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Characteristics of a Phosphatidylinositol Exchange Activity of Soybean Microsomes[1]

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ABSTRACT

Microsome fractions from hypocotyls of dark-grown soybean (*Glycine max* [L.] Merrill) seedlings incorporated *myo*-inositol into phosphatidylinositol by an exchange reaction stimulated by Mn^{2+} (optimum at 10 mM) and cytidine nucleotides (CMP = CDP ≃ CTP) but not by Mg^{2+} or nucleotides other than cytidine nucleotides. The activity was membrane associated, with an optimum pH of 8, stimulated by auxin, and inhibited by certain thiol reagents or by heating above 40°C. With radioactive inositol, phosphatidylinositol was the only radioactive product. That turnover was by *myo*-inositol exchange was verified from experiments where unlabeled inositol replaced already incorporated inositol with approximately the same kinetics as for the incorporation of label. Both the incorporation and the displacement reactions were stimulated by Mn^{2+} and CMP and both were responsive to auxin with comparable dose dependency. Corresponding exchange activities with choline or ethanolamine were not observed. The phosphatidylinositol-*myo*-inositol exchange activity was low or absent from plasma membrane, tonoplast, and mitochondria enriched fractions. The activity co-localized on free-flow electrophoresis and aqueous two-phase partition with NADPH cytochrome *c* reductase and latent IDPase, markers for endoplasmic reticulum and Golgi apparatus, respectively. With microsomes incubated with both ATP and inositol, polyphosphoinositides were unlabeled demonstrating separate locations for the inositol exchange and phosphatidylinositol kinase reactions. Thus, the auxin-responsive inositol turnover activity of soybean membranes is distinct from the usual de novo biosynthetic pathway. It is not the result of a traditional D-type phospholipase and appears not to involve plasma membrane-associated polyphosphoinositide metabolism. It most closely resembles previously described phosphatidylinositol-*myo*-inositol exchange activities of plant and animal endoplasmic reticulum.

Phosphatidylinositol metabolism of soybean membranes assayed *in vitro* has been shown to be influenced by auxin (17) but the specific enzyme system involved was not characterized. In animals, phosphorylated derivatives of PI[3] are involved in signal transduction (1, 2, 15) and it has been established recently that plant cell cultures incorporate externally supplied radioactive *myo*-inositol into several phosphoinositides, mainly, PI, PIP, and PIP_2 (4, 8). The kinases involved in the formation of the latter are located predominantly in the plasma membrane (22). The object of this study was to characterize the auxin-stimulated phosphatidylinositol turnover in comparison with other reactions affecting PI status of plant membranes.

MATERIALS AND METHODS

Plant Material. Seeds of soybean (*Glycine max* (L) Merr., var. Wayne) were soaked in deionized water 4 to 6 h, planted in moist vermiculite, and grown 4 to 5 d in darkness. Two cm long segments, cut 5 mm below the cotyledons, were harvested under normal laboratory conditions and used for isolation of membranes.

Membrane Isolations. Six g of hypocotyl segments were homogenized for 60 to 90 s with a mechanized razor blade chopper (16) in 5 ml of ice cold medium (0.30 M sucrose, 10 mM KCl, 1.0 mM $MgCl_2$, 25 mM Tris/Mes [pH 7.5]). The homogenate was filtered through one layer of Miracloth (Chicopee Mills), and the residue was washed once with 5 ml of homogenization medium. The combined filtrates were centrifuged for 10 min at 10,000 g_{max} (Sorvall HB-4 rotor) and the resulting supernatant was centrifuged for 30 min at 60,000 g_{max} (Beckman SW-28 rotor). The pellet was resuspended in assay medium (0.25 M sucrose, 10 mM KCl, 25 mM Tris/Mes [pH 8.0]).

For subcellular localization studies, the 60,000*g* pellet obtained from 30 to 40 g of hypocotyl segments, was washed in buffer, resuspended and subjected to a free-flow electrophoretic separation as described previously (23) or was drained and subjected to partitioning in a two-phase system (6.4% [w/w] Dextran T500 [Pharmacia], 6.4% [w/w] Polyethylene Glycol 3400 [Union Carbide], 0.25 M sucrose, 5 mM potassium phosphate buffer [pH 6.8] and a plasma membrane fraction isolated according to Kjellbom and Larsson (13).

***Myo*-Inositol Incorporation.** PI-*myo*-inositol exchange was assayed at 25°C for various times as indicated for individual experiments. The reaction mixture contained 0.23 M sucrose, 7.5 mM KCl, 20 mM Tris/HCl (pH 8.0), and [2-^{3}H]-*myo*-inositol (9–26 μ Ci, specific activity 0.9 mCi–16 Ci/mmol, New England Nuclear) and unlabeled *myo*-inositol, divalent cations, cytidine nucleotides, and other additions as indicated in table and figure legends. The amount of protein present in the reaction mixture was within the linear range of the reaction. For incorporation studies, the reaction volume was 200 μl and the reactions were terminated by transfer of 50 μl portions of 2 ml of chloroform: methanol:0.2 M KCl in water (10:20:7). For experiments involving use of prelabeled membranes, the chase reaction volume was 100 or 200 μl and the reaction was terminated by the addition of 2 ml of the chloroform-methanol-0.2 M KCl mixture.

For tissue incorporation of [^{3}H]-*myo*-inositol, portions of 10 hypocotyl segments each were incubated in 1 ml of potassium phosphate buffer 5 mM, 0.1% (w/v) glucose and 10 μCi-labeled compound for 18 h.

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[3] Abbreviations: PI, phosphatidylinositol; PIP phosphatidylinositol monophosphate; PIP_2, phosphatidylinositol diphosphate.

Lipid Extraction and Measurement of Radioactivity. Lipids were extracted from membrane fractions assayed for turnover activities in chloroform:methanol:aqueous 0.2 M KCl (10:20:7, by volume) for at least 1 h at 0 to 4°C after which chloroform and 0.2 M KCl were added (3). The resulting bottom layer was removed and the remaining upper layer was washed once with an equal volume of bottom layer from a freshly prepared mixture of chloroform:methanol:aqueous 0.2 M KCl (2:2:1.8, by volume). The combined lipid-containing bottom layers were washed three times with equal volumes of fresh upper layer and transferred to a scintillation vial. When the solvent had evaporated, radioactivity was determined in the presence of ASC (Amersham) using a Packard Model 3255 liquid scintillation spectrometer. For thin layer chromatography, the combined bottom layers were dried under nitrogen, dissolved in 50 to 100 μl chloroform, and applied to silica gel HR thin layer plates (Merck). Development in the first direction was in chloroform:methanol:28% ammonium hydroxide (13:5:1, by volume) and in the second direction chloroform:acetone:methanol:acetic acid:water (6:8:2:2:1, by volume) (5). The developed plates were exposed to iodine to localize the lipids, followed by autoradiography. The identification of PI was based on co-chromatography of the radioactive material with authentic PI.

For labeling experiments, all values are the means of the lipid extracts of three equal portions from the same sample minus a zero time incubation. For chase experiments, the values are means of two or three different samples from the same prelabeled microsomal membrane fraction, minus a zero chase time. The experiments were repeated two (Tables 3 and 4, Fig. 6) or three or more (all other data) times using different membrane isolates with qualitatively similar results.

For experiments on formation of phosphorylated PIs, membrane fractions or tissue segments were extracted in an acid medium (24) as described (22). The lipid extracts were separated on TLC plates (solvent system chloroform:methanol:25% ammonium hydroxide:water, 45:45:3.5:10, v/v) (24) and bands scraped off the plates and radioactivity was determined as described above. Authentic PI, PIP, and PIP_2 (Sigma) were used as standards.

Protein Determination. The protein contents of the samples were determined according to Lowry *et al.* (14) with BSA as standard.

RESULTS

When incubated with [^{3}H]-*myo*-inositol, the soybean microsomal fraction incorporated radioactivity into chloroform soluble material in a manner proportional to time of incubation (Fig. 1A) and amount of membrane protein added to the incubation mixture (Fig. 1B). Two-dimensional TLC of the chloroform extracted products followed by autoradiography verified that the only radiolabeled compound co-migrated with authentic PI (not illustrated). Unincorporated [^{3}H]-*myo*-inositol accounted for less than 0.5% of the total radioactivity of the lipid extract (Fig. 2B). In contrast to tissue labeled for 18 h with [^{3}H]-*myo*-inositol, where PIP_2, PIP, and PI represented approximately 2, 2, and 55% of all labeled material of the chromatographed lipid extracts (Fig. 2A), no zones (<0.2% of total radioactivity) corresponded to PIP_2 or PIP with the incubated membranes (Fig. 2B). Of the total radioactivity, 95% was present as PI and the distribution of radioactivity was unaffected by incubation with ATP.

Mn^{2+} was required for activity and the optimum concentration was 10 mM (Fig. 1C). The Mn^{2+} could not be substituted by Mg^{2+}, Cu^{2+}, Zn^{2+}, or Ca^{2+}. The exchange activity was eliminated by 5 mM EDTA either in the presence or absence of 10 or 20 mM $MnCl_2$. The pH optimum was 8.0 (Fig. 1D).

Incorporation was not dependent upon, but was stimulated by, the addition of cytidine nucleotides (Tables I and II). The optimum stimulation by CMP occured at 10 to 25 μM nucleotide, while the optimum stimulation by CDP or CTP was at 50 to 100 μM.

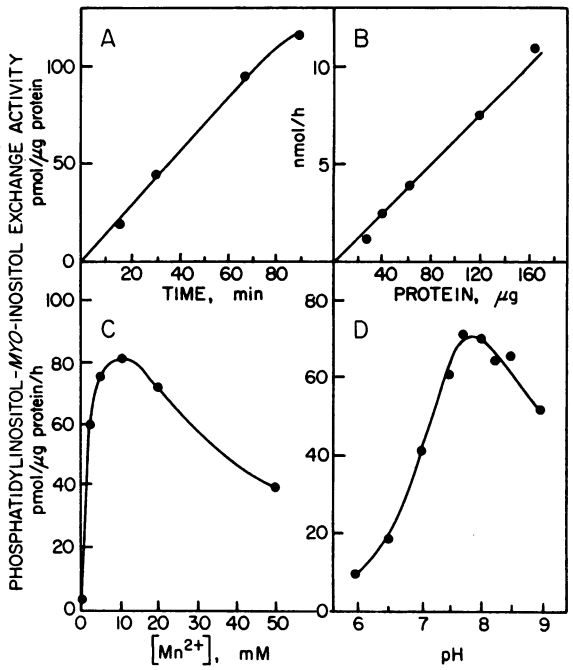

FIG. 1. Characteristics of PI-*myo*-inositol exchange of microsomal membranes isolated from hypocotyls of dark-grown soybean. Reaction medium: 0.23 M sucrose, 7.5 mM KCl, 10 mM $MnCl_2$, 0.5 mM *myo*-inositol (final specific activity of [2-^{3}H]-*myo*-inositol 91 mCi/mmol), 25 mM CMP, and 20 mM Tris/HCl (pH 8.0). A, Time of incubation. B, Amount of protein. Incubation time of 20 min. C, Mn^{2+} concentration. The reaction medium contained 0.23 M sucrose 7.5 mM KCl, 3 μM *myo*-inositol (final specific activity of [2-^{3}H]-*myo*-inositol 15.6 Ci/mmol), and 20 mM Tris/HCl (pH 8.0). Incubation time 10 min. D, Effect of pH. Incubation time 30 min.

The activity was saturable by *myo*-inositol with an apparent K_m of 64 μM (Fig. 3). Additions of 0.1 mM phosphatidic acid or 0.1 mM diacylglycerol resulted in reaction rates of 59 and 77%, respectively, of the control rate.

The [^{3}H]-*myo*-inositol incorporation reaction was almost completely inhibited by 0.1 mM *p*-chloromercuribenzoate, and inhibited about 90% by 1 mM *N*-ethylmaleimide but resisted inhibition by α-iodoacetamide and iodoacetic acid (Table III). Diethyl ether but not Triton X-100 (0.1%) inhibited. Activity was lost by heating above 40°C for 10 min.

That the activity was an exchange of inositol between PI and free inositol was verified by the displacement of incorporated radioactive inositol by unlabeled inositol (Table IV; Figs. 4 and 5). When membranes were prelabeled with [^{3}H]-*myo*-inositol and then transferred to nonradioactive *myo*-inositol, the specific activity of the inositol label of the membranes decreased with approximately the same rate as it entered (Fig. 4). Correspondingly, the incorporation and displacement of radioactivity were affected similarily by auxin. For experiments of Figure 5, membranes were incubated 10 min without or with varying concentrations of IAA or 2,4-D in the presence of [^{3}H]-*myo*-inositol to measure auxin effects on incorporation (upper curves). Labeled membranes previously untreated with auxin were then transferred to solutions containing unlabeled *myo*-inositol and different concentrations of IAA and 2,4-D to measure displacement (lower curves). The magnitude and concentration dependency of both reactions were similar.

Under conditions where the nucleotide independent exchange

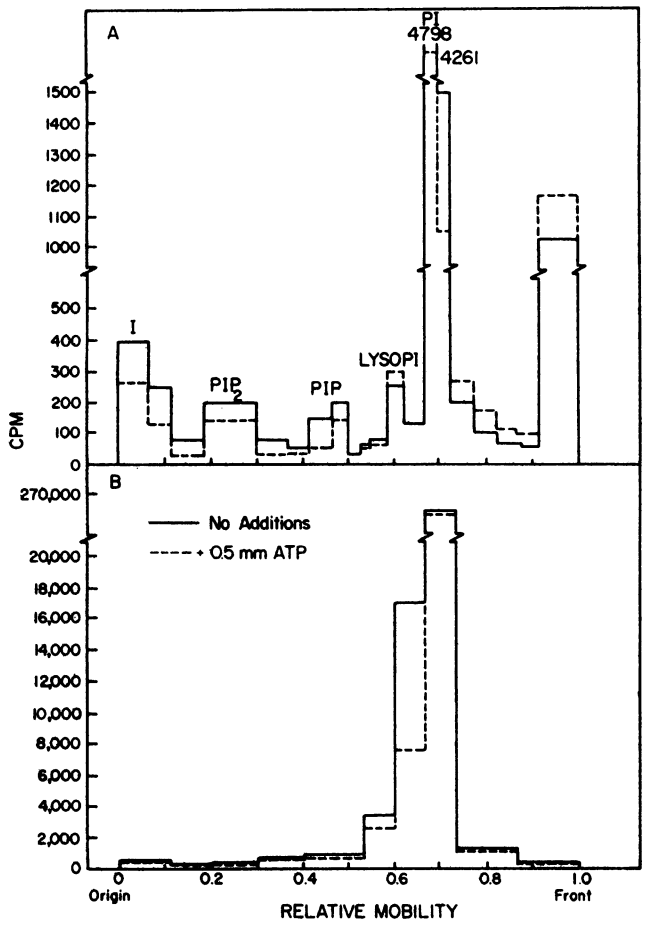

FIG. 2. TLC of lipid extracts: A, Hypocotyl segments labeled for 18 h in a solution containing 10 μ Ci [2-^{3}H]-*myo*-inositol. Results from two separate experiments run in parallel are shown. B, Isolated microsomes incubated for min as described in Figure 1 but with only radioactive [2-^{3}H]-*myo*-inositol (1 μCi) added. The solid bars represent no additions, the dashed bars represent addition of 0.5 mM ATP to the incubation medium. The chromatography plates were developed in chloroform-methanol-25% ammonium hydroxide-water 45:45:3.5:10, v/v) and the regions indicated were scraped from the plates and analyzed for radioactivity (lysoPI = lysophosphatidylinositol).

Table I. *Effects of Cytidine Nucleotides and Mg^{2+} and Mn^{2+} on PI-myo-inositol Exchange Activities of Microsomal Membrane Fraction of Dark-Grown Soybean Hypocotyls*

Reaction medium: 0.23 M sucrose, 7.5 mM KCl, 3 μM [2-^{3}H]-*myo*-inositol (specific activity, 15.6 Ci/mmol), 20 mM Tris/HCl (pH 8.0), and nucleotides and salts as indicated. Incubation time, 10 min. ND, not determined.

Nucleotide Added	PI-*myo*-Inositol Exchange Activity		
	None	10 mM $MgCl_2$	10 mM $MnCl_2$
	pmol/μg protein·h		
None	1	0.4	17
50 μM CMP	ND[a]	5	51
50 μM CDP	ND	5	51
50 μM CTP	ND	6	78

[a] Not determined.

activity for *myo*-inositol was optimal, no appreciable exchange activity was observed with radioactive choline or ethanolamine (Fig. 6). While 10 to 15% of the *myo*-inositol present in the reaction mixture was incorporated into the membrane lipid

Table II. *Effects of Cytidine and Nucleotides on PI-myo-Inositol Exchange Activity of Microsomal Fraction of Dark-Grown Soybean Hypocotyls*

Reaction mixture: 0.23 M sucrose, 7.5 mM KCl, 10 mM $MnCl_2$, 0.5 mM *myo*-inositol (specific activity of [2-^{3}H]-*myo*-inositol, 91 mCi/mmol), 20 mM Tris/HCl (pH 8.0), and additions (50 μM) as indicated. Incubation time was 20 min.

Additions	PI-*myo*-Inositol Exchange Activity
	pmol/μg protein·h
None	5
CMP	46
CDP	51
CTP	27
Cytidine	9
AMP	tr[a]
cAMP	3
ATP	tr
GMP	tr
IMP	2
TMP	1
UMP	0.3

[a] Trace.

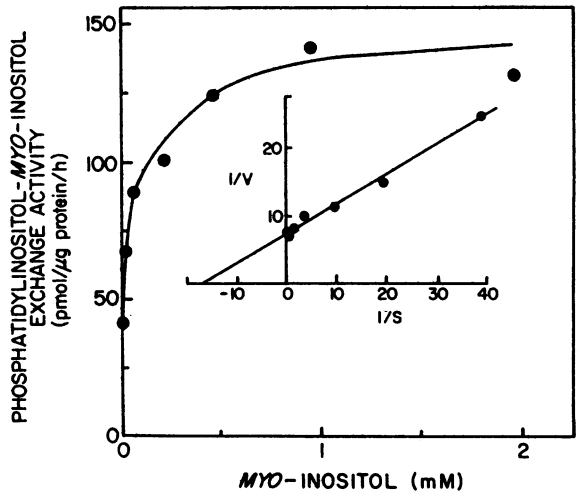

FIG. 3. Relationships of substrate concentration to activity of PI-*myo*-inositol exchange of microsomal membranes of dark-grown soybean hypocotyls. Reaction medium as in Figure 1C. Incubation time, 30 min.

fraction during a 10 min incubation, incorporation of choline or ethanolamine was less than 0.1% of the substrate present in the reaction mixture.

In studies of the subcellular distribution of the activity, a dominant plasma membrane localization was ruled out on the basis of several criteria. With aqueous two-phase partition of the microsomal fraction, the bulk of the activity (>99%) partitioned with the lower phase, whereas total plasma membranes were evenly distributed approximately equally between the upper and lower phases. Membranes collected from the washed upper phase were > 90% plasma membrane-derived, yet the total activity of the PI-*myo*-inositol exchange of this fraction was only 0.1% that of the mixed membranes of the lower phase.

Similarily, when microsomal membrane fractions were separated by preparative free-flow electrophoresis (Table V), the plasma membrane rich fraction E contained only about 1% of the total PI-*myo*-inositol exchange activity. The activity did not correlate with sterol glycoside synthesis, an activity concentrated in plasma membranes, nor was it concentrated in the tonoplast-rich fraction A. The distribution of PI-*myo*-inositol exchange

Table III. *Effects of Thiol Reagents, Ether, Triton X-100, and Low and High Temperatures on PI-myo-Inositol Exchange Activities of Microsomal Membranes of Soybean Hypocotyls*

Reaction mixture: 0.23 M sucrose, 7.5 mM KCl, 10 mM $MnCl_2$, 0.5 mM *myo*-inositol (specific activity of [2-^{3}H]-*myo*-inositol, 91 mCi/mmol), 25 μM CMP, 20 mM Tris/HCl (pH 8.0). Incubation time was 30 min.

Treatment	Concentration	Relative Activity
None		100
+ *p*-Chloromercuribenzoiz acid	0.1 mM	1
+ Iodoacetic acid	1 mM	101
+ α-Iodoacetamide	1 mM	81
+ *N*-ethylmaleimide	1 mM	10
+ Diethylether	40% (by volume)	2
+ Triton X-100	0.1% (by volume)	95
Incubation on ice		4
50°C for 10 min		56
100°C for 10 min		8

Table IV. *PI-myo-Inositol Exchange Activity Measured as Release of Radioactivity from Soybean Microsomal Membranes Prelabeled with 6 μM [2-^{3}H]-myo-Inositol (Specific Activity 15.6 Ci/mmol)*

The prelabel and reaction mixtures contained 0.23 M sucrose, 7.5 mM KCl, 10 mM $MnCl_2$, 20 mM Tris/HCl (pH 8.0), and nucleotides and labeled and unlabeled *myo*-inositol as indicated. The washing steps included dilution with reaction medium (first washing with 5 mM unlabeled *myo*-inositol, second and third washings were without *myo*-inositol) and centrifugation for 20 min at 45,000g_{max}.

Treatment	Exchange Activity		
	None	50 μM CMP	50 μM CTP
	cpm/μg protein·30 min		
1 h 6 μM [2-^{3}H]-*myo*-inositol			
+ 3 Washes (= prelabel)	1448		
+ 15 Min 0.5 mM unlabeled *myo*-inositol	1404	1225	1312
+ 30 Min 0.5 mM unlabeled *myo*-inositol	1377	1247	1055

activity correlated positively with that of latent IDPase and that of NADPH-Cyt *c* reductase but not with succinate INT reductase. Thus an endoplasmic reticulum and/or Golgi apparatus localization for the PI-*myo*-inositol exchange activity was indicated.

DISCUSSION

The incorporation of [^{3}H]-*myo*-inositol into PI of microsomal membranes of soybean hypocotyls showed characteristics closely resembling those of a PI-*myo*-inositol exchange activity described for animal cells (9, 10, 18, 26) and subsequently by Sexton and Moore (25) for an endoplasmic reticulum-enriched fraction of castor bean endosperm. This activity, measured as incorporation of [^{3}H]-*myo*-inositol into PI, was shown to have an optimum pH of about 8, to require Mn^{2+}, and was stimulated by cytidine nucleotides (25). Sexton and Moore (25) concluded that all effects of cytidine nucleotides could be attributed to CMP and that the stimulatory effects of CTP and CDP were effected by their breakdown to CMP. We show the reaction to be an exchange of free inositol with the inositol present in PI, providing further evidence that it represents an unusual form of phospholipid metabolism not necessarily leading to net synthesis of PI.

Exchange of the inositol headgroup of PI as result of the action of a traditional phospholipase D (6, 7, 11, 12, 19) was ruled out by several considerations. Both *in situ* and *in vitro*, activity of

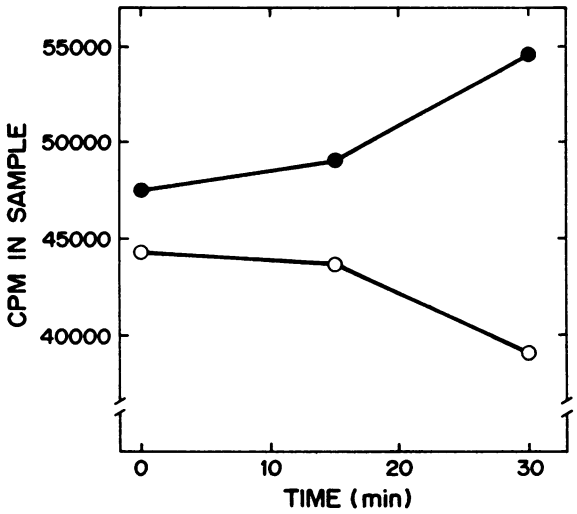

FIG. 4. PI-*myo*-inositol exchange activity of a microsomol membrane fraction from dark-grown soybean hypocotyls. The membrane fraction was prelabeled with [2-^{3}H]-*myo*-inositol for 30 min as described in Table V. After three washes (see Table IV), the membranes were incubated either with 10 μM [2-^{3}H]-*myo*-inositol, specific activity 15.6 Ci/mmol) (●), or 10 μM unlabeled *myo*-inositol ○).

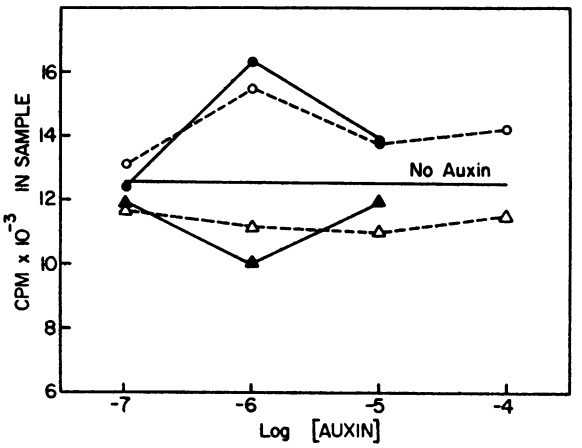

FIG. 5. PI-*myo*-inositol exchange activity of a microsomal membrane fraction from dark-grown soybean hypocotyls. The membranes were incubated with radioactive *myo*-inositol for 10 min as described in Table IV without (horizontal bar) or (upper curves) with varying concentrations of 2,4-D (●) or IAA (○). A portion of the membranes not treated with auxin was washed three times, and incubated for 10 min with 2,4-D (▲) or IAA (△) as above to determine auxin effects on the displacement reaction (lower curves).

phospholipase toward PI was either absent or negligible, phosphatidylcholine and phosphatidylethanolamine being the substrates preferred (20, 21, 27), whereas we found exchange activity only with inositol. Neither choline nor ethanolamine showed exchange activity. Phospholipase D had a maximum activity below pH 7 (19, 21, 27) and was stimulated by membrane solubilizing agents such as diethylether (11, 20). In contrast, PI-*myo*-inositol exchange was optimal at pH 8, inhibited by diethylether and unaffected by Triton X-100. The effects of thiol reagents on the PI-*myo*-inositol exchange activity partially resembled the effects of these substances on a traditional phospholipase D. Phospholipase D has been reported to be inhibited by 0.1 mM *p*-mercuribenzoate, but to be little affected by 1 mM of two other thiol reagents, α-iodoacetamide and iodoacetic acid (27). The same concentrations of these reagents had corresponding effects on the PI-*myo*-inositol exchange activity. However, with respect

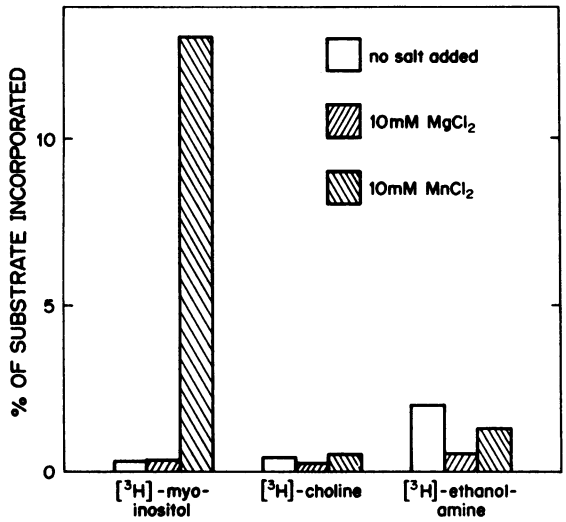

FIG. 6. Phospholipid headgroup exchange activity. Microsomal membranes of soybean hypocotyls were incubated for 10 min in 0.23 M sucrose, 7.5 mM KCl, 20 mM Tris/HCl (pH 8.0), and 4.8 μM [^{3}H]-labeled substrate (specific activities: [2-^{3}H]-*myo*-inositol, 15.6 Ci/mmol; [methyl-^{3}H]-choline chloride, 78 Ci/mmol; [1-^{3}H]-ethanolamine hydrochloride, 8.8 Ci/mmol), with or without 10 mM $MgCl_2$ or $MnCl_2$ as indicated. Incubation time, 10 min.

to inhibition by 12 mM-*N*-ethylmalemide, the enzyme activities again were found to differ. The PI-*myo*-inositol exchange activity was inhibited to about 90%, whereas phospholipase D was inhibited by this compound by less than 5% (27). Additionally, the 100,000*g* supernatant fractions from soybean hypocotyls contained little or no PI-*myo*-inositol exchange activity, while phospholipase D occurred mainly as a soluble enzyme (19).

The significance of this turnover reaction to hormone action remains obscure. The loss of PI from a microsomal membrane fraction isolated from hypocotyls of dark-grown soybeans was shown to be promoted by 2,4-D in a concentration-dependent manner over the growth promoting range of hormone concentrations (17). The reaction characterized in the present work is similar in that auxins (IAA and 2,4-D) promote both *myo*-inositol incorporation and *myo*-inositol displacement such that the same activity appears to be involved for both reactions. A relationship between the exchange activity and polyphosphoinositide metabolism is unlikely since they appear to occur in different membrane fractions. Phosphorylation of PI and of PIP in membranes isolated from shoots or roots of dark-grown wheat occur mainly in a fraction of isolated plasma membranes (22); results with soybean are similar. The PI-*myo*-inositol exchange activity could be considered largely absent from the plasma membrane from our results as determined by two subcellular fractionation methods, preparative free-flow electrophoresis and aqueous polymer two-phase partition. We therefore do not consider the exchange mechanism stimulated by auxins to be connected with PI and PIP kinases, both of which are active *in vitro* in plasma membranes isolated from soybean hypocotyls (AS Sandelius, M Sommarin, unpublished data). The findings from free-flow electrophoresis exclude also the tonoplast and mitochondria as major sites for PI-*myo*-inositol exchange activity. Sexton and Moore (25) concluded that the activity from castor bean endosperm was located in the endoplasmic reticulum. Thus the activity of inositol exchange in PI of soybean has many characteristics in common with the activity reported previously by Sexton and Moore (25) for castor bean endosperm and by others (9, 10, 18, 26) for animal tissues. The PI-*myo*-inositol exchange activity is separated spatially from the polyphosphoinositide metabolism of the plasma membrane and represents a major mechanism for PI-*myo*-inositol turnover with the internal endomembranes of plant cells.

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LITERATURE CITED

1. BERRIDGE MJ 1986 Cell signalling through phospholipid metabolism. J Cell Sci Suppl 4: 137–153
2. BERRIDGE MJ, RF IRVINE 1984 Inositol triphosphate, a novel second messenger in cellular signal transduction. Nature 312: 315–321
3. BLIGH EG, WJ DYER 1959 A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37: 911–917
4. BOSS WJ, MO MASSELL 1985 Polyphosphoinosotides are present in plant tissue culture cells. Biochem Biophys Res Commun 132: 1018–1023
5. BOUHOURS JF, H GUIGARD 1979 Free ceramide, sphingomyelin, and glucosylceramide of isolated rat intestinal cells. J Lipid Res 20: 897–907
6. GALLIARD T 1971 Enzymic deacylation of lipids in plants. The effects of free fatty acids on the hydrolysis of phospholipids by the lipolytic acyl hydrolase of potato tubers. Eur J Biochem 21: 90–98
7. GALLIARD T 1974 Techniques for overcoming problems of lipolytic enzymes and lipoxygenases in the preparation of plant organelles. Methods Enzymol 31: 520–528
8. HEIM S, KG WAGNER 1986 Evidence of phosphorylated phosphatidylinositols in the growth cycle of suspension cultured plant cells. Biochem Biophys Res Commun 134: 1175–1181
9. HOLUB BJ 1974 The Mn^{2+}-activated incorporation of inositol into molecular species of phosphatidylinositol of rat liver microsomes. Biochim Biophys Acta 369: 111–122
10. JUNGALWALA FB, N FREINKEL, RMC DAWSON 1971 The metabolism of phosphatidylinositol in the thyroid gland of the pig. Biochem J 123: 19–33
11. KATES M 1955 Hydrolysis of lecithin by plant plastid enzymes. Can J Biochem 35: 575–589
12. KATES M 1970 Plant phospholipids and galactolipids. *In* R Paoletti, D Kritchevsky, eds, Advances in Lipid Research. Academic Press, New York, pp 225–265
13. KJELLBOM P, C LARSSON 1984 Preparation and polypeptide composition of cholophyll-free plasma membranes from leaves of light-grown spinach and barley. Physiol Plant 62: 501–509
14. LOWRY OH, NJ ROSENBROUGH, AL FARR, RJ RANDALL 1951 Protein measurement by the Folin phenol reagent. J Biol Chem 193: 265–273
15. MICHELL RH, SS JAFFERJI, LM JONES 1977 Advances in the experimental medicine and biology. The possible involvement of phosphatidylinositol breakdown in the mechanism of stimulus-response coupling at receptors which control cell-surface calcium gates. Adv Exp Med Biol 83: 447–464
16. MORRÉ DJ 1971 Isolation of Golgi apparatus. Methods Enzymol 22: 130–148
17. MORRÉ DJ, B GRIPSHOVER, A MONROE, JT MORRÉ 1984 Phosphatidylinositol turnover in isolated soybean membranes stimulated by the synthetic growth

Table V. *Distribution of PI-myo-Inositol Turnover and Marker Enzyme Activities among Pooled Fractions from Free-Flow Electrophoresis Separations*

The detailed procedures for marker enzyme determinations are given by Sandelius *et al.* (23).

	Membrane Fraction					
	Microsomes	A	B	C	D	E
PI-*myo*-inositol turnover, (pmol/μg protein·h)	65	17	83	90	10	8
Sterol glycoside synthetase, (cpm/h·μg protein)	23	23	16	12	12	112
Succinate-INT reductase, (pmol/h·μg protein)	800	162	346	1356	474	66
NADPH-Cyt *c* reductase, (pmol/h·μg protein)	8.2	2.4	7.8	10.2	5.8	3.9
Latent IDPase, (nmol/h·μg protein)	6.3	2.5	5.9	8.2	<1	<1
Protein (mg/50 g fresh wt)	17.6	1.0	2.0	8.9	1.8	1.2

hormone 2,4-dichlorophenoxyacetic acid. J Biol Chem 259: 15364–15368
18. PAULUS H, EP KENNEDY 1960 The enzymatic synthesis of inositol monophosphatide. J Biol Chem 235: 1303–1311
19. QUARLES RH, RMC DAWSON 1969 The distribution of phospholipase D in developing and mature plants. Biochem J 112: 787–794
20. ROUGHAN PG, CR SLACK 1976 Is phospholipase D really an enzyme? A comparison of in situ and in vitro activities. Biochim Biophys Acta 431: 86–95
21. ROUGHAN PG, CR SLACK, R HOLLAND 1978 Generation of phospholipid artefacts during extraction of developing soybean seeds with methanolic solvents. Lipids 13: 497–503
22. SANDELIUS AS, M SOMMARIN 1986 Phosphorylation of phosphatidylinositols in isolated plant membranes. FEBS Lett 201: 282–286
23. SANDELIUS AS, C PENEL, G AUDERSET, A BRIGHTMAN, M MILLARD, DJ MORRÉ 1986 Isolation of highly purified fractions of plasma membrane and tonoplast from the same homogenate of soybean hypocotyls by free-flow electrophoresis. Plant Physiol 81: 177–185
24. SCHACHT J 1978 Purification of polyphosphoinositides by chromatography on immobilized neomycin. J Lipid Res 19: 1063–1067
25. SEXTON JC, TS MOORE 1981 Phosphatidylinositol synthesis by a Mn^{2+}-dependent exchange enzyme in castor bean endosperm. Plant Physiol 68: 18–22
26. TAKENAWA T, K EGAWA 1977 CDP-diglyceride:inositol transferase from rat liver. Purification and properties. J Biol Chem 242: 477–484
27. YANG SF, S FREER, AA BENSON 1967 Transphosphatidylation by phospholipase D. J Biol Chem 242: 477–484