A Receptor and G-protein-regulated Polyphosphoinositide-specific Phospholipase C from Turkey Erythrocytes

I. PURIFICATION AND PROPERTIES*

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Eighty-three percent of polyphosphoinositide-specific phospholipase C activity was recovered in a cytosolic fraction after nitrogen cavitation of turkey erythrocytes. This activity has been purified approximately 50,000-fold when compared to the starting cytosol with a yield of 1.7-5.0%. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the phospholipase C preparation revealed a major polypeptide of 150 kDa. The specific activity of the purified enzyme was 6.7-14.0 μmol/min/mg of protein with phosphatidylinositol 4,5-bisphosphate or phosphatidylinositol 4-phosphate as substrate. Phospholipase C activity was markedly dependent on the presence of Ca+++. The phospholipase C showed an acidic pH optimum (pH 4.0). At neutral pH, noncyclic inositol phosphates were the major products formed by the phospholipase C, while at pH 4.0, substantial formation of inositol 1:2-cyclic phosphate derivatives occurred. Properties of the purified 150-kDa turkey erythrocyte phospholipase C were compared with the approximately 150-kDa phospholipase C-α and -γ isoenzymes previously purified with respective sizes determined by SDS-PAGE analysis of 65, 150, 145, 85, and 85 kDa. The entire primary sequence of PLC-α, -β, -γ, and -δ has been determined by cDNA cloning (10-14). Although overall homology is low, PLC-β, -γ, and -δ contain two domains of significant sequence similarity, possibly involved in catalysis. The isoenzymes share some common properties. When assayed with substrates presented as components of phospholipid vesicles or of mixed phospholipid and detergent micelles, each isoenzyme displays dependence on calcium for activity and can hydrolyze all three inositol lipids. However, substrate selectivity is critically dependent on both the composition of the substrate preparation used and on the concentration of Ca++ and pH of the assay medium. The polyphosphoinositides are better substrates for these enzymes than is phosphatidylinositol under physiologically relevant conditions of Ca++ and pH. The molecular properties of members of the family of PLC isoenzymes identified to date recently have been reviewed by Rhce et al (9).

The mechanism(s) by which extracellular signals increase intracellular PLC activity are not yet fully understood. G-proteins play established roles in the stimulatory and inhibitory coupling of cell surface receptors to adenylate cyclase and the regulation of cGMP phosphodiesterase by rhodopsin in retinal rod outer segments (Ref. 15). A substantial body of evidence supports the idea that a G-protein couples cell surface receptors to PLC (Ref. 16). This proposal does not exclude alternative regulatory mechanisms; certain growth factor receptors may modulate PLC activity through a re-

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The abbreviations used are: PLC, phospholipase C; G-protein, guanine nucleotide binding protein; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; PtdIns(4,5)P2, phosphatidylinositol 4,5-bisphosphate; PtdIns4P, phosphatidylinositol 4-phosphate; PtdIns, phosphatidylinositol; Ins(1,4,5)P3, d-myo-inositol 1,4,5-trisphosphate; Ins(1,4)P2, d-myo-inositol 1,4-bisphosphate; Ins(1,2-cyclic,4)P2, d-myo-inositol 1,2-cyclic 4,5-bisphosphate; Ins(1,2-cyclic,4,5)P3, d-myo-inositol 1,2-cyclic 4,5,6-trisphosphate; EGTA, ethylenebis(oxyethylenenitrilo)tetraacetic acid; DTPA, diethylene-triaminepentaacetic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; PMSF, phenylmethylsulfonyl fluoride; FPLC, fast protein liquid chromatography; HPLC, high performance liquid chromatography.
been directly implicated in receptor-stimulated generation of purinergic receptor and G-protein-mediated regulation of properties of a X0-kDa PLC from turkey erythrocytes are agonists in a guanine nucleotide-dependent manner. The activity of this enzyme can be regulated by P2y-purinergic receptor with ['Hlinositol-labeled turkey erythrocyte ghosts, the activity and detergent dependence between the purified PLC are an excellent model cell-free system for studying P2y-phospholipase C (18-21). None of the identified isoenzymes of PLC has recently demonstrated receptor-catalyzed tyrosine-specific phosphorylation (17).

Ghosts prepared by hypotonic lysis of turkey erythrocytes are an excellent model cell-free system for studying P2y-purinergic receptor and G-protein-mediated regulation of PLC (18-21). None of the identified isoenzymes of PLC has been directly implicated in receptor-stimulated generation of inositol lipid-derived second messengers. Turkey erythrocytes offer a homogeneous and readily available source from which we are currently attempting to identify and purify components of the PLC-dependent signalling system. In this paper, we describe the purification and properties of a polyphosphoinositide-specific PLC from the cytosolic fraction of turkey erythrocytes and demonstrate differences in substrate selectivity and detergent dependence between the purified PLC and two mammalian PLCs of similar molecular weight. In the following paper, we demonstrate that, when reconstituted with [1H]inositol-labeled turkey erythrocyte ghosts, the activity of this enzyme can be regulated by P2y-purinergic receptor agonists in a guanine nucleotide-dependent manner.

**Experimental Procedures and Results**

Results related to the purification and general catalytic properties of a 150-kDa PLC from turkey erythrocytes are presented below in Miniprint form. Table 1 summarizes results from a typical purification. A silver-stained SDS-polyacrylamide gel of fractions from a final step of Mono Q anion exchange chromatography and corresponding measurements of PLC activity for each fraction is shown in Fig. 6. On preliminary characterization of the enzyme, we obtained results that were in some respects markedly different from those reported for two similarly sized PLC isoenzymes (PLC-β and -γ) previously purified from bovine brain (6). We therefore undertook a direct comparison of the turkey erythrocyte PLC with PLC-β and -γ.

**Effect of Ca**2+ on Turkey Erythrocyte PLC Activity

The purified PLC was incubated with either PtdIns(4,5)P2, PtdIns4P, or PtdIns under standard assay conditions except that the free ionized calcium concentration in the incubations was varied using calcium EGTA buffers. For the lower values (0.05-0.15 mM), the actual Ca**2+ concentration was measured in parallel incubations using the fluorescent indicator, Fura-2 (33). Activity against the polyphosphoinositides was markedly dependent on Ca**2+, with half-maximal activation occurring at approximately 70 nM Ca**2+ with both substrates (Fig. 9). The PLC displayed some activity in the absence of added Ca**2+. This activity was 8% and 26% of the maximal Ca**2+-stimulated activity observed with PtdIns4P and PtdIns(4,5)P2, respectively. Under the range of assay conditions used, the rate of phosphatidylinositol hydrolysis observed with the purified PLC was at most one-hundredth of that displayed with the polyphosphoinositides as substrates.

**Comparison of the Turkey Erythrocyte PLC with PLC-β and PLC-γ**

**Ca**2+ Dependence and Substrate Selectivity—The high degree of substrate selectivity observed with the 150-kDa turkey erythrocyte PLC differs from that observed for PLC-β and -γ (6), although it should be noted that Katan and Parker (5) reported that a preparation of PLC (subsequently shown to be PLC-β, Ref. 11) purified from detergent extracts of bovine brain membranes hydrolyzed pure polyphosphoinositides some 30-fold faster than PtdIns. Since these earlier observations were made using dispersions of pure inositol lipids and no detergent, it was important to compare directly the turkey erythrocyte PLC under conditions identical with those previously used. As such, substrate was prepared as previously described (6) and consisted of dispersed sonicates of pure PtdIns or PtdIns(4,5)P2 with no detergent or other phospholipids present, each at a concentration of 0.1 mM. The activity of the purified turkey erythrocyte PLC was compared with PLC-β and -γ (Fig. 10). Hydrolysis of PtdIns by all three

<table>
<thead>
<tr>
<th>Table 1: Purification of turkey erythrocyte PLC</th>
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<tr>
<td>Purification activity for each fraction</td>
</tr>
<tr>
<td>Step</td>
</tr>
<tr>
<td>------</td>
</tr>
<tr>
<td>Cytosol</td>
</tr>
<tr>
<td>Resuspended (NH₄)₂SO₄ precipitate</td>
</tr>
<tr>
<td>Q-Sepharose</td>
</tr>
<tr>
<td>Hydroxylapatite</td>
</tr>
<tr>
<td>Heparin-Sepharose</td>
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<tr>
<td>Sephacyrl S-300</td>
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<td>Mono Q</td>
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</table>

* Protein was determined by colorimetric hemoglobin assay.  
* Protein was determined by the method of Bradford (29).  
* Protein was determined by determination of protein absorbance at 280 nm.

**Fig. 6. Examination of the purified PLC by SDS-PAGE.** Fractions 50-60 from a final step of Mono Q anion exchange chromatography were examined by SDS-PAGE, and proteins were detected by silver staining as described under “Experimental Procedures.” The lower panel shows PLC activity (nmol/μl/5 min) determined in each fraction using the methods described under “Experimental Procedures.”
Purification and Properties of Turkey Erythrocyte Phospholipase C

Effect of Sodium Cholate on PLC Activity—The effect of increasing concentrations of sodium cholate on the activity of purified PLC-β, PLC-γ, and the turkey erythrocyte PLC was investigated. The substrate preparation used for these experiments was phospholipid vesicles composed of PtdIns(4,5)P₂, phosphatidylserine, and phosphatidylethanolamine in a molar ratio of 1:1:1 prepared as described above. The concentration of PtdIns(4,5)P₂ was 0.1 mM, and the concentration of Ca²⁺ was 10⁻⁶ M. As previously reported by Ryu et al., the activity of PLC-β and PLC-γ sharply increased in activity as the concentration of detergent was increased with maximal activity attained at 0.05% w/v (Fig. 11). Activity declined rapidly at higher detergent concentrations. By contrast, no effect of 0.05% Na⁺ cholate on the turkey erythrocyte PLC was observed. Higher concentrations of cholate increased activity of the turkey erythrocyte PLC with half-maximal activation occurring at around 0.2% w/v and maximal activity attained at 0.4% w/v. The absolute magnitudes of the maximal increases in enzymic activity observed for PLC-β, PLC-γ, and the turkey erythrocyte PLC were similar (3.11-, 4.21-, and 4.42-fold, respectively).

DISCUSSION

A 150-kDa PLC has been purified from the cytosolic fraction of turkey erythrocytes. When assayed with exogenously provided substrates, the properties of this enzyme are broadly similar to those described for the other identified isoenzymes of PLC (9 and references therein). That is, activity is dependent on Ca²⁺ and displays an acidic pH optimum, and the enzyme can form both cyclic and noncyclic inositol phosphates although only the latter products are detectable at neutral pH. The specific activity of the turkey erythrocyte PLC when assayed with exogenously provided polyphosphoinositide substrates is comparable to that reported for the other isoenzymes of PLC (9). Two isoenzymes of PLC with molecular weights similar to the turkey erythrocyte PLC (PLC-β and PLC-γ) have been purified and their cDNAs have been cloned (5, 6, 10, 11). In accord with published findings (6), we find that these isoenzymes of PLC display similar specific activities for hydrolysis of pure PtdIns and PtdIns(4,5)P₂. However, when directly compared with these isoenzymes of PLC under identical conditions, the turkey erythrocyte PLC hydrolyzes PtdIns(4,5)P₂ approximately 100 times faster than PLC-β and PLC-γ displays high selectivity for polyphosphoinositides over PtdIns.
times faster than PtdIns. Further divergence in properties between the 150 kDa turkey erythrocyte PLC, PLC β, and PLC-γ is also observed with the effect of sodium cholate on rates of substrate hydrolysis. Thus, when added to assay mixtures containing substrates in mixed phospholipid vesicles, low concentrations of sodium cholate (0.05% w/v) stimulate PLC-β and γ and have little effect on the turkey erythrocyte PLC, while stimulation of the turkey erythrocyte enzyme occurs with higher concentrations (>0.15% w/v) of detergent that are non-stimulatory or inhibitory to PLC-β and PLC-γ. It should be noted that, when dispersed in aqueous solution, the polyphosphoinositides have been observed to form micellar structures while PtdIns forms larger vesicular structures (4, 42) so catalytic discrimination between substrates in these forms may represent a substrate aggregation state preference of the PLCs rather than an absolute substrate selectivity. The basis of the effect of anionic bile salt detergents on PLC activity is also poorly understood. Differential effects of detergent have been noted for isoenzymes of phospholipase A2 and variously ascribed to differential susceptibility to inhibition by detergent monomers, to differences in enzyme binding to substrate-containing detergent and phospholipid micelles, and to effects on "surface dilution" of substrate in the micelle (see Ref. 45 for review). Nevertheless, in the comparative experiments described above, the turkey erythrocyte PLC can be distinguished from PLC-β and -γ, and further studies of the structure and function of these enzymes will be required to uncover the basis of this distinction.

As an initial step in this process, by immunoblotting, we have found that the turkey erythrocyte PLC does not react with mixtures of monoclonal antibodies raised against either of these proteins. Based on a number of considerations, the existence of further isoenzymes of PLC with certain sequence similarities to, yet significant divergence from, PLC-β and -γ is not unexpected. Ohita et al. (39) report the sequence of a cDNA derived from a human lymphocyte cDNA library that potentially encodes a protein with some homology to PLC-γ. We have found that antibodies raised against a recombinant form of the protein encoded by this cDNA would not react with the turkey erythrocyte PLC. The genetic lesion of the stock Drosophila visual mutant NorpA involves a mutation of a gene that potentially encodes a protein with considerable sequence similarity to PLC-β and γ (40). Finally, an isoenzyme of PLC with an estimated size of 143 kDa has been purified from bovine platelets (41). No information is available to suggest the relationship of this isoenzyme of PLC to the turkey erythrocyte-derived PLC or indeed to the other identified isoenzymes of PLC.

Previous work from our laboratory has investigated the properties of a receptor- and G-protein-regulated PLC present in turkey erythrocyte ghosts (18–20). The substrate selectivity and propensity to form cyclic inositol phosphate products displayed by this receptor- and guanine nucleotide-activated PLC when acting on endogenously labeled substrates are similar to those of the purified PLC determined using exogenously provided substrates. However, the significance of this similarity is questioned by a series of original observations by Irvine and co-workers (35–37) made using a crude preparation of PLC (38, see also Ref. 9 for review) and further studies on the effect of substrate phospholipid composition on the activity of the 150-kDa turkey erythrocyte PLC are clearly warranted. In broad agreement with the results discussed above, we note that although it shows high activity against substrates presented as mixed phospholipid and detergent micelles, the turkey erythrocyte PLC is considerably less active when incubated with turkey erythrocyte ghosts containing radiolabeled substrates. However, under appropriate conditions, we have found that hormonal activators can stimulate the purified turkey erythrocyte PLC to hydrolyze polyphosphoinositol components of turkey erythrocyte ghost membranes, and this phenomenon is discussed in the following paper (44).

In summary, further work is required to establish the relationship of the turkey erythrocyte PLC to the other members of the family of PLC isoenzymes. The generation of antibodies directed against the turkey erythrocyte PLC and ultimately molecular cloning and the determination of its primary sequence will be important steps in this process.

Acknowledgments—We would like to thank Drs. Sue Goo Rhee, Jim Putney, Arlene Hughes, Debra Horstman, Steve Shears, Len Stephens, Robin Irvine, and Gene Scarborough for much invaluable advice and assistance. We are indebted to Ron Frers and Carolina Turkeys Inc., Mount Olive, NC, for providing unlimited quantities of turkey blood.

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purification and properties of turkey erythrocyte phospholipase c

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supplementary material to a receptor and g-protein-coupled phospholipase c from turkey erythrocytes

1. purification and properties

by andrew j. morriss, garry l. waldo, peter downes, and t. kenneth harden

experimental procedures

materials: Hendry-linked glass beads were prepared from glass-coated
silica gel beads (lot no. 2433) purchased from Fisher Scientific, Fairlawn, nj, using the method of
fmsa and o'connor (1985). Dextran blue (lot no. 4265) was purchased from mce and used as
a marker with gel-bead columns. Erythrocytes from turkey (meleagris gallopavo) were obtained
at a local market in minneapolis, mn, at the rate of 3 kg per week.

preparation of phospholipase c: [125i]-phosphatidylcholine (specific
radioactivity 4.5 mci/mg) was prepared from egg yolk phosphatidylcholine (3 mg/ml)
(wet weight) that was prepared from egg yolk obtained from a local market in
minneapolis, mn, using the method of chung and harden (1984) with
minor modifications. The samples were assayed for phospholipase c activity using the method
of harden et al. (1988). The purified enzyme was stored at -80°c.

preparation of turkey erythrocyte phospholipase c: turkey erythrocyte
phospholipase c was purified from turkey erythrocytes using the method of
harden et al. (1988). The enzyme was stored at -80°c.

preparation of turkey erythrocyte phospholipase c from turkey erythrocytes:
1. purification of turkey erythrocyte phospholipase c

2. purification of turkey erythrocyte phospholipase c from turkey erythrocytes:

3. purification of turkey erythrocyte phospholipase c from turkey erythrocytes:
Purification and Properties of Turkey Erythrocyte Phospholipase C

Table 3. Products formed by the PLC at pH 4.6 and pH 7.5

<table>
<thead>
<tr>
<th>pH of incubation</th>
<th>Product 1</th>
<th>Product 2</th>
<th>Product 3</th>
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<tr>
<td>4.6</td>
<td>1.5</td>
<td>1.5</td>
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</table>

**Table 4.** Purification of PLC by chromatography on O-phosphatase-Sepharose. Fractions were pooled and applied to a column of O-phosphatase-Sepharose (1.6 x 5.0 cm). The column was washed with buffer containing 1.0 M NaCl followed by 5.0 M NaCl. The eluant was collected in 0.5 M fractions.

**Figure 1.** Purification of PLC by chromatography on O-phosphatase-Sepharose. Fractions were pooled and applied to a column of O-phosphatase-Sepharose (1.6 x 5.0 cm). The column was washed with buffer containing 1.0 M NaCl followed by 5.0 M NaCl. The eluant was collected in 0.5 M fractions.

**Figure 2.** Purification of PLC by chromatography on O-phosphatase-Sepharose. Fractions were pooled and applied to a column of O-phosphatase-Sepharose (1.6 x 5.0 cm). The column was washed with buffer containing 1.0 M NaCl followed by 5.0 M NaCl. The eluant was collected in 0.5 M fractions.

**Figure 3.** Purification of PLC by chromatography on O-phosphatase-Sepharose. Fractions were pooled and applied to a column of O-phosphatase-Sepharose (1.6 x 5.0 cm). The column was washed with buffer containing 1.0 M NaCl followed by 5.0 M NaCl. The eluant was collected in 0.5 M fractions.

**Figure 4.** Purification of PLC by chromatography on O-phosphatase-Sepharose. Fractions were pooled and applied to a column of O-phosphatase-Sepharose (1.6 x 5.0 cm). The column was washed with buffer containing 1.0 M NaCl followed by 5.0 M NaCl. The eluant was collected in 0.5 M fractions.

**Table 5.** Purification of PLC by chromatography on O-phosphatase-Sepharose. Fractions were pooled and applied to a column of O-phosphatase-Sepharose (1.6 x 5.0 cm). The column was washed with buffer containing 1.0 M NaCl followed by 5.0 M NaCl. The eluant was collected in 0.5 M fractions.

**Table 6.** Purification of PLC by chromatography on O-phosphatase-Sepharose. Fractions were pooled and applied to a column of O-phosphatase-Sepharose (1.6 x 5.0 cm). The column was washed with buffer containing 1.0 M NaCl followed by 5.0 M NaCl. The eluant was collected in 0.5 M fractions.

**Table 7.** Purification of PLC by chromatography on O-phosphatase-Sepharose. Fractions were pooled and applied to a column of O-phosphatase-Sepharose (1.6 x 5.0 cm). The column was washed with buffer containing 1.0 M NaCl followed by 5.0 M NaCl. The eluant was collected in 0.5 M fractions.

**Table 8.** Purification of PLC by chromatography on O-phosphatase-Sepharose. Fractions were pooled and applied to a column of O-phosphatase-Sepharose (1.6 x 5.0 cm). The column was washed with buffer containing 1.0 M NaCl followed by 5.0 M NaCl. The eluant was collected in 0.5 M fractions.

**Table 9.** Purification of PLC by chromatography on O-phosphatase-Sepharose. Fractions were pooled and applied to a column of O-phosphatase-Sepharose (1.6 x 5.0 cm). The column was washed with buffer containing 1.0 M NaCl followed by 5.0 M NaCl. The eluant was collected in 0.5 M fractions.

**Table 10.** Purification of PLC by chromatography on O-phosphatase-Sepharose. Fractions were pooled and applied to a column of O-phosphatase-Sepharose (1.6 x 5.0 cm). The column was washed with buffer containing 1.0 M NaCl followed by 5.0 M NaCl. The eluant was collected in 0.5 M fractions.

**Table 11.** Purification of PLC by chromatography on O-phosphatase-Sepharose. Fractions were pooled and applied to a column of O-phosphatase-Sepharose (1.6 x 5.0 cm). The column was washed with buffer containing 1.0 M NaCl followed by 5.0 M NaCl. The eluant was collected in 0.5 M fractions.

**Table 12.** Purification of PLC by chromatography on O-phosphatase-Sepharose. Fractions were pooled and applied to a column of O-phosphatase-Sepharose (1.6 x 5.0 cm). The column was washed with buffer containing 1.0 M NaCl followed by 5.0 M NaCl. The eluant was collected in 0.5 M fractions.

**Table 13.** Purification of PLC by chromatography on O-phosphatase-Sepharose. Fractions were pooled and applied to a column of O-phosphatase-Sepharose (1.6 x 5.0 cm). The column was washed with buffer containing 1.0 M NaCl followed by 5.0 M NaCl. The eluant was collected in 0.5 M fractions.

**Table 14.** Purification of PLC by chromatography on O-phosphatase-Sepharose. Fractions were pooled and applied to a column of O-phosphatase-Sepharose (1.6 x 5.0 cm). The column was washed with buffer containing 1.0 M NaCl followed by 5.0 M NaCl. The eluant was collected in 0.5 M fractions.

**Table 15.** Purification of PLC by chromatography on O-phosphatase-Sepharose. Fractions were pooled and applied to a column of O-phosphatase-Sepharose (1.6 x 5.0 cm). The column was washed with buffer containing 1.0 M NaCl followed by 5.0 M NaCl. The eluant was collected in 0.5 M fractions.

**Table 16.** Purification of PLC by chromatography on O-phosphatase-Sepharose. Fractions were pooled and applied to a column of O-phosphatase-Sepharose (1.6 x 5.0 cm). The column was washed with buffer containing 1.0 M NaCl followed by 5.0 M NaCl. The eluant was collected in 0.5 M fractions.

**Table 17.** Purification of PLC by chromatography on O-phosphatase-Sepharose. Fractions were pooled and applied to a column of O-phosphatase-Sepharose (1.6 x 5.0 cm). The column was washed with buffer containing 1.0 M NaCl followed by 5.0 M NaCl. The eluant was collected in 0.5 M fractions.

**Table 18.** Purification of PLC by chromatography on O-phosphatase-Sepharose. Fractions were pooled and applied to a column of O-phosphatase-Sepharose (1.6 x 5.0 cm). The column was washed with buffer containing 1.0 M NaCl followed by 5.0 M NaCl. The eluant was collected in 0.5 M fractions.

**Table 19.** Purification of PLC by chromatography on O-phosphatase-Sepharose. Fractions were pooled and applied to a column of O-phosphatase-Sepharose (1.6 x 5.0 cm). The column was washed with buffer containing 1.0 M NaCl followed by 5.0 M NaCl. The eluant was collected in 0.5 M fractions.

**Table 20.** Purification of PLC by chromatography on O-phosphatase-Sepharose. Fractions were pooled and applied to a column of O-phosphatase-Sepharose (1.6 x 5.0 cm). The column was washed with buffer containing 1.0 M NaCl followed by 5.0 M NaCl. The eluant was collected in 0.5 M fractions.

**Table 21.** Purification of PLC by chromatography on O-phosphatase-Sepharose. Fractions were pooled and applied to a column of O-phosphatase-Sepharose (1.6 x 5.0 cm). The column was washed with buffer containing 1.0 M NaCl followed by 5.0 M Nacl. The eluant was collected in 0.5 M fractions.

**Table 22.** Purification of PLC by chromatography on O-phosphatase-Sepharose. Fractions were pooled and applied to a column of O-phosphatase-Sepharose (1.6 x 5.0 cm). The column was washed with buffer containing 1.0 M NaCl followed by 5.0 M NaCl. The eluant was collected in 0.5 M fractions.
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Figure 4. Purification of PLC by chromatography on Sephacyr S-300 HR. Fractions containing PLC activity from the Sephacryl S-300 HR column were pooled, applied to a column of Sephacyr S-100 HR, the column was eluted and fractions collected and assayed for PLC activity. The section of protein determined by absorbance at 280 nm is also illustrated.

Figure 5. Purification of PLC by chromatography on Mono-Q. Fractions containing PLC activity from the Sephacryl S-300 HR column were pooled and applied to a Mono-Q HR 5/5 column. The column was washed, eluted, and fractions collected and assayed for PLC activity. The section of protein determined by absorbance at 280 nm is also illustrated.

Figure 6. Initial rate of PLC activity, [PtdIns(4,5)P_2] (S) (μM) vs. PLC activity (μmol/min/mg).

Figure 7 (a). Determination of the apparent K_m of the purified PLC for PtdIns(4,5)P_2.

Purified PLC was incubated with (a) PtdIns(4,5)P_2 or (b) PtdIns(4,5)P_3 as described in the text and the initial rate of formation of Ins(1,4,7)P_3 determined. The inset is a plot of substrate concentration divided by initial rate plotted against substrate concentration.

Figure 8. pH dependence of PLC activity. PLC activity against either PtdIns(4,5)P_2 (top panel) or PtdIns(4,5)P_3 (bottom panel) was determined at the pH of the incubations as described in the text. The initial rate of formation of Ins(1,4,7)P_3 was determined. The inset is a plot of substrate concentration divided by initial rate plotted against substrate concentration.