Identification of group X secretory phospholipase A_2 as a natural ligand for mouse phospholipase A_2 receptor

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Abstract Phospholipase A_2 receptor (PLA_2R) mediates various biological responses elicited by group IB secretory phospholipase A_2 ($sPLA_2$-IB). The recently cloned group X $sPLA_2$ ($sPLA_2$-X) possesses several structural features characteristic of $sPLA_2$-IB. Here, we detected a specific binding site of $sPLA_2$-X in mouse osteoblastic MC3T3-E_1 cells. Cross-linking experiments demonstrated its molecular weight (180 kDa) to be similar to that of PLA_2R. In fact, $sPLA_2$-X was found to bind the recombinant PLA_2R expressed in COS-7 cells, and its specific binding detected in mouse lung membranes was abolished by the deficiency of PLA_2R. These findings demonstrate $sPLA_2$-X to be one of the high-affinity ligands for mouse PLA_2R.

Key words: Phospholipase A_2; Secretory phospholipase A_2; Group X secretory phospholipase A_2; Phospholipase A_2 receptor; Lipid mediator

1. Introduction

Secretory phospholipase A_2s ($sPLA_2$s) are a growing family of enzymes that hydrolyze the *sn*-2 fatty acid ester bonds of glycerophospholipids to produce free fatty acids and lysophospholipids [1,2]. $sPLA_2$s have several common characteristics including a relatively low molecular mass (13–18 kDa), the presence of 6 to 8 disulfide bridges and an absolute catalytic requirement for millimolar concentrations of Ca^{2+} [3,4]. At present, mammalian $sPLA_2$s are classified into nine different groups depending on the primary structure characterized by the number and positions of cysteine residues [3,5–9]. Among them, the group II subfamily (IIA, IID, IIE and V) is thought to play a role in the production of several lipid mediators especially in the delayed phase of the cell activation process, because their expression levels are up-regulated under various inflammatory conditions [6,7,10,11]. In contrast, group IB $sPLA_2$ ($sPLA_2$-IB) has long been thought to be a digestive enzyme, given its abundance in the pancreas. However, the discovery of the PLA_2 receptor (PLA_2R) has expanded our concept of $sPLA_2$-IB [12]. In addition to its digestive function, $sPLA_2$-IB exerts various biological responses, including cell proliferation and lipid mediator releases, through binding to PLA_2R [13]. The PLA_2R is composed of a large extracellular portion including a characteristic tandem repeat of eight carbohydrate recognition domains, and its overall molecular organization is related to a unique member of the C-type animal lectin family, including the macrophage mannose receptor [14]. Our recent studies with PLA_2R-deficient mice have demonstrated its critical role in the production of pro-inflammatory cytokines during the progression of endotoxic shock [15,16]. In the ligand–receptor relationship, there is a strict species specificity and $sPLA_2$-IB was identified as an endogenous ligand of PLA_2R, at least in rats and mice [17]. Group IIA $sPLA_2$ ($sPLA_2$-IIA) can also bind PLA_2R with about 10-fold lower affinity compared to $sPLA_2$-IB [17], whereas group IID $sPLA_2$ can not act as a ligand in spite of its structural similarities with $sPLA_2$-IIA [18].

The recently cloned group X $sPLA_2$ ($sPLA_2$-X) possesses several structural features characteristic of other $sPLA_2$s [5]. It has 16 cysteine residues located at positions characteristic of both $sPLA_2$-IB and $sPLA_2$-IIA, an amino acid C-terminal extension that is typical of $sPLA_2$-IIA, and a propeptide sequence attached at NH_2-terminals similar to $sPLA_2$-IB. We and other groups have recently shown that $sPLA_2$-X possesses a powerful hydrolyzing activity toward phosphatidylcholine and elicits a marked release of arachidonic acid from several intact cell membranes [19,20]. Considering the structural similarities of $sPLA_2$-X with $sPLA_2$-IB and IIA, knowing about its potency as a ligand for PLA_2R is indispensable for understanding its physiological and pathological functions. In the present study, we purified mouse $sPLA_2$-X and examined its binding characteristics in mouse osteoblastic MC3T3-E_1 cells that possess high levels of native PLA_2R [21]. Further binding studies in recombinant PLA_2R-expressing cells and in PLA_2R-deficient mice also demonstrated that $sPLA_2$-X is one of the high-affinity ligands for murine PLA_2R.

2. Materials and methods

2.1. Materials

Sodium [^{125}I]iodine (carrier-free, 3.7 GBq/ml) was purchased from Amersham Pharmacia Biotech. Purified recombinant human $sPLA_2$-IB, IIA and X were prepared as described previously [19]. Porcine pancreatic $sPLA_2$-IB was obtained from Boehringer Mannheim. Disuccinidyl suberate (DSS) was purchased from Pierce Chemical Co. Indoxam was synthesized at Shionogi Research Laboratories [22].

2.2. Recombinant expression and purification of mouse pro- and mature $sPLA_2$-X

Mouse $sPLA_2$-X cDNA was isolated from mouse spleen by polymerase chain reaction based on the human $sPLA_2$-X sequence [5], which was then inserted into pcDNA3.1(+) (Invitrogen) to construct the expression plasmid. Transfection into Chinese hamster ovary cells,

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Abbreviations: PLA_2, phospholipase A_2; $sPLA_2$, secretory phospholipase A_2; $sPLA_2$-IB, group IB $sPLA_2$; PLA_2R, phospholipase A_2 receptor; $sPLA_2$-IIA, group IIA $sPLA_2$; $sPLA_2$-X, group X $sPLA_2$; DSS, disuccinidyl suberate; SDS-PAGE, SDS-polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline

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selection of stably expressing clones, collection of the conditioned medium and purification with an antibody-coupled affinity column were performed as described for human $sPLA_2$-X [19], based on the cross-reactivity of anti-human $sPLA_2$-X antibody with mouse enzyme. Analysis of the final reverse-phase high performance liquid chromatography preparations by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) revealed the existence of two species of $sPLA_2$-X, and NH_2-terminal sequence analysis with an Applied Biosystems Procise Sequencer revealed them to be a mature form of $sPLA_2$-X (13 kDa) and its pro-form (14 kDa) having a propeptide sequence at the NH_2 terminus.

2.3. Binding experiments

Iodination of mouse $sPLA_2$-X and porcine $sPLA_2$-IB was performed by the chloramine T method as described previously [23] and the specific radioactivity of ^{125}I-$sPLA_2$-X and ^{125}I-$sPLA_2$-IB were 1200 and 1100 cpm/fmol, respectively. MC3T3-E_1 cells (ATCC) were cultured in 24-well plates in 10% FCS/DMEM. At confluence, the cells were washed three times with phosphate-buffered saline (PBS) and incubated with various concentrations of mouse ^{125}I-$sPLA_2$-X in 0.4 ml of the binding buffer (Hanks' balanced salt solution, pH 7.4, containing 0.1% BSA) for 2 h at 4°C. After the incubation, cells were washed with PBS three times, and the cell-bound radioactivity was measured. Specific binding was determined as the differences between the presence and absence of 100 nM unlabelled mouse $sPLA_2$-X. Displacement experiments were performed by incubating with 1.5 nM mouse ^{125}I-$sPLA_2$-X in the presence of various concentrations of $sPLA_2$ proteins under the same assay conditions as described above. The IC_{50} value was evaluated from the inhibition curves as the concentration that inhibits half of the specific binding.

In separate experiments, the binding potency of $sPLA_2$-X to mouse recombinant PLA_2R was examined as follows. The cDNA encoding mouse PLA_2R was transiently introduced into COS-7 cells with FuGene 6 reagent (Roche Diagnostics), and the binding activity of ^{125}I-$sPLA_2$-X was evaluated as described above. The inhibitory effect of indoxam on $sPLA_2$-X binding to PLA_2R-expressing COS-7 cells was examined by incubating with 2 nM ^{125}I-$sPLA_2$-X in the presence of various concentrations of indoxam.

The generation of PLA_2R-deficient mice was described in our previous paper [15] and they were backcrossed to C57BL/6J mice more than eleven times. C57BL/6J mice matched for gender and age were used as wild-type mice. Preparation of the crude lung membranes and their binding assay with ^{125}I-$sPLA_2$-X (2 nM) was performed according to the same protocol as the $sPLA_2$-IB binding study described in the previous report [15]. Specific binding was determined as the differences between the presence and absence of unlabeled 100 nM mouse PLA_2-X.

2.4. Affinity cross-linking experiment

Confluent MC3T3-E_1 cells grown in 10-cm-diameter dishes were incubated in 6 ml of binding medium containing 2 nM ^{125}I-$sPLA_2$-X with or without 100 nM unlabelled mouse $sPLA_2$-X for 2 h at 4°C. In separate experiments, the cells were incubated with 2 nM ^{125}I-$sPLA_2$-IB with or without 500 nM unlabelled porcine $sPLA_2$-IB. After washing three times with Hanks' medium, pH 7.6, cells were incubated in 8 ml of Hanks' medium containing 0.25 mM DSS for 30 min at room temperature. The reaction was stopped by the addition of Tris/HCl (final 50 mM, pH 7.4). The cells were harvested and collected by centrifugation. They were lysed by adding 50 μl of 1% Triton X-100 containing a mixture of protease inhibitors (1 mM phenylmethylsulfonyl fluoride, 2 mM EDTA and 20 μg/ml leupeptin). After centrifugation at 10 000 × *g* for 15 min, the resultant supernatant was separated by SDS–PAGE with a 4–20% gradient SDS–polyacrylamide gel (Daiichi Pure Chemical, Tokyo). After electrophoresis, the gel was dried and exposed to the imaging plate and read with a Fujix BAS2000 bio-image analyzer.

3. Results

3.1. ^{125}I-$sPLA_2$-X binding to MC3T3-E_1 cells

We prepared a highly active radioligand of mouse $sPLA_2$-X and examined its binding characteristics in MC3T3-E_1 cells that possess high levels of native PLA_2R on the cell surface [21]. As shown in Fig. 1A, a saturable and specific binding of

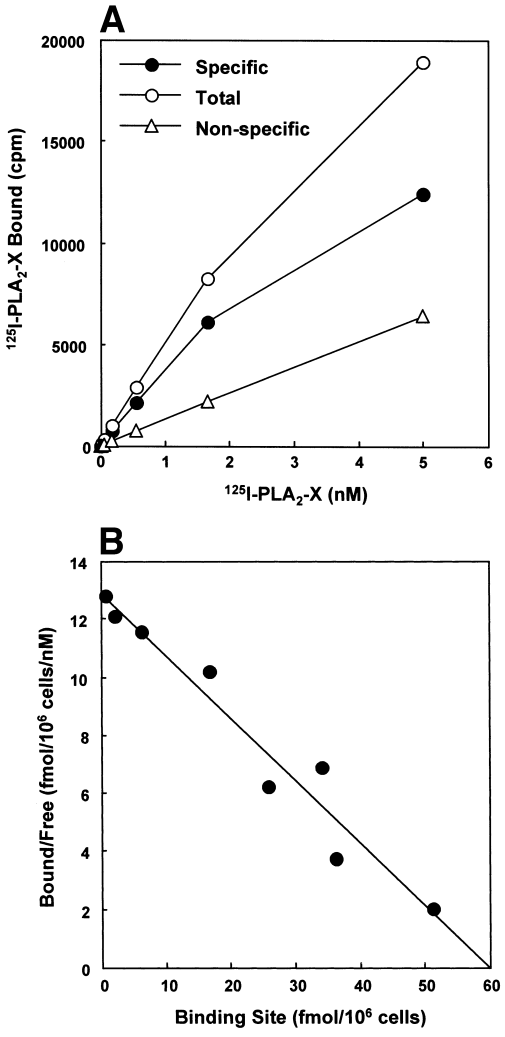

Fig. 1. Equilibrium binding of ^{125}I-$sPLA_2$-X to MC3T3-E_1 cells. A: Saturation isotherm of ^{125}I-$sPLA_2$-X binding to MC3T3-E_1 cells. Specific binding was calculated by subtracting the non-specific binding from the total binding. B: Scatchard plot of the specific ^{125}I-$sPLA_2$-X binding. Each point represents the mean of duplicate measurements. The data are representative of three experiments.

mouse ^{125}I-$sPLA_2$-X was detected. Scatchard analysis of these data (Fig. 1B) demonstrated the existence of a single class of binding sites with an equilibrium dissociation constant (K_d) of 4.6 nM and a maximum binding capacity (B_{max}) of 59.7 fmol/10^6 cells (about 36 000 binding sites/cell), which coincided with the B_{max} value reported in the porcine $sPLA_2$-IB binding to the PLA_2R in MC3T3-E_1 cells [21]. Next, the specificity of the $sPLA_2$-X binding site was examined by evaluating the relative inhibitory potencies of various $sPLA_2$ proteins. As for the mouse $sPLA_2$-X species, pro-$sPLA_2$-X blocked the binding with about 10-fold weaker potency than the mature form of $sPLA_2$-X (Fig. 2). Porcine and human $sPLA_2$-IB also strongly blocked the $sPLA_2$-X binding with IC_{50} values of 1.14 and 1.71 nM, respectively. Inversely, mouse $sPLA_2$-X blocked the porcine $sPLA_2$-IB binding to the PLA_2R in MC3T3-E_1 cells (data not shown). In both cases, human $sPLA_2$-IIA showed a much weaker suppressive effect.

In order to characterize a high-affinity binding site of ^{125}I-$sPLA_2$-X, we performed the affinity labeling experiments using a homobifunctional cross-link reagent, DSS. As shown in

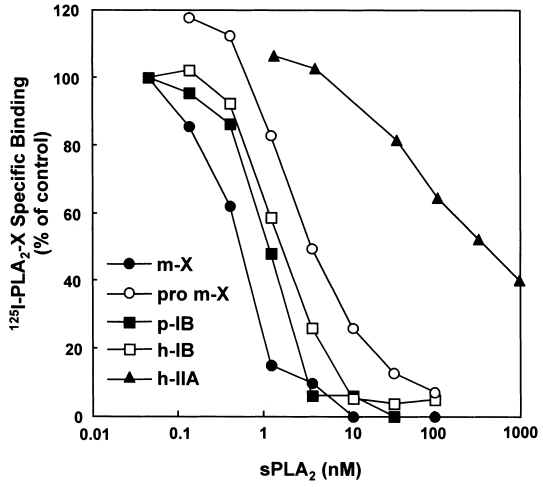

Fig. 2. Displacement of ^{125}I-sPLA$_2$-X binding by sPLA$_2$ proteins in MC3T3-E$_1$ cells. MC3T3-E$_1$ cells were incubated with 1.5 nM ^{125}I-sPLA$_2$-X in the presence of various concentrations of mouse mature sPLA$_2$-X (m-X), pro-sPLA$_2$-X (pro m-X), porcine sPLA$_2$-IB (p-IB), human sPLA$_2$-IB (h-IB), and human sPLA$_2$-IIA (h-IIA) and the specific binding activity was measured. The results are expressed as the percentage of ^{125}I-sPLA$_2$-X specific binding in the absence of these sPLA$_2$s. Each point represents the mean of duplicate measurements. The data are representative of three experiments.

Fig. 3, the ^{125}I-sPLA$_2$-X binding complex was specifically detected at an apparent molecular mass of 200 kDa only in the treatment with DSS (lanes 4 and 6), which was completely abolished in the presence of an excess amount of unlabeled sPLA$_2$-X protein (lane 3) as well as porcine sPLA$_2$-IB (data not shown). In a similar cross-linking experiment, the cross-

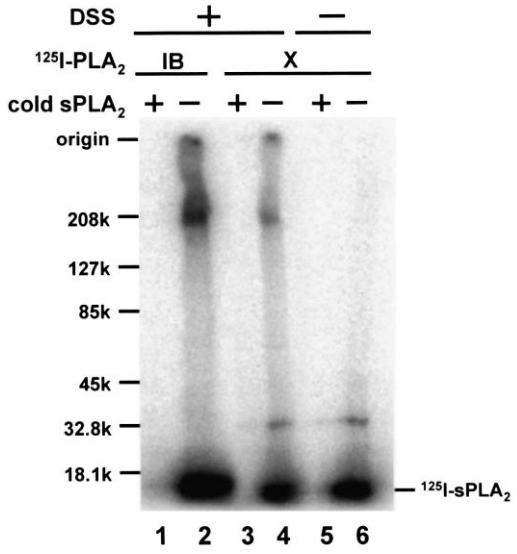

Fig. 3. Characterization of the sPLA$_2$-X binding site by affinity cross-linking. Binding assays were carried out at 4°C for 2 h by incubating MC3T3-E$_1$ cells with 2 nM ^{125}I-porcine sPLA$_2$-IB in the presence (lane 1) or absence (lane 2) of 500 nM of unlabelled porcine sPLA$_2$-IB, or with ^{125}I-mouse sPLA$_2$-X in the presence (lanes 3 and 5) or absence (lanes 4 and 6) of 100 nM of unlabelled mouse sPLA$_2$-X. At the end of incubation, the cells were washed and treated (lanes 1–4) or untreated (lanes 5 and 6) with 0.25 mM DSS for 30 min and then analyzed as described in Section 2 under the reduced conditions. Molecular weight markers are indicated on the left.

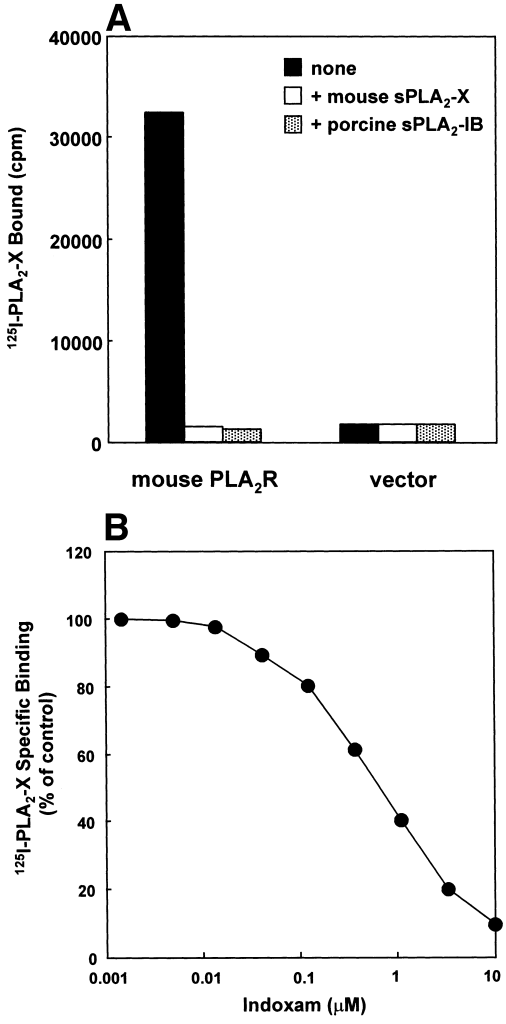

Fig. 4. ^{125}I-mouse sPLA$_2$-X binding to mouse PLA$_2$R expressed in COS-7 cells. A: COS-7 cells transfected with PLA$_2$R cDNA or vector alone were incubated with 2 nM ^{125}I-mouse sPLA$_2$-X in the absence (none) or presence of 300 nM of unlabelled mouse sPLA$_2$-X or porcine sPLA$_2$-IB for 2 h at 4°C, and the binding activity was determined. B: PLA$_2$R-expressing COS-7 cells were incubated with 1.5 nM ^{125}I-sPLA$_2$-X in the presence of various concentrations of indoxam, and the specific binding activity was measured. The results are expressed as the percentage of ^{125}I-sPLA$_2$-X specific binding in the absence of indoxam. Each point represents the mean of duplicate measurements. The data are representative of three experiments.

linked complex composed of sPLA$_2$-IB (14 kDa) and PLA$_2$R (180 kDa) showed the same molecular weight in MC3T3-E$_1$ cells (lanes 1 and 2). Thus, the binding specificity and biochemical features of the sPLA$_2$-X binding site strongly suggest its relation to PLA$_2$R.

3.2. *^{125}I-sPLA$_2$-X binding to recombinant PLA$_2$R*

In order to verify the identity of the sPLA$_2$-X binding protein, we examined the binding potency of mouse sPLA$_2$-X to the recombinant mouse PLA$_2$R transiently expressed in COS-7 cells. As shown in Fig. 4A, a high amount of specific sPLA$_2$-X binding was detected in the PLA$_2$R-expressing COS-7 cells in contrast to its absence in the mock-transfected cells. The sPLA$_2$-X binding was also blocked by porcine sPLA$_2$-IB and the specific sPLA$_2$-IB binding detected in the PLA$_2$R-express-

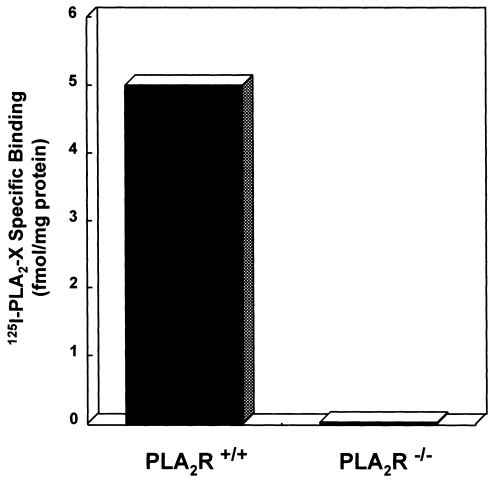

Fig. 5. sPLA$_2$-X binding activity in mouse lung membrane fractions. Specific binding activity of ^{125}I-sPLA$_2$-X in crude lung membranes prepared from wild-type (+/+) or PLA$_2$R-deficient mice (−/−) was examined as described in Section 2. Data represent the mean value for five mice.

ing cells was completely blocked by mouse sPLA$_2$-X (data not shown). As shown in Fig. 4B, a sPLA$_2$ specific inhibitor indoxam [22] suppressed the sPLA$_2$-X binding to mouse PLA$_2$R in a dose-dependent manner with an IC_{50} value of 670 nM. Thus, these findings demonstrate that mouse sPLA$_2$-X is one of the natural ligands of mouse PLA$_2$R.

3.3. *^{125}I-sPLA$_2$-X binding to mouse lung membranes*

To check for the existence of other binding components of sPLA$_2$-X in mice, we examined the binding activity of ^{125}I-sPLA$_2$-X in the crude lung membrane fractions and found a considerable amount of its specific binding (Fig. 5). The binding was saturable, specific for sPLA$_2$-X and displaceable by sPLA$_2$-IB (data not shown). In PLA$_2$R-deficient mice that we have recently generated [15], there was no significant binding in the prepared lung membranes, demonstrating that PLA$_2$R represents the specific binding site for sPLA$_2$-X in mice.

4. Discussion

Since the discovery of PLA$_2$R, sPLA$_2$s have been thought to act as signaling molecules for various biological responses in addition to their enzymatic activities [12,13]. In the present study, sPLA$_2$-X was identified as a natural ligand of mouse PLA$_2$R in native and recombinant receptors. Since there is a strict species specificity in the ligand–receptor relationship, the binding affinity of sPLA$_2$-X for mouse PLA$_2$R cannot be directly compared with those of sPLA$_2$-IB and IIA due to the lack of these mouse enzymes. In previous binding studies with snake venom *Oxyuranus scutellatus* toxin 1 as a radioligand, mouse sPLA$_2$-IB showed a similar binding affinity for mouse PLA$_2$R with human sPLA$_2$-IB, whereas mouse sPLA$_2$-IIA had about 6- to 10-fold lower affinity [17]. In the present work, mouse sPLA$_2$-X showed a three-fold higher affinity compared to human sPLA$_2$-IB in MC3T3E$_1$ cells (Fig. 2), suggesting that sPLA$_2$-X is one of the high-affinity ligands for mouse PLA$_2$R. Pro-sPLA$_2$-X had a weaker binding potency than its mature form, indicating that proteolytic removal of the propeptide is required for the high-affinity receptor binding, as in the case of sPLA$_2$-IB [24]. Because some inbred mouse strains have a natural mutation in the sPLA$_2$-IIA gene [25,26], the contribution of sPLA$_2$-IIA as a PLA$_2$R ligand could be much lower in mice.

We have previously shown a pivotal role of PLA$_2$R in the promotion of pro-inflammatory cytokine synthesis during murine endotoxic shock, based on the findings of reduced cytokine production in PLA$_2$R-deficient mice as well as in indoxam-administered mice [15,16]. In the present study, the sPLA$_2$-X specific binding was completely abrogated by the deficiency in PLA$_2$R (Fig. 5) and by the addition of indoxam (Fig. 4B). Furthermore, our recent immunohistochemical analysis with sPLA$_2$-IIA-deficient mice revealed co-localization of sPLA$_2$-X and PLA$_2$R in alveolar type II pneumocytes, which are known to play a role in the production of various cytokines and lipid mediators (Ikeda et al., unpublished observation). These findings suggest a potential involvement of sPLA$_2$-X in the receptor-mediated regulation of cytokine synthesis under inflammatory conditions. Since sPLA$_2$-X induces a potent release of fatty acids leading to eicosanoid formation in several inflammatory cells [19,20], the potential role of PLA$_2$R in this process should be examined in future work. In particular, PLA$_2$R might play a role in the internalization and degradation of sPLA$_2$-X to suppress its powerful enzymatic activity, as reported for snake venom sPLA$_2$s [27]. Finally, further elucidation of PLA$_2$R-mediated biological responses should enable us to assign more precise biological roles to the growing family of mammalian sPLA$_2$s.

References

[1] Vadas, P. and Pruzanski, W. (1986) Lab. Invest. 55, 391–404.
[2] Arita, H., Nakano, T. and Hanasaki, K. (1989) Prog. Lipid Res. 28, 273–301.
[3] Tischfield, J.A. (1997) J. Biol. Chem. 272, 17247–17250.
[4] Lambeau, G. and Lazdunski, M. (1999) Trends Pharmacol. Sci. 20, 162–170.
[5] Cupillard, L., Koumanov, K., Mattei, M.-G., Lazdunski, M. and Lambeau, G. (1997) J. Biol. Chem. 272, 15745–15752.
[6] Ishizaki, J., Suzuki, N., Higashino, K., Yokota, Y., Ono, T., Kawamoto, K., Fujii, N., Arita, H. and Hanasaki, K. (1999) J. Biol. Chem. 274, 24973–24979.
[7] Suzuki, N., Ishizaki, J., Yokota, Y., Higashino, K., Ono, T., Ikeda, M., Fujii, N., Kawamoto, K. and Hanasaki, K. (2000) J. Biol. Chem. 275, 5785–5793.
[8] Valentin, E., Ghomashchi, F., Gelb, M.H., Lazdunski, M. and Lambeau, G. (1999) J. Biol. Chem. 274, 31195–31202.
[9] Valentin, E., Ghomashchi, F., Gelb, M.H., Lazdunski, M. and Lambeau, G. (2000) J. Biol. Chem. 275, 7492–7496.
[10] Kudo, I., Murakami, M., Hara, S. and Inoue, K. (1993) Biochim. Biophys. Acta 117, 217–231.
[11] Sawada, H., Murakami, M., Enomoto, A., Shimbara, S. and Kudo, I. (1999) Eur. J. Biochem. 263, 826–833.
[12] Ohara, O., Ishizaki, J. and Arita, H. (1995) Prog. Lipid Res. 34, 117–138.
[13] Hanasaki, K. and Arita, H. (1999) Arch. Biochem. Biophys. 372, 215–223.
[14] Ishizaki, J., Hanasaki, K., Higashino, K., Kishino, J., Kikuchi, N., Ohara, O. and Arita, H. (1994) J. Biol. Chem. 269, 5897–5904.
[15] Hanasaki, K., Yokota, Y., Ishizaki, J., Itoh, T. and Arita, H. (1997) J. Biol. Chem. 272, 32792–32797.
[16] Yokota, Y., Hanasaki, K., Ono, T., Nakazato, H., Kobayashi, T. and Arita, H. (1999) Biochim. Biophys. Acta 1438, 213–222.
[17] Cupillard, L., Mulherkar, R., Gomez, N., Kadam, S., valentin, E., Lazdunski, M. and Lambeau, G. (1999) J. Biol. Chem. 274, 7043–7051.
[18] Valentin, E., Koduri, R.S., Scimeca, J.-C., Carle, G., Gelb, M.H., Lazdunski, M. and Lambeau, G. (1999) J. Biol. Chem. 274, 19152–19160.

[19] Hanasaki, K., Ono, T., Saiga, A., Morioka, Y., Ikeda, M., Kawamoto, K., Higashino, K., Nakano, K., Yamada, K., Ishizaki, J. and Arita, H. (1999) J. Biol. Chem. 274, 34203–34211.
[20] Bezzine, S., Koduri, R.S., Valentin, E., Murakami, M., Kudo, I., Ghomashchi, F., Sadilek, M., Lambeau, G. and Gelb, M.H. (2000) J. Biol. Chem. 275, 3179–3191.
[21] Tohkin, M., Kishino, J., Ishizaki, J. and Arita, H. (1993) J. Biol. Chem. 268, 2865–2871.
[22] Hagishita, S., Yamada, M., Shirahase, K., Okuda, T., Murakami, Y., Itoh, Y., Matsuura, T., Wada, M., Kato, T., Ueno, M., Chikazawa, Y., Yamada, K., Ono, T., Teshirogi, I. and Ohtani, M. (1996) J. Med. Chem. 39, 3636–3658.
[23] Hanasaki, K. and Arita, H. (1992) J. Biol. Chem. 267, 6414–6420.
[24] Kennedy, B.P., Payette, P., Mudgett, J., Vadas, P., Pruzanski, W., Kwan, M., Tang, C., Rancourt, D.E. and Cromlish, W.A. (1995) J. Biol. Chem. 270, 22378–22385.
[25] Arita, H., Hanasaki, K., Nakano, T., Oka, S. and Matsumoto, K. (1991) J. Biol. Chem. 266, 19139–19141.
[26] MacPhee, M., Chepenic, K.P., Liddell, R.A., Nelson, K.K., Siracusa, L.D. and Buchberg, A.M. (1995) Cell 81, 957–966.
[27] Zvaritch, E., Lambeau, G. and Lazdunski, M. (1996) J. Biol. Chem. 271, 250–257.