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Identification of a phosphatidic acid-preferring phospholipase A_1 from bovine brain and testis

(lysophosphatidic acid/lysophospholipase/Triton X-100 micelles/phospholipase D/diacylglycerol kinase)

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ABSTRACT **Recent experiments in several laboratories have provided evidence that phosphatidic acid functions in cell signaling. However, the mechanisms that regulate cellular phosphatidic acid levels remain obscure. Here we describe a soluble phospholipase A_1 from bovine testis that preferentially hydrolyzes phosphatidic acid when assayed in Triton X-100 micelles. Moreover, the enzyme hydrolyzes phosphatidic acid molecular species containing two unsaturated fatty acids in preference to those containing a combination of saturated and unsaturated fatty acyl groups. Under certain conditions, the enzyme also displays lysophospholipase activity toward lysophosphatidic acid. The phospholipase A_1 is not likely to be a lysosomal enzyme because its optimum pH is 7.5–8.5. Furthermore, it is probably not a general lipid metabolic enzyme because high levels of activity are found in mature testis and brain but no measurable activity is seen in liver, spleen, or heart. The fact that the activity of the phospholipase A_1 in mature bovine testis is >10-fold higher than that in newborn calf testis raises the possibility that the enzyme may play a regulatory role in spermatogenesis or sperm function.**

A growing body of evidence suggests that phosphatidic acid (PA) may be a second messenger in agonist-stimulated cell activation. In a number of cells, PA levels increase dramatically in response to a wide variety of agonists (1). Furthermore, PA can affect a number of cellular processes. In IIC9 fibroblasts, PA generated by α-thrombin stimulation caused appreciable and sustained actin polymerization, an effect shown not to be mimicked by diacylglycerol (DG) (2). In neutrophils, agonist-stimulated PA, but not DG, was shown to stimulate the generation of H_2O_2 (3). *In vitro*, PA stimulated specifically and substantially (>10-fold) a phosphatidylinositol (PI) kinase and phospholipase C-γ1 (4, 5).

The production of agonist-stimulated PA can occur by several mechanisms, one being hydrolysis of phosphatidylcholine (PC) or PI by enzymes with phospholipase D (PLD) activity (1, 6). PLD activities can be stimulated by a number of mechanisms *in vivo* and *in vitro* (7–9) and at least two isozymes are known to exist (10). PA can also be generated by a two-step mechanism involving phospholipase C hydrolysis of either PI or PC followed by phosphorylation of the resulting DG by one of several DG kinase (DGK) isozymes (11).

How PA signals are attenuated is not known. PA generated during the PI cycle is thought to be reconverted to PI (12). In addition, a portion of the PLD-generated PA may be converted into DG by either of two PA phosphohydrolases (13, 14). Recent experiments have suggested, however, that PLD-generated PA may not be converted to DG when IIC9 fibroblasts are stimulated by thrombin (15). Another potential mechanism for attenuation of PA signals might involve hydrolysis by phospholipase A (PLA) activities, producing lysophosphatidic acid (LPA). A PA-specific PLA_2 has been reported (16), but detailed information about this enzyme is lacking. Another possibility is that a PLA_1 might metabolize PA to *sn*-2-LPA. Several PLA_1 activities have been identified in mammalian tissues, including one found in rat liver plasma membrane (17), another found in rat brain cytosol (18, 19), and others found in lysosomes (20, 21). None of these enzymes, however, displays a preference for PA as a substrate.

In the present study, we identify and characterize a cytosolic PLA_1 with a strong preference for PA. Under a limited set of conditions, this enzyme can also hydrolyze LPA. The fact that this PLA_1 shows high levels of activity in mature testis and brain but not in liver, heart, or spleen suggests that it may play a specific role in these tissues.

MATERIALS AND METHODS

Preparation of High-Speed Supernatant (HSS) from Bovine Tissues. Tissues, frozen in liquid nitrogen immediately upon removal from the animal, were purchased from Pel-Freez Biologicals. All subsequent procedures were carried out at 4°C except where mentioned otherwise. The frozen tissue was cut into 2- to 5-cm cubes and extraction buffer (3 ml/g) was added [25 mM 3-[*N*-morpholino]propanesulfonic acid, pH 7.2/5 mM EDTA/5 mM benzamidine/1 mM EGTA/1 mM dithiothreitol (DTT)/1 mM phenylmethylsulfonyl fluoride/leupeptin (2 μg/ml)/aprotinin (2 μg/ml)]. The mixture was homogenized then centrifuged at 800 × *g* for 10 min. The resulting low-speed supernatant was centrifuged at 235,000 × *g* for 60 min to obtain a HSS.

Fractionation of HSS by Mono Q Fast Protein Liquid Chromatography. HSS (45 mg of protein) was loaded onto a Mono Q 5/5 Fast Protein Liquid Chromatography column (Pharmacia) equilibrated in 25 mM 3-[*N*-morpholino]propanesulfonic acid, pH 7.2/1 mM EGTA/1 mM DTT. After the column was washed with buffer containing 100 mM KCl, a 50-ml 100–400 mM KCl gradient was applied, followed by a wash with buffered 1 M KCl. All steps were carried out at a flow rate of 0.8 ml/min and fractions were collected at 2-min intervals.

Synthesis of [^{32}P]PA. [^{32}P]PA was synthesized from the corresponding DG, which was either purchased [*sn*-1,2-dioleoyl-DG or *sn*-1-palmitoyl-2-oleoyl-DG from Avanti or *sn*-1-stearolyl-2-arachidonoyl (18:0/20:4)-DG from Sigma] or synthesized from the corresponding PC purchased from

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Abbreviations: cmc, critical micelle concentration; DG, diacylglycerol; DGK, diacylglycerol kinase; 18:0/20:4, *sn*-1-stearoyl-2-arachidonoyl; DTT, dithiothreitol; HSS, high-speed supernatant; LPA, lysophosphatidic acid; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PLA, phospholipase A; PLD, phospholipase D; PS, phosphatidylserine; TX100, Triton X-100.

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Avanti Polar Lipids (22). Four micromoles of anhydrous DG was resuspended in 720 μl of 50 mM imidazole hydrochloride, pH 6.6/50 mM LiCl/12.5 mM $MgCl_2$/1 mM EGTA/1% octyl glucoside/2 mM DTT, and the reaction was started by the addition of 100 μl of [γ-^{32}P]ATP (40.5 mM; 0.1 Ci/mmol; 1 Ci = 37 GBq; New England Nuclear) and 180 μl of *Escherichia coli* DGK (Calbiochem). After 1 hr at 25°C, the reaction was stopped by the addition of 3 ml of $CHCl_3$/MeOH, 1:1 (vol/vol), followed by 1 ml of 1% perchloric acid/MeOH, 1:7 (vol/vol), and 1 ml of $CHCl_3$. The mixture was vortex mixed and centrifuged at 200 × *g* for 2 min, and the lower phase was evaporated under argon.

The ^{32}P-labeled PA was purified using a 3-ml Bond Elut PRS column (Varian) preequilibrated with $CHCl_3$ under gravity flow. The PA was applied to the column in 1 ml of $CHCl_3$. The column was washed successively with 8 vol of $CHCl_3$/MeOH at 100:1 (vol/vol), 99:1, and 98:2. PA was eluted in the 98:2 fraction. Solvent was removed from this fraction by evaporation under argon and the residue was resuspended in 1 ml of toluene/ethanol, 1:1 (vol/vol), and stored at −20°C. Analysis of the PA by TLC (silica 60 plate, Merck) in $CHCl_3$/acetone/MeOH/acetic acid/water, 6:8:2:2:1 (vol/vol), revealed one radioactive I_2-staining spot that comigrated with authentic PA.

Assay of PLA Activity on PA. [^{32}P]PA was solubilized in 5% (wt/vol) Triton X-100 (TX100) (Boehringer Mannheim) in assay buffer (50 mM Tris·HCl, pH 8.0/100 mM KCl/1 mM EGTA/1 mM DTT/0.1 mM diethylenetriaminepentaacetic acid). The reaction was started by adding 10 μl of this solution to 90 μl of assay buffer containing the enzyme (usually peak fraction from Mono Q of mature testis HSS). Reactions were carried out for 20 min and stopped by the addition of 250 μl of $CHCl_3$/MeOH, 2:1, containing 0.1 M trifluoroacetic acid, carrier LPA (50 μg/ml), and carrier PA (25 μg/ml). After a brief centrifugation, 165 μl of the resulting upper phase was removed and analyzed by liquid scintillation counting to determine the amount of ^{32}P present, which was always <1% of the product generated. The lower phase was dried in a SpeedVac (Savant). The pellet was dissolved in 25 μl of $CHCl_3$/MeOH, 1:1, and 20 μl of this solution was spotted onto a reverse-phase silica TLC plate (J. T. Baker), which was developed in MeOH/4 M ammonium formate, pH 6.7, 98:2 (vol/vol). LPA and PA spots were detected by exposure of the plate to x-ray film (Kodak), the radioactive spots were scraped, and radioactivity was measured by scintillation counting (Beckman counter) in 5 ml of Ecolume (ICN). The assay was linear for both time (up to 30 min) and protein (up to 9 μg of Mono Q peak fraction) for all PA concentrations tested. The pH dependence of the PLA activity was determined using 0.5 mol % di-oleoyl-PA at pH values of 4.0 (potassium formate or sodium lactate), 4.5 and 5.0 (potassium acetate), 6.0 (2[*N*-morpholino]ethanesulfonic acid or histidine), 6.5 (piperazine-*N*,*N*′-bis-[2-ethanesulfonic acid]), 7.0 and 7.2 (3-[*N*-morpholino]propanesulfonic acid), 7.5, 7.75, 8.0, 8.25, and 8.5 (Tris·HCl or triethanolamine), 9.0 (2-[*N*-cyclohexylamino]ethanesulfonic acid), and 10.0 (3-[cyclohexylamino]-1-propanesulfonic acid).

Modification of PLA Assay for Other Lipids. Radioactive lipids used were as follows: *sn*-1-stearoyl-2-arachidonoyl[5, 6, 8, 9, 11, 12, 14, 15-^{3}H]PI or -PC (NEN); 1,2-dioleoyl-3-phosphatidyl[3-^{14}C]serine (PS) (Amersham); 1,2-[1-^{14}C]-dioleoyl-PC (NEN); 1,2-dioleoyl-3-phosphatidyl[2-^{14}C]ethanolamine (PE) (Amersham); and DG made by phospholipase C digestion of [^{14}C]PC (22). Unlabeled lipids were purchased from Avanti Polar Lipids. All assay steps were similar to those for PA except that separation of products was conducted on Silica 60 TLC plates in the following solvents: $CHCl_3$/MeOH/acetic acid/water, 25:15:4:2 (vol/vol), for PS; $CHCl_3$/acetone/MeOH/acetic acid/water, 6:8:2:2 (vol/vol), for PC; $CHCl_3$/MeOH/water, 65:25:4 (vol/vol), for PE; hexane/diethyl ether/MeOH/acetic acid, 60:50:5:2 (vol/vol), for DG. PLA activity was determined by scintillation counting of the scraped lysophospholipid or fatty acid bands.

PLA Assay Using Sonicated Vesicles of PA or PI. The procedure was similar to that described in ref. 18. Briefly, 10 μl of assay buffer was added to 10 nmol of dry 18:0/20:4-[^{32}P]PA or 18:0/20:4-[^{3}H]PI and the mixture was bath-sonicated (Branson) for three 15-sec periods at 30-sec intervals. Reactions were started by adding the vesicle suspension to 90 μl of assay buffer containing the appropriate amount of enzyme. Products were analyzed as described above.

LPA Hydrolysis Assay. ^{32}P-containing LPAs were produced from their respective monoglycerides (Serdary Research Laboratories, London, ON, Canada) as described (23) except that *E. coli* DGK was used as the enzyme source. The desired quantity of [^{32}P]LPA was solubilized in 1 mM TX100. Then 2 μl of this solution was added to 98 μl of a mixture of the other assay components to start the assay. Final buffer concentrations were the same as for the PA hydrolysis assay except that TX100 was 20 μM. The assay was conducted for 20 min at 25°C and then stopped in the same solvent used to stop the PA hydrolysis assay. Radioactivity in an aliquot of the upper phase was measured to determine the amount of aqueous ^{32}P produced. Identity of the aqueous ^{32}P-containing compound as glycerol 3-phosphate was established by polyethylenimine-cellulose TLC (Merck) in 500 mM ammonium formate, pH 3.5/100 mM LiCl.

Analysis of Phospholipase Acyl Chain Specificity. Incubations and lipid extractions were done as described above using 0.5 mol % 18:0/20:4-[^{32}P]PA. After extraction of the lipid, the lower phase was dried down under argon and the residue was resuspended in 1 ml of $CHCl_3$. The sample was then applied to a Varian Bond Elut PRS column equilibrated in $CHCl_3$. After the column was washed with 8 ml of $CHCl_3$/MeOH, 98:2, LPA was eluted with 4 ml $CHCl_3$/MeOH, 95:5. The LPA was dried down and methylated as described (23). The methylated LPA was applied to a reverse-phase TLC plate (Whatman), which was developed in acetonitrile/isopropanol/MeOH/water, 45:25:15:15 (vol/vol), for 1 hr. Radioactive bands were detected by exposure of the TLC plate to Kodak X-Omat film. Control assays were carried out using *Crotalus durissus terrificus* PLA_2 or *Rhizopus arrhizus* lipase (Sigma). The methylated LPA products produced by these two enzymes migrated identically to authentic methylated 18:0– or 20:4-LPA, respectively. Authentic LPA standards were produced as described above.

Additional Methods. Protein concentrations were assayed using the bicinchoninic acid method (Pierce). KCl concentrations of chromatography buffers were analyzed using a conductivity meter (Radiometer Copenhagen). The concentrations of purchased phospholipids were routinely analyzed by phosphate assay (24) and their purities were verified by the TLC systems mentioned above. Hill coefficients were calculated by fitting the data points to the Hill equation.

RESULTS

When the HSS from mature bovine testis was fractionated by Mono Q chromatography and the resulting fractions were assayed for their ability to produce [^{32}P]LPA from di-oleoyl-PA, one peak of activity was identified (Fig. 1*A*). A similar peak was identified by Mono Q fractionation of HSS from bovine brain but not from liver, spleen, heart, or serum (Fig. 1*B*). These results suggest that the identified PLA activity is involved in a process particular to testis and brain and not in general lipid biosynthetic or degradative processes common to all cells. In addition, mature testis contained 10-fold more activity than did newborn calf testis, introducing the possibility that the enzyme is involved in spermatogenesis or sperm function.

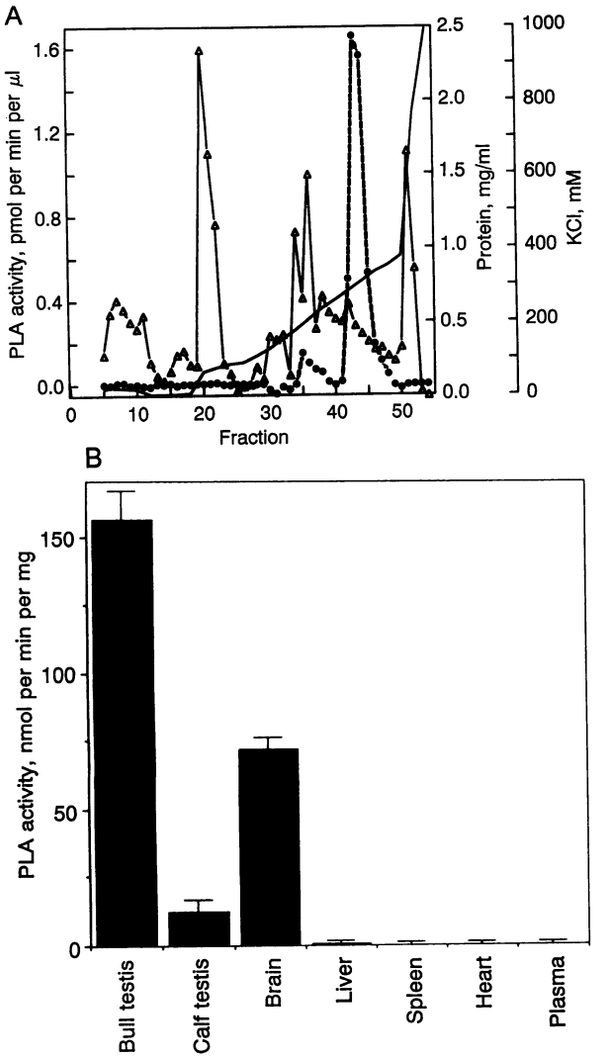

FIG. 1. Identification of PLA activity in a bovine tissue HSS fraction. (*A*) HSS from mature bovine testis (45 mg of protein) was loaded onto a Mono Q column and proteins were eluted with a KCl gradient. The resulting fractions were assayed for PLA activity using 0.5 mol % dioleoyl-[^{32}P]PA in TX100 micelles as substrate. Circles with dashed line, PLA activity; triangles with solid line, protein concentration; solid line, KCl concentration. (*B*) Specific activity of PLA in Mono Q-fractionated HSS from several bovine tissues. Results in *A* and *B* are representative of two experiments.

Further characterization of the enzyme was carried out using material corresponding to the peak activity fraction obtained by Mono Q chromatography of mature testis HSS. The acyl chain specificity of the PLA was determined by incubating 18:0/20:4-[^{32}P]PA with the PLA followed by analysis of the products by reverse-phase TLC. The only product observed was ^{32}P-labeled arachidonoyl-LPA (Fig. 2). A similar result was obtained when the PLA was incubated with 18:0/[^{3}H]20:4-PA (data not shown). Thus, the phospholipase identified is of the A_1 class and will hereafter be referred to as PA-PLA$_1$.

PA-PLA$_1$ hydrolytic activity varied depending on the PA molecular species used as a substrate. As shown in Fig. 3*A*, PAs containing two unsaturated fatty acyl chains (diunsaturated PA) were markedly better substrates than those in which the *sn*-1 fatty acid was saturated. As one example, *sn*-1,2-diarachidonoyl-PA was at least an 8-fold better substrate for PA-PLA$_1$ than was 18:0/20:4-PA. Likewise, dioleoyl-PA was hydrolyzed three times faster than *sn*-1-palmitoyl-2-oleoyl-PA. Diunsaturated PA molecular species

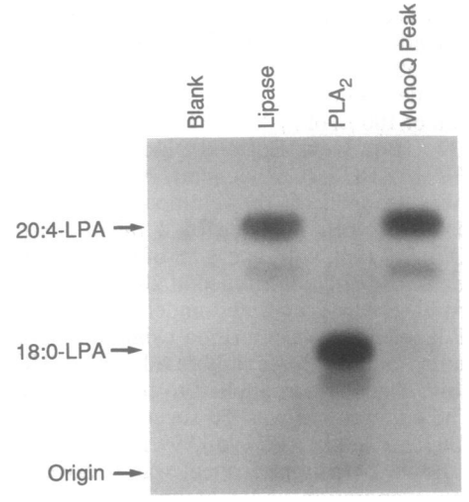

FIG. 2. Determination of PLA acyl chain specificity. The 18:0/20:4-[^{32}P]PA was used as substrate and the Mono Q peak was the enzyme source. After a 20-min reaction time, the LPA was methylated and the products were analyzed by reverse-phase TLC. PLA$_2$ from *Crotalus durissus terrificus* was used as a control, producing 18:0-[^{32}P]LPA. Similarly, lipase from *Rhizopus arrhizus* produced 20:4-[^{32}P]LPA. Mono Q peak fraction produced only 20:4-[^{32}P]LPA, identifying it as a PLA$_1$. Results are representative of two experiments.

may thus be physiologically important substrates for PA-PLA$_1$.

All PA substrates displayed sigmoidal kinetics (Fig. 3*A*), with a Hill coefficient of 3. In contrast, a much lower effect of increasing substrate concentrations was seen when the anionic phospholipid, PS, was used as a substrate, and none was observed with zwitterionic phospholipids (PC or PE) (Fig. 3*C*). The basis for the increases in PA-PLA$_1$ activity observed with PA and PS remains to be determined.

Various PLA$_1$ activities have been reported. One of these enzymes hydrolyzes several different phospholipids, as well as mono-, di-, and triglycerides, with comparable affinities (17). Another, identified in rat brain cytosol, hydrolyzes PI at a 6-fold higher rate than it hydrolyzes PA (18). In contrast to these two enzymes, PA-PLA$_1$ displayed a preference for PA over a number of other phospholipids. When 18:0/20:4-containing phospholipids were used as substrates at 0.5–1 mol % in the TX100 micelle assay, PA-PLA$_1$ hydrolyzed PA 5-fold faster than PC and 3-fold faster than PI (Fig. 3*B*). Furthermore, when a sonicated phospholipid assay similar to the one described (18) was used, PA-PLA$_1$ activity from testis or brain was two times greater toward 18:0/20:4-PA than toward the corresponding molecular species of PI (data not shown). When di-oleoyl-containing lipids were used as substrates at low concentrations (0.5–1 mol %) in TX100 micelle assays, PA was hydrolyzed 5, 7.5, 10, and 20 times faster than PS, PE, PC, and DG, respectively, and 20 times faster than *sn*-1-oleoyl-LPA (Fig. 3*C Inset*). At 10 mol % di-oleoyl-containing substrate, the enzyme's preference for PA increased to 10-fold over PS and 50-fold over the other lipids tested (Fig. 3*C*). PA-PLA$_1$ thus displays a strong preference for PA as a substrate over a number of glycerolipids, suggesting that it is a distinctive enzyme.

Interestingly, PA-PLA$_1$ displayed potent lysophospholipase activity toward *sn*-1-oleoyl-LPA under certain conditions. When a submicellar concentration of TX100 was used, LPA hydrolysis was linear to a concentration of 1 μM LPA, at which point the activity leveled off and then decreased precipitously to become almost undetectable at 5 μM LPA (Fig. 4*A*). Concentrations of TX100 above its critical micelle concentration (cmc) strongly inhibited the lysophospholipase

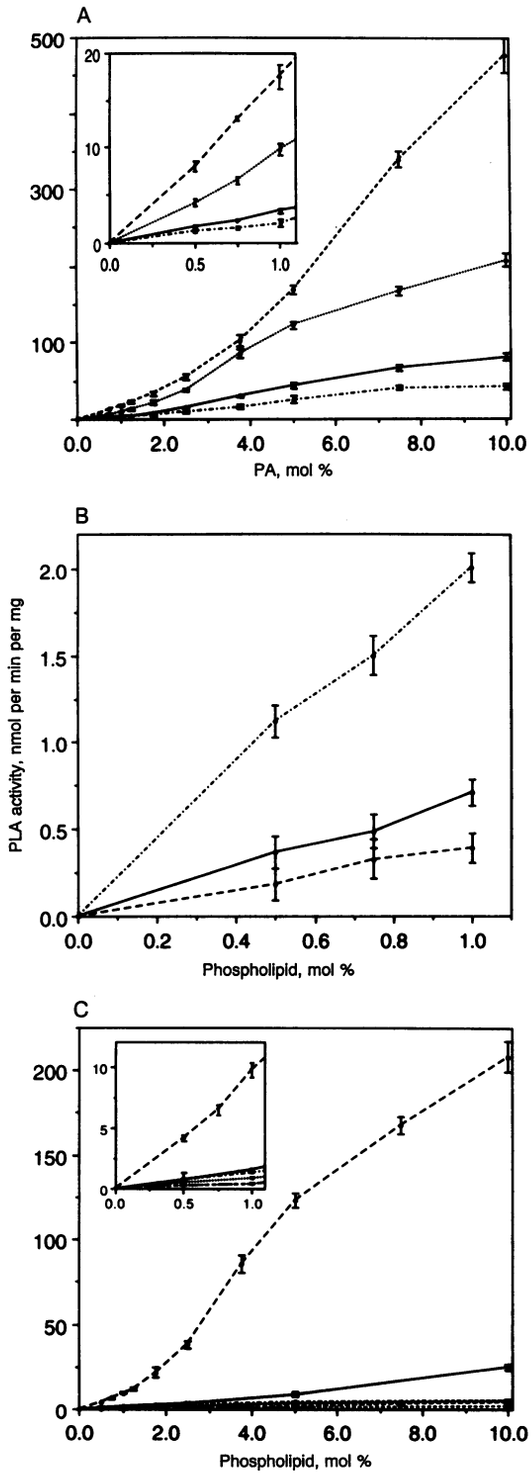

FIG. 3. Substrate preference of PA-PLA$_1$. (*A*) PA-PLA$_1$ hydrolysis of various PA molecular species. The di-arachidonoyl (dashes), di-oleoyl (dots), palmitoyl/oleoyl (solid line), and 18:0/20:4 (dots and dashes) molecular species of [^{32}P]PA were compared as substrates for PA-PLA$_1$ (Mono Q peak fraction) in the TX100 micelle assay system. Enzyme-specific activity (expressed as nmol of substrate hydrolyzed per min per mg of protein) was determined by quantitating the amount of [^{32}P]LPA produced. (*B*) The 18:0/20:4 molecular species of PA (dots and dashes), PI (solid line), and PC (dashes) were compared as substrates for PA-PLA$_1$. (*C*) The di-oleoyl molecular species of PA (dashes), PS (solid), PE (dots and dashes), PC (dots), and DG (short dashes) and *sn*-1-oleoyl-LPA (long dashes) were compared as substrates for PA-PLA$_1$. (*Inset*) PLA activity at low substrate mol %. Results are representative of two (PC, PE, and PI) or three (PA and PS) experiments carried out in duplicate.

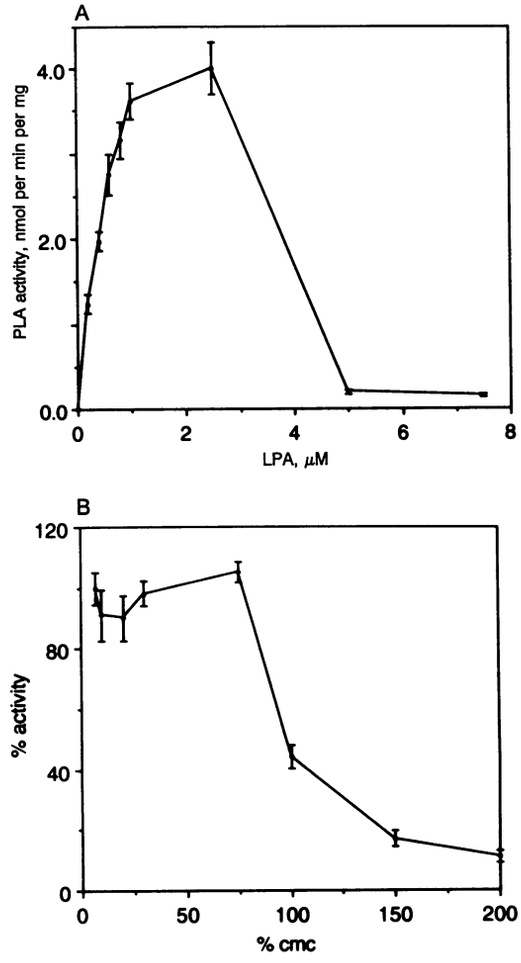

FIG. 4. LPA hydrolysis by PA-PLA$_1$. (*A*) Degradation of *sn*-1-oleoyl-[^{32}P]LPA by Mono Q peak fraction in the presence of submicellar TX100 (20 μM; cmc = 290 μM) expressed as nmol of LPA hydrolyzed per min per mg of protein. After a 10-min reaction time, assays were stopped in acidified organic solution and the ^{32}P remaining in the aqueous portion was determined and shown to be glycerol 3-phosphate. (*B*) Effect of TX100 on LPA breakdown. Reactions were conducted as in *A* but with various amounts of TX100 present. Results are representative of two experiments done in duplicate.

activity of PA-PLA$_1$ (Fig. 4*B*). In addition, both phospholipid vesicles and bovine serum albumin inhibited LPA hydrolysis in a concentration-dependent manner (data not shown). One possible explanation for these results is that PA-PLA$_1$ acts as a lysophospholipase only when LPA is in aqueous solution and is inhibited when the concentration of aqueous LPA is reduced by micelle formation or by interaction with vesicles or proteins. In addition, the loss of lysophospholipase activity at micromolar LPA concentrations (Fig. 4*A*) might be due to binding and inactivation of PA-PLA$_1$ on the highly charged micelle surface. The cmc for LPA under these assay conditions is not known, but the cmc for similar lysophospholipids is in the low micromolar range (25).

Several other properties of the enzyme were examined. The peak fraction from Mono Q-fractionated testis HSS displayed measurable transacylase activity in the TX100 assay when di-oleoyl-PA and *sn*-2-oleoyl-LPA were used as donor and acceptor, respectively (data not shown). However, this transacylase activity occurred at a 2000-fold lower rate than did PA hydrolysis, suggesting the enzyme's true role to be that of a phospholipase. In addition, the pH optimum measured for PA-PLA$_1$ was between 7.5 and 8.5 (data not shown), clearly distinguishing this PLA$_1$ from those found in lysosomes, whose pH optima are in the range of 4–5.5 (20). Finally,

PA-PLA$_1$ activity was not affected by either calcium or magnesium chloride at concentrations ranging from 10 μM to 5 mM when either 0.5 or 5 mol % di-oleoyl-PA was used as a substrate (data not shown).

DISCUSSION

The PLA$_1$ identified in this study appears to differ from earlier identified PLA$_1$ activities (17–21) in terms of its tissue distribution (limited to the brain and mature testis), substrate specificity (preference for PA), and pH optimum (around pH 8). However, none of these enzymes has been purified to homogeneity and the assays used differ. Full purification of each enzyme will be necessary to determine whether each activity represents a different polypeptide.

The interaction of PA-PLA$_1$ with the membrane surface is a prerequisite to the enzyme's catalytic function. Specific phospholipids may regulate this interaction, as is the case for a number of proteins (26). For example, acidic phospholipids might enhance the binding of PA-PLA$_1$ to the cytosolic surface of a membrane and this might stimulate the rate of phospholipid hydrolysis. Indeed, the apparent stimulation of PA-PLA$_1$ activity by high concentrations of PA observed in the TX100 micelle assay system (Fig. 3*A*) might be due to just such an effect. Other phospholipids may stimulate even more effectively or possibly inhibit this interaction, providing clues as to possible physiological regulation of the enzyme's activity. To address these issues, a unilamellar vesicle assay system should be useful, as has been found for other PLA activities (27).

The limited tissue distribution of PA-PLA$_1$ (Fig. 1*B*) suggests that the enzyme may play a special role in brain and mature testis. Thus PA-PLA$_1$ might attenuate agonist-generated PA signals such as those mentioned earlier (2–5) or signals that affect vesicle transport processes (28). In this light, our observation that PA-PLA$_1$ preferentially hydrolyzes diunsaturated PA (Fig. 3*A*) may be of special interest. Certain diunsaturated phospholipids, including PA, are thought to destabilize lipid bilayers and affect vesicle budding or fusion mechanisms (29, 30). If PA-PLA$_1$ preferentially hydrolyzes diunsaturated PA *in vivo*, it might attenuate this type of "signal" and restore bilayer stability. The LPA generated by the PA-PLA$_1$ reaction might be used to reform phospholipids originally hydrolyzed in response to stimulation or, if it accumulates in excess, it might be hydrolyzed to glycerol 3-phosphate and fatty acid.

As mentioned previously, a number of mechanisms generate PA during cell signaling (1, 6–11). Several of these mechanisms operate in brain and testis. One DGK isozyme localizes to neurons of the corpus striatum and another is found in glial cells (11). In the testis, a cytosolic DGK is upregulated 10-fold and a membrane-bound, arachidonate-specific DGK is upregulated 6-fold during maturation (31). Less is known about specific PLD isozymes in these tissues, although PLD activity has been detected in brain (10) and sperm (32).

In conclusion, a PA-specific PLA$_1$ has been identified. Its limited tissue distribution and substrate specificity suggest that it may play a role in a specific regulatory process.

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