Characterization of *Arabidopsis* secretory phospholipase A_2-γ cDNA and its enzymatic properties[1]

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Abstract Plant secretory phospholipases A_2 ($sPLA_2$s) probably play important roles in phospholipid signaling based on the data reported from other organisms, but their functions are poorly understood because of the lack of cloned $sPLA_2$ genes. In this study, we cloned and characterized an *Arabidopsis* secretory phospholipase A_2-γ ($AtsPLA_2$-γ) cDNA, and examined its enzymatic properties. The recombinant protein of $AtsPLA_2$-γ showed maximal enzyme activity at pH 8.0, and required Ca^{2+} for activity. Moreover, $AtsPLA_2$-γ showed *sn*-2 position specificity but no prominent acyl preference, though it showed head group specificity to phosphatidylethanolamine rather than to phosphatidylcholine. $AtsPLA_2$-γ was found to predominate in the mature flower rather than in other tissues, and subcellular localization analysis confirmed that $AtsPLA_2$-γ is secreted into the intercellular space.

Key words: Secretory phospholipase A_2; $AtsPLA_2$-γ; Phospholipid; Lysophospholipid; Phosphatidylethanolamine; Phosphatidylcholine

1. Introduction

Secretory phospholipases A_2 ($sPLA_2$s) are a family of Ca^{2+}-dependent disulfide-rich 13–18 kDa enzymes, which catalyze the hydrolysis of the *sn*-2 position of membrane glycerophospholipids to release free fatty acids (FFAs) and lysophospholipids [1,2]. These enzymes, nuclear-encoded and targeted to the extracellular region, play a critical role in phospholipid signaling [2,3]. In mammals, $sPLA_2$ is involved in the liberation of arachidonic acid as the precursor of eicosanoid. Pancreatic $sPLA_2$ was the first mammalian $sPLA_2$ identified, and plays a central role in the digestion of dietary phospholipids. Moreover, non-pancreatic mammalian $sPLA_2$, the first well-known $sPLA_2$ and a member of the group IIA enzyme family, is expressed at high levels as a bactericidal agent during inflammation in human tears [2,4]. Recently, several putative plant $sPLA_2$s have been cloned and investigated. Two cDNAs encoding $sPLA_2$ were isolated from rice [1], and another $sPLA_2$ cDNA was reported in our previous study on mature carnation flowers [5]. Plant $sPLA_2$, a 14 kDa enzyme, was purified from developing elm seeds and showed *sn*-2 position specificity and millimolar Ca^{2+} dependence [6]. More recently, our group reported that plant $sPLA_2$ plays important roles in cell elongation and shoot gravitropism via auxin signaling in *Arabidopsis* [7]. Even though it has been speculated that plant $sPLA_2$s play roles in various plant biological mechanisms [1,5–7], the exact cellular functions of plant $sPLA_2$s have not been fully elucidated, mainly because of the delay of availability of the cloned and identified $sPLA_2$ genes.

As stated in our previous papers [5,7], plant $sPLA_2$ seems to be involved in auxin signaling as well as flower maturation. In the present study, we report upon the cloning, spatial expression, cellular localization, and recombinant enzyme analysis of a novel *Arabidopsis* $sPLA_2$. In accordance with the human $sPLA_2$ nomenclature system and the alternative plant phospholipase nomenclature system, we have named this novel $sPLA_2$ *Arabidopsis thaliana* secretory phospholipase A_2-γ ($AtsPLA_2$-γ) based on its chromosomal location in *Arabidopsis* [4,7].

2. Materials and methods

2.1. Molecular cloning of $AtsPLA_2$-γ from Arabidopsis

Using two gene-specific primers QRT-A2-γ-S (5′-GAAGTGCAGCAACACCTGCATTGCACAG) and QRT-A2-γ-A (5′-TTTTCTCCGTTAGAGTGTTTAATAGATTTGC), a partial cDNA encoding $AtsPLA_2$-γ was amplified from the total RNA of mature *Arabidopsis* flowers by reverse transcription polymerase chain reaction (RT-PCR) using Superscript™ II reverse transcriptase (Invitrogen) and Pyrobest® DNA polymerase (TaKaRa). Full-length cDNA corresponding to $AtsPLA_2$-γ (At4g29460) was amplified from the RACE (rapid amplification of cDNA ends) cDNA pool of mature *Arabidopsis* flowers made by using a Marathon™ cDNA amplification kit (Clontech). The 5′-end of the $AtsPLA_2$-γ transcript was confirmed using a modified cRACE as a primer extension method [8]. The open reading frame (ORF) of $AtsPLA_2$-γ was subcloned into *Eco*RV-cut pCR2.1-TOPO (Invitrogen) and transferred into XL1-Blue strain (Stratagene).

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[1] The nucleotide sequence(s) reported in this paper has been registered at GenBank™/EBI Data Bank with accession number(s) AY148346

Abbreviations: $AtsPLA_2$-γ, *Arabidopsis thaliana* secretory phospholipase A_2-γ; PC, phosphatidylcholine; PE, phosphatidylethanolamine; LysoPC, lysophosphatidylcholine; LysoPE, lysophosphatidylethanolamine; FFA, free fatty acid; JA, jasmonic acid; LA, linolenic acid; PLD, phospholipase D

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2.2. Analysis of the distribution of AtsPLA$_2$-γ in Arabidopsis *tissues*

Total RNA was extracted from *Arabidopsis* mature flowers, cauline leaves, rosette leaves, inflorescences, and roots using a Nucleospin® RNA Plant kit (Macherey-Nagel, Germany). 3 μg of total RNA was used as a template for each RT reaction, and random decamers were used as primers. The previously used gene-specific primers (QRT-γ-S and QRT-γ-A) and internal standard 18S rRNA primers/competimers (2:8, v/v) obtained from the Quantum*mRNA*™ Universal 18S Internal Standards kit (Ambion) were used for quantitative RT-PCR. The PCR conditions used were: 35 cycles of denaturing for 1 min at 95°C, annealing for 30 s, and polymerizing for 1 min at 73°C, followed by 10 min of elongation at 73°C. The annealing temperature of this reaction was decreased by 2°C every fifth cycle from 64°C to a 'touchdown' at 58°C, which equated to a total of 20 cycles [9].

2.3. Subcellular localization of AtsPLA$_2$-γ

The *Bam*HI fragments harboring signal peptide and the ORF of AtsPLA$_2$-γ were respectively amplified with two sets of primer pairs: GFP-γ-5B (5′-CGGGATCCAGATGATCACCGGGCTTGCTTTG) and GFP-γ-3S (5′-CGGGATCCGGCTGCTAACGACGGCGAGGAG), and GFP-γ-5B and GFP-γ-3B (5′-CG*GGATCC*ATATATTTAAAGAAGCAGAGACTTTGGAGCC). After digestion with *Bam*HI, two fragments were fused in frame to the *Bam*HI-restricted site of the N-terminus of green fluorescence protein (GFP) in psmGFP vector [10]. Plasmid DNA from each construct was then introduced into onion epidermal cells using the biolistic PDS-1000 gene delivery system (Bio-Rad) with 1100-p.s.i. rupture disks and 1.0-μm gold microcarriers. The bombarded onion epidermal cells were then incubated in the dark on a solid Murashige and Skoog medium (Duchefa) at 23°C for 24 h. For further analysis, the bombarded onion epidermal cells were plasmolyzed by incubation for 24 h on the same medium containing 30 g/l of sucrose and 180 mg/l of KH_2PO_4 and examined under a fluorescence microscope using UV-blue light excitation (Zeiss) [11]. psmGFP was used as the constitutively expressed GFP control.

2.4. Expression of recombinant AtsPLA$_2$-γ in Escherichia coli

To construct a vector expressing the fused DsbC-sPLA$_2$-γ [premature form], the AtsPLA$_2$-γ coding fragment was amplified with two primers, ET-γ-5B (5′-CGGGATCCGATGATCACCGGGCTTGCTTTG) and ET-γ-3H (5′-CCAAGCTTTATATTTAAAGAAGCAGAGACTTTGGAGCCT), digested with *Bam*HI and *Hin*dIII, and subcloned into the cohesive site of pET-40b(+) vector (Novagen). The construct encoding the fused DsbC-sPLA$_2$-γ [mature form] was obtained by amplifying AtsPLA$_2$-γ, without the fragment encoding the transit peptide, using two primers, ET-γ-5M (5′-CGGGATCCCCAGGAGAAGTGCAGCAACACCTG) and the above-described ET-γ-3H, digested with *Bam*HI and *Hin*dIII, and inserted into the corresponding site of pET-40b(+) vector. These two constructs were introduced into *E. coli* BL21(DE3)pLysS (Novagen) and used to overexpress fusion proteins using the IPTG induction system. The target proteins, premature or mature types of sPLA$_2$-γ, which linked through an enterokinase (EK) cleavage site to DsbC, were isolated from the total crude extracts of *E. coli* using a RoboPop™ Ni-NTA His·Bind® purification kit (Novagen). The fused target protein was then cleaved using an EK mix, which contained 1 U of EK and 1× EK-reaction buffer (Invitrogen), to detach and refold the AtsPLA$_2$-γ [premature or mature form] from DsbC. The purified protein mixtures were quantified using bovine serum albumin as a standard (pET System Manual, Novagen).

2.5. Assay of recombinant AtsPLA$_2$-γ activity

Recombinant sPLA$_2$-γ activity was determined by measuring the release of radioactive lysophospholipid and FFA from: 1,2-di[1-^{14}C]-palmitoyl-phosphatidylcholine (PC), 1-palmitoyl-2-[1-^{14}C]palmitoyl-PC, 1-palmitoyl-2-[1-^{14}C]linoleoyl-PC, and 1-palmitoyl-2-[1-^{14}C]linoleoyl-phosphatidyl-ethanolamine (Amersham Pharmacia Biotech). After treating the fused recombinant sPLA$_2$-γ (20 μg) with EK mix in a final volume of 30 μl at 37°C for 3 h, the resultant mixture containing EK, DsbC, and free recombinant sPLA$_2$-γ was assayed for enzyme activity. To prepare the substrate, 625 nCi of each of the radioactive phospholipids was mixed with 3 μmol of unlabeled 1-palmitoyl-2-linoleoyl-PC (Avanti Polar Lipids) in chloroform, dried under a N_2 stream, and emulsified in 1 ml of sterile water by sonication for 20 s. The standard enzyme assay mixture contained 50 mM Tris–HCl (pH 8.0), 10 mM $CaCl_2$, 0.05% Triton X-100, 20 μl of the above substrates, and 30 μl of sPLA$_2$-γ mixture in a total volume of 200 μl. The reactions were carried out at 30°C for an appropriate time up to 1.5 h, in buffer with various pH levels and Ca^{2+} concentrations. Reactions were stopped by adding 750 μl of chloroform/methanol (2:1, v/v) followed by 200 μl chloroform and 200 μl KCl (2 M). After vortexing and separation by centrifugation, the chloroform phase was dried, dissolved in chloroform and spotted on to a TLC plate (silica gel G), which was developed with chloroform/methanol/NH_4OH/water (65:39:4:4, v/v/v/v). Lipids on the plates were visualized by brief exposure to iodine vapor; the spots corresponding to the lipid standard FFA, lysophospholipid, and phospholipid were then scraped into vials, and their radioactivities were determined by using a scintillation counter.

3. Results and discussion

3.1. Molecular cloning and sequence analysis of AtsPLA$_2$-γ from Arabidopsis

Searches for sPLA$_2$ homologs based on the previously published carnation sPLA$_2$ cDNA sequence enabled us to identify four putative members (At2g06925, At2g19690, At4g29460, and At4g29470) of sPLA$_2$ in the *Arabidopsis thaliana* 'Col-0' genome (Genomic database at the National Center for Biotechnology Institute). Two of these homologs (At2g06925 and At2g19690) were located on chromosome #2, and the other family members (At4g29460 and At4g29470) were found to be tied to each other with the same orientation on chromosome #4, indicating that duplication and divergence probably occurred in this region during the course of evolution. Thus, we report here on the cDNA of At4g29460. The cloned full-length cDNA sequence was registered at GenBank with accession number AY148346, and named *Arabidopsis* secretory PLA$_2$-γ (AtsPLA$_2$-γ) [7]. The full-length cDNA sequence of AtsPLA$_2$-γ was found to be 791 nucleotides long with an ORF consisting of 564 nucleotides from 7 to 570 (data not shown), encoding a protein of 187 amino acids. This was found to contain a predicted signal peptide of 25 amino acids from the initial methionine. The resultant mature form of sPLA$_2$-γ thus contains 162 amino acids and has a theoretical molecular mass of 17 505.12 Da and a p*I* of 8.25 [12]. A comparison of its deduced amino acid sequences with other plant sPLA$_2$s, showed that AtsPLA$_2$-γ has relatively low homology in terms of its overall amino acid sequences, but that catalytic site and Ca^{2+}-binding loop are highly conserved (Fig. 1). Twelve cysteine residues, known to form structural disulfide bonds, also exist in the corresponding positions of the other known sPLA$_2$s. In the His/Asp dyad, the histidine residue and the linked aspartate were found to be well conserved. This histidine has been suggested to function as a base for the deprotonation of ester carbonyl carbons of the substrate, and the β-carboxyl group of the aspartate has been suggested to interact with Ca^{2+} cofactor [13]. It is believed that AtsPLA$_2$-γ is a group XI sPLA$_2$ member, as was recently reported for other sPLA$_2$s, and supported by their structural homogeneities.

3.2. Spatial expression of AtsPLA$_2$-γ

To confirm the distribution of AtsPLA$_2$-γ expression, total RNAs from various tissues were used in Northern blot analysis by using the 3′-UTR (untranslated region) of AtsPLA$_2$-γ as a labeled probe. However, signals were barely detectable due to the low abundance of AtsPLA$_2$-γ mRNA (data not shown). Therefore, quantitative RT-PCR was performed in

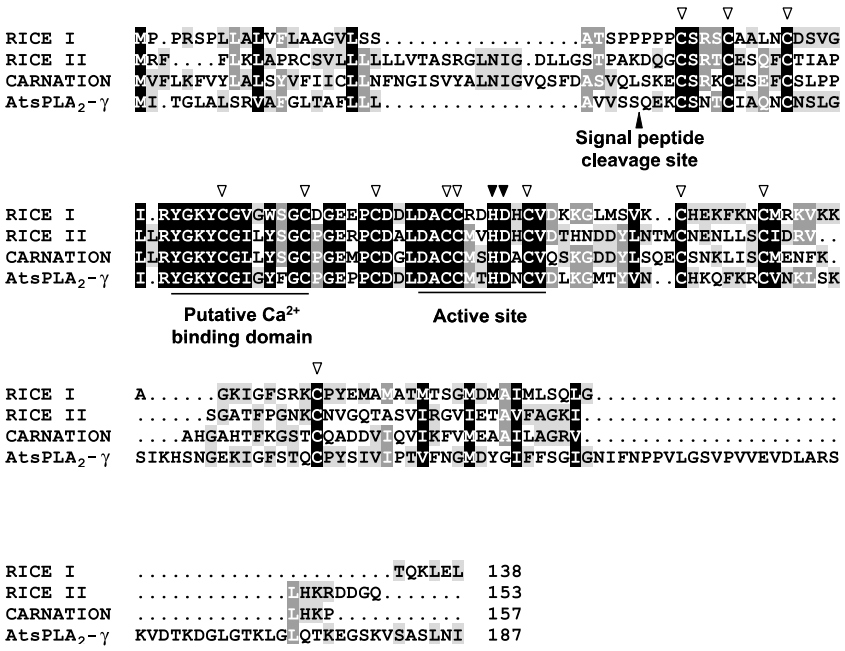

Fig. 1. Alignment of the deduced amino acid sequences of sPLA$_2$s. The ORF of AtsPLA$_2$-γ was aligned with the amino acid sequences of previously published rice-I, rice-II and carnation sPLA$_2$s using Clustal W (DNAstar). The arrowhead indicates the predicted cleavage site of the signal peptide. The putative Ca^{2+}-binding domain and the active site, which are found in other sPLA$_2$s, are underlined. The opened triangles and closed triangles indicate the positions of the conserved cysteine residues and the catalytic His/Asp dyad, respectively.

the condition of concomitant amplification with the internal standard 18s rRNA (Ambion). The 243-bp fragment corresponding to AtsPLA$_2$-γ and the 315-bp fragment corresponding to 18s rRNA were amplified simultaneously in the same reaction (Fig. 2). AtsPLA$_2$-γ was found to be strongly transcribed in mature flowers but weakly expressed in other tissues, suggesting that AtsPLA$_2$-γ has an important role in flower tissue.

3.3. Subcellular localization of AtsPLA$_2$-γ

Using the pSORT (http://psort.ims.u-tokyo.ac.jp) and TargetP (http://www.cbs.dtu.dk/services/TargetP) programs, we found that the cleavage site of the signal peptide in AtsPLA$_2$-γ protein is located between the 25th and 26th amino acid sequences. To determine the subcellular localization of AtsPLA$_2$-γ, green fluorescence protein (GFP) gene was fused to the C-terminus of AtsPLA$_2$-γ ORF or to its predicted signal peptide sequences, and two fused constructs were then introduced into onion epidermal cell by biolistic particle bombardment (Fig. 3b,c). The GFP fused with the AtsPLA$_2$-γ ORF was detected in the intercellular space, indicating that it is expressed and secreted from the sublayer of onion epidermal cells. In the case of signal peptide-fused GFP, it was also found to be secreted into the intercellular region (Figs. 3e,f,h,i), while the control GFP alone remained in the cytoplasm (Fig. 3a,d,g). In addition, after the onion epidermal cells had been plasmolyzed, we could observe that the majority of the GFP was located outside the cytosol (data not shown).

3.4. Recombinant expression and the functional analysis of AtsPLA$_2$-γ

Two *E. coli* expression vectors, DsbC-sPLA$_2$-γ [premature form] containing the signal peptide and DsbC-sPLA$_2$-γ [mature form] not containing the signal peptide, were constructed to determine whether the recombinant AtsPLA$_2$-γ protein has functional sPLA$_2$ activity. However, the premature form of sPLA$_2$-γ was 23% less active than the mature form in terms of PC hydrolysis, which might have been due to a structural rearrangement of the enzyme during maturation. On the other hand, DsbC alone had no hydrolytic activity (data not shown). Thus, the DsbC-fused mature form of sPLA$_2$-γ was used in further study because it had higher enzyme activity. The mature form of sPLA$_2$-γ was separated from DsbC protein by enterokinase treatment. This de novo dissociated mature form of sPLA$_2$-γ in this EK reaction mixture had a higher enzymatic activity than the other fusion proteins tested, and

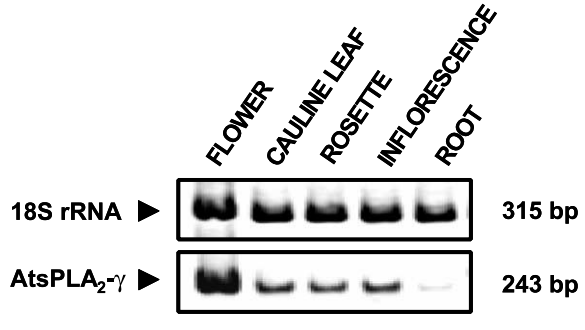

Fig. 2. Spatial expression of AtsPLA$_2$-γ by quantitative RT-PCR. Total RNAs were extracted from 30-day old *Arabidopsis* plants, and 3 μg of this total RNA, obtained from different tissues, was converted to cDNA with random decamer (Ambion). Two gene-specific primers (QRT-A2-γ-S and QRT-A2-γ-A) and internal standard 18S rRNA primers/competimers (2:8, v/v), in one tube, were subjected to semi-quantitative RT-PCR.

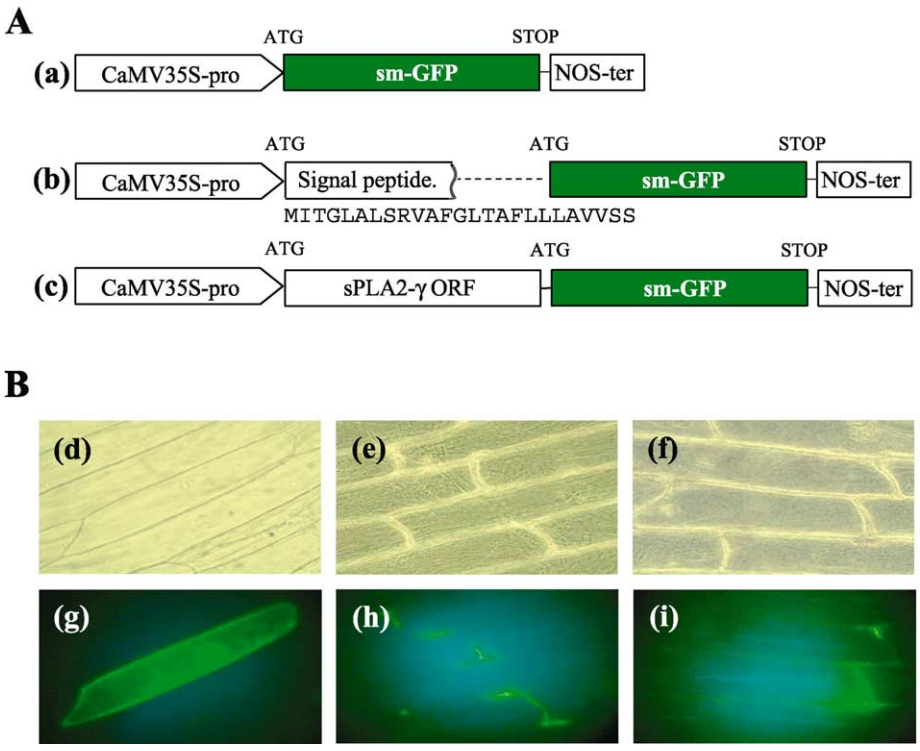

Fig. 3. Subcellular localization of AtsPLA$_2$-γ in onion epidermal cells. A: A map of the three constructs used for cellular localization analysis. A construct encoding only GFP was used as a control (panel a), GFP was fused to the C-terminus of the putative signal peptide sequence of AtsPLA$_2$-γ (panel b), or to the C-terminus of the full ORF of AtsPLA$_2$-γ (panel c). B: Subcellular localization of GFP-fused sPLA$_2$-γ. Panels d and g correspond to panel a; panels e and h to panel b; panels f and i to panel c. Upper panels d–f are bright-field images of onion epidermal cells; lower panels g–i show the cytolocalizations of GFP and GFP-fusion proteins by fluorescence microscopy under UV-blue light excitation (Zeiss).

was thus selected for the subsequent enzymatic activity determinations. The catalytic properties of sPLA$_2$-γ were investigated with respect to time, pH, and Ca^{2+} concentration to characterize the conditions required for its enzymatic activity. Free mature sPLA$_2$-γ hydrolyzed approximately 30% of total PC in 1.5 h, in a linear fashion, under the reaction conditions used (Fig. 4A), and its activity peaked near pH 8.0 (Fig. 4B). In terms of the effect of Ca^{2+} concentration on enzyme activity, sPLA$_2$-γ activity continuously increased on treatment with an increase of the $CaCl_2$ concentration to 10 mM. In this study (Fig. 4C), AtsPLA$_2$-γ was found to be secreted into the intercellular space, which is acidic with a pH of 5.0–6.0. However, we found by in vitro assay that almost all other sPLA$_2$s have an optimum pH range in the alkaline region, near pH 8.0, regardless of organism, which indicates that sPLA$_2$-γ is inactivated in the intercellular region [2,6]. However, it has been suggested from animal studies that monomeric sPLA$_2$, the inactive form, is converted to the dimeric active sPLA$_2$ with the aid of the negatively charged membrane and by interaction of the sPLA$_2$-mediated lipid products [14–16]. Thus, it can be speculated that AtsPLA$_2$ is secreted into the intercellular space, then changed with its structural conformation for optimal activity. This suggests that the activity of sPLA$_2$s can be regulated at a post-translational stage as well as at the transcriptional stage.

sn-2 position specificity and the acyl preference of sPLA$_2$-γ for PC were also examined (Fig. 4D). In this analysis, it was found that sPLA$_2$-γ hydrolyzed 1-palmitoyl-2-[1-^{14}C]linoleoyl-PC and 1-palmitoyl-2-[1-^{14}C]palmitoyl-PC, and that its activity was slightly higher when the acyl group of PC was a linoleoyl group rather than a palmitoyl group at the *sn*-2 position. In addition, radioactive lysophosphatidylcholine (lysoPC) was detected to a negligible extent (less than 1%), indicating that the enzyme does not act at the *sn*-1 position of PC. In a control experiment, the sPLA$_2$ activity was not detected after incubation with EK alone. These results suggest that AtsPLA$_2$-γ shows *sn*-2 position specificity, but has a negligible acyl preference for production of linolenic acid (LA). It is believed that the biosynthetic pathway of jasmonic acid (JA) is initiated by the production of LA, which arises from the hydrolysis of phospholipids, as catalyzed by PLA$_2$ [17,18]. Although the acyl preference of AtsPLA$_2$-γ toward the linoleoyl group at the *sn*-2 position of phospholipids seems unremarkable, the amount of linoleoyl group at the *sn*-2 position of the major phospholipids, at least in the plasma membrane, is significant [19,20]. However, it is difficult to predict whether the LA produced by the catalytic activity of AtsPLA$_2$-γ acts as a precursor for the biosynthesis of JA in response to environmental signals, without data about the transportation of FFAs from the membrane to the plastid, in which the JA biosynthetic pathway is present [18]. The head group specific activity of AtsPLA$_2$-γ was also examined using 1-palmitoyl-2-[1-^{14}C]linoleoyl-PC and 1-palmitoyl-2-[1-^{14}C]linoleoyl-phosphatidylethanolamine (PE) as substrates, which are the most abundant phospholipids integrated into plant plasma membranes. Using the same reaction conditions for 1 h, about 90% of the total PE was converted to FFA and lysophosphatidylethanolamine (lysoPE), whereas 9.5% of the PC was hy-

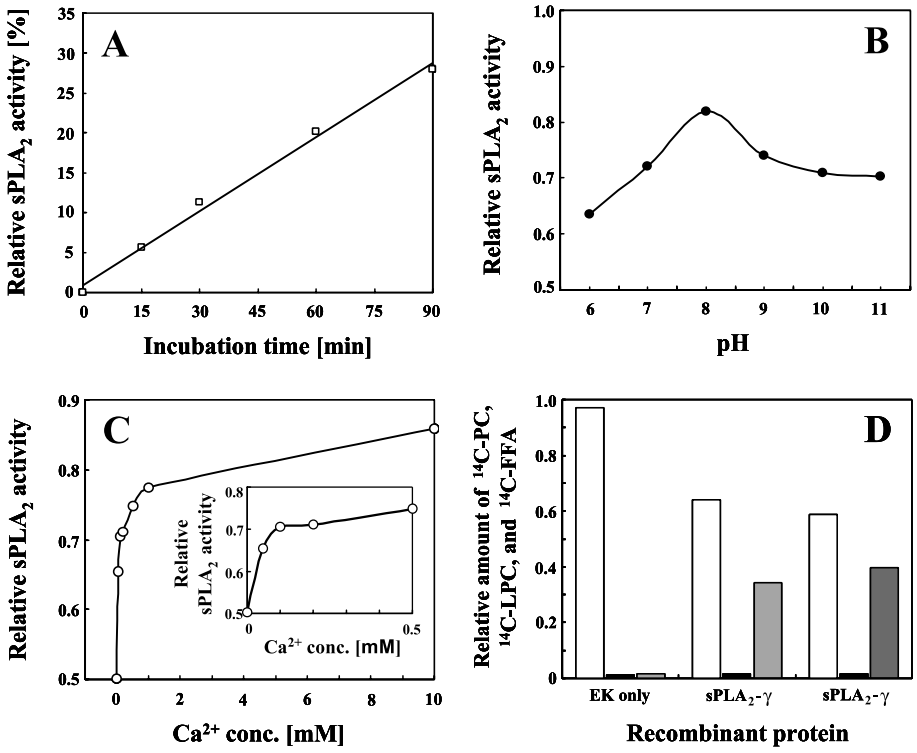

Fig. 4. Enzymatic activities of the recombinant AtsPLA$_2$-γ. A: Substrate sensitivity of the recombinant AtsPLA$_2$-γ. An enzyme mixture containing enterokinase, DsbC, and free recombinant AtsPLA$_2$-γ [mature form] was incubated with 1,2-di[1-^{14}C]palmitoyl-PC as a substrate in Tris–HCl buffer (pH 8.0), and 10 mM of $CaCl_2$ for various periods of time up to 1.5 h at 30°C. B: pH dependence of the recombinant AtsPLA$_2$-γ. The enzyme mixture was incubated with PC in buffer with various pH levels and 10 mM $CaCl_2$ for 1.5 h at 30°C. Data represent the relative activities. C: The effect of Ca^{2+} concentration on recombinant AtsPLA$_2$-γ. The enzyme mixture was incubated with PC in Tris–HCl buffer (pH 8.0) with various concentrations of $CaCl_2$ for 1.5 h at 30°C. For the above experiments, the radioactivities of the released fatty acids, lysoPC, and PC were quantified and amounts of hydrolysis products from the substrate PC were determined. D: *sn*-2 position specificity and acyl preference of the recombinant AtsPLA$_2$-γ. Two substrates, 1-palmitoyl-2-[1-^{14}C]palmitoyl-PC and 1-palmitoyl-2-[1-^{14}C]linoleoyl-PC, were incubated with the enzyme mixture in Tris–HCl buffer (pH 8.0) with 10 mM $CaCl_2$ for 1.5 h at 30°C. The open bar indicates ^{14}C-PC and the closed bar indicates ^{14}C-lysoPC. Palmitoic acid and LA are indicated by a light gray and a dark gray bar, respectively. Radioactivities are expressed as a proportion of the sum of the three fractions. All experiments were repeated in triplicate; standard deviations are not indicated because they were negligible.

drolyzed to lysoPC and FFA. In a control experiment, 1,2-di[1-^{14}C]palmitoyl-PC was found to be hydrolyzed into two products, lysoPC and FFA, to a similar extent (Table 1). These results suggest that AtsPLA$_2$-γ is a real sPLA$_2$, as in these respects it resembles previously purified sPLA$_2$s, and that this enzyme has head group specificity rather than an acyl preference. It has been reported that phospholipase D (PLD) is a key enzyme, which mediates the degradation of membrane phospholipids during the initial stages of plant senescence, and that its activity is inhibited by lysoPE [21]. Therefore, because AtsPLA$_2$-γ enzyme has relatively higher activity to PE, it is possible that it may play an important role in regulation of PLD-mediated senescence by releasing its product lysoPE [21,22].

Plant PLA$_2$ enzymes appear to be associated with plant biological mechanisms, such as auxin signaling, wounding-induced signal transduction and pathogenesis. However, almost all studies have focused on other PLA$_2$ enzymes [23–29]. Recently, several cDNAs encoding plant sPLA$_2$s have been cloned and characterized [1,5–7], but little is known about the biological functions of the sPLA$_2$ enzymes in plants. Our results lead us to hypothesize that AtsPLA$_2$-γ may play a certain role in flower tissues and PLD-mediated senescence. Further work is being undertaken by our group along these lines. We hope that the cloning and characterization of AtsPLA$_2$-γ will make it possible to elucidate the cellular functions of sPLA$_2$-γ in plant phospholipid-derived signal transduction.

Table 1
Head group specific activity of the recombinant AtsPLA$_2$-γ

Substrate	Recovered [^{14}C] radioactivity	
	Lysophospholipids	Free fatty acids
	Percentage of total (S.D.)[a]	
L-3-PC, 1-palmitoyl-2-[1-^{14}C]linoleoyl	0.47 (0.09)	9.51 (0.28)
L-3-PE, 1-palmitoyl-2-[1-^{14}C]linoleoyl	0.67 (0.05)	89.31 (3.18)
L-3-PC, 1,2-di[1-^{14}C]palmitoyl	9.80 (0.44)	10.36 (0.66)

[a]S.D. was obtained from three independent in vitro assays ($n = 3$).

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