Characterization of a salt-extractable phosphatidylinositol synthase from rat pituitary-tumour membranes

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Solubilization of phosphatidylinositol (PtdIns) synthase (CDP-diacylglycerol: *myo*-inositol 3-phosphatidyltransferase, EC 2.7.8.11) from rat pituitary (GH₃) tumours was investigated. PtdIns synthase activity was partially extracted from crude membranes by 3 M-KCl. Prior separation of membranes revealed that a greater proportion of plasma-membrane PtdIns synthase activity was salt-extractable than was endoplasmic-reticulum activity. The activity of the salt-extracted enzyme was maximized by low concentrations of 3-(3-cholamidopropyl)dimethylammonio-1-propanesulphonate (CHAPS; 0.5 mM), Triton X-100 (0.1 mM) or a phospholipid mixture (0.05 mg/ml), but higher concentrations of detergents were inhibitory. The activity of salt-extracted PtdIns synthase was 0.25 ± 0.08 nmol/min per mg of protein. Salt-extracted PtdIns synthase activity was dependent on Mg²⁺ (maximal at 0.1 mM) and Mn²⁺ (maximal at 5 mM), and its pH optimum was in the range 7.0–7.5. The apparent K_m for *myo*-inositol (in the presence of 0.1 mM-CDP-diacylglycerol) was 0.06 mM, and that for CDP-diacylglycerol (at 0.1 mM-*myo*-inositol) was 0.21 mM. Salt-extracted PtdIns synthase activity was potently inhibited by Ca²⁺ (50% inhibition at 1 μ M), with over 90% inhibition at 10 μ M-Ca²⁺. These data imply the existence of two forms of membrane-associated PtdIns synthase, namely salt-extractable and salt-resistant, with different intracellular localizations. The salt-extractable form of this enzyme may be a useful preparation for further characterization and purification of mammalian PtdIns synthase.

INTRODUCTION

The phospholipase C-mediated hydrolysis of the polyphosphoinositides (PPI) is now widely accepted to be an early event in the mechanism of signal transduction for a wide range of hormones and neurotransmitters [for reviews see Abdel-Latif (1986) and Berridge (1987)]. Although our knowledge of the characteristics of inositol phospholipid metabolism has grown rapidly with the realization of the importance of this messenger-transduction system, our understanding of the interactions and regulation of the individual enzymes involved is still relatively poor. This applies particularly to the phosphatidylinositol (PtdIns) synthase (CDP-diacylglycerol: *myo*-inositol 3-phosphatidyltransferase, EC 2.7.8.11), an enzyme which is functionally linked to the size of the hormone-sensitive PPI pool and to the rate of resynthesis of the inositol lipids during stimulation. The activity of PtdIns synthase is thus likely to influence both the magnitude and the duration of inositol lipid hydrolysis which occurs during stimulation, and hence is central to the regulation of signal transduction.

Previous studies in a number of cell types have suggested that a single enzyme is responsible for the PtdIns synthase activity (Agranoff *et al.*, 1958; Paulus & Kennedy, 1960), and that it resides primarily in the endoplasmic reticulum and Golgi (Van Golde *et al.*, 1974; Williamson & Morre, 1976; Jelsema & Morre, 1978; Batenburg *et al.*, 1985; Bleasdale *et al.*, 1985). However, more recent evidence (Imai & Gershengorn, 1987*a,b*) suggests that considerable activity also resides in the plasma membrane of rat pituitary-tumour (GH₃)

cells. This enzyme activity displays different kinetic characteristics from the endoplasmic-reticulum activity, implying the existence of two PtdIns synthase enzyme activities in this cell type. These findings have potentially important consequences for our understanding of the regulation of PtdIns synthesis, because they raise the possibilities that this enzyme can be regulated by changes in phospholipid content of the plasma membrane, or directly by interaction with other enzymes of inositol lipid metabolism. In addition, such an intracellular location would circumvent the need for PtdIns-transfer proteins to provide new PtdIns to replace that hydrolysed, and provide a functional basis for the existence of metabolically compartmentalized pools of PtdIns.

The purpose of this study was to solubilize and characterize the PtdIns synthase activity from GH₃ cells. To do this, a variety of detergents and other factors were tested in order to solubilize the enzyme activity. Of the conditions examined, incubation with high salt was the most effective factor in allowing recovery of enzyme activity in a soluble form. Moreover, the salt-extractable form was recovered in greater amounts from the plasma membrane than from the endoplasmic-reticulum fractions. These results clearly indicate the existence of two forms of PtdIns synthase activities with dissimilar locations within the cell.

EXPERIMENTAL

Materials

All chemicals were reagent grade or better. Phospholipids, *myo*-inositol, bovine serum albumin, digitonin,

Abbreviations used: PPI, polyphosphoinositides; PtdIns, phosphatidylinositol; CDP-DG, CDP-diacylglycerol; CHAPS, 3-(3-cholamidopropyl)-dimethylammonio-1-propanesulphonate.

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octyl glucoside, Triton X-100, Nonidet P-40 and CHAPS [3-(3-cholamidopropyl)dimethylammonio-1-propane-sulphonate] were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. *myo*-[2-³H]inositol was purchased from New England Nuclear, Boston, MA, U.S.A.

Growth of cells and tumours

GH₃ cells (rat pituitary) were grown as described previously (Imai & Gershengorn, 1987b). GH₃ tumours were grown subcutaneously in female Wistar Furth rats (12–16 weeks old) (Pryor-Jones *et al.*, 1986). Tumours were initiated by inoculation of 10×10^6 GH₃ cells.

Extraction of PtdIns synthase activity from GH₃ tumours

GH₃ tumours were excised from animals when they were 3–5 cm in diameter (weight 20–30 g). Connective tissue was removed, and the tumours were pooled in lysis buffer (1 mM-EGTA, 2 mM-EDTA, 0.5 mM-NaHCO₃, 4 mM-dithiothreitol, 1 mM-phenylmethanesulphonyl fluoride and 10 mM-Hepes, pH 7.5, 4 °C) and homogenized with a Sorvall tissue homogenizer. Cells were disrupted by nitrogen decompression by using a Parr Bomb of 2000 lb/in² (14 MPa). All subsequent steps were performed at 4 °C. After this treatment, the homogenate was centrifuged (450 g for 10 min) to pellet cell debris and nuclei. The supernatant was then centrifuged (30 000 g for 30 min) to yield the crude membrane fraction. PtdIns synthase activity was routinely extracted from the pellet by using 3 M-KCl in the absence of detergent, in a buffer also containing 100 mM-Tris/HCl, pH 7.5, 1 mM-EGTA, 1 mM-phenylmethanesulphonyl fluoride and 1 mM-dithiothreitol. Typically 15 ml of buffer was used per g wet wt. of crude membrane preparation. Membranes and solubilization buffer were stirred vigorously for 1 h to aid extraction, and finally centrifuged (100 000 g for 1 h). The supernatant was transferred to dialysis tubing (molecular-mass cut-off, 12–14 kDa) and dialysed overnight at 4 °C against a sufficient volume of 100 mM-Tris/HCl (pH 7.5)/1 mM-EGTA/0.4 mM-dithiothreitol so that the final concentration of KCl was approx. 0.3 M. During this process, some protein was precipitated, and this was removed by centrifugation (30 000 g for 15 min). The pellet was washed once by rehomogenization and centrifugation with 0.3 M-KCl/100 mM-Tris/HCl, (pH 7.5)/1 mM-EGTA/0.4 mM-dithiothreitol and the supernatant obtained was combined with the previous one. The combined supernatants were then dialysed with a sufficient volume of 100 mM-Tris/HCl (pH 7.5)/1 mM-EGTA/0.4 mM-dithiothreitol/20 % (v/v) glycerol to give a final concentration of 0.14 M-KCl. The extracted preparation was then stored frozen at –70 °C until it was used.

Extraction of PtdIns synthase from the plasma membrane and endoplasmic reticulum of GH₃ cells

In order to obtain a reproducible separation of endoplasmic reticulum and plasma-membrane fractions, it was necessary to use GH₃ cells rather than tumours. These have the advantages that they can be easily lysed and are completely homogeneous. Cells were treated exactly as described by Imai & Gershengorn (1987a), and the membrane pellet obtained was applied to a discontinuous sucrose gradient consisting of 30, 36, 41, 45 and 50 % (w/v) sucrose in lysis buffer. The upper and

lower two bands were collected as the plasma-membrane and endoplasmic-reticulum fractions respectively (Imai *et al.*, 1986). The fractions were washed with lysis buffer and extracted as described above.

Enzyme assay

The standard assay was performed in 140 mM-KCl/100 mM-Tris/HCl (pH 7.5)/6 % glycerol/1 mM-EGTA/3 mM-MgCl₂/3 mM-MnCl₂ containing 0.1 mM-*myo*-[2-³H]inositol (approx. 4×10^4 d.p.m./nmol), 0.1 mM-CDP-DG and 50 µg of membrane protein in a total volume of 0.1 ml at 37 °C for 20 min. In some experiments the composition of the assay buffer was altered, and in these cases the relevant conditions are given in the legend. For experiments in which the free Ca²⁺ concentration was varied, free Ca²⁺ was measured by using quin 2, assuming a *K*_d of 115 nM for Ca²⁺ in the presence of 1 mM-Mg²⁺ (Tsien *et al.*, 1982).

CDP-DG was added to the assay either alone or in combination with detergent or phospholipid. Before addition to the assay system, CDP-DG was dispersed by sonication in the presence of the detergent or phospholipid in assay buffer. In all studies the phospholipid composition was kept constant and contained 22 % (w/w) phosphatidylserine, 22 % (w/w) phosphatidylcholine, 22 % (w/w) phosphatidylethanolamine, 12 % (w/w) sphingomyelin and 22 % (w/w) cholesterol. This mixture is similar to that believed to exist in the inner leaflet of the plasma membrane, except that it contains no PtdIns (Dawson *et al.*, 1980).

The reactions were terminated by adding 1 ml of chloroform/methanol/conc. HCl (100:100:1, by vol.), and the phases were separated with 0.2 ml of 10 mM-EDTA. The upper phase was transferred to separate tubes and the lower phase was washed with 1 ml of pre-equilibrated upper phase. Enzyme activity was routinely quantified by measurement of radioactivity incorporated into the chloroform-soluble lipid product. For the determination of inositol phosphate accumulation, the upper phase was washed with pre-equilibrated lower phase and dried under N₂. Dowex AG1-X2 [formate form; 0.5 ml of 50 % (w/v) in 5 mM-*myo*-inositol] was then added, and the resin was washed with 4 × 3 ml of 5 mM-*myo*-inositol. Inositol phosphates were eluted with 0.5 ml of 1 M-ammonium formate/0.1 M-formic acid. Samples (0.4 ml) were counted for radioactivity in 10 ml of scintillant (Formula 963-NEN).

Protein was determined by the method of Bradford (1976), with bovine serum albumin as a standard.

RESULTS

Solubilization of PtdIns synthase

The crude extranuclear-membrane pellet contained most of the PtdIns synthase activity present in GH₃ tumours, with approx. 2 % also present in the soluble fraction and 1 % in the crude nuclear fraction. Initial trials indicated that Triton X-100 (1 %), Nonidet P-40 (0.5 %) and octyl glucoside (5 %) were ineffective in solubilizing PtdIns synthase activity. CHAPS (0.1 %) and digitonin (0.5 %) were partially successful, solubilizing 10 % and 5 % of the activity, respectively; higher concentrations of CHAPS were inhibitory. Treatment with increasing concentrations of KCl did, however, lead to significant extraction of PtdIns synthase activity.

High-salt extraction of PtdIns synthase activity was dependent on both the volume (results not shown) and the concentration of KCl used (Table 1). The optimal volume and concentration of KCl (≥ 3 M) resulted in a 20–30% yield of synthase activity in a soluble form,

Table 1. Effects of KCl on the extraction from crude membranes and on the activity of PtdIns synthase in the soluble form

[KCl] (M)	Extracted* (% of maximal)	Activity† (% of maximal)
0.14	n.d.‡	100
0.20	—	68 ± 4
0.40	—	35 ± 3
0.60	—	29 ± 2
0.80	—	23 ± 4
1.00	25 ± 3	19 ± 3
1.50	43 ± 21	—
2.00	74 ± 17	21 ± 4
2.50	85 ± 3	—
3.00	95 ± 7	5 ± 5
3.50	100 ± 20	—

* GH₃-cell tumours were grown as described in the Experimental section and the crude membrane fractions were treated with various concentrations of KCl for 1 h at 4 °C. After dialysis to 0.14 M-KCl, each sample was assayed as described in the Experimental section. Results represent means ± S.D. of triplicate determinations from a typical experiment repeated four times. Data are presented in terms of the maximal solubilization achieved (25% of total activity).

† PtdIns synthase activity was extracted with 3 M-KCl and assayed in the presence of various concentrations of KCl. Similar results were obtained with NaCl. Results represent the cumulated data from four experiments (means ± S.E.M.), expressed as a percentage of the maximum enzyme activity assayed in the presence of 0.14 M-KCl.

‡ Not detected.

although the activity remaining in the pellet was typically less than 35% of the original activity. Thus the total activity measured in the extracted pellet plus that in the soluble fraction was always less than that measured in the crude membranes. However, it is not possible to determine whether the high-salt treatment resulted in the loss of PtdIns synthase, or whether the soluble form of the enzyme is less active. This loss of activity leads to some uncertainty in assessing the efficiency of extraction, and it is possible that the values reported are underestimates of the recovery. During dialysis, a significant amount of protein precipitates and some synthase activity remain associated with this precipitate. The degree of precipitation and association of synthase activity with the precipitate were dependent on the ionic strength of the buffer, and this factor dictated the final ionic strength used during dialysis. Most synthase activity was apparently recovered from the precipitate by resuspension of the pelleted material and centrifugation. Once extracted, the enzyme in the soluble form was stable for up to 2 weeks at 4 °C or for up to 2 months at –70 °C. The stability of the enzyme was enhanced by the presence of glycerol (20%, v/v), KCl (140 mM), and Mg²⁺ and Mn²⁺ (both 3 mM) during storage.

Prior separation of the crude membranes into plasma-membrane and endoplasmic-reticulum fractions revealed that the high-salt-extractable portion of enzyme activity was different in the two intracellular locations, even though each fraction contained approximately equal initial activity. After high-salt treatment, 58% of the initial activity remained in the endoplasmic-reticulum fraction, whereas only 17% remained in the plasma-membrane fraction. In contrast, recovery of the soluble form from the plasma-membrane fraction was 230% of that recovered from the endoplasmic-reticulum fraction.

The high-salt-extractable enzyme was further characterized because its enrichment in the plasma membrane suggested that it may play an important role

Fig. 1. Effect of detergents and phospholipids on PtdIns synthase activity

CHAPS (a), Triton X-100 (b) or a phospholipid mixture consisting of 22% (w/w) phosphatidylserine, 22% (w/w) phosphatidylcholine, 22% (w/w) phosphatidylethanolamine, 12% (w/w) sphingomyelin and 22% (w/w) cholesterol (c) were pre-mixed with the CDP-DG and dispersed by brief sonication. PtdIns synthase activity was then assayed for 20 min as described in the Experimental section. Results represent the cumulated data from four experiments (means ± S.E.M.), expressed as a percentage of the maximal enzyme activity.

in the synthesis of PtdIns utilized in PPI-mediated messenger transduction.

Properties of salt-extractable PtdIns synthase

PtdIns synthase activity was linear with time and protein concentration under the assay conditions employed. Specific activity was 0.25 ± 0.08 nmol/min per mg of protein.

In contrast with the degree of extraction, enzyme activity was markedly inhibited by concentrations of KCl or NaCl above 0.14 M (Table 1). The salts were equally inhibitory.

The effects of detergents and phospholipid on enzyme activity were investigated by the dispersion of the detergent or phospholipid mixture with CDP-DG by sonication. Under these conditions, CHAPS, Triton X-100 or the phospholipid mixture produced stimulation of enzyme activity at low concentrations, and inhibition at higher concentrations (Fig. 1). The optimal concentration of CHAPS was 0.5 mM, giving a CDP-DG/CHAPS molar ratio of 1:5, whereas that of Triton X-100 was 0.1 mM, giving a CDP-DG/Triton X-100 molar ratio of 1:1. For the mixture of phospholipids, there was a broader range, from 0.05 to 0.15 mg/ml (w/w ratio with CDP-DG of 2–6:1), of concentrations giving maximal activities. In subsequent experiments CDP-DG was added pre-mixed with phospholipid at a 2:1 (w/w) ratio.

The pH optimum of the synthase was determined by using acetate (pH 3–6), Tris (pH 7–8) and bicarbonate (pH 9–11) buffers (results not shown). The pH optimum was in the range 7.0–7.5.

The effects of bivalent cations on PtdIns synthase activity were measured (Fig. 2). Both Mg^{2+} and Mn^{2+} stimulated PtdIns synthase activity, although with quite different potencies. Mg^{2+} showed a pronounced stimulation at relatively low concentration, with a peak at 0.1 mM, and inhibition at higher concentrations. Mn^{2+} stimulation of activity was maximal at 5 mM, and there was no inhibition up to 50 mM- Mn^{2+} . The maximal stimulations evoked by Mn^{2+} and Mg^{2+} were comparable in magnitude.

Experiments to determine the kinetic parameters of

Fig. 2. Effect of Mg^{2+} or Mn^{2+} on PtdIns synthase activity

Mg^{2+} (○) or Mn^{2+} (●) was added to the assay buffer, and PtdIns synthase activity was assayed under standard conditions. Results represent the cumulated data (means $\pm$ S.E.M.) from four experiments carried out in triplicate, expressed as a percentage of the maximal enzyme activity.

Fig. 3. Kinetic analysis of the PtdIns synthase activity

myo-Inositol (a) or CDP-DG (b) concentrations were varied at constant specific activity, and enzyme activity was assayed under standard assay conditions as described in the text. Data are plotted in the form s/v versus s . Results represent the cumulated data from four experiments carried out in triplicate; units of v are nmol/min per mg of protein.

salt-extractable PtdIns synthase were conducted by using uniform mixed-micelle substrates of phospholipid and CDP-DG at a ratio of 2:1 (w/w). The synthase activity exhibited linear kinetics with respect to both *myo*-inositol (Fig. 3a) and CDP-DG (Fig. 3b), although *myo*-inositol displayed characteristics of substrate inhibition above 1 mM (results not shown). *myo*-Inositol concentration was varied either at constant specific activity, or by varying the specific activity and multiplying by suitable factors. Both methods produced essentially identical results, with an apparent K_m for *myo*-inositol (at 0.1 mM-CDP-DG) of 0.06 mM, and for CDP-DG (at 0.1 mM-*myo*-inositol) of 0.21 mM.

The effect of Ca^{2+} on PI synthase activity was determined by using Ca^{2+} /EGTA buffers. Under the conditions of the assay (1 mM- Mg^{2+}), Ca^{2+} was extremely inhibitory, displaying high potency and efficacy. Ca^{2+} inhibition of PtdIns accumulation was 50% at approx. 1 μ M- Ca^{2+} , and was over 90% at 10 μ M (Fig. 4). In order to verify that this effect was caused by inhibition of PtdIns synthase rather than stimulation of PtdIns degradation by a phospholipase C, a separate series of experiments was carried out. In these experiments, 10 mM-LiCl was included in the assay buffer to prevent the hydrolysis of inositol phosphates to inositol. (Li^+ , up to 20 mM, had no influence on PtdIns synthase activity; results not shown.) [^3H]PtdIns was synthesized *in vitro* in

Fig. 4. Effect of Ca^{2+} on PtdIns synthase activity

Free Ca^{2+} concentrations were varied by using Ca^{2+} /EGTA buffers in the presence of 1 mM- Mg^{2+} without Mn^{2+} . Ca^{2+} concentrations were confirmed by measurement using quin 2. Results represent the cumulated data (means $\pm$ S.E.M.) from four experiments carried out in triplicate, expressed as percentages of the maximal enzyme activity.

the standard assay system for 1 h, and Ca^{2+} was then added to give the same free Ca^{2+} concentrations as above. The incubation was continued for a further 20 min, and [^3H]PtdIns and [^3H]inositol phosphates were measured. Under these conditions, 1 μM - Ca^{2+} or greater inhibited any further increase in [^3H]PtdIns, but there was no accumulation of [^3H]inositol phosphates.

DISCUSSION

In attempts to solubilize mammalian PtdIns synthase, previous investigators have used a variety of detergents and tissues, including Triton X-100 in rat brain (Ghalayini & Eichberg, 1985), sodium cholate in rat liver (Takenawa & Egawa, 1977; Takenawa *et al.*, 1977) and n-octyl glucopyranoside in dog pancreas (Parries & Hokin-Neaverson, 1984). Their findings that different detergents yielded more-active enzyme preparations from one tissue and less-active ones from others have made it very difficult to delineate and compare the properties of these PtdIns synthases. In an attempt to overcome this difficulty, we succeeded for the first time in extracting PtdIns synthase in an active, soluble, form by using high salt alone. We found that 3 M-NaCl or -KCl led to the recovery, after dialysis, of 20–30% of PtdIns synthase activity in the soluble form. However, high-salt treatment also led to a net decrease in total measurable synthase activity that may indicate that the true degree of extraction may be underestimated. This could arise if the native PtdIns synthase is more active when it is associated with cellular membranes, or if the soluble form of the enzyme is no longer capable of access to its substrates. High-salt treatment extracted only a portion of PtdIns synthase activity even under optimal conditions, implying that at least two forms of the enzyme exist that are associated with membranes in different ways. It was noteworthy that a higher proportion of PtdIns synthase activity was extracted by high salt from the plasma-membrane fraction than from the endoplasmic-reticulum fraction. This predominant plasma-membrane location of the high-salt-extractable

enzyme is consistent with the idea that this enzyme may be of particular importance to PPI messenger transduction, where it may be responsible for formation and maintenance of the stimulus-sensitive pool of PtdIns, while the salt-resistant form could generate the stimulus-insensitive pool. By this mechanism, stimulus-sensitive and insensitive pools of PtdIns (Fain & Berridge, 1979; Billah & Lapetina, 1982; Monaco & Woods, 1983; Koreh & Monaco, 1986; Monaco, 1987) could be generated in a functional way within the plasma membrane without the need for discrete anatomic compartmentalization.

The effects of detergents and phospholipids on salt-extracted PtdIns synthase activity found in the present study are similar to observations in previous studies of mammalian enzymes, namely that low concentrations of detergents activate, whereas higher concentrations inhibit, the activity of mammalian PtdIns synthases. Carman & Matas (1981) demonstrated similar biphasic effects of Triton X-100 on PtdIns synthase isolated from yeast. They showed, moreover, that maximal activation occurred at a specific CDP-DG/Triton X-100 ratio, and suggested that the role of the detergent or phospholipid mixture is to form uniform mixed micelles with CDP-DG and thereby provide a surface for catalysis (Carman & Dowhan, 1979).

The mammalian enzymes displayed similar characteristics with respect to Mn^{2+} -dependency and K_m values for CDP-DG. Other characteristics of the high-salt-extracted PI synthase activity from GH_3 cells, however, were different from the properties of the enzymes solubilized from rat liver (Takenawa & Egawa, 1977), rat brain (Ghalayini & Eichberg, 1985) or dog pancreas (Parries & Hokin-Neaverson, 1984). These differences were most obvious in three areas: the pH optima, the K_m for *myo*-inositol and the dependency on Mg^{2+} .

The pH optimum for the high-salt-extracted enzyme from GH_3 tumours was in the range 7.0–7.5, whereas those for the enzymes from rat liver (Takenawa & Egawa, 1977), rat brain (Ghalayini & Eichberg, 1985) or dog pancreas (Parries & Hokin-Neaverson, 1984) were all in the range 8.5–9.0. The K_m for *myo*-inositol for the enzyme characterized here was at 0.06 mM, considerably lower than that for the enzyme activities from rat liver (2.5 mM), rat brain (4.6 mM) or dog pancreas (0.76 mM). The Mg^{2+} optimum (0.1 mM) for the high-salt-extracted enzyme was considerably lower than the values reported for the other enzymes obtained by detergent solubilizations, which had Mg^{2+} optima around 10 mM. Taken together with the marked inhibition of the salt-extracted enzyme activity by detergent, these data suggest that at optimal assay conditions used for the detergent-solubilized preparations the salt-extracted enzyme would show little activity. Since conditions that optimize activity of detergent-solubilized enzymes have been used to assay PtdIns synthase activity during cell-fractionation studies, this could explain the failure of some workers to detect significant PtdIns synthase activity in the plasma membrane (Van Golde *et al.*, 1974; Williamson & Morre, 1976; Jelsema & Morre, 1978). Lastly, the characteristics of the high-salt-extracted enzyme are similar to those of the unextracted enzyme within GH_3 -cell membranes (Imai & Gershengorn, 1987a,b) and are more compatible with the probable physiological conditions than are the detergent-solubilized activities.

The effects of sub-micromolar concentrations of Ca^{2+}

to inhibit PtdIns synthase reported here are similar to those found by Fain & Berridge (1979), Egawa *et al.* (1981), Takenawa & Nagai (1982) and Imai & Gershengorn (1987a). The observation that PtdIns synthase is inhibited by Ca^{2+} within the concentration range present in the cytoplasm of intact cells suggests that this inhibition is significant *in situ*. In fact, this conclusion is consistent with the available data for intact GH_3 cells. In GH_3 cells, re-synthesis of PtdIns is not enhanced during the first 2 min of stimulation by thyrotropin-releasing hormone (Imai & Gershengorn, 1986). This time period coincides with the peak in intracellular free Ca^{2+} concentrations stimulated by this hormone (Gershengorn, 1986), and is thus consistent with a direct inhibitory role of Ca^{2+} on PtdIns synthesis.

In conclusion, we have presented evidence of a novel salt-extractable form of PtdIns synthase that is present in plasma membranes and endoplasmic reticulum of GH_3 pituitary cells. The ability to obtain this enzyme in a soluble form without detergents suggests that it will be a useful preparation for further characterization and purification of mammalian PtdIns synthase. Lastly, although the physiological role for this form of the enzyme is unknown, its predominance within the plasma membrane is consistent with it being involved in generating the pool of PtdIns that is used for signal transduction.

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