL} \)-like activity, \( \text{PLA}_2 \) activities toward POPA of 0.21 and 0.41 nmol/min/mg at pH 8.5 and 5.5, respectively, and the activity hydrolyzing monooleoylglycerol of 3.34 nmol/min/mg at pH 8.5. Note that the \( \text{PLA}_2 \) specific activity at pH 5.5 was greater than at pH 8.5, suggesting that this PA-\( \text{PLA}\_2/\text{MGL} \)-like activity could be involved more significantly in producing LPA than \( \text{PLA}_2 \) being active at alkaline pH. Under the assay conditions used group IIA \( \text{PLA}_2 \)s, which prefers anionic phospholipids including PA (4, 8), accounted for most of the measurable \( \text{PLA}_2 \) activity at pH 8.5 in platelet homogenate (8). PA-\( \text{PLA}\_2/\text{MGL} \) is a candidate for enzymes producing LPA in the testis that was one of the rich sources of LPA (37).

This study provides evidence for the ability of PA-\( \text{PLA}\_2/\text{MGL} \) to hydrolyze LBPA, a unique anionic phospholipid localized specifically in intravesicular vesicles of late endosomes (11), at the optimal pH of 5.5, which is interestingly the same as a typical pH value of the lumen of late endosomes (38). LBPA is unstable because of 2,3-acyl migration catalyzed by acid or base and chromatographic silica supports. This hinders isolation and structural determination of its natural form(s) (39). In this study we used LBPA-rich lipids, extracted from lysosomal frac-



REFERENCES

1. Moolenaar, W. H., Kraneenberg, O., Postma, F. R., and Zondag, G. C. (1997) Mol. Pharmacol. 58, 1188–1196.
2. Contos, J. J. A., Ishii, L., and Chun, J. (2000) Mol. Pharmacol. 58, 1188–1196.
3. Gaitis, F., Fourcade, O., Le Balle, F., Gueguen, G., Gaige, B., Gassama-Diagne, A., Faurel, J., Salles, J. P., Mauro, G., Simon, M. F., and Chap, H. (1997) FEBS Lett. 410, 54–58.
4. Snitko, Y., Yoon, E. T., and Cho, W. (1997) Biochem. J. 321, 757–774.
5. Thomson, F. J., and Clark, M. A. (1995) Biochem. J. 306, 305–309.
Purification and Properties of a Phospholipase A2/Lipase Preferring Phosphatidic Acid, Bis(monoacylglycerol) Phosphate, and Monoacylglycerol from Rat Testis
Masafumi Ito, Urbain Tchoua, Mitsuhiro Okamoto and Hiromasa Tojo

J. Biol. Chem. 2002, 277:43674-43681. doi: 10.1074/jbc.M202817200 originally published online September 9, 2002

Access the most updated version of this article at doi: 10.1074/jbc.M202817200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 46 references, 20 of which can be accessed free at http://www.jbc.org/content/277/46/43674.full.html#ref-list-1