Phosphatidylserine-specific Phospholipase A₁ Stimulates Histamine Release from Rat Peritoneal Mast Cells through Production of 2-Acyl-1-lyso phosphatidylserine*

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Hiroyuki Hosono‡, Junken Aoki‡§, Yuki Nagai‡, Koji Bando‡, Mayuko Ishida‡, Ryo Taguchi‡, Hiroyuki Arai‡, and Keizo Inoue‡∥

From the ‡Graduate School of Pharmaceutical Sciences, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan and the §Faculty of Pharmaceutical Sciences, Nagoya City University, 3-1 Tanabe-dori, Mizuho-ku, Nagoya, Aichi 467-0027, Japan

Lysophosphatidylserine (1-acyl-2-lyso-PS) has been shown to stimulate histamine release from rat peritoneal mast cells (RPMC) triggered by FcεRI (high affinity receptor for IgE) cross-linking, although the precise mechanism of lyso-PS production has been obscure. In the present study we show that phosphatidylserine-specific phospholipase A₁ (PS-PLA₁) stimulates histamine release from RPMC through production of 2-acyl-1-lyso-PS in the presence of FcεRI cross-linker. The potency of 2-acyl-1-lyso-PS was almost equal to that of 1-acyl-2-lyso-PS. A catalytically inactive PS-PLA₁, in which an active serine residue (Ser₁⁶⁶) was replaced with an alanine residue did not show such activity.

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In this study, the role of two phospholipase A$_2$, PS-PLA$_1$, and sPLA$_2$-IIA, in the mast cell activation was investigated. The results indicate that PS-PLA$_1$ is indeed capable of producing lyso-PS from intact membranes and is involved in mast cell activation. Our findings indicate that PS-PLA$_1$ plays a key role in the production of bioactive lysophospholipid, lyso-PS.

**EXPERIMENTAL PROCEDURES**

**Materials**—Bovine brain PS and di-oleyl PS were purchased from Avanti Polar Lipids (Alabaster, AL), and Rhizopus delemar lipase was obtained from Seikagaku Corporation (Tokyo, Japan). The rat monoclonal anti-2,4-dinitrophenylated Ascaris (DNP-As) IgE antibody (21) and DNP-As (22) were kindly donated by Dr. Kiyoto Hara (Kissei Pharmaceutical Co. Ltd., Nagano, Japan). Anti-human Fas monoclonal antibody (CH-11, 200 g/ml) was kindly donated by Dr. Toshiro Omori (Seikagaku Corporation, Tokyo, Japan). The rat monoclonal anti-Fas monoclonal antibody (CH-11) was purchased from Medical & Biological Laboratories Co., Ltd. (Nagoya, Japan). All other chemicals were purchased from Sigma.

**Mast Cells—**RPMC from the peritoneal cavity of male Wistar rats, weighing 200–250 g, were purified with Percoll (Amersham Pharmacia Biotech, Uppsala, Sweden) as described previously (23). Purified RPMC were suspended in HEPES-buffered Tyrode solution (137 mM NaCl, 2.7 mM KCl, 12 mM HEPES, 1 mM MgCl$_2$, 2 mM CaCl$_2$, 5.6 mM dextrose, and 0.01% bovine serum albumin, pH 7.4). The purity of the RPMC in the final preparation was >95%, as estimated by toluidine blue staining. We used the cells recovered from the peritoneal cavity as “crude RPMC.” Using Wright-Giemsa staining we confirmed that the crude peritoneal cells (about 2 x 10$^7$ cells per rat) consisted mainly of mononuclear leukocytes (macrophages and monocytes) with a minor population of other cells such as mast cells and polymorphonuclear leukocytes (neutrophils). The ratio of mononuclear leukocytes, polymorphonuclear leukocytes, and other cells was typically 94:1:5. Thus in the crude RPMC preparation there are about 20 times as many other cells as mast cells.

**Cell Culture—**Human Jurkat T cells were maintained in RPMI 1640 medium containing 5% fetal calf serum in an atmosphere of 5% CO$_2$. To induce apoptosis, the cells were incubated with serum-free RPMI 1640 medium in the presence of anti-Fas monoclonal antibody (CH-11, 20 ng/ml) for 4 h at 37 °C. In the co-culture system, we mixed 1 x 10$^4$ of purified RPMC and 2 x 10$^4$ Jurkat cells.

**Determination of Apoptotic Cells—**PS exposure was measured by the binding of FITC-labeled annexin V using the Annexin V-FITC Kit (Immunotech). Briefly, 5 x 10$^4$ cells (apoptotic cells or rat peritoneal cells) were washed with ice-cold phosphate-buffered saline and resuspended in 495 µl of binding buffer. Annexin V-FITC (5 µl) was added to the suspension and incubated for 10 min on ice in the dark. The cells were analyzed by flow cytometry using EPICS XL flow cytometer (Beckman Coulter).

**Assay of Histamine Release from RPMC—**The RPMC were washed once and resuspended at cell density of 5 x 10$^4$ RPMC/ml (0.2 ml) in HEPES-buffered Tyrode solution and sensitized with 10 µg/ml monoclonal IgE anti-DNP-As for 30 min at 37 °C. After two washes in HEPES-buffered Tyrode solution, the cells were stimulated with 60 µg/ml DNP-As at 37 °C in the presence of lyso-PS or recombinant PS-PLA$_1$. After 15 min, 1.2 ml of ice-cold HEPES-buffered Tyrode solution was added to terminate the reaction, and the reaction mixture was centrifuged at 800 x g for 5 min at 4 °C. Histamine in the supernatant was determined by the fluorometric assay of Shore et al. (24). Histamine release was calculated as a percentage of the total cell histamine, and values are the means ± S.E. of three independent experiments.

**Preparation of 2-Acyl-1-lyso-PS and 1-Acyl-2-lyso-PS—**Diacyl-PS (5 µmol) was incubated with R. delemar lipase (20 mg/ml; Seikagaku-kogyo, Tokyo, Japan) or phospholipase A$_2$ (from porcine pancreas, Roche Molecular Biochemicals) in 50 ml Tris maleate buffer, pH 5.7, in the presence of 0.25 volume of diethyl ether, at 37 °C for 2 h. After extraction of free fatty acids with diethyl ether/petroleum ether (1:1; v:v), four times, the remaining lyso-PS was extracted by the method of Bligh and Dyer (25) and used as 2-acetyl-1-lyso-PS. The 2-acetyl-1-lyso-PS was stored at ~80 °C in chloroform/methanol (2:1; v:v) and used within 24 h to avoid fatty acid migration (26).

**Preparation of PS-PLA$_1$ and sPLA$_2$-IIA—**Recombinant rat PS-PLA$_1$ was prepared as described previously (11). Briefly, SF9 cells (8 x 10$^5$ cells/ml) in a spinner flask were infected with recombinant rat PS-PLA$_1$ baculovirus (multiplicity of infection = 10) and incubated for 96 h at 27 °C. The proteins were purified from the culture supernatant of the infected cells by sequential passages through a DEAE ion exchange column, a heparin-Sepharose column, and a blue Sepharose column as described previously (11). Approximately 400 µg of recombinant PS-

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**FIG. 1.** Dose-response curves showing 2-acetyl-1-lyso-PS enhanced IgE-antigen (A) and ConA-stimulated (B) histamine release from RPMC. Purified RPMC (1 x 10$^4$) from the peritoneal cavity of rats were pretreated with anti-DNP-As IgE/DNP-As (A) or ConA (B) and then incubated at 37 °C in the presence of 1-acetyl(oleoyl)-2-lyso-PS (closed circles) or 2-acetyl(oleoyl)-1-lyso-PS (open circles). Histamine release in the absence of antigen (A) or in the absence of ConA (B) is shown by the closed squares (1-acetyl-2-lyso-PS) and open squares (2-acetyl-1-lyso-PS), respectively. Histamine release is expressed as percent of the total cell histamine, and values are the means ± S.E. of three independent experiments.

**FIG. 2.** Dose-response curve showing effect of PS-PLA$_1$ on ConA-stimulated histamine release from RPMC in the presence of PS liposomes. Purified RPMC (1 x 10$^4$) were incubated for 15 min with 100 µg/ml of ConA, and various concentrations of PS liposomes, in the presence (1 µg/ml) (closed circles) or absence (open circles) of PS-PLA$_1$. Histamine release is expressed as percent of the total cell histamine, and values are the means ± S.E. of three independent experiments.
PLA$_1$ was recovered from 1 liter of infected culture fluid. The specific activity of purified recombinant PS-PLA$_1$ was 2.8 nmol/min/µg of protein for PS. PS-PLA$_1$ belongs to the lipase family, and alignment of amino acid sequences of PS-PLA$_1$ with other members of the lipase family positions 124 of rat PS-PLA$_1$ cDNA (11)) and 5’-GAGGCCCCAAGCCCATTTTCCAGTC (nucleotide positions 1348–1371 of rat PS-PLA$_1$), were then used as primers for a second PCR. The resulting PCR product was introduced into EcoRI/HindIII sites of the baculovirus transfer vector pFASTBAC1 plasmid (Invitrogen), and production of recombinant baculovirus was performed according to the manufacturer’s instructions (Bac-to-Bac system). The mutant PS-PLA$_1$ proteins were expressed and purified as described above. sPLA$_2$-IIA protein was purified from culture medium of activated rat platelets as described previously (17). The specific activity of purified sPLA$_2$-IIA was 32 nmol/min/µg of protein.

Monoclonal Antibodies against Rat PS-PLA$_1$—Rat PS-PLA$_1$ cDNA (encoding amino acids Val$_{27}^{27}$–Val$_{456}^{456}$) was ligated into the BamHI/HindIII sites of the pET21c vector (pET system, Novagen). After the plasmid had been introduced into Escherichia coli strain BL21 (DE3) (Novagen), protein expression was induced with 1 mM isopropyl-β-D-thiogalactopyranoside. Rat PS-PLA$_1$ polypeptides were recovered in the insoluble fraction. The cell pellet was sonicated for 20 min on ice using a tip-type sonicator. After sonication, protein was recovered by ultracentrifugation at 100,000 × g. The insoluble pellet was dissolved in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer and subjected to 10% acrylamide SDS-PAGE. After electrophoresis, proteins were transferred to nitrocellulose filters. The filters were blocked with 5% (w/v) skimmed milk and 0.05% (v/v) Tween 20, incubated with anti-rat PS-PLA$_1$ monoclonal antibodies (ascites prepared from clone 15D12, diluted 1:1000) in Tris-buffered saline containing 5% skimmed milk and 0.05% Tween 20, and then treated with anti-mouse IgG-horseradish peroxidase. Proteins bound to the antibodies were visualized with an enhanced chemiluminescence kit (ECL, Amersham Pharmacia Biotech).

Western Blotting—Protein samples were separated by SDS-PAGE and transferred to nitrocellulose filters using the Bio-Rad protein transfer system. The filters were blocked with Tris-buffered saline containing 5% (w/v) skimmed milk and 0.05% Tween 20, incubated with anti-rat PS-PLA$_1$, monoclonal antibodies (ascites prepared from clone 15D12, diluted 1:1000) in Tris-buffered saline containing 5% skimmed milk and 0.05% Tween 20, and then treated with anti-mouse IgG-horseradish peroxidase. Proteins bound to the antibodies were visualized with an enhanced chemiluminescence kit (ECL, Amersham Pharmacia Biotech).

Lipid Preparation—Apoptotic human Jurkat T cells (2.3 × 10$^8$ cells) or rat peritoneal cells (2.4 × 10$^8$ cells in the presence of ConA (100 µg/ml)) were incubated with serum-free RPMI 1640 medium in the presence or absence of recombinant PS-PLA$_1$ (1.8 µg/ml) for 30 min at 37°C. Cholesterol and phospholipids in the cell supernatants were extracted by the
method of Bligh and Dyer (25) at acidic pH (2.5). Under these conditions, acidic lysophospholipids such as lyso-PS and lysophosphatidic acid were recovered from the organic phase. The extracted lipids were dried, dissolved in chloroform/methanol (1:1), and used for a functional bioassay and mass spectrometry analysis. The recovery of lipids was monitored by adding a trace amount of 1-[14C]oleoyl-lyso-PS to the samples. Under these conditions, recovery of 1-[14C]oleoyl-lyso-PS was always >95%.

**MS Analysis**—Lipid extracts from the cell culture supernatant were analyzed with a Quattro II mass spectrometer (Micromass, Manchester, United Kingdom) equipped with an electrospray ion source (ESI) as described previously (27). Aliquots (2 μl) of samples (100–200 pmol/μl) dissolved in chloroform/methanol (2:1) were introduced by means of a flow injector into the ESI chamber at a flow rate of 2 μl/min. The eluting solvent used was acetonitrile/methanol/water (2:3:1) containing 0.1% ammonium formate, pH 6.4. The mass spectrometer was operated in the positive and negative ion modes. Nitrogen gas at a temperature of 80°C and a flow rate of 12 liter/min was used for drying. In most cases, the capillary voltage was set at 3.7 kV, and the cone voltage was set at 30 V, both in the positive and negative ion modes. For MS/MS experi-

![Fig. 6](http://www.jbc.org/)

**Fig. 6.** Detection of lyso-PS in the supernatant of PS-PLA1-treated cells. A, dose-response curve of standard lyso-PS. Purified RPMC (1 x 10⁶ cells) were incubated at 37°C for 15 min with 100 μg/ml of ConA in the presence of each dose of lyso-PS. Histamine release is expressed as percent of the total cell histamine, and values are the means ± S.E. of three independent experiments. B, detection of lyso-PS activity in the culture supernatant after incubating the cells with PS-PLA1. Human Jurkat T cells that were pretreated with anti-Fas antibody (closed and open squares) or crude RPMC (in the presence of 100 μg/ml of ConA, closed and open triangles) were incubated with HEPES-buffered Tyrode solution (see "Experimental Procedures") both in the presence (closed squares and triangles) or absence (open squares and triangles) of recombinant PS-PLA1 (1 μg/ml). After PS-PLA1, in the culture supernatant was heat-inactivated (60°C, 10 min), the ability of the culture supernatant to stimulate histamine release from ConA-primed RPMC (purified) was determined. Histamine release is expressed as percent of the total cell histamine, and values are the means ± S.E. of three independent experiments. C–J, the ESI-MS spectra of phospholipids extracted from the apoptotic human Jurkat cells (C–F) or RPMC (G–J) after they were treated with recombinant PS-PLA1. Human Jurkat T cells that were pretreated with anti-Fas antibody or RPMC were incubated with HEPES-buffered Tyrode solution both in the presence (1.8 μg/ml) or absence of recombinant PS-PLA1. Both supernatants and cell fractions were analyzed. The result from the negative ion scanning mode is shown. Full-scale on the y axes for both PS-PLA1 (+) and (−) corresponds to 10,000 eV for C–F and 8,000 eV for G–J. The major ions and their identities are 494.3 (16:1-lyso-PS) and 522.4 (18:1-lyso-PS).
**RESULTS**

2-Acyl-1-lyso-PS Enhances Antigen-dependent Activation of RPMCs—The ability of 2-acyl-1-lyso-PS to stimulate antigen-dependent mast cell activation was determined. Purified RPMCs primed with anti-DNP monoclonal IgE were incubated with DNP-As in the presence of lyso-PS. Like 1-oleoyl-2-lyso-PS, 2,0-oil-1-lyso-PS was able to stimulate histamine release from IgE-antigen-stimulated mast cells (Fig. 1A). The dose-dependent curve showed that 2-oleoyl-1-lyso-PS was as potent as 1-oleoyl-2-lyso-PS at stimulating histamine release. Neither lyso-PS was able to enhance histamine release in the absence of antigen. Similar results were obtained when ConA was used instead of IgE-antigen complexes (Fig. 1B). 2-Acyl-1-lyso-PS and 1-acyl-2-lyso-PS were also prepared from bovine brain-derived PS, with different acyl chains at the sn-1 and sn-2 positions. The activity of these lyso-PS was almost identical to that of 2-oleoyl-1-lyso-PS (data not shown), indicating that the fatty acid moiety does not affect the activity of lyso-PS. It is generally accepted that the acyl chain at the sn-2 position of 2-acyl-lyso-phospholipids easily migrates to the sn-1 position, resulting in production of 1-acyl-lyso-phospholipids (26). To determine whether 2-acyl-1-lyso-PS is really active against mast cells, ConA-primed mast cells were incubated with PS liposomes for 15 min in the presence or absence of PS-PLA1, and histamine release from the cells was determined. PS is known to stimulate mast cell activation at a concentration above 2 × 10^{-6} M. However, at lower concentrations, PS alone was unable to stimulate histamine release from ConA-primed RPMCs. As shown in Fig. 2, PS stimulates histamine release from RPMCs only in the presence of recombinant PS-PLA1 in a dose-dependent manner. This shows that the 2-acyl-1-lyso-PS produced from PS by PS-PLA1 is active against RPMCs, since the migration of the acyl chain takes several hours (26).

**PS-PLA1 Enhances Histamine Release from RPMCs**—Next we examined the effect of recombinant PS-PLA1 on histamine release from IgE-antigen-stimulated mast cells in the absence of PS liposomes. As shown in Fig. 3, when the purified RPMCs, which were pretreated with anti-DNP monoclonal IgE, were incubated with DNP-As in the presence of recombinant PS-PLA1 (5 µg/ml), the amount of histamine released increased. A similar result was obtained when ConA was used instead of IgE-antigen for FcεRI cross-linking (Fig. 3). PS-PLA1 alone did not show such an activity. This result suggests that lyso-PS is produced from PS on the RPMC membrane by PS-PLA1. When we analyzed the crude RPMC, we found that they contain many cells other than mast cells (see *Experimental Procedures*). Thus, it is possible that lyso-PS is produced from PS not only on mast cells but also on the other cells that reside near the mast cells. To test this possibility, we first used co-culture system in which purified RPMCs were mixed with apoptotic cells. PS is located mainly in the inner leaflet of lipid bilayers in normal cells. Thus, the accessibility of PS-PLA1 to the substrate on the plasma membrane seems to be limited. It is generally accepted that PS appears on the cell surface in apoptotic cells or cytokine-activated cells. As shown in Fig. 4C, exposure of PS is limited in normal Jurkat cells, but it is evident in apoptotic (anti-Fas antibody-treated) Jurkat cells. When the purified RPMCs were mixed with apoptotic Jurkat cells, the ability of PS-PLA1 to stimulate histamine release from the mast cells increased significantly (Fig. 4A). These results clearly indicate that PS-PLA1 hydrolyzes PS exposed on apoptotic cells and produces lyso-PS. Next, we used crude RPMCs instead of purified RPMCs and subjected them to a histamine assay in the presence of ConA and PS-PLA1. Exposure of PS was limited in crude RPMC population but was enhanced after cells were treated with ConA (Fig. 4D). As shown in Fig. 4B, when the crude mast cells were used, the ability of PS-PLA1 to stimulate ConA-induced histamine release from RPMCs increased significantly. The crude mast cell preparation consisted mainly of mononuclear leukocytes (macrophages and monocytes) with a minor population of other cells such as polymorphonuclear leukocytes (neutrophils) (see *Experimental Procedures*), and together they were about 20 times more numerous than RPMCs. Thus again in this system it was shown that PS-PLA1 efficiently stimulates FcεRI-dependent histamine release from RPMCs in the presence of PS-exposing cells. It was also shown that a catalytically inactive mutant PS-PLA1 has no enzyme activity (Fig. 5A) and is unable to stimulate histamine release from RPMC (Fig. 5B). This result confirmed that catalytic activity of PS-PLA1 is required to enhance histamine release from RPMCs. All these data strongly indicate that PS-PLA1 can hydrolyze PS efficiently when it appears on the surface of apoptotic cells and that lyso-PS is actually derived from cells surrounding mast cells in vivo.  

**Detection of Lyso-PS Produced by PS-PLA1**—We further examined whether lyso-PS itself is actually produced by PS-PLA1 from the cell membranes. As shown in Fig. 6A, the ability of the cell supernatant from the apoptotic cells to stimulate ConA-induced histamine release from RPMCs was greatly enhanced when the apoptotic cells or crude RPMCs were treated with PS-PLA1. From the standard curve of lyso-PS (Fig. 6A), it is estimated that ~8 nmol or the equivalent of lyso-PS was recovered from 2.3 × 10^{6} cells. This corresponds to 4% of the total PS of the cells (data not shown). Lipids in the cell supernatant were further analyzed by ESI-MS. In good agreement with the activity data (Fig. 6B), signals with m/z values of 522.4, and 494.3, which correspond to lyso-PS with oleic acid (18:1, m/z 522.4, and palmitoleic acid (16:1, m/z 494.3), respectively, were detected in the lipid fraction from supernatant of apoptotic Jurkat T cells (Fig. 6C) and crude RPMC (ConA-treated, Fig. 6G) incubated with PS-PLA1. The identity of the peak (m/z 522.4) as 18:1-lyso-PS was confirmed by MS/MS analysis of the daughter ions (data not shown). The signals were not detected in the absence of PS-PLA1 treatment (Fig. 6D and H). Interestingly the lipids were detected almost exclusively in the cell supernatant and not in the cell fraction (Fig. 6, E and I).
can be concluded that the lyso-PS detected is 2-acyl-1-lyso-PS, since acyl chains at the sn-2 position of phospholipids are rich in unsaturated fatty acids.

Heparin Blocks the Effect of PS-PLA1—Like other lipases, PS-PLA1 has an affinity for heparin (11). We next examined whether such affinity for heparin is important for mast cell activation by PS-PLA1. As shown in Fig. 7, 50 ng/ml of heparin completely blocked the histamine release from crude RPMC. However, heparin did not inhibit the ability of PS-PLA1 to hydrolyze PS in vitro nor lyso-PS-induced histamine release from RPMC (data not shown). This result indicates that an association between PS-PLA1 and the cell surface membrane via heparan sulfate proteoglycan is important for the cellular function of the enzyme.

PS-PLA1 Stimulates Mast Cell Activation More Efficiently than sPLA2-IIA—We finally examined the ability of sPLA2-IIA to stimulate RPMC activation and compared it with that of PS-PLA1, since sPLA2-IIA is capable of producing lyso-PS in vitro. sPLA2-IIA efficiently hydrolyzes anionic phospholipids such as phosphatidylethanolamine and PS in vitro, and it does not hydrolyze phospholipids on intact membranes. When the same amounts of each enzyme were separately added to crude RPMC in the presence of FcRI cross-linker, PS-PLA1 was much more potent than sPLA2-IIA in inducing histamine release (Fig. 5A). A similar result was obtained when purified RPMC were co-cultured with apoptotic Jurkat cells in the presence of FcRI cross-linker and PS-PLA1, although at high concentrations, sPLA2-IIA significantly stimulated histamine release (Fig. 5B).

DISCUSSION

The present study was undertaken to determine whether PS-PLA1 participates in the activation of RPMC. Three results of this study indicate that PS-PLA1 stimulates histamine release from RPMC through the production of 2-acyl-1-lyso-PS: 1) 1-acyl-2-lyso-PS has almost the same ability as 2-acyl-1-lyso-PS to stimulate IgE-antigen-induced histamine release from RPMC; 2) recombinant PS-PLA1, but not mutant PS-PLA1, stimulated IgE-antigen-induced histamine release from RPMC; and 3) lyso-PS with unsaturated fatty acids was detected by MS analysis after apoptotic cells or crude RPMC were incubated with recombinant PS-PLA1. In addition, the present results, summarized below, provide some insights into how the enzyme acts on cell membranes.

Lyso-PS Production by PS-PLA1, and Its Regulation—PS-PLA1 was effective in stimulating histamine release from purified RPMC co-cultured with apoptotic Jurkat T cells (Fig. 4A) or from crude RPMC (Fig. 4B). In addition, lyso-PS with unsaturated fatty acids was actually detected in the cell supernatant by mass spectrometry. These results clearly show that PS-PLA1 produces lyso-PS by hydrolyzing PS exposed on the surface of peritoneal cells or apoptotic cells, although, like other phospholipases, it does not act on intact cell membranes. They also suggest that a key factor regulating lyso-PS production is the availability of PS on the cell surface. It is well accepted that apoptotic cells expose PS on the surface. In addition, PS exposure is enhanced in activated platelets and cytokine-stimulated or ConA-treated cells (this study). Thus it can be concluded that PS exposed on such cells may be a target for PS-PLA1 in vivo. In fact, we previously detected lyso-PS with unsaturated fatty acids in activated rat platelets, which abundantly express PS-PLA1 (13). We also observed that the activated rat platelets stimulated IgE-antigen- or nerve growth factor-induced histamine release from RPMC. Lyso-PS has also been reported to be present in vivo: it has been detected in the aqueous humor of the eyes (28) and is produced by rat peritoneal cells or at the site of tissue injury (10). It is possible that PS-PLA1 is responsible for the production of such lyso-PS in vivo. In this study, we showed that lyso-PS, produced by PS-PLA1, was almost exclusively recovered from cell supernatant. In our previous work we have found that lyso-PS applied to cells is immediately converted to PS by acylation (5). The result may explain why lyso-PS was not detected in the cell fractions.

Another factor regulating lyso-PS production is the expression of PS-PLA1 in vivo. We previously showed that rat platelets are a major source of PS-PLA1 (11) and that PS-PLA1 is detected in various human tissues (14). In addition, preliminary experiments in our laboratory have shown that expression of PS-PLA1 can be induced in inflammatory sites and in several tissues by various inflammatory stimuli. Our preliminary results show that the concentrations of PS-PLA1 in the supernatant of activated rat platelets, rat plasma, and the peritoneal cavity of rats injected intraperitoneally with casein are 400, 5, and 150 ng/ml, respectively. Thus the amount of PS-PLA1 used in this study is close to the physiological levels, and it is possible that the enzyme stimulates mast cells in vivo.

We showed that PS-PLA1, which has an affinity for heparin, completely lost its ability to stimulate mast cells in the presence of heparin (Fig. 7). Many lipases, such as hepatic lipase and lipoprotein lipase, have an affinity for heparin and function on the cell surface of hepatocyte or adipocyte by interacting with heparan sulfated proteoglycan. It has been also demonstrated that sPLA2-IIA is attached to the cell surfaces by its C-terminal heparin-binding domain and that the attachment is essential for prostaglandin biosynthesis (29). This may be also the case with PS-PLA1.

PS-PLA1 Produces Lyso-PS More Efficiently than sPLA2-IIA—From the data presented here, we assume that sPLA2-IIA
is less potent than PS-PLA₁ in producing lyso-PS (Fig. 8). Living cells are normally resistant but become susceptible during apoptosis to the enzyme. Unlike PS-PLA₁, sPLA₂-IIA acts on other phospholipids than PS. This is a possible reason why sPLA₂-IIA is less potent than PS-PLA₁ in producing lyso-PS. This is a possible reason why sPLA₂-IIA alone has been reported to stimulate histamine release from RPMC provided that the concentration of the enzyme is high enough (30). Indeed, a concentration of sPLA₂-IIA >20 µg/ml was required to induce histamine release from RPMC by sPLA₂-IIA alone. Conversely, the effect of PS-PLA₁ was observed at a concentration of about 50 ng/ml in the presence of FcεRI cross-linker (Fig. 8). The effect of sPLA₂-IIA has also been reported to be independent of lyso-PS, because histamine release triggered by sPLA₂-IIA can be observed in the absence of FcεRI cross-linkers (30). Thus, the two PLAs act against mast cells in a very different manner, although we cannot exclude the involvement of another secretory PLA₂ that has recently been identified (31).

In summary, PS-PLA₁ was found to stimulate histamine release from RPMC. PS is normally present on the inner leaflet of lipid bilayers, and there is evidence to suggest that PS is exposed to the outer surface of apoptotic cells, dead cells, and cytokine-stimulated cells. PS-PLA₁ hydrolyzes PS on the outer membrane of such cells producing 2-acyl-1-lyso-PS, a lipid messenger of mast cells. The synthetic pathways for lipid mediators such as lysophosphatidic acid, platelet-activating factor, and sphingosine-1-phosphate have not been fully solved yet. In this sense it can be said that PS-PLA₁ is the first enzyme that has been shown to produce bioactive lysophospholipid, although further studies are definitely required to demonstrate the role of this enzyme in vivo.

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Phosphatidylserine-specific Phospholipase A₂ Stimulates Histamine Release from Rat Peritoneal Mast Cells through Production of 2-Acyl-1-lysophosphatidylserine

Hiroyuki Hosono, Junken Aoki, Yuki Nagai, Koji Bandoh, Mayuko Ishida, Ryo Taguchi, Hiroyuki Arai and Keizo Inoue

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