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# Purification of two distinct types of phosphoinositide-specific phospholipase C from rat liver

## Enzymological and structural studies

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Two kinds of phosphoinositide-specific phospholipase C (PLC) were purified from rat liver by acid precipitation and several steps of column chromatography. About 50% of the activity could be precipitated when the pH of the liver homogenate was lowered to pH 4.7. The redissolved precipitate yielded two peaks, PLC I and PLC II, in an Affi-gel Blue column, and each was further purified to homogeneity by three sequential h.p.l.c. steps, which were different for the two enzymes. The purified PLC I and PLC II had estimated $M_r$ values of 140000 and 71000 respectively on SDS/polyacrylamide-gel electrophoresis. Both enzymes hydrolysed phosphatidylinositol (PI), phosphatidylinositol 4-phosphate (PIP) and phosphatidylinositol 4,5-bisphosphate ($PIP_2$) in a $Ca^{2+}$- and pH-dependent manner. PLC I was most active at 10 $\mu$M- and 0.1 mM-$Ca^{2+}$ for hydrolysis of PI and $PIP_2$ respectively, whereas PLC II showed the highest activity at 5 mM- and 10 $\mu$M-$Ca^{2+}$ for that of PI and $PIP_2$ respectively. The optimal pH of the two enzymes also differed with substrates or $Ca^{2+}$ concentration, in the range pH 5.0–6.0. Hydrolysis of phosphoinositides by these enzymes was completely inhibited by $Hg^{2+}$ and was affected by other bivalent cations. From data obtained by peptide mapping and partial amino acid sequencing, it was clarified that PLC I and PLC II had distinct structures. Moreover, partial amino acid sequences of three proteolytic fragments of PLC I completely coincided with those of PLC-148 [Stahl, Ferenz, Kelleher, Kriz & Knopf (1988) Nature (London) 332, 269–272].

## INTRODUCTION

It has been well demonstrated that various kinds of neurotransmitters, hormones and growth factors induce an enhancement of phosphoinositide turnover, which is followed by generation of intracellular second messengers (for reviews see refs. [1,2]). In this signal-transduction system, the activation of phosphoinositide-specific phospholipase C (PLC) is induced with receptor stimulation and plays a crucial role in generating second-messenger molecules. Majerus and co-workers (reviewed in ref. [3]) suggested distinct physiological roles in PLC-mediated phosphatidylinositol (PI) hydrolysis and phosphatidylinositol 4,5-bisphosphate ($PIP_2$) hydrolysis, namely that PI hydrolysis by PLC produces enough 1,2-diacylglycerol to activate protein kinase C, whereas $PIP_2$ hydrolysis by PLC predominantly results in an elevation of intracellular $Ca^{2+}$ concentration through generation of inositol 1,4,5-trisphosphate. It might therefore be possible that different PLC enzymes mediate hydrolysis of different substrates. In addition to the above function of PLC, it has been suggested that inositol phospholipids are important in anchoring proteins into the membrane, and that PLC releases these proteins into the cytosol [4]. Several PLC enzymes have been identified from mammalian tissues [5–21], and studies on characterization of PLC enzymes have suggested the multiplicity of PLC enzymes in mammalian tissues [6,8,16,18,21]. Although some explanations, including degradation of the high-$M_r$ PLC and oligomerization of the low-$M_r$ PLC, are possible to account for the multiplicity of the enzyme, it still remains to clarify the physiological function of each PLC molecule in cellular responses against external stimuli. In the present study, we found a structural heterogeneity of PLC enzyme in rat liver, established purification methods for two forms of PLC and examined their biochemical properties.

## MATERIALS AND METHODS

### Materials

PI (soya bean) was purchased from Serdary Research Laboratories. PIP and $PIP_2$ were isolated by the method of Schacht [22] from bovine spinal cord. Phosphatidylethanolamine was from Avanti Polar Lipids. [$^3$H]PI (16.3 Ci/mmol) and [$^3$H]PIP (1 Ci/mmol) were from Amersham. [$^3$H]$PIP_2$ (2 Ci/mmol) was from New England Nuclear. *Staphylococcus aureus* V8 protease and the serine proteinase inhibitors PMSF and DFP were from Sigma. *Achromobacter lyticus* proteinase I (Lysilendopeptidase) was from Wako Pure Chemical Industries (Japan). Affi-gel Blue, a silver staining kit and a protein assay kit were purchased from

Abbreviations used: PLC, phosphoinositide-specific phospholipase C; PI, phosphatidylinositol; PIP, phosphatidylinositol 4-phosphate; $PIP_2$, phosphatidylinositol 4,5-bisphosphate; PMSF, phenylmethanesulphonyl fluoride; DFP, di-isopropyl fluorophosphate.

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Bio-Rad Laboratories. Two h.p.l.c. columns, TSKgel SP-5PW (2.15 cm × 15 cm) and TSKgel G3000SW (2.5 cm × 40 cm), were from Tosoh (Japan). Mono Q (HR 5/5) and Mono S (HR 5/5) were from Pharmacia. Vydac $C_{18}$ (0.4 cm × 15 cm) reverse-phase h.p.l.c. columns were from Separations Group (U.S.A.).

### Assay for PLC activity

For measurement of PLC activity during the purification steps, substrate embedded in small unilamellar vesicles was prepared as described by Wilson *et al.* [23]. Assays were performed in a 50 $\mu$l reaction mixture containing 0.5 mM-[$^3$H]PI (800 d.p.m./nmol), 0.2 mM-phosphatidylethanolamine, 2 mM-$CaCl_2$, 1 mg of bovine serum albumin/ml, 50 mM-sodium acetate (pH 5.5) and a sample of the fraction. For the measurement of $Ca^{2+}$-dependent activities, $Ca^{2+}$–EGTA buffer was used to stabilize the free calcium concentration [24] and the reaction was done in 50 mM-Mes/NaOH (pH 6.5) because EGTA had a weak capacity to chelate $Ca^{2+}$ at pH 5.5. For the study of effect of pH or substrates on enzyme activity, a variety of buffer systems or small unilamellar vesicles containing various concentrations of PI, PIP or $PIP_2$ were used instead. Details are described in the Figure legends. The mixture was incubated at 37 °C for 10 min, the reaction was terminated by adding 2 ml of chloroform/methanol (2:1, v/v), and inositol 1-monophosphate was extracted with 0.5 ml of 1 M-KCl. Otherwise, cleaved inositol polyphosphates were extracted with 0.5 ml of 1 M-HCl. A sample (0.7 ml) of the upper aqueous phase was removed for measurement of radioactivity. Products of hydrolysis by PLC were identified by using Dowex 1 anion-exchange resin, as described by Berridge *et al.* [25].

### Purification procedure

**Step 1: preparation of liver extracts.** Rat liver (500 g) was homogenized in 1500 ml of TE buffer [10 mM-Tris/HCl (pH 7.4), 1 mM-EDTA, 0.1 mM-PMSF and 0.1 mM-DFP] with a Waring blender. The homogenate was centrifuged at 100000 ***g*** for 1 h and the supernatant was collected. The supernatant was adjusted to pH 4.7 with 5 M-acetic acid and then incubated for 1 h on ice. Precipitates were collected by centrifugation (12000 ***g***, 1 h) and dissolved in 300 ml of TE buffer. The solution was adjusted to pH 6.3 with 5 M-NaOH and was centrifuged (12000 ***g***, 1 h) to remove insoluble proteins. The resultant supernatant was further adjusted to pH 7.4 with 5 M-NaOH and to 0.1 M-NaCl by adding solid NaCl. This final preparation was used as starting material for the purification.

**Step 2: Affi-gel Blue column chromatography.** The supernatant from the previous step was applied to an Affi-gel Blue column (3 cm × 15 cm), equlibrated with TES buffer (TE buffer containing 0.1 M-NaCl). After washing the column with 500 ml of TES buffer, proteins were eluted from the column with a 0.1–2.0 M-NaCl gradient in TE buffer (1.0 litre); 15 ml fractions were collected. Fractions corresponding to peak I (PLC I) and II (PLC II), shown in Fig. 1, were pooled separately.

**Step 3 for PLC I: cation-exchange h.p.l.c.** The fractions containing PLC I (150 ml) of the previous step were dialysed against 2 litres of MEG buffer [25 mM-Mes/NaOH (pH 6.6), 1 mM-EDTA, 10 % glycerol and 0.1 mM-PMSF] at 4 °C and applied to a SP-5PW h.p.l.c. column (2.15 cm × 15 cm) equilibrated with MEG buffer. The column was eluted at a flow rate of 4 ml/min with a linear 0–0.3 M-NaCl gradient in MES buffer (160 ml). Activity was eluted at 0.14 M-NaCl, and the corresponding fractions were pooled.

**Step 4 for PLC I: gel-filtration h.p.l.c.** The pooled fractions (30 ml) of step 3 for PLC I were concentrated to 6 ml by reverse dialysis against 30 % poly(ethylene glycol) 6000. A portion of concentrated fraction was subjected to gel filtration on G3000SW (2.5 cm × 40 cm) equilibrated with TESG buffer [20 mM-Tris/HCl (pH 7.4), 1 mM-EDTA, 0.1 M-NaCl, 10 % glycerol and 0.1 mM-PMSF]. Flow rate was 2 ml/min. Activity was eluted at 58 min, and the corresponding fractions were pooled.

**Step 5 for PLC I: Mono Q anion-exchange h.p.l.c.** The pooled fractions (45 ml) from triplicate batches of G3000SW chromatography were applied directly to a Mono Q HR5/5 column, equilibrated with TESG buffer, which was then eluted at a flow rate of 1 ml/min with a linear 0.1–0.4 M-NaCl gradient in TESG buffer (20 ml). Activity was eluted at 0.2 M-NaCl and the fractions in the peak were pooled and stored in portions at −80 °C.

**Step 3 for PLC II: cation-exchange h.p.l.c.** The fractions corresponding to PLC II (90 ml) from step 2 were dialysed against 1 litre of MEG buffer and applied to a SP-5PW column (2.15 cm × 15 cm), equilibrated with MEG buffer, which was eluted at a flow rate of 4 ml/min with a linear 0–0.4 M-NaCl gradient (160 ml). Activity was eluted at 0.2 M-NaCl and fractions were pooled.

**Step 4 for PLC II: Mono S cation-exchange h.p.l.c.** After dilution of pooled fractions from the previous step with an equal volume of MEG buffer, the sample was applied to Mono S HR5/5 equilibrated with MEG buffer, and eluted at a flow rate of 1 ml/min with a linear 0–0.3 M-NaCl gradient (30 ml). Activity was eluted at 0.2 M-NaCl and fractions were pooled.

**Step 5 for PLC II: anion-exchange h.p.l.c.** The active fractions containing PIC II (3 ml) were dialysed against 200 ml of TESG buffer and applied to Mono Q HR5/5 equilibrated with the same buffer. The elution was carried out with a linear 0.1–0.3 M-NaCl gradient in TESG buffer (20 ml). Activity was eluted at 0.2 M-NaCl, and the fractions in the peak were pooled and stored in portions at −80 °C.

### Peptide mapping on polyacrylamide gel

The final preparations of PLC I (20 $\mu$g) and PLC II (10 $\mu$g) were subjected to SDS/7.5 %-polyacrylamide-gel electrophoresis [27] and electroblotted on to nitrocellulose filters. After transfer, proteins were stained with 0.1 % Ponceau S in 1 % acetic acid, and excess stain was removed with 1 % acetic acid as described by Aebersold *et al.* [26]. The regions corresponding to PLC were cut into small pieces and transferred to small tubes. After removal of Ponceau S with 100 mM-Tris/HCl (pH 9.0), the filter pieces were incubated for 30 min at 37 °C in 1 ml of 1 % acetic acid containing 0.5 % polyvinylpyrrolidone-40. Excess of the latter was removed by washing five times with water. PLC on the nitrocellulose pieces was digested as follows. (1) Lysilendopeptidase:

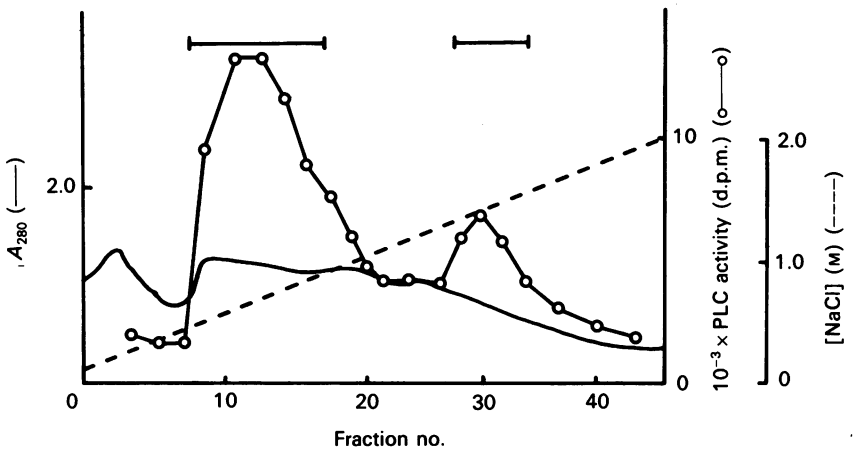


**Fig. 1. Affi-gel Blue column chromatography of PLC**

The PLC fraction after pH precipitation was subjected to Affi-gel Blue column chromatography as described in the Materials and methods section; 15 ml fractions were collected. A portion (5 $\mu$l) of each fraction was assayed for PLC activity.

50 $\mu$l of 100 mM-Tris-HCl (pH 9.0) containing 2 $\mu$g of Lysilendopeptidase. (2) V8 protease: 50 $\mu$l of 100 mM-Tris/HCl (pH 7.4) containing 2 $\mu$g of V8 protease. Incubation was carried out at 37 °C for 18 h. Then 25 $\mu$l of 0.15 M-Tris/HCl (pH 6.8) containing 10% 2-mercaptoethanol, 4.5% SDS and 20% glycerol was added to the reaction mixture, and boiled for 5 min. The whole reaction mixture was analysed by SDS/15%-polyacrylamide-gel electrophoresis, and then the gel was stained with a silver staining kit.

### Partial amino acid sequencing

Internal amino acid sequences were determined by the technique of Aebersold *et al.* [26]. Final preparations of PLC I (100 $\mu$g) and PLC II (50 $\mu$g) were subjected to SDS/polyacrylamide-gel electrophoresis, electroblotted and stained with Ponceau S as described above. The filter pieces containing PLC were incubated at 37 °C for 12 h in 200 $\mu$l of 100 mM-Tris/HCl (pH 9.0), 5% acetonitrile and 6 $\mu$g of Lysilendopeptidase. The supernatant was directly applied to Vydac $C_{18}$ (0.4 cm × 15 cm) reverse-phase h.p.l.c. The peptide fragments were eluted at a flow rate of 1 ml/min with a linear 0–50% (v/v) acetonitrile gradient containing 0.1% trifluoroacetic acid in 50 min. Amino acid sequences of proteolytic fragments were determined by a gas-phase sequencer (Applied Biosystems model 470A).

## RESULTS

### Purification of PLC I and PLC II

About 60% of the total PLC activity was recovered in the soluble fraction of rat liver. Since this soluble fraction was too turbid to be used for column chromatography, we chose the isoelectric-sedimentation procedure as first step of this purification. In brief, the pH of the soluble fraction was lowered to 4.7, and it was incubated for 1 h on ice. The resultant precipitates were dissolved in the pH 6.3 buffer, and insoluble particles were removed from the preparation. Then the supernatant was adjusted to pH 7.4 and used as a starting material for this purification. Half of the soluble PLC activity was recovered in the fraction precipitated at pH 4.7, though only 10% of soluble protein was precipitated by the treatment. This sedimentation procedure seemed to be more effective than the $(NH_4)_2SO_4$ precipitation method and is useful to achieve successful purification.

The enzyme preparation yielded two peaks, PLC I and PLC II, in the first Affi-gel Blue column chromatography (Fig. 1). These two peaks were also obtained when the crude extract was directly applied on Affi-gel Blue. This result indicates that the acid-precipitation procedure neither affects the resolution of the two peaks nor produces any loss of the PLC peaks, suggesting a heterogeneity of PLC enzyme in rat liver. After the first

**Table 1. Purification of PLC I and PLC II from rat liver**

| Purification step | PLC | Total protein (mg) | Total activity ($\mu$mol/min) | Specific activity ($\mu$mol/min per mg) | Purification (fold) | Yield (%) |
|---|---|---|---|---|---|---|
| Cytosol | I+II | 60800 | 198 | 0.00326 | 1.00 | – |
| pH precipitation | I+II | 5840 | 96.5 | 0.0165 | 5.06 | – |
| Affi-gel Blue | I | 555 | 64.2 | 0.116 | 35.6 | 100 |
| | II | 500 | 27.0 | 0.0539 | 16.5 | 100 |
| SP-5PW | I | 35.6 | 16.9 | 0.477 | 146 | 26.3 |
| | II | 10.0 | 8.01 | 0.801 | 246 | 29.7 |
| G3000SW | I | 4.05 | 9.89 | 2.45 | 751 | 15.4 |
| Mono S | II | 1.10 | 1.95 | 1.77 | 543 | 7.23 |
| Mono Q | I | 0.236 | 3.55 | 15.1 | 4630 | 5.53 |
| | II | 0.021 | 0.305 | 14.5 | 4450 | 1.13 |

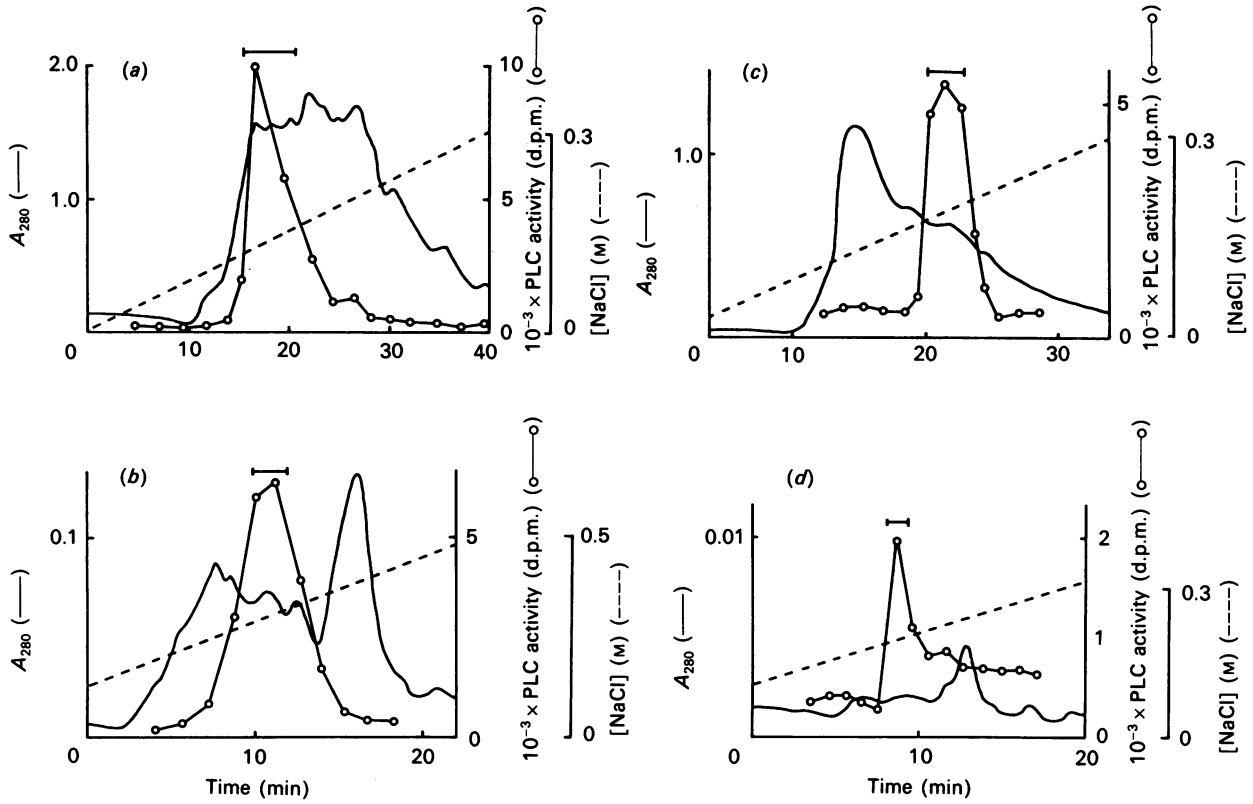


**Fig. 2. H.p.l.c. steps of PLC I and PLC II**

Peak I and peak II fractions after Affi-gel Blue were applied to SP-5PW cation-exchange h.p.l.c. [step 3 for PLC I (*a*) and PLC II (*c*)]. The final step of the purification was carried out by the use of Mono Q anion-exchange h.p.l.c. for PLC I (*b*) and PLC II (*d*). Details are described in the Materials and methods section.

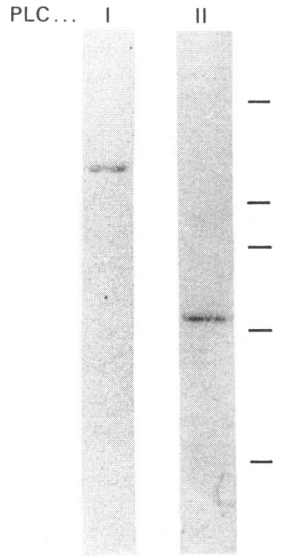


**Fig. 3. Purity of PLC I and PLC II**

Samples of final preparations (10 $\mu$l of PLC I and 40 $\mu$l of PLC II) was subjected to SDS/polyacrylamide (7.5%)-gel electrophoresis. The gel was stained with Coomassie Brilliant Blue. Bars indicate $M_r$ standards: from top, myosin heavy chain (205000), $\beta$-galactosidase (116000), phosphorylase *b* (97000), albumin (68000) and ovalbumin (45000).

column, we employed a h.p.l.c. system to decrease the purification time and increase the reproducibility (Fig. 2). After Affi-gel Blue column chromatography, activity was detected as a single peak in each h.p.l.c. step (Fig. 2). These two enzymes were purified to homogeneity by different procedures, which were not interchangeable.

Our procedure achieved 4630- and 4450-fold purifications and 5.5% and 1.8% recoveries of PLC I and PLC II respectively. The purification steps are summarized in Table 1. The purities of the final preparations were checked by SDS/polyacrylamide-gel electrophoresis (Fig. 3), and PLC I and PLC II were found to have apparent $M_r$ values of 140000 and 71000 respectively. The fact that PLC I was coincidentally eluted from G3000SW with a dimer of bovine serum albumin (results not shown) is also consistent with the $M_r$ estimation by SDS/polyacrylamide-gel electrophoresis. PLC II activity, however, was not recovered from G3000SW, probably owing to non-specific interaction.

### Biochemical properties of purified PLC I and PLC II

Since the two PLC enzymes showed complete dependency on $Ca^{2+}$ for their activity, we first examined the effect of $Ca^{2+}$ concentrations on PLC activity. Free $Ca^{2+}$ concentration was stabilized by using $Ca^{2+}$–EGTA buffer as described in ref. [24]. As shown in Fig. 4, when PI was used as a substrate, PLC I had an optimal concentration of 10 $\mu$M- or 0.1 mM-$Ca^{2+}$, whereas PLC II required more than 1 mM-$Ca^{2+}$. With $PIP_2$, PLC I and PLC II were

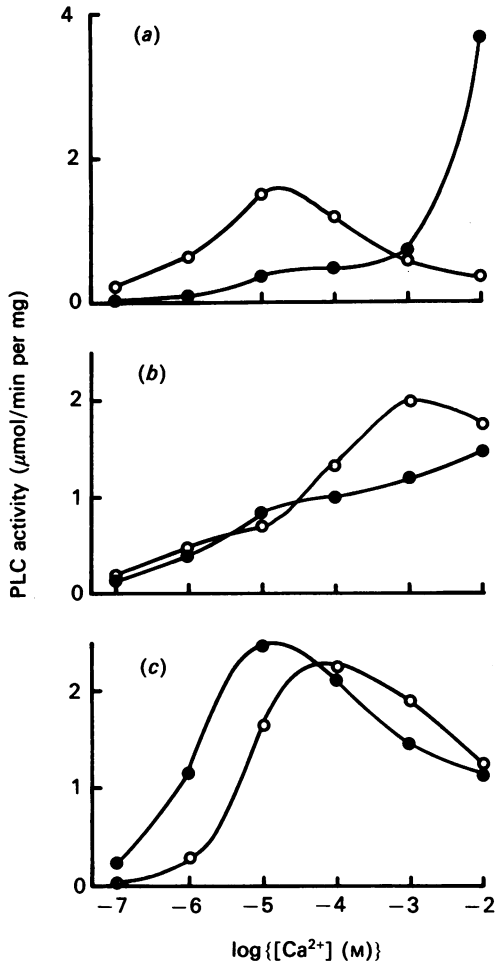


**Fig. 4. Effect of $Ca^{2+}$ on rate of hydrolysis of PI (*a*), PIP (*b*) and $PIP_2$ (*c*)**

PLC I (○) or PLC II (●) activity was measured in the buffer containing various concentrations of $Ca^{2+}$ and 100 μM-PI, -PIP or -$PIP_2$ as substrate. Various free $Ca^{2+}$ concentrations were prepared by using Ca–EGTA buffer [24]. The assay buffer was adjusted to pH 6.5.

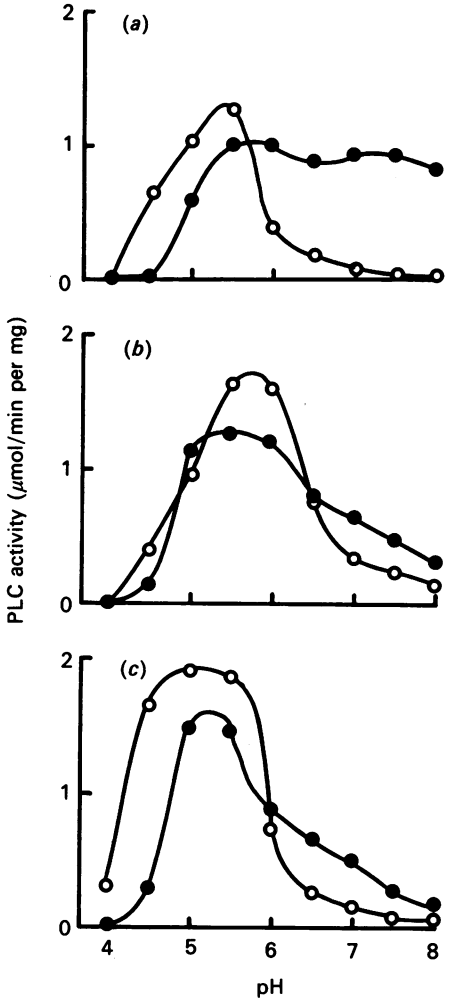


**Fig. 5. Effect of pH on rate of hydrolysis of PI (*a*), PIP (*b*) and $PIP_2$ (*c*)**

PLC I (○) or PLC II (●) activity was determined at various pH values. Sodium acetate (pH 4.0–5.5), Mes/NaOH (pH 5.5–7.0) and Tris/HCl (pH 7.0–8.0) were used to obtain the indicated pH values. The reaction mixture also contained 2 mM-, 1 mM- and 0.1 mM-$Ca^{2+}$ for PLC activity against PI, PIP and $PIP_2$ respectively.

maximally activated by 0.1 mM- and 10 μM-$Ca^{2+}$ respectively. Through the entire concentration ranges of $Ca^{2+}$ examined, neither enzyme could hydrolyse phosphatidylethanolamine, phosphatidylcholine, phosphatidylserine or phosphatidic acid. Activities of both enzymes were totally inhibited by the addition of $Hg^{2+}$ (10 μM) to the reaction mixture, suggesting that a thiol group may play an important role in PLC activity. Adding $Mn^{2+}$, $Cu^{2+}$, $Cd^{2+}$ or $Mg^{2+}$ (0.1 mM) also inhibited $Ca^{2+}$-dependent PLC activity, probably owing to competitive inhibition of $Ca^{2+}$ binding to the enzymes. These two enzymes could hydrolyse three kinds of inositol phospholipid (PI, PIP and $PIP_2$) in a pH-dependent manner (Fig. 5). PLC I had a pH optimum at acidic range (pH 4.5–6.0) against all three substrates, whereas PLC II had a wide pH optimum (pH 5.0–8.0). The effect of substrate concentrations on PLC activity was also studied. Phosphoinositide hydrolysis by PLC I and PLC II was increased in a dose-dependent manner with respect to substrate concentrations up to 0.2 mM, until it reached a plateau at 0.3 mM. The $K_m$ values of the two enzymes for all three phosphoinositides were very similar, 0.12–0.15 mM. The $V_{max.}$ values of PLC I for PI, PIP and $PIP_2$ were 15.1, 18.3 and 20.2 μmol/min per mg of protein respectively, and those of PLC II were 17.2, 20.7 and 23.2 μmol/min per mg of protein respectively. Furthermore, it was confirmed that both PLC I and PLC II produced inositol mono- and tris-phosphate with PI and $PIP_2$ as substrates, respectively, by the method of Berridge *et al.* [25].

### Structural distinction between PLC I and PLC II

To elucidate the differences between PLC I and PLC II, PLC preparations were analysed by peptide mapping technique by SDS/polyacrylamide-gel electrophoresis and reverse-phase h.p.l.c. of proteolytic fragments and showed quite different patterns (Figs. 6 and 7). These data indicate that PLC I and PLC II have different amino acid sequences from each other. Some peptide fragments eluted from reverse-phase h.p.l.c. were further applied to

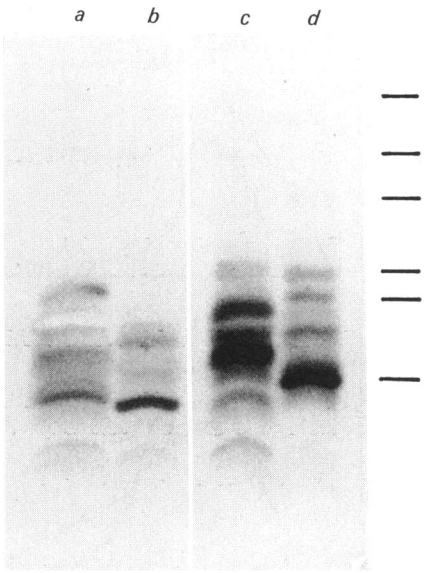


**Fig. 6. Peptide mapping of PLC I and PLC II by SDS/polyacrylamide-gel electrophoresis**

PLC I (*a*, *c*) and PLC II (*b*, *d*) were digested with Lysil-endopeptidase (*a*, *b*) or V8 protease (*c*, *d*) as described in the Materials and methods section. Bars indicate $M_r$ standards: from top, trypsin (24000), myoglobin (16900), CNBr-treated myoglobin fragment I + II (14400), I (8160), II (6210), and III (2510).

an automated gas-phase sequencer (Fig. 7). Three amino acid sequences of proteolytic fragments, I-1, I-2 and I-3, were entirely coincident with deduced amino acid sequences at positions 752–762, 466–481 and 1128–1139 respectively, of PLC-148 [28] (Fig. 7*a*). On the other hand, two fragments of PLC II had quite different sequences from that of PLC-148 (Fig. 7*b*).

## DISCUSSION

In the present study, we established a purification method for two types of PLC, PLC I and PLC II, from rat liver and examined not only their biochemical properties but also their structural distinction. One of the crucial points of purification method is an acid precipitation of the crude extract. This step was very effective for PLC purification, because of an excellent recovery and no bad effect on chromatographic preparation of the enzyme. This result is consistent with the previous report by Ryu *et al.* [14]. An $(NH_4)_2SO_4$ precipitation procedure is not so suitable, because of the low recovery of PLC activity. Introduction of a h.p.l.c. system is also necessary for successful purification, owing to a decrease in purification time and an improvement of reproducibility.

The $M_r$ values of PLC I and PLC II from rat liver were estimated to be 140000 and 71000 respectively, by SDS/polyacrylamide-gel electrophoresis. PLC I seems to be a monomeric form, but not a dimer of PLC II, considering

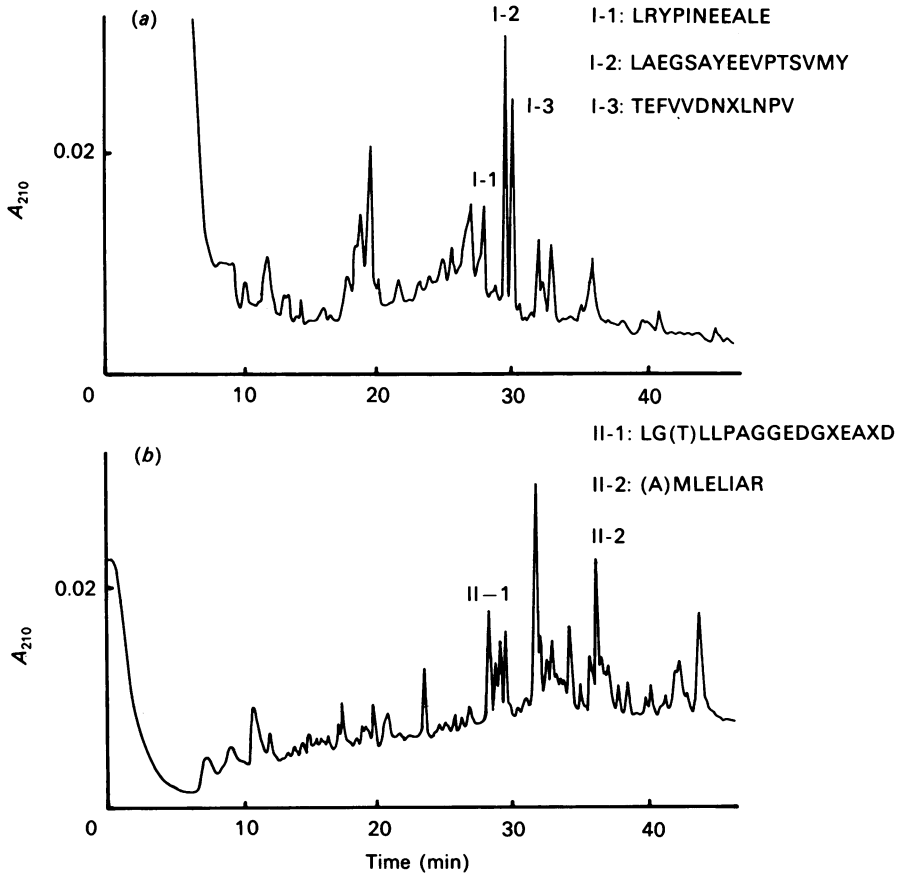


**Fig. 7. Peptide mapping of PLC I (*a*) and PLC II (*b*) by reverse-phase h.p.l.c., and amino acid sequences of the peptide fragments**

PLC I and PLC II were digested with Lysilendopeptidase as described in the Materials and methods section. Amino acid residues are represented by one-letter code: X represents an unidentified amino acid residue. Amino acids in parentheses are not confirmed.

the results from SDS/polyacrylamide-gel electrophoresis of the denatured form, gel filtration, peptide mapping and also amino acid sequencing.

In this paper, we describe the differences of amino acid sequences between two types of PLC from rat liver. Furthermore, from the sequence data, it was suggested that PLC I of rat liver was the same molecule as PLC II of bovine brain [14,28]; however, PLC II of rat liver was a distinct molecule. PLC II may be the same molecule as a PLC form with $M_r$ of 69000 which was reported previously [5].

On the basis of studies of characterization [6,16,18,21] and molecular cloning [28] of PLC enzymes, including our present report, it becomes clear that heterogeneity of PLC enzymes is present in a number of tissues, including liver. Such heterogeneity of PLC enzymes suggests that each PLC molecule plays distinct physiological roles [3]. It has been also proposed that some kinds of guanine nucleotide-binding proteins (G proteins) modulate PLC activity [30,31]. In several tissues, pertussis toxin was effective to inhibit the PLC-mediated $PIP_2$ degradation [32–37], but not in other tissues [38–40]. Therefore, it seems likely that each type of PLC enzyme is coupled, depending on the structural difference, with a specific G protein and receptor in individual cells. These ideas may suggest that PLC I and PLC II are regulated by different G proteins or other modulators [28,29] and activated in different pathways in cellular activation. To elucidate the above possibilities, molecular cloning of PLC I and PLC II of rat liver would be helpful.

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