A Cytosolic Phospholipase A2 from Potato Tissues Appears to Be Patatin

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Phospholipase (PL) A2 is involved in signal transduction in the resistance reaction that is induced in potato by inoculation of an incompatible race of Phytophthora infestans, the late blight fungus, or by treatment with fungal elicitor hyphal wall components (Kawakita et al. 1993). In this study, PLA2 in the soluble fraction from potato tuber was purified. The following results suggested that the enzyme was, in fact, patatin: (1) the molecular mass of the purified enzyme was 40 kDa, the same as that of patatin; (2) the pi of the purified enzyme was approximately 4.75, which corresponds to that of patatin; and (3) the amino-terminal amino acid sequence of the purified enzyme showed a high degree of homology to that of patatin. Patatin is known as a storage protein of the potato tuber and it has been shown to have esterase activity. However, other enzymatic activities and the function(s) of patatin are unknown. We investigated the PLA2 activities of the purified patatin. The PLA2 activity of the patatin was much higher than the PLA1 activity, even though the protein exhibited both activities. The PLA2 activity of the enzyme was particularly apparent when phosphatidylcholine with linoleic acid at the sn-2 position was used as substrate. Lower activity was observed with phosphatidylcholine with palmitic acid, oleic acid and arachidonic acid at the sn-2 position.

Key words: Acyl specificity — Patatin — Phospholipase A2 (EC 3.1.1.4) — Potato tuber — Resistance reaction — Signal transduction.

Phospholipase A2 (PLA2; EC 3.1.1.4) is a lipolytic enzyme that catalyzes the hydrolysis of the sn-2 fatty acyl ester linkage in diacylphospholipids, liberating free fatty acids and lysophospholipids. Activation of PLA2 appears to be involved in a series of resistance responses that can be induced in potato tubers (Doke and Nishimura 1988, Kawakita et al. 1993). In slices of potato tuber inoculated with an incompatible race of Phytophthora infestans Mont. de Bary, the late blight fungus, the generation of the superoxide anion is enhanced, with subsequent transient activation of PLA2. After activation of this enzyme, membrane-bound lipoxygenase is also activated and intracellular lipid peroxides are generated prior to the accumulation of rishitin, a potato phytoalexin (Doke and Nishimura 1988).

In animal cells, arachidonic acid is released from the sn-2 position of glycerol in membrane phospholipids by an activated form of PLA2 to initiate the synthesis of two potent classes of inflammatory mediators, the prostaglandins and leukotrienes (Flower and Blackwell 1976, Needleman et al. 1986, Samuelsson et al. 1987). Lysophospholipids can serve as precursors to an other mediator of inflammation, platelet-activating factor (Hanahan 1986). Thus, PLA2 in animal cells is a key enzyme in the initial step in the arachidonic acid cascade that leads to the generation of these inflammatory mediators. By contrast, membrane phospholipids in plant cells consist not of arachidonic acid but of linoleic or linolenic acid at the sn-2 position of glycerol. Some peroxidation products of unsaturated fatty acids have been shown to induce the accumulation of phytoalexins (Bostock and Stermer 1989). Thus, it seems likely that such bioactive metabolites of linoleic or linolenic acid in plant cells, corresponding to those of arachidonic acid in animal cells, might play an important role in induction of the hypersensitivity reaction. PLA2 in plant cells is a likely candidate for a key enzyme in these processes. However, little is known about the nature and function of PLA2 in plant cells.

In the present study, we purified a protein with PLA2 activity from a soluble fraction of potato tuber, and, from the molecular mass, pi and the amino-terminal amino acid sequence of the purified enzyme, we concluded that the enzyme was identical to patatin. Then, we investigated the PLA2 and PLA1 activities of the purified enzyme in detail. The possible functional role of patatin is discussed.

Materials and Methods

Plant material—Tubers of potato (Solanum tuberosum L.) cultivar Irish cobbler were used in this study. Plants were harvested under standard agricultural conditions at the Nagoya University farm and tubers were stored at 4°C until use.

Chemicals—C14-NBD-PC was purchased from Avanti Polar Lipids (Birmingham, AL, U.S.A.). 1-Palmitoyl-2-[1-14C]linoleoyl PC, 1-palmitoyl-2-[1-14C]palmitoyl PC, 1-palmitoyl-2-[1-14C]oleoyl PC, 1-palmitoyl-2-[1-14C]arachidonoyl PC and 1-14C]palmitic acid were obtained from New England Nuclear. Lipase (from Rhizopus arthrius) was from Boehringer Mannheim and phospholipase A2 (from bee venom) was from Sigma Chemical Co.
Purification of cytosolic phospholipase A\textsubscript{2} from potato tuber

Discs of potato tubers were homogenized and the soluble fraction was prepared as described previously (Kawakita et al. 1993). The soluble fraction was brought to 40% saturation with ammonium sulfate and centrifuged at 14,000 \times g for 15 min. The supernatant was brought to 60% saturation with ammonium sulfate. After centrifugation, the resulting pellet was suspended in buffer A [50 mM MOPS-KOH buffer (pH 7.6) containing 1 mM EGTA] and dialyzed against the same buffer overnight. This fraction was then applied to a column (2.1 cm i.d. \times 35 cm) of DEAE-Sephadex A-25 (Pharmacia) that has been pre-equilibrated with buffer A. The column was washed with 500 ml of buffer A and PL\textsubscript{A2} was eluted with a 620-ml linear gradient from 0 to 0.3 M NaCl in buffer A at a flow rate of 0.8 ml min\textsuperscript{-1}. Active fractions were pooled, lyophilized and loaded onto a column (2.1 cm i.d. \times 30 cm) of Butyl Toyopearl 650 (Tosoh Co., Japan) that has been pre-equilibrated with buffer A supplemented with 0.8 M (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4}. After washing of the column with 500 ml of the same buffer, PL\textsubscript{A2} were eluted by a 420-ml reverse gradient from 0.8 to 0 M (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4} in buffer A at a flow rate of 0.5 ml min\textsuperscript{-1}, with subsequent elution with 240 ml of buffer A without (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4}. Active fractions were pooled and dialyzed against buffer A overnight. The dialyzed solution was applied to a 5-ml of Econo-Pac Q column (Bio-Rad) that has been pre-equilibrated with buffer A and was part of an FPLC system (Pharmacia). Protein was eluted with a 90-ml gradient from 0 to 0.3 M NaCl in buffer A at a flow rate of 1 ml min\textsuperscript{-1}. Active fractions were pooled and dialyzed against buffer A overnight. This preparation of enzyme was lyophilized and stored at \textdegree C. The entire procedure was carried out at 4\textdegree C.

Assays of phospholipases A\textsubscript{1} and A\textsubscript{2}—PL\textsubscript{A2} activity in fractions after column chromatography was assayed with phospholipid C\textsubscript{2}-NBD-PC as a fluorometric substrate (Wittenauer et al. 1984). The assay mixtures contained 50 mM Tris-HCl (pH 8.5), 3 mM CaCl\textsubscript{2} and an appropriate amount of each fraction in a final volume of 1,990 \mu l. The reaction was started by the addition of 10 \mu l of a solution of 5 \mu g ml\textsuperscript{-1} C\textsubscript{2}-NBD-PC and fluorescence was monitored continuously as a function of time, with excitation at 470 nm and emission at 540 nm.

To quantify the PL\textsubscript{A2} activity and PL\textsubscript{A1} activity of the purified enzyme, the amounts of radioactivity in linoleic acid generated from 1-palmitoyl-2-[\textsuperscript{14}C]linoleoyl PC and in palmitic acid generated from 1-[\textsuperscript{14}C]palmitoyl-2-linoleoyl PC, respectively, were measured by the method of Natori et al. (1983) with some modifications. A solution of radiolabeled PC was adjusted to a final concentration of 0.2 mM with unlabeled PC and evaporated to dryness in vacuo. The residue was suspended in 45 \mu l of 50 mM Tris-HCl buffer (pH 8.5) that contained 0.05% Triton X-100 and 3 mM CaCl\textsubscript{2}. The suspension was sonicated and the reaction was started by addition of 5 \mu l of the preparation of enzyme (0.8 \mu g protein ml\textsuperscript{-1}). The mixture was incubated at 37\textdegree C for 15 min. The reaction was stopped by addition of 300 \mu l of a mixture of chloroform and methanol (2:1, v/v) at 0\textdegree C. The mixture was agitated on a vortex mixer for 30 s and then centrifuged at 4,000 \times g for 5 min to separate the two layers. The chloroform layer was evaporated to dryness in vacuo and the residue was dissolved in 50 \mu l of the mixture of chloroform and methanol. The extracted lipid was applied to a silica gel thin-layer plate (LKD2; 2 mm thick; Whatman) and the plate was developed with a mixture of chloroform, methanol and water (65:25:4, v/v/v). Radioactivity on the plate was located and quantitated with a radioanalytic imaging system (AMBlS).

Preparation of 1-[\textsuperscript{14}C]palmitoyl-2-linoleoyl phosphatidylcholine—1-[\textsuperscript{14}C]Palmitoyl-2-linoleoyl PC was prepared as described by Lands and Merckl (1963) with some modifications.

Unlabeled PC was suspended in 1 ml of 25 mM Tris-maleate buffer (pH 6.4) that contained 10 mM CaCl\textsubscript{2} and 0.05% Triton X-100. The reaction was started by addition of 300 units of lipase (from Rhizopus arrhizus) and incubated at 37\textdegree C for 2 h. Lipids were extracted by the method of Bligh and Dyer (1959). The lipid fraction was applied to a silica gel thin-layer plate (LKD2; 2 mm thick; Whatman) and the plate was developed with a mixture of chloroform, methanol and water (65:25:4, v/v/v). The plate was sprayed with a 0.01% solution of primuline in 80% acetone for visualization of products and the spots corresponding to lysophosphatidylcholine (LPC) were scraped off. Lyso-PC were recovered and supplemented with 0.5 \mu mol linoleic acid and 1.9 MBq [\textsuperscript{14}C]palmitic acid. The mixture was evaporated to dryness in vacuo, and then the residue was suspended in 200 \mu l of 250 mM phosphate buffer (pH 7.4) that contained 250 mM MgCl\textsubscript{2} and 250 mM ATP. This mixture was sonicated and the reaction was started by addition of 0.4 mM CoA and a microsomal fraction from porcine liver (0.2 mg of protein). After a 3-h of incubation at 37\textdegree C, lipids were extracted and 1-[\textsuperscript{14}C]palmitoyl-2-linoleoyl PC was purified by thin-layer chromatography as described above.

Amino-terminal amino acid sequence analysis—The amino-terminal sequence of the purified enzyme protein (0.1 mg) was determined by repeated cycles of Edman degradation in an automated sequencer (model 476A; Applied Biosystems). The amino acid sequence of the purified enzyme was compared with those of other proteins in Gene Bank.

Electrophoresis and quantitation of protein—For SDS-PAGE, a solution of protein was mixed with Laemmli's sample buffer, boiled for 3 min, and then analyzed by electrophoresis on a 7.5% polyacrylamide gel as described by Laemmli (1970). The gel was stained with CBB IEF-PAGE was performed on a 4% polyacrylamide gel (IEF-PAGE mini pl 4–10; TEFCO) according to the manufacturer's instructions. Concentrations of protein were determined, with bovine serum albumin as the standard, by the dye-binding method with a kit from Bio-Rad according to the manufacturer's instructions.

Results

Purification of cytosolic phospholipase A\textsubscript{2} from potato tuber—Less than 10% of the original PL\textsubscript{A2} activity in the potato tuber homogenate was present in the membrane fraction. However, substantial PL\textsubscript{A2} activity was recovered in the soluble fraction, as detected with C\textsubscript{2}-NBD-PC as substrate (data not shown). Therefore, purification of PL\textsubscript{A2} was performed using the soluble fraction from potato tuber. As shown in Table 1, an overall purification with a yield of 36% was achieved. The final preparation had a specific activity of 34.9 \mu mol (mg protein)\textsuperscript{-1} min\textsuperscript{-1}.

After chromatography on the Econo-Pac Q column, the preparation of enzyme was analyzed by SDS-PAGE to determine its purity. When the gel was stained with CBB, the band of only one protein, with a molecular mass of 40 kDa, was detected in the preparation (Fig. 1A). The preparation was also analyzed by IEF-PAGE. After electrophoresis, the gel was cut longitudinally. One portion of the gel was stained with CBB and the other portion was sliced into 5-mm segments. The proteins in the segments were eluted...
from the polyacrylamide gel by incubation at 4°C with 0.1 M Tris-HCl buffer (pH 8.5) overnight, and PLA₂ activity in each eluent was determined. The location of the only protein band that had been stained with CBB corresponded to the segment in which PLA₂ activity was detected (Fig. 1B, C). The pl value of the protein was estimated to be approximately 4.75.

Amino-terminal amino acid sequence of the purified enzyme—The amino-terminal amino acid sequence of the purified sample after chromatography on the Econo-Pac Q column revealed a very high degree of homology to the sequence of patatin (Park et al. 1983), through the first 22 residues (Fig. 2). Therefore, it seems very likely that the purified enzyme was patatin. In addition to the homology in sequence of patatin (Park et al. 1983), through the first 22 residues (Fig. 2), therefore, it seems very likely that the purified enzyme was patatin. In addition to the homology in terms of amino acid sequence, the molecular weight and pl value of the purified enzyme were in good agreement with those of patatin. Glycine and glutamic acid were present in equimolar amounts at position 3 in the amino-terminal amino acid sequence, indicating the possible presence of at least two isoforms in the purified preparation.

Phospholipase activities of the purified enzyme—Patatin accounts for up to 40% of the soluble protein in potato tubers (Racusen and Foote 1980) and it is thought to be the main storage protein of the tuber. It has been shown that patatin has activities of enzymes that are known to be involved in lipid metabolism, namely, lipid acyl hydrolase activity and acyl transferase activity (Racusen 1984, 1986, Rosahl et al. 1987). PLA₂ and PLA₁, which releases fatty acids from sn-1 positions of phospholipids, are also lipid acyl hydrolases. Therefore, we investigated the PLA₂ and PLA₁ activities of the purified enzyme in detail.

A commercially available radiolabeled substrate, 1-palmitoyl-2-[1-¹⁴C]linoleoyl PC, was used for assays of PLA₂ activity. To investigate PLA₁ activity, a PC with [1-¹⁴C]palmitic acid at the sn-1 position was synthesized as described in Materials and Methods and used as the substrate for the assay. After the products of the reaction had been separated by TLC, PLA₂ activity was determined by calculating the radioactivity of linoleic acid released from 1-palmitoyl-2-[1-¹⁴C]linoleoyl PC, and PLA₁ activity was determined from the amount of palmitic acid released from 1-[1-¹⁴C]palmitoyl-2-linoleoyl PC. In order to determine the positions of free fatty acids after chromatography, each substrate was hydrolyzed by lipase from Rhizopus arthrius, which has PLA₁ activity, or by PLA₂ from bee venom and subjected to chromatography on a TLC plate at the same time, to serve as a control (Fig. 3). The purified enzyme released linoleic acid from the sn-2 position (Fig. 3A) and palmitic acid from the sn-1 position (Fig. 3B), indicating that the protein had both PLA₂ and PLA₁ activities. As shown in Figure 4, the rates of reactions catalyzed by the PLA₂ and PLA₁ activities were constant for the first 15 min of incubation but PLA₂ activity was higher than PLA₁ activity. On the basis of these results, reaction mix-

### Table 1 Purification of soluble phospholipase A₂ from potato tuber

<table>
<thead>
<tr>
<th>Protein</th>
<th>Total activity (^a) (µmol min⁻¹)</th>
<th>Specific activity (^a) (µmol (mg protein)⁻¹ min⁻¹)</th>
<th>Purification (-fold)</th>
<th>Yield (%)</th>
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<tbody>
<tr>
<td>Soluble fraction</td>
<td>761</td>
<td>468</td>
<td>0.62</td>
<td>1.0</td>
</tr>
<tr>
<td>40–60% (NH₄)₂SO₄</td>
<td>358</td>
<td>419</td>
<td>1.17</td>
<td>1.9</td>
</tr>
<tr>
<td>DEAE-Sephadex</td>
<td>72.8</td>
<td>261</td>
<td>3.59</td>
<td>5.8</td>
</tr>
<tr>
<td>Butyl-Toyopearl</td>
<td>20.0</td>
<td>184</td>
<td>9.27</td>
<td>15.1</td>
</tr>
<tr>
<td>Econo Pac Q</td>
<td>7.0</td>
<td>168</td>
<td>34.9</td>
<td>56.8</td>
</tr>
</tbody>
</table>

\(^a\) PLA₂ activity was determined by calculating the amount of radioactivity in linoleic acid released from 0.2 mM 1-palmitoyl-2-linoleoyl PC. The reaction mixture contained 3 mM CaCl₂ and 0.05% Triton X-100 in 50 mM Tris-HCl buffer (pH 8.5). Each fraction was added to the reaction mixture which was then incubated at 37°C for 15 min.
The purified enzyme Lys Leu Gly Glu Met Val Thr Val Leu Ser Gly Gly Gly Gln Pro Ala

Superior

Thr Lys

LaChipper

Thr Gly

Fig. 2 Comparison of amino-terminal amino acid sequences. The amino-terminal amino acid sequence of the purified enzyme from the Irish cobbler cultivar was compared with those of patatins purified from two other potato cultivars, Superior and LaChipper (Park et al. 1983). Dots represent identical amino acids.

nature were incubated for 15 min in all subsequent experiments.

The enzyme had maximum PLA$_2$ activity between pH 7.5 and 8.5, and the activity dropped dramatically when the pH was below 7.0. The PLA$_1$ activity displayed a broad pH profile (Fig. 5). Thus, Tris-HCl buffer (pH 8.5) was used for standard assays.

In a previous report (Kawakita et al. 1993), it was shown that a partially purified PLA$_2$ activity was stimulated by millimolar levels of Ca$^{2+}$ ions. Thus, the dependence on Ca$^{2+}$ ions of the purified enzyme was investigated at pH 8.5 with Ca$^{2+}$/EGTA buffers. Both the PLA$_2$ and the PLA$_1$ activity of the purified sample had a similar response to CaCl$_2$ (Fig. 6). The activities increased at concentrations of CaCl$_2$ above 0.1 mM and reached a maximum at 3 mM, although PLA$_1$ activity was very low when compared to PLA$_2$ activity.

Kinetic analysis of phospholipase A$_2$ and A$_1$ activities of the purified enzyme—For kinetic analyses, the PLA$_2$ and PLA$_1$ activities of the purified enzyme were measured at various concentrations of substrate PC, in the presence and in the absence of 3 mM CaCl$_2$. When PLA$_2$ activity was determined in the absence of Ca$^{2+}$ ions, substrate inhibition was observed at concentrations above 0.4 mM, but this phenomenon was not observed in the presence of Ca$^{2+}$ ions. Similarly, PLA$_1$ activity was inhibited at concentrations of substrate above 0.2 mM (Fig. 7A). The apparent $K_m$ and $V_{max}$ values were determined by extrapolation of the linear portion of the graphs of s/v against s (Fig. 7B) and they are given in Table 2.

Acyl specificity of the phospholipase A$_2$ activity of the purified enzyme—The characterization of the PLA$_2$ activity of the purified enzyme was performed using PC with linoleic acid at the sn-2 position as substrate. The acyl specificity of the PLA$_2$ activity of the purified enzyme was also investigated. In this experiment, molecular species of PC with a labeled fatty acid, namely, palmitic acid (16:0), oleic acid (18:1), linoleic acid (18:2) or arachidonic acid (20:4), at the sn-2 position were used as substrates for the assay. As shown in Figure 8, more linoleic acid was released from PC by the purified enzyme than oleic acid and palmitic acid, with arachidonic acid being released at the lowest rate of all.
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Fig. 5 Dependence on pH of PLA activities of the purified enzyme. The reaction mixture contained 0.2 mM substrate, 3 mM CaCl\textsubscript{2} and 0.05% Triton X-100 in 50 mM MES-KOH, MOPS-KOH or Tris-HCl buffer, at pH 5.5 to 6.5, pH 6.5 to 7.5, and pH 7.5 to 9.5, respectively. PLA\textsubscript{1} (○) and PLA\textsubscript{2} (●) activities were determined as the amount of radioactivity found in palmitic acid released from 1-[1\textsuperscript{14}C]palmitoyl PC and in linoleic acid released from 2-[1\textsuperscript{14}C]linoleoyl PC, respectively. Each plotted value represents the mean of results from three independent experiments and the bars indicate standard deviations.

Discussion

In a previous study (Kawakita et al. 1993), we partially purified PLA\textsubscript{2} from the soluble fraction of potato tuber by precipitation with ammonium sulfate and column chromatography on DEAE-Sephadex. Further purification was achieved by column chromatography on Butyl Toyopearl and Econo-Pac Q. The resultant preparation gave one major band after SDS-PAGE, which corresponded to a molecular mass of about 40 kDa. The purified enzyme was the major protein present in the preparation. This result indicated that a constitutive protein with PLA\textsubscript{2} activity was abundant in the soluble fraction. We concluded that the pu-

Fig. 6 Effects of CaCl\textsubscript{2} at various concentrations on PLA activities of the purified enzyme. PLA\textsubscript{1} (○) and PLA\textsubscript{2} (●) activities were determined as the amount of radioactivity found in palmitic acid released from 1-[1\textsuperscript{14}C]palmitoyl PC and in linoleic acid released from 2-[1\textsuperscript{14}C]linoleoyl PC, respectively. Each plotted value represents the mean of results from three independent experiments and the bars indicate standard deviations.

Fig. 7 Kinetic analysis of PLA activities of the purified enzyme. (A) Effects of PC on PLA\textsubscript{1} activity (○, ○) and PLA\textsubscript{2} activity (●, ●) were determined at various concentrations of 1-[1\textsuperscript{14}C]palmitoyl PC and 2-[1\textsuperscript{14}C]linoleoyl PC, respectively, in the presence (●, ●) and in the absence (○, ○) of 3 mM CaCl\textsubscript{2}. Each plotted value represents the mean of results from three independent experiments and the bars indicate the standard deviations. (B) Graph of \( s/v \) versus \( s \) for PLA\textsubscript{2} activity in the presence (●) and in the absence (○) of 3 mM CaCl\textsubscript{2}.

Fig. 8 Acyl specificity of the PLA\textsubscript{2} activity of the purified enzyme. The acyl specificity of the PLA\textsubscript{2} activity was determined with PC that contained palmitic acid (16:0), oleic acid (18:1), linoleic acid (18:2) or arachidonic acid (20:4) at the sn-2 position. 1,2-[1\textsuperscript{14}C]palmitoyl PC; 2,2-[1\textsuperscript{14}C]oleoyl PC; 3,2-[1\textsuperscript{14}C]linoleoyl PC; 4,2-[1\textsuperscript{14}C]arachidonoyl PC. Each value represents the mean of results from three independent experiments and the bars indicate standard deviations.
Apparent values were determined by extrapolation of the linear portion of curves since apparent inhibition was observed.

Kinetic parameters of PLA activities of the purified enzyme

<table>
<thead>
<tr>
<th>PLA₁ activity&lt;sup&gt;a&lt;/sup&gt;</th>
<th>3 mM CaCl₂</th>
<th>K&lt;sub&gt;m&lt;/sub&gt;&lt;sup&gt;c&lt;/sup&gt; (mM)</th>
<th>V&lt;sub&gt;max&lt;/sub&gt;&lt;sup&gt;c&lt;/sup&gt; (µmol (mg protein)&lt;sup&gt;−1&lt;/sup&gt; min&lt;sup&gt;−1&lt;/sup&gt;)</th>
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<tr>
<td>-</td>
<td>-0.08</td>
<td>0.04</td>
<td></td>
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<tr>
<td>+</td>
<td>-0.09</td>
<td>0.11</td>
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<tr>
<th>PLA₂ activity&lt;sup&gt;b&lt;/sup&gt;</th>
<th>3 mM CaCl₂</th>
<th>K&lt;sub&gt;m&lt;/sub&gt;&lt;sup&gt;c&lt;/sup&gt; (mM)</th>
<th>V&lt;sub&gt;max&lt;/sub&gt;&lt;sup&gt;c&lt;/sup&gt; (µmol (mg protein)&lt;sup&gt;−1&lt;/sup&gt; min&lt;sup&gt;−1&lt;/sup&gt;)</th>
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<tbody>
<tr>
<td>-</td>
<td>-0.02</td>
<td>2.07</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>0.16</td>
<td>5.56</td>
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<sup>a</sup> PLA₁ activity was determined by calculating the radioactivity in palmitic acid released from 1-[<sup>14</sup>C]palmitoyl PC.

<sup>b</sup> PLA₂ activity was determined by extrapolating the amount of linoleic acid released from 2-[<sup>14</sup>C]linoleoyl PC.

<sup>c</sup> Apparent K<sub>m</sub> and V<sub>max</sub> values were determined by extrapolation of the linear portion of curves since apparent inhibition was observed at high concentrations of substrates.

The observation that the PLA₂ activity was demonstrated preferentially against linoleic acid at the sn-2 position suggests a role for patatin in the release of linoleic acid from the phospholipids in the plasma membrane of potato cells. Oxidized linoleic acid elicits the production of phytoalexin in potato (unpublished data). Thus, the liberation of linoleic acid might be an important step in production of derivatives such as endogenous elicitors. An unlikely possibility is that patatin releases arachidonic acid, an elicitor in the production of phytoalexin in potatoes (Bostock et al. 1981) from the membrane phospholipids of <i>P. infestans</i>. This possibility seems unlikely because of the lower PLA₂ activity of patatin against arachidonic acid at the sn-2 position.

We showed here that the PLA₂ activity of patatin depended on pH: it was apparent at pH 7.5 to 9.0 but minimal below pH 7.0. Patatin is localized mainly in the vacuoles of tubers (Sonnensward et al. 1989, 1990), and the pH of vacuoles is known to be rather low. Therefore, the PLA₂ activity of patatin is likely to be regulated by its localization at the subcellular level. It is possible that patatin shows no PLA₂ activity under acidic conditions when localized constitutively in the vacuole and that PLA₂ becomes active under basic conditions when the enzyme is translocated to the cytosol in response to attack by a pathogen or when it is released from broken cells.

The PLA₂ activity of patatin was activated by Ca²⁺ ions (Fig. 6). We showed previously that a partially purified PLA₂ activity was also stimulated by Ca²⁺ ions (Kawakita et al. 1993). In addition, we suggested that the partially purified PLA₂ was activated by a protein kinase (Kawakita et al. 1993). Moreover, cytosolic PLA₂ is activated by protein kinase C in animal cells (McIntyre et al. 1987, Asaoka et al. 1992). Therefore, it seems likely that the activation of the PLA₂ activity of patatin is caused by phosphorylation, but we have no direct evidence for this possibility at present. Perhaps, activation of patatin by Ca²⁺ ions and/or phosphorylation occurs during translocation from the cytosol.
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