Catalytic properties of a purified phosphatidylinositol-4-phosphate kinase from rat brain

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A phosphatidylinositol-4-phosphate (PIP) kinase activity was purified from rat brain extract through several chromatographic steps to yield an active preparation (specific activity 1 μmol of ^{32}P incorporated into phosphatidylinositol 4,5-bisphosphate/min per mg of protein) with an apparent molecular size of 100–110 kDa in the native form. The isolated PIP kinase required Mg^{2+} (optimally 20–30 mM) for its activity and was not influenced by Ca^{2+} . The enzyme used ATP (K_m 25 μM) and GTP (K_m 133 μM) as phosphate sources and appeared specific for PIP (K_m 3.3 $\mu\text{g}/\text{ml}$) as the lipid substrate. The PIP-phosphorylation reaction was inhibited by micromolar concentrations of heparin [ID_{50} (concn. giving 50% inhibition) 2 $\mu\text{g}/\text{ml}$] and the flavonoid quercetin (ID_{50} 0.2 μM). Whereas heparin behaves as a competitive inhibitor to PIP, quercetin was competitive towards ATP (or GTP). Phosphorylation of the preparation by a highly active purified protein kinase C did not detectably alter PIP kinase activity. Whereas 12-*O*-tetradecanoylphorbol acetate and various phospholipids had no effect, phosphatidylserine elicited a dose-dependent activation of PIP activity. This suggests that a phosphatidylserine–PIP kinase interaction may be considered as a possible regulatory process at the cell-membrane level.

INTRODUCTION

Increased membrane phosphoinositide turnover has been established as a major transduction mechanism in response to a number of cellular effectors, including hormones, neurotransmitters and growth factors (Michell *et al.*, 1984; Berridge, 1984; Berridge & Irvine, 1984; Majerus *et al.*, 1984). Occupancy of the corresponding specific membrane receptor appears to trigger the hydrolytic breakdown of PIP_2 through the activation of a specific phosphodiesterase (phospholipase C). The resulting diacylglycerol and inositol 1,4,5-trisphosphate are suggested to act as second messengers, activating phospholipid/ Ca^{2+} -sensitive protein kinase (protein kinase C) and Ca^{2+} mobilization respectively, and ultimately contributing to the cell response.

However, PIP_2 belongs to a phosphoinositide metabolic cycle, considered to be mostly associated with the inner leaflet of the plasma membrane (Irvine, 1982). It follows that the available PIP_2 pool results from an equilibrium between a degradative pathway involving mono- and di-esterases and anabolic steps reconstituting PIP_2 by phosphorylation of PI and PIP by PI kinase and PIP kinase (Berridge, 1984; Irvine, 1982). Although its link with receptor activation is not yet fully understood, the degradative pathway of PIP_2 and the corresponding specific phospholipase C activity have been most often considered as a key regulatory step (Berridge, 1984; Berridge & Irvine, 1984). However, PI kinase and PIP kinase should also be examined as potential targets in the regulation of phosphoinositide turnover (Michell *et al.*, 1984; Berridge, 1984; Irvine, 1982).

Interest in this area has been widened by the finding

that PI kinase and PIP kinase activities may be associated with the tyrosine kinase oncogenes of the src family (Macara *et al.*, 1984; Sugimoto *et al.*, 1984; MacDonald *et al.*, 1985) and certain growth-factor receptors (Thompson *et al.*, 1985). These observations have led to the suggestion that increased phosphoinositide turnover might be implicated in the mechanism of oncogene-induced cell transformation (Macara *et al.*, 1984; Sugimoto *et al.*, 1984). Other reports (De Chaffoy de Courcelles *et al.*, 1984; Halenda & Feinstein, 1984) described the activation of inositol lipid phosphorylation in human platelets after treatment with an active tumour promoter, phorbol ester, supporting the idea that PI kinase and PIP kinase may be the targets of specific regulation.

As a prerequisite to the study of regulation of PI kinase and PIP kinase in the intact cell, we have prepared a partially purified PIP kinase from rat brain. We report herein some properties of this preparation which appeared sensitive *in vitro* to several effectors of potential interest. This may be especially the case for the selective activation of PIP kinase by phosphatidylserine. On the other hand, treatment of the enzyme preparation by a purified protein kinase C under phosphorylating conditions had no detectable effect on its activity.

MATERIALS AND METHODS

Materials

$[\alpha\text{-}^{32}\text{P}]\text{ATP}$ (20 Ci/mmol) and $[\gamma\text{-}^{32}\text{P}]\text{GTP}$ (10 Ci/mmol) were from Amersham. PI, PIP, PIP_2 , phosphatidylserine, TPA, quercetin and heparin were purchased from Sigma. DEAE-cellulose DE52 and

Abbreviations used: PI, phosphatidylinositol; PIP, phosphatidylinositol 4-phosphate; PIP_2 , phosphatidylinositol 4,5-bisphosphate; TPA, 12-*O*-tetradecanoylphorbol 13-acetate.

phosphocellulose P-11 were obtained from Whatman. Ultrogel AcA 44 was from IBF (France), and silica-gel t.l.c. plates were from Merck.

Protein kinase C was prepared in homogeneous form, by the following procedure (Vilgrain *et al.*, 1985). Briefly, the enzyme from rat brain tissue soluble extract was purified by DEAE-cellulose chromatography (Vilgrain *et al.*, 1984), gel filtration over Ultrogel AcA 44, fast liquid chromatography through a Mono Q column (Sahyoun *et al.*, 1983) and phenyl-Sepharose hydrophobic affinity chromatography (Kikkawa *et al.*, 1982). The preparation had a specific activity of 36 μmol of ^{32}P incorporated/min per mg of enzyme with histone H1 as substrate under standardized conditions (Vilgrain *et al.*, 1984).

Determination of PIP kinase activity

PIP kinase activity was assayed at 22 °C in a 40 μl final reaction volume, consisting of 30 μl of 20 mM-Tris/HCl buffer, pH 7.5, containing 0.1 mM- $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ (radioactivity 100–200 c.p.m./pmol) and 20 mM- MgCl_2 , to which was added 10 μl of a 200 μM -PIP suspension in 100 mM-Tris/HCl buffer, pH 7.5, previously sonicated for 3 min at 30 °C. The reaction was started by the addition of ATP and was terminated 5 min later by addition of 400 μl of ice-cold chloroform/methanol/12 M-HCl (800:400:3, by vol.). These incubation conditions allowed linear incorporation of $[\text{P}^{32}]\text{P}_i$ into PIP for at least 20 min. The lipids were extracted after addition of carrier polyphosphoinositides and separated by t.l.c. as described by Billah & Lapetina (1982). The lipids were detected with iodine vapour, and ^{32}P -labelled spots were detected by autoradiography on Kodak Royal X Omat film. After the spots were scraped from the plates, the amount of radioactivity in PIP_2 was estimated by liquid-scintillation counting in Aquasol (New England Nuclear) scintillation mixture.

Slab gel electrophoresis

This was performed in 12% -polyacrylamide gels in the presence of 0.1% SDS, as described by Laemmli & Favre (1973).

Preparation of soluble and particulate rat brain extracts

Rat brain tissue (24 g) was homogenized in 8 vol. of 20 mM-Tris/HCl buffer, pH 7.5, containing 2 mM-EDTA, 10 mM-EGTA, 0.25 M-sucrose and 50 mM- β -mercaptoethanol. The homogenate was centrifuged at 105000 g for 30 min at 4 °C and the supernatant I was kept. The resulting pellet was resuspended in 20 vol. of homogenization buffer and centrifuged under the same conditions to yield supernatant II. The supernatants I and II were pooled and are referred to as 'soluble fraction'. The final pellet was extracted for 20 min at room temperature in homogenization buffer containing 0.5% Triton X-100. The mixture was centrifuged for 20 min at 15000 g, and the resulting supernatant is referred to as 'particulate extract'.

Partial purification of PIP kinase from rat brain

Rat brain tissue was homogenized at 4 °C in 8 vol. of 20 mM-Tris/HCl buffer, pH 7.5, containing 2 mM-EDTA, 10 mM-EGTA, 0.25 M-sucrose, 50 mM- β -mercaptoethanol and 0.5% Triton X-100. The homogenate was incubated for 20 min at room temperature and sonicated (Sonicator Ultrasons-Annemasse, at a 60 V setting) for 2 min at

0 °C. The extract was ultracentrifuged at 105000 g for 30 min and the resultant supernatant was used as starting material for the partial purification of the enzyme. This was done essentially as described by Van Dongen *et al.* (1984), with the following modifications. After the DEAE-cellulose step, the enzyme was concentrated by Amicon ultrafiltration and chromatographed on a column (1.6 cm $\times$ 95 cm) of Ultrogel AcA 44 equilibrated in 20 mM-Tris/HCl, pH 7.5, containing 2 mM-EDTA, 2 mM-EGTA, 50 mM- β -mercaptoethanol, 0.15 M-NaCl and 2% (v/v) glycerol (buffer A). The enzyme activity was eluted as a symmetrical peak, and the corresponding fractions were pooled and directly applied to a column (2 cm $\times$ 3.2 cm) of phosphocellulose equilibrated in buffer A. The column was developed with a 0.15–1.2 M-NaCl linear gradient in buffer A; the enzyme was eluted at 0.8 M-NaCl. The active fractions were concentrated by ultrafiltration and stored in small portions at -80 °C.

RESULTS

PIP kinase activity in soluble and particulate rat brain extracts

Soluble fraction (105000 g supernatant) and soluble extract from the particulate fraction (105000 g pellet) obtained from a Triton-treated rat brain homogenate were analysed for their PIP kinase content after DEAE-cellulose chromatography. As illustrated in Fig. 1, both preparations contained a PIP kinase activity, eluted from the column between 0.2 M and 0.3 M along the NaCl gradient. When the total activity recovered from each fraction was taken into account, it was calculated that the enzyme exhibited a bimodal distribution, of about 7:3 in favour of the particulate fraction.

Partial purification of PIP kinase activity

PIP kinase was partially purified from rat brain extract by the three chromatographic steps described in the Materials and methods section. Figs. 2 and 3 illustrate the profiles of the enzyme activity eluted from the filtration and phosphocellulose columns respectively. Although at this stage the enzyme was separated from the bulk of proteins and its activity was highly enriched, electrophoretic analysis of the pooled active fractions showed that the preparation was not homogeneous (Fig. 3, inset). Coomassie Blue staining revealed the presence of a major component of apparent M_r 45000, together with several minor additional stained bands. When the Ultrogel AcA 44 column used in the second step of purification was calibrated with proper protein markers (Fig. 2), it could be calculated that the symmetrical peak of PIP kinase activity behaved as a moiety of 100–110 kDa apparent molecular mass. The final purified kinase preparation used in further experiments had a specific activity of 1 μmol of ^{32}P incorporated in PIP/min per mg of protein, and could be stored at -80 °C for at least 3 months without detectable loss of activity.

Catalytic properties of the purified brain PIP kinase

Ion requirement. Mg^{2+} appeared to be an essential cofactor for PIP kinase activity; as shown in Fig. 4, its optimal concentration was 20–30 mM. Mn^{2+} could not substitute for Mg^{2+} , whereas Ca^{2+} (1 mM) had no detectable effect on the PIP kinase activity in the presence of 10 mM- Mg^{2+} .

Fig. 1. PIP kinase activity in the soluble and particulate fractions of rat brain

Rat brain tissue (24 g) was treated as indicated in the Materials and methods section to yield a soluble fraction and a particulate extract. The latter was diluted 10-fold with homogenization buffer before analysis. The soluble fraction (a) and the extract of the particulate fraction (b) were transferred on to DEAE-cellulose columns (2 cm $\times$ 5 cm) equilibrated in 20 mM-Tris/HCl, pH 7.5, containing 2 mM-EDTA, 2 mM-EGTA, 50 mM- β -mercaptoethanol and 2% glycerol. The columns were developed with a linear 0–0.4 M-NaCl gradient (—) in the same buffer. Fractions (1.5 ml) were collected and assayed for PIP kinase activity (●).

Substrate specificity. The purified kinase activity was assayed with diacylglycerol, phosphatidylinositol, PIP or PIP_2 as the lipid substrate, under standardized conditions in the presence of 100 μM - $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ and 10 mM- Mg^{2+} . Incorporation of ${}^{32}\text{P}$ was detectable only with PIP as the lipid substrate. The enzyme thus exhibited a strong lipid specificity as a PIP kinase. Under these conditions, with increasing amounts of PIP in the reaction and from Lineweaver–Burk plots of the data, a K_m value of 3.3 $\mu\text{g}/\text{ml}$ and a V_{max} of 1.1 $\mu\text{mol}/\text{min}$ per mg of protein were calculated for PIP.

The nucleotide specificity was not examined with either $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ or $[\gamma\text{-}^{32}\text{P}]\text{GTP}$ as the phosphate donor in the PIP kinase assay. As illustrated in Fig. 5, the kinase could readily utilize GTP as a phosphate source with an efficiency over 50% that of ATP at the same concentrations. When PIP kinase activity was determined with a set

Fig. 2. Chromatography of PIP kinase on Ultrogel AcA 44

Fractions from the DEAE-cellulose column (Fig. 1) containing PIP kinase activity were pooled and concentrated to 3 ml by Amicon ultrafiltration and chromatographed on a column (1.6 cm $\times$ 95 cm) of Ultrogel AcA 44 equilibrated and eluted with buffer A. The elution was carried out at a flow rate of 15 ml/h, and 2 ml fractions were collected. A_{280} (—) and PIP kinase activity (●) were measured in the eluted fractions. The arrows indicate the exclusion volume (V_0) and the total volume (V_t) of the column and the elution behaviour of the protein markers run under the same conditions for column calibration, with their M_r values ($\times 10^{-3}$) indicated: human IgG (150); bovine serum albumin (68); ovalbumin (45); carbonic anhydrase (30).

of different concentrations of either ATP or GTP, Lineweaver–Burk plots of the data yielded K_m values of 25 and 133 μM respectively, and V_{max} values of 1.08 and 0.07 μmol of ${}^{32}\text{P}$ incorporated/min per mg of protein could be determined for ATP and GTP respectively. Competitive experiments using mixtures of $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ and various unlabelled nucleoside triphosphates were conducted; the results, given in Table 1, confirmed that GTP behaved as an analogue of ATP in the reaction. In addition, UTP and CTP significantly inhibited ${}^{32}\text{P}$ transfer from ATP, thus suggesting that the PIP kinase has a rather broad specificity for the nucleoside phosphate source.

Effectors of PIP kinase activity. Several compounds known to affect selectively the protein phosphotransferase reaction catalysed by different protein kinases were examined for their effect on PIP kinase activity. Polyamines have been shown to activate cyclic nucleotide-independent type II casein kinase (G type) (Cochet & Chambaz, 1983). As illustrated in Fig. 6, PIP kinase was strongly activated in the presence of micromolar concentrations of spermine; at 2 mM- Mg^{2+} , optimal PIP kinase activity was observed with 100–200 μM -spermine. The polycation thus apparently substitutes, at least partly, for Mg^{2+} in the reaction.

On the other hand, heparin (Feige *et al.*, 1980) and the

Fig. 3. Phosphocellulose column chromatography of PIP kinase

Active fractions from the Ultrogel AcA 44 column (Fig. 2) were pooled and fractionated by phosphocellulose chromatography as described in the Materials and methods section. The proteins were eluted by a linear 0.15–1.2 M-NaCl gradient (----) (●) A_{280} and (○) PIP kinase activity were measured in the eluted fractions. Active fractions (25–32) were pooled and concentrated by ultrafiltration. A sample containing 15 μ g of protein was analysed by SDS/polyacrylamide-gel electrophoresis, followed by Coomassie Blue staining (inset).

Fig. 4. Influence of Mg^{2+} on PIP kinase activity

PIP kinase activity was assayed as described in the Materials and methods section in the presence of different Mg^{2+} concentrations.

Fig. 5. Kinetics of PIP kinase activity in the presence of two different nucleotide donors

PIP kinase was measured at different time intervals with either $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ (○) or $[\gamma\text{-}^{32}\text{P}]\text{GTP}$ (●) as nucleotide donor in the reaction.

flavonoid quercetin (Cochet *et al.*, 1982) have been shown to be selective inhibitors of casein kinase G. Fig. 7 shows that both compounds were potent blockers of the PIP kinase reaction; ID_{50} values (concn. giving 50% inhibition) of 2 μ g/ml and 0.2 μ M were determined for heparin and quercetin respectively. More detailed study

aiming to define the nature of the inhibitory properties of the two compounds disclosed that, whereas heparin behaved as a competitive inhibitor of the lipid substrate PIP in the reaction, quercetin was competitive toward the nucleotide substrate ATP (results not shown). An apparent inhibitory constant of 0.2 μ M was calculated for

Table 1. Effect of different nucleoside triphosphates on PIP kinase activity

The reaction was performed with 50 μM -[γ - ^{32}P]ATP in the presence of 500 μM of different unlabelled nucleoside triphosphates.

Addition	^{32}P incorporated (c.p.m./min)
None (control)	40 000
ATP	7 500
GTP	8 500
UTP	18 500
CTP	26 000

Fig. 6. Influence of spermine on PIP kinase activity

The enzyme was assayed as described in the Materials and methods section, except that the Mg^{2+} concentration was scaled down to 2 mM. The spermine concentration in the medium was varied from 0 to 2 mM.

quercetin; since the K_m of the enzyme for ATP was found to be about 25 μM , it may be inferred that quercetin exhibits an affinity for the nucleotide site about two orders of magnitude higher than ATP itself.

Since the major part (approx. 70%) of PIP kinase activity was found associated with the particulate fraction (100 000 g pellet), the influence of several lipid membrane components and potential lipidic messengers were examined. As illustrated in Fig. 8, phosphatidylcholine, phosphatidylglycerol and PI did not affect PIP kinase activity in the range of concentrations tested. Phosphatidylethanolamine weakly favoured the kinase reaction at high concentrations. The striking observation was the high sensitivity of the enzyme to phosphatidylserine. This phospholipid elicited a strong concentration-dependent activation of the kinase, which reached a plateau above 100 μg of phosphatidylserine/ml under these conditions. Potential phospholipid-derived messengers, i.e. diacylglycerol (diolein) and inositol 1,4,5-trisphosphate, as well as TPA had no detectable effect on the enzyme activity. Diacylglycerol did not affect the phosphatidylserine activation curve, whether Ca^{2+} was present or not.

Fig. 7. Effect of heparin and quercetin on PIP kinase activity

The enzyme was incubated with different concentrations of heparin (a) or quercetin (b) and assayed as described in the Materials and methods section.

Fig. 8. Effect of different phospholipids on PIP kinase activity

The enzyme was incubated with increasing concentrations of different phospholipids (Δ , phosphatidylcholine; $\circ$, phosphatidylglycerol; $\times$, phosphatidylinositol; $\blacktriangle$, phosphatidylethanolamine; $\bullet$, phosphatidylserine) and assayed as described in the Materials and methods section.

In another set of experiments, the PIP kinase preparation was incubated with a highly purified protein kinase C from rat brain, under optimal conditions for protein kinase C activity, i.e. in the presence of Ca^{2+} , phosphatidylserine and diolein (Vilgrain *et al.*, 1984). None of these experiments disclosed any detectable change in the PIP kinase-specific activity, whereas at the same time protein kinase C was fully active in control experiments with either histones or cytochrome *P*-450 (Vilgrain *et al.*, 1985) as protein substrates. It may be concluded that, under conditions *in vitro*, as used in the present work, PIP kinase activity is not likely to be regulated by a direct phosphorylation/dephosphorylation mechanism involving protein kinase C.

DISCUSSION

The first aim of this work was the characterization of some functional features of a phospholipid kinase taking part in what can be considered as the anabolic side of the phosphoinositide cycle, namely catalysing the formation of PIP_2 from PIP. Special interest was devoted to the identification of molecular effectors of potential value in future study designed to examine the implication of this enzyme in the mechanism of cell response involving activation of phosphoinositide metabolism.

In agreement with previous reports (Irvine, 1982), PIP kinase activity was found to exhibit a bimodal distribution between particulate and soluble fractions from rat brain homogenate. However, whereas a 7:3 ratio of enzyme activity was found in this work, in favour of the particulate fraction, in previous studies (Kai *et al.*, 1968) about 80% of the PIP kinase activity was recovered in the cytosol fraction. Although we have no explanation for this discrepancy, the gentle homogenization conditions and the solubilization procedure used in the present work might have contributed to a higher enzyme recovery from membrane components.

After three different chromatographic steps, the enzyme obtained from rat brain extract was far from homogeneous; however, the preparation was devoid of any detectable PI kinase activity and appeared specific for PIP as the lipid substrate. To our knowledge, PIP kinase has not yet been obtained in highly purified form (Cooper & Hawthorne, 1976; Shaikh & Palmer, 1977; Desmukh *et al.*, 1984), although work with partially purified preparations has resulted in the proposal that the enzyme may be the target of a regulation in the intact cell (Jolles *et al.*, 1980; Van Dongen *et al.*, 1985). Gispen and co-workers (Jolles *et al.*, 1980; Van Dongen *et al.*, 1985) have described a brain PIP kinase preparation which co-purified as a complex with a 50 kDa phosphoprotein (B-50 protein), and a series of reports from his group resulted in the proposal that B-50 could be a modulator of PIP kinase through a shuttle between a phosphorylated (inhibitory) and a dephosphorylated form (Van Dongen *et al.*, 1985). Purified B-50 protein was suggested to be phosphorylated by the phospholipid/ Ca^{2+} -sensitive protein kinase (protein kinase C) (Aloyo *et al.*, 1983). Our preparation exhibited 45 and 50 kDa protein moieties under denaturing conditions; however, we have no evidence that either of them could represent the B-50 protein. In addition, incubation of the preparation with purified and highly active protein kinase C had no effect on its PIP kinase activity. Clearly, under the conditions used, these observations would not suggest the presence of a regulatory B-50 protein in our preparation and would not support the possibility of a regulation of PIP kinase activity by a direct phosphorylation involving protein kinase C.

Among the molecular structures showing an effect on PIP kinase activity *in vitro*, some may represent potentially valuable tools for further study of the implication of PIP kinase in the cellular machinery. This is the case for heparin and the flavonoid quercetin, which appeared to be potent blockers of the kinase activity at micromolar concentrations. However, it may be recalled that heparin as well as quercetin are also selective inhibitors of a particular type of protein kinase, namely the cyclic nucleotide-independent casein kinase of type II, also termed casein kinase G (Cochet & Chambaz, 1983).

In addition, quercetin, as well as other related flavonoids, are known inhibitors of enzymic activities involving a nucleotide substrate, such as ATPase systems (Graziani *et al.*, 1977). Therefore, possibly non-specific effects should be kept in mind in this context if these drugs are to be employed with intact cells. The inhibitory properties and the mechanism of inhibition by heparin and quercetin of PIP kinase, i.e. the competitive effect toward the substrate and the phosphate donor (ATP) respectively, are interestingly reminiscent of their mechanisms of inhibition of casein kinase G (Cochet & Chambaz, 1983). Another property of casein kinase G, namely the use of GTP almost as well as ATP as the source of phosphate, was also a catalytic character of our PIP kinase preparation. All these somewhat similar catalytic properties may suggest that the active site of both PIP kinase and casein kinase G could present some common structural and/or functional features.

A striking observation in this work was the finding that PIP kinase activity was highly sensitive to the presence of phosphatidylserine, whereas other phospholipids were without effect. The dose-dependent activation of the enzyme by phosphatidylserine observed *in vitro* may suggest a potential regulatory mechanism of PIP kinase activity in the intact cell, since both components may associate in the inner leaflet of the plasma membrane. In this regard, it has been shown that crude PIP kinase from brain tissue was associated with various lipids, including phosphatidylserine (Kai *et al.*, 1968). This property is reminiscent of the phospholipid-dependence of protein kinase C, whose activity is selectively supported by phosphatidylserine and which may shuttle between a cytosolic and a membrane-associated form (Nishizuka, 1984). However, whereas protein kinase C is optimally activated in the presence of phosphatidylserine and dicylglycerol, this is not the case for our PIP kinase preparation. On the other hand, the PIP kinase activity most significantly involved in polyphosphoinositide metabolism in intact cells may be that confined in the membrane compartment (Seyfred *et al.*, 1985). In such an environment, PIP kinase will in fact act not on an isolated PIP substrate but on PIP included in a mixed lipid bilayer, including phosphatidylserine, as well as other phospholipids. One practical suggestion coming from these observations is that PIP kinase may be better routinely assayed in the presence of phosphatidylserine when effectors of its activity are to be examined *in vitro*. It remains to be seen whether cellular effectors such as active phorbol esters, which have been shown to activate PIP_2 generation in stimulated cells (De Chaffoy de Courcelles *et al.*, 1984; Halenda & Feinstein, 1984) (and which showed no direct effect on our preparation *in vitro*), may influence the subcellular distribution of PIP kinase, as observed for protein kinase C in various cell systems (Kraft & Anderson, 1983).

This work was possible thanks to the support of INSERM (U-244), CNRS (Unité Alliée), the Association pour la Recherche sur le Cancer and the Fondation pour la Recherche Médicale. We thank Michelle Keramidas for excellent technical assistance and Sonia Lidy for excellent secretarial assistance.

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Received 7 October 1985/17 January 1986; accepted 25 February 1986