Characterization of partially purified phospholipase C from human platelet membranes

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A phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5) P_2]-hydrolytic activity was found to be present in the human platelet membrane fraction, with 20 % of the total activity of the homogenate. The membrane-associated phospholipase C activity was extracted with 1 % deoxycholate (DOC). The DOC-extractable phospholipase C was partially purified approx. 126-fold to a specific activity of 0.58 μ mol of PtdIns(4,5) P_2 cleaved/min per mg of protein, by Q-Sepharose, heparin-Sepharose and Ultrogel AcA-44 column chromatographies. This purified DOC-extractable phospholipase C had an M_r of approx. 110 000, as determined by Ultrogel AcA-44 gel filtration. The enzyme exhibits a maximal hydrolysis for PtdIns(4,5) P_2 at pH 6.5 in the presence of 0.1 % DOC. The addition of 0.1 % DOC caused a marked activation of both PtdIns(4,5) P_2 and phosphatidylinositol (PtdIns) hydrolyses by the enzyme. The enzyme hydrolysed PtdIns(4,5) P_2 and PtdIns in a different Ca^{2+} -dependent manner; the maximal hydrolyses for PtdIns(4,5) P_2 and PtdIns were obtained at 4 μ M- and 0.5 mM- Ca^{2+} respectively. In the presence of 1 mM- Mg^{2+} , PtdIns(4,5) P_2 -hydrolytic activity was decreased at all Ca^{2+} concentrations examined, but PtdIns-hydrolytic activity was not affected.

INTRODUCTION

In a variety of cell types, agonist stimulation leads to the rapid hydrolysis of polyphosphoinositides by phospholipase C (EC 3.1.4.10) to produce two intracellular messengers, diacylglycerol and inositol trisphosphate (Berridge & Irvine, 1984; Nishizuka, 1984). Phospholipase C enzymes that have been well characterized are soluble proteins (Irvine & Dawson, 1978; Takenawa & Nagai, 1981; Hofmann & Majerus, 1982; Hakata *et al.*, 1982; Low & Weglicki, 1983) and are not polyphosphoinositide-specific (Rittenhouse, 1983; Wilson *et al.*, 1984; Graff *et al.*, 1984; Irvine *et al.*, 1984; Nakanishi *et al.*, 1985; Banno *et al.*, 1986a; Low *et al.*, 1986). Several studies have shown that degradation of [3 H]PtdIns(4,5) P_2 (endogenous substrate) in isolated membranes prelabelled with *myo*-[3 H]inositol is caused by GTP and/or its non-hydrolysable analogues (Litosch *et al.*, 1985; Cockcroft & Gomperts, 1985; Smith *et al.*, 1985; Wallace & Fain, 1985; Gonzales & Crews, 1985; Uhing *et al.*, 1986; Straub & Gershengorn, 1986). Guanine-nucleotide-dependent phospholipase C activation has also been demonstrated by using exogenous [3 H]PtdIns(4,5) P_2 in membranes from feline-sarcoma-virus-transformed cells (Jackowski *et al.*, 1986), rat liver (Melin *et al.*, 1986), and blowfly salivary glands (Litosch & Fain, 1985). These findings indicating guanine-nucleotide-mediated stimulation of polyphosphoinositide-specific phospholipase C in membranes have led us to consider that the membrane-bound phospholipase C enzymes may be distinct from the soluble enzymes.

In platelets, most of the phospholipase C activity is reported to be localized in the cytosol (Billah *et al.*, 1980; Rittenhouse, 1983; Siess & Lapetina, 1983). Furthermore, our and other recent studies have indicated that the cytosolic phospholipase C enzymes also are activated by guanine nucleotides in human platelets (Baldassare &

Fisher, 1986; Banno *et al.*, 1986b; Deckmyn *et al.*, 1986). On the other hand, Rittenhouse (1983) suggested the presence of a particulate-bound phospholipase C showing preferential hydrolysis of PtdIns(4,5) P_2 . The recent evidence has indicated that the agonist-stimulated phosphoinositide hydrolysis in platelets is catalysed by a membrane-bound phospholipase C in the absence of Ca^{2+} (Baldassare & Fisher, 1986) or in the presence of Ca^{2+} and DOC (Plantavid *et al.*, 1986; Hrbolich *et al.*, 1987; Rock & Jackowski, 1987). However, in those experiments, the low concentration of endogenously radiolabelled PtdIns(4,5) P_2 made it difficult to characterize in detail the properties of the membrane-bound phospholipase C. Accordingly, in the present study, we have solubilized a phospholipase C with 1 % DOC from the membrane fraction of human platelets, partially purified it by successive column chromatographies, and then characterized its properties as compared with those of cytosolic enzymes.

MATERIALS AND METHODS

Materials

PtdIns (soya bean) and PtdIns(4,5) P_2 (bovine brain) were obtained from Sigma Chemical Co. [3 H]PtdIns (sp. radioactivity 16.3 Ci/mmol) was obtained from Amersham Corp. [3 H]PtdIns(4,5) P_2 (sp. radioactivity 3.5 Ci/mmol) was obtained from New England Nuclear.

Preparation of particulate fraction and extraction of phospholipase C from human platelets

Outdated human platelet concentrate was centrifuged at 200 *g* for 5 min to remove contaminating red blood cells. Platelets were then collected and washed twice with Tris/bicarbonate buffer, pH 6.8, containing 5 mM-EGTA, by centrifugation at 2000 *g* for 15 min. The washed platelets were resuspended in 20 mM-Tris/maleate buffer (pH 7.0)/2 mM-EGTA/1 mM-phenyl-

methanesulphonyl fluoride (buffer A), and were lysed by sonication in an ice/water bath with a micro-probe (Branson Sonifier, B-12) for 4×15 s. Unbroken platelets were removed by centrifugation (3500 *g* for 10 min) and the supernatant was taken as the platelet homogenate. The particulate and soluble fractions were obtained by centrifugation of the platelet homogenate at 105000 *g* for 60 min. The particulate fraction was washed once with buffer A. The washed pellet was suspended in 2 M-KCl in buffer A and stirred for 12 h at 4 °C. Buffer A was then added to dilute the KCl solution to 1 M, and the suspension was centrifuged for 1 h at 105000 *g* to yield a salt extract and a residual pellet. The residual pellet was resuspended in buffer A containing 2 mM-dithiothreitol and 1% DOC. After stirring for 2 h at 4 °C, the suspension was centrifuged at 105000 *g* for 60 min to obtain a DOC extract.

Purification of the DOC-extractable phospholipase C

Q-Sepharose column chromatography. The enzyme fraction solubilized by DOC (DOC-extractable enzyme) was partially purified as follows. Proteins precipitated with 25–55 % -satd. $(\text{NH}_4)_2\text{SO}_4$ were dissolved in 20 mM-Tris/HCl, pH 7.4, containing 0.1 M-NaCl, 2 mM-dithiothreitol, 0.5 % sodium cholate and 0.5 mM-phenylmethanesulphonyl fluoride (buffer B) and dialysed overnight at 4 °C against the same buffer. The dialysed solution was applied to a Q-Sepharose (superfine grade; Pharmacia Fine Chemicals) column (2.5 cm $\times$ 20 cm) which had been equilibrated with buffer B. The column was washed with 50 ml of the same buffer, and then elution was performed with a linear concentration gradient of NaCl from 0.1 to 0.4 M in 600 ml of buffer B and then with 200 ml of 0.4 M-NaCl in buffer B. Most of the bound phospholipase C activity was eluted between 0.2 M- and 0.3 M-NaCl (Fig. 1a), and active fractions were pooled and dialysed against buffer B containing 1 mM-EDTA.

Heparin-Sepharose column chromatography. The dialysed sample from the Q-Sepharose step was applied to a heparin-Sepharose column (2.0 cm $\times$ 20 cm) equilibrated with buffer B containing 1 mM-EDTA, and the column was washed with the same buffer. The column was eluted with a linear gradient of 0.1–0.6 M-NaCl in buffer B (total 600 ml). Most of the applied protein was not bound to the column, whereas the phospholipase C activity was bound, and was eluted between 0.3 M- and 0.4 M-NaCl as a single peak (Fig. 1*b*). Active fractions were pooled and concentrated to 2 ml in an Amicon concentrator (YM-10 membrane) for the subsequent purification step.

Ultrogel AcA-44 column chromatography. The concentrated sample obtained above was then subjected to gel filtration on a column (2.5 cm $\times$ 90 cm) of Ultrogel AcA-44 equilibrated with buffer C (20 mM-Tris/HCl, 0.3 M-NaCl, 1 mM-dithiothreitol, 0.5% sodium cholate and 1 mM-phenylmethanesulphonyl fluoride, pH 7.4). Phospholipase C activity was eluted as a single peak, whose position was between aldolase (M_r 150 000) and bovine serum albumin (M_r 66 200) markers (Fig. 1c). Active fractions were pooled and concentrated by Amicon concentrator to a small volume and stored at -80°C .

Table 1. Phospholipase C activity in the membrane and cytosol fractions from human platelets

The various fractions were prepared as described in the Materials and methods section. The membrane and cytosolic fractions were obtained by centrifugation of the platelet homogenate at 105 000 *g* for 1 h. The membrane fraction was then washed twice with hypo-osmotic Tris buffer. The supernatants obtained after two washings were combined with the initial supernatant fraction from the homogenate. The washed membrane fraction was treated with 2 M-KCl, and the supernatant was obtained by centrifugation at 105 000 *g* for 1 h (KCl-extract). The unextracted residual pellet was then treated with 1 % DOC, and the supernatant (DOC-extract) and the pellet (DOC-pellet) were separated by the same centrifugation. PtdIns hydrolysis was assayed at pH 6.5 in the presence of 1 mM-Ca²⁺ and 0.1 % DOC, and PtdIns(4,5)P₂ hydrolysis at pH 6.5 in the presence of 6 μ M-Ca²⁺ and 0.1 % DOC. Values are means $\pm$ S.D. for the three separate experiments.

Fraction	PtdIns hydrolysis		PtdIns(4,5) P_2 hydrolysis		Lactate dehydrogenase	
	Specific activity (nmol/mg of protein)	Activity recovered (%)	Specific activity (nmol/mg of protein)	Activity recovered (%)	Specific activity (nmol/mg of protein)	Activity recovered (%)
<i>a</i>) Homogenate	1.31 $\pm$ 0.43	100	4.25 $\pm$ 1.40	100	20.0 $\pm$ 3.8	100
<i>b</i>) Cytosol	2.15 $\pm$ 0.27	99.8 $\pm$ 2.1	4.34 $\pm$ 0.56	79.6 $\pm$ 4.9	24.0 $\pm$ 5.4	98.3 $\pm$ 1.5
<i>c</i>) Membrane	0.22 $\pm$ 0.08	2.7 $\pm$ 0.8 (% of the initial membrane activity)	4.69 $\pm$ 0.59	20.4 $\pm$ 3.6 (% of the initial membrane activity)	—	— (% of the initial membrane activity)
<i>(c-1)</i> KCl-extract	0.26 $\pm$ 0.10	46.7 $\pm$ 11.0	2.32 $\pm$ 0.59	23.9 $\pm$ 4.5	2.30 $\pm$ 0.5	73.2 $\pm$ 5.8
<i>(c-2)</i> DOC-extract	0.21 $\pm$ 0.06	48.2 $\pm$ 7.3	7.60 $\pm$ 2.51	72.7 $\pm$ 10.5	0.31 $\pm$ 0.1	25.3 $\pm$ 8.9
<i>(c-3)</i> DOC-pellet	0.05 $\pm$ 0.02	3.9 $\pm$ 1.5	1.29 $\pm$ 0.37	5.9 $\pm$ 3.5	—	—

Fig. 1. Chromatography of the DOC-extractable phospholipase C on columns of (a) Q-Sepharose, (b) heparin-Sepharose and (c) Ultrogel AcA-44

(a) The DOC extract was applied to a Q-Sepharose column (2.5 cm $\times$ 20 cm) which had been equilibrated with buffer B as described in the Materials and methods section. The column was washed with the same buffer, followed by elution with a linear concentration gradient of 0.1–0.4 M-NaCl. The active fractions, indicated by a bar, were pooled. The phospholipase C activity was assayed as described in the Materials and methods section. $\bullet$, PtdIns(4,5) P_2 hydrolysis in the presence of 6 μ M- Ca^{2+} and 0.1% DOC at pH 6.5; $\blacktriangle$, PtdIns hydrolysis in the presence of 1 mM- Ca^{2+} and 0.1% DOC at pH 7.0; $\circ$, A_{280} ; —, [NaCl]. (b) The pooled enzyme solution was applied to the heparin-Sepharose column (2.0 cm $\times$ 20 cm) equilibrated with buffer B containing 1 mM-EDTA. The enzyme was eluted with a linear concentration gradient of 0.1–0.6 M-NaCl. The active fractions, indicated by a bar, were pooled and concentrated to a small volume. The enzyme activity was assayed as described in (a). (c) The concentrated enzyme solution was applied to the Ultrogel AcA-44 column (2.5 cm $\times$ 90 cm), followed by elution with buffer C as described in the Materials and methods section. The enzyme activity was assayed as described in (a).

Assay for phospholipase C activity

Phospholipase C was assayed by measuring the formation of radioactive inositol phosphates from [3H]-

PtdIns (16.6 μ Ci/nmol) or [3H]PtdIns(4,5) P_2 (3.5 μ Ci/nmol) as described previously (Banno *et al.*, 1986a). The reaction mixture contained Tris/maleate buffer (0.1 M), pH 6.5, 50 mM-KCl, sodium deoxycholate (1 mg/ml),

Table 2. Purification of the DOC-extractable phospholipase C from human platelet membranes

Phospholipase C activity was assayed with PtdIns(4,5) P_2 as substrate as described in the Materials and methods section.

Steps	Total protein (mg)	Total activity (nmol/min)	Specific activity (nmol/min per mg of protein)	Purification (fold)	Yield (%)
Membrane fraction	547.5	2518.5	4.6	1	100
DOC-extract	251.9	1914.1	7.6	1.6	76.3
Q-Sepharose	69.7	1317.2	18.9	4.1	52.3
Heparin-Sepharose	5.7	969.6	170.5	37.1	38.5
Ultrogel AcA-44	0.89	516.3	578.4	125.7	20.5

[3 H]PtdIns(4,5) P_2 (25000 d.p.m.; 0.1 mM) or [3 H]PtdIns (30000 d.p.m.; 0.25 mM) and enzyme preparation in a final volume of 100 μ l. Where indicated, free Ca^{2+} concentrations were maintained by using Ca^{2+} /EGTA buffers containing 2 mM-EGTA (final concn.) and the appropriate amount of $CaCl_2$ to give the desired Ca^{2+} concentration (Raaffaub, 1960). Other variations to this basic assay are indicated in the legends. In some experiments, small unilamellar vesicles consisting of total platelet lipids or phosphatidylethanolamine plus [3 H]-PtdIns(4,5) P_2 (27000 d.p.m./assay) were prepared by sonication in 50 mM-Hepes, pH 7.4, as described by Wilson *et al.* (1984).

Other assays

Lactate dehydrogenase activity was assayed by the method of Bergmeyer & Bernt (1974). The A_{340} was measured by a Hitachi spectrophotometer.

Protein was measured by the Bio-Rad protein assay, with bovine serum albumin as standard.

RESULTS AND DISCUSSION

Despite numerous studies, the intracellular distribution of phospholipase C is still controversial. It is not proved, but only suggested, that most of the enzyme activity for hydrolysis of PtdIns [and PtdIns(4,5) P_2] is located in the cytosol, and the rest of phospholipase C, showing a preferential hydrolysis of PtdIns(4,5) P_2 , is associated with membranes. Whereas the cytosolic phospholipase C has been purified to homogeneity from several types of cells, e.g. liver (Takenawa & Nagai, 1981), seminal gland (Hofmann & Majerus, 1982), platelets (Hakata *et al.*, 1982) and brain (Ryu *et al.*, 1986), only sparse information is available for isolation of the membrane-bound phospholipase C enzyme (Wang *et al.*, 1986).

As for platelets, much less is known about the membrane-bound phospholipase C. In this context, we are far from a full understanding of the activation mechanism of phospholipase C coupled with receptor-mediated signal transduction in the platelet.

Distribution of phospholipase C in human platelets

To examine the distribution of phospholipase C in human platelets, the enzyme activity was measured with exogenous substrates, [3 H]PtdIns and [3 H]PtdIns(4,5) P_2 , for the membrane and soluble fractions. As shown in Table 1, PtdIns-hydrolytic activity was entirely recovered in the cytosolic fraction, with only very low activity

(2.7 ± 0.8 %) left in the residual pellet. Lactate dehydrogenase activity was also measured in order to estimate cytosolic contamination in the membrane fraction. Both PtdIns-hydrolytic activity and lactate dehydrogenase activity were exclusively located in the cytosolic fraction. A small percentage of PtdIns-hydrolytic activity in the membrane fraction could be accounted for by cytosolic contamination, as suggested by Siess & Lapetina (1983).

On the other hand, for PtdIns(4,5) P_2 hydrolysis most of the activity (79.6 ± 4.9 %) was recovered in the cytosolic fraction, and 20.4 ± 3.6 % was associated with the membrane fraction which had been thoroughly washed with the buffer. The hydrolytic activity of this membrane fraction was much greater for PtdIns(4,5) P_2 than for PtdIns, 0.22 ± 0.08 and 4.69 ± 0.59 nmol/mg of protein for PtdIns and PtdIns(4,5) P_2 respectively. Subsequently, we attempted to solubilize phospholipase C activity from the membranes. About half of the membrane-associated PtdIns hydrolytic activity was released by extraction with 2 M-KCl, and 23.9 ± 4.5 % of its PtdIns(4,5) P_2 -hydrolytic activity was extracted by the same treatment. The residual pellet was then treated with 1 % DOC, and 72.7 ± 10.5 % of total PtdIns(4,5) P_2 -hydrolytic activity was solubilized, suggesting that the phospholipase C hydrolysing PtdIns(4,5) P_2 is tightly associated with membranes. This DOC-extractable enzyme shows a much higher selectivity for PtdIns(4,5) P_2 hydrolysis, 0.21 ± 0.06 nmol/mg of protein for PtdIns and 7.6 ± 2.5 nmol/mg of protein for PtdIns(4,5) P_2 . Taken together, it is conceivable that phospholipase C enzymes showing differences in intracellular distribution and substrate preference may be present in the human platelet. For further characterization, the DOC-extractable enzyme was further purified by Q-Sepharose, heparin-Sepharose and Ultrogel AcA-44 column chromatographies.

Purification and properties of DOC-extractable phospholipase C

The DOC-extractable phospholipase C was eluted as a single peak of activity for both PtdIns(4,5) P_2 and PtdIns hydrolyses through the purification steps employed (Fig. 1). The purification process of the DOC-extractable enzyme is summarized in Table 2, showing approx. 20 % recovery of the initial activity in the membrane fraction. The final step gave rise to the enzyme fraction with a specific activity of 578.4 ± 10.5 and 13.2 ± 3.8 nmol cleaved/min per mg of protein for PtdIns(4,5) P_2 and

Fig. 2. pH-dependency of the partially purified cytosolic and DOC-extractable phospholipase C activities for PtdIns and PtdIns(4,5) P_2 hydrolyses

The buffer used was Tris/maleate (pH 4.0–8.0). The DOC-extractable and cytosolic enzymes eluted from the Ultrogel AcA-44 columns were used as the partially purified enzyme preparations. The enzyme activity was assayed as described in the Materials and methods section. Values are the means of three separate experiments performed in the presence (●) or the absence (○) of added DOC (0.1 %).

PtdIns respectively. The M_r of the partially purified enzyme was approx. 110000, as determined on a calibrated Ultrogel AcA-44 column.

The pH optima for PtdIns and PtdIns(4,5) P_2 hydrolyses by the partially purified DOC-extractable enzyme and the cytosolic enzyme were measured in the presence or absence of added DOC (0.1 %) (Fig. 2). The PtdIns(4,5) P_2 hydrolysis showed different pH optima in the absence or presence of 0.1 % of DOC (Figs. 2a and c). Both enzymes had pH optima in the acid region. However, in the presence of DOC the breakdown of PtdIns(4,5) P_2 was maximal at neutral pH: pH 6.5 for the partially purified DOC-extractable enzyme and pH 7.3 for the cytosolic enzyme. The pH/activity profiles of PtdIns hydrolysis are considerably different from those of PtdIns(4,5) P_2 hydrolysis. The partially purified cytosolic phospholipase C had an optimum pH of 7.0 in the absence of DOC, whereas the enzyme activity was decreased by addition of 0.1 % DOC and the optimal pH was shifted to 6.5 (Fig. 2d). The inhibitory effect of DOC on the cytosolic hydrolytic activity for exogenous

PtdIns was consistent with the observation by Siess & Lapetina (1983). On the other hand, it should be noted that PtdIns hydrolysis by the partially purified DOC-extractable phospholipase C was remarkably enhanced by 0.1 % DOC, with maximal activity at pH 7.3 (Fig. 2b).

As shown in Fig. 3, maximal hydrolytic activities for both PtdIns(4,5) P_2 and PtdIns catalysed by the partially purified DOC-extractable phospholipase C were obtained at 1 mg of DOC/ml, whereas at lower concentrations of DOC (less than 0.5 mg/ml) no significant activity for PtdIns hydrolysis was detectable. These results suggest that the membrane-bound phospholipase C requires some factor(s) that favour its interaction with the substrate in a manner similar to DOC under physiological conditions.

The effect of phospholipids on the activity of the partially purified DOC-extractable phospholipase C was examined. The hydrolysis of PtdIns(4,5) P_2 was enhanced 3-fold in liposomes consisting of phosphatidylethanolamine and PtdIns(4,5) P_2 (10:1 molar ratio).

Fig. 3. Effect of DOC on the partially purified DOC-extractable phospholipase C activity for PtdIns(4,5) P_2 hydrolyses

The enzyme activity was measured as described in the Materials and methods section. ●, PtdIns(4,5) P_2 hydrolysis in the presence of $6\ \mu\text{M}$ - Ca^{2+} , at pH 6.5; ○, PtdIns hydrolysis in the presence of $1\ \text{mM}$ - Ca^{2+} , at pH 7.0. Results are expressed as means of three separate experiments.

Conversely, a decrease in the activity for PtdIns(4,5) P_2 hydrolysis to approx. 20% was observed when a 5-fold molar excess of platelet total membrane lipids was added to PtdIns(4,5) P_2 , probably owing to phosphatidylcholine. Phosphatidylcholine is known to inhibit phospholipase C activity (Irvine *et al.*, 1979; Hofmann & Majerus, 1982).

Previous studies with various cell types have reported that cytosolic phosphatidylinositol-specific phospholipase C is Ca^{2+} -dependent, requiring millimolar concentration of Ca^{2+} to obtain the maximal activity for PtdIns hydrolysis, whereas at lower Ca^{2+} concentration (micromolar) the enzyme preferentially hydrolyses polyphosphoinositides (Wilson *et al.*, 1984; Irvine *et al.*, 1984; Nakanishi *et al.*, 1985; Wang *et al.*, 1986). In our previous work, we demonstrated in human platelets that a cytosolic phospholipase C required a higher Ca^{2+} concentration for hydrolysis of PtdIns than for PtdIns(4,5) P_2 (Banno *et al.*, 1986a). Such Ca^{2+} -dependency was also seen in the partially purified DOC-extractable phospholipase C; the maximal activity for PtdIns hydrolysis was obtained at $0.5\ \text{mM}$ - Ca^{2+} and for PtdIns(4,5) P_2 hydrolysis at $4\ \mu\text{M}$ - Ca^{2+} (Fig. 4). Higher Ca^{2+} concentrations (over $0.1\ \text{mM}$) were rather inhibitory for PtdIns(4,5) P_2 hydrolysis. When the activity was assayed in the presence of $1\ \text{mM}$ - MgCl_2 and $80\ \text{mM}$ - KCl (physiological conditions) (Irvine *et al.*, 1984), PtdIns(4,5) P_2 hydrolytic activity was decreased at all free Ca^{2+} concentrations examined (Fig. 4a), but PtdIns hydrolytic activity was not affected (Fig. 4b).

Although it is still premature to assess the presence of membrane-associated phospholipase C which hydrolyses preferentially PtdIns(4,5) P_2 , the results obtained in this

Fig. 4. Effect of Ca^{2+} concentration on the partially purified DOC-extractable phospholipase C activity

The enzyme activity was measured with PtdIns(4,5) P_2 (●, ■) and PtdIns (○, □) as substrates in the presence (■, □) or absence (●, ○) of $1\ \text{mM}$ - MgCl_2 , as described in the Materials and methods section. Results are expressed as means of three separate experiments.

work lead us to propose that phospholipase C exists in membranes and plays a critical role in signal transduction in the receptor-activated human platelet. In order to prove this proposal, a highly purified membrane phospholipase C is needed for antibody production which would enable immunological identification of cytosolic enzymes.

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