JOURNAL OF LIPOSOME RESEARCH
Vol. 14, Nos. 3 & 4, pp. 141–153, 2004

Effects of D-Myo-Inositol 1-Phosphate on the Transfer Function of Phosphatidylinositol Transfer Protein α

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ABSTRACT

The lipid metabolite D-myo-inositol-1-phosphate is shown to increase the phospholipid transfer activity of phosphatidylinositol transfer protein α from liposomal and liver microsomal membranes. Dose-response curves indicated substantial enhancements of transfer in the low mM range that upon normalization were independent of membrane composition or the identity of the transferred phospholipid. The unnormalized effect is potentiated by anionic membrane surface charge and substantial membrane phosphatidylethanolamine content consistent with alterations of the protein's membrane binding affinity and alterations of surface electrostatic interactions as contributing factors.

Key Words: Lipid transfer; Lithium; Phosphatidylethanolamine; Polyphosphoinositide signaling; Signal transduction.

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DOI: 10.1081/LPR-200029889
0898-2104 (Print); 1532-2394 (Online)

INTRODUCTION

Phosphatidylinositol transfer protein α (PITP-α) is a bifunctional phosphatidylinositol (PtdIns) and phosphatidylcholine (PtdCho) transfer protein [reviewed in Refs. (1,2)]. It is ubiquitous in mammalian cells and eukaryotes. PITP-α has critical roles in polyphosphoinositide signaling (3), in constitutive and regulated vesicular trafficking among intracellular organelles (4,5) and in secretory trafficking pathways (6,7). Its transfer activity is strongly dependent on the lipid composition of the membrane, the structure and charge of polar head groups, headgroup spacing and acyl-chain composition (8–11). Cellular levels of polar lipid metabolites are coupled to PITP-α expression (12,13). Polar lipid metabolites have received increased scrutiny for their diagnostic use in evaluating cellular function in disease states and for accumulating indications that may exert biological activity upon their pathogenic progression [e.g., Refs. (14–18)].

A variety of polar PtdIns and PtdCho lipid metabolites were screened for their activity toward PITP-α-mediated phospholipid transfer in our laboratory. Two were identified as eliciting substantial responses in an in vitro phospholipid transfer assay. L-α-glycerylphosphorylcholine, as discussed elsewhere (19), was shown to be inhibitory. D-myo-inositol 1-phosphate (Ins(1)*P*), the subject of this report, was found to be stimulatory. The possibility is raised that elevations of D-myo-inositol-1-phosphate may have consequences for cells and may provide a means for pharmacologic intervention.

MATERIALS AND METHODS

Materials

L-α-[myo-inositol-2-^{3}H]-PtdIns (> 90% 1-stearoyl-2-arachidonoyl) was obtained from NEN Life Science Products Inc (Boston, MA). 1-Palmitoyl-2-[1-^{14}C]oleoyl-PtdCho, [1α, 2α-H]-cholesteryl oleate (CholOle) and [1-^{14}C]-cholesteryl oleate were obtained from Amersham Pharmacia Biotech (Arlington Heights, IL). Myo-[2-^{3}H]inositol was from American Radiolabeled Chemicals (St. Louis, MO).

1-Palmitoyl-2-oleoyl-3-*sn*-phosphatidylcholine (PamOlePtdCho), 1-palmitoyl-2-oleoyl-3-*sn*-phosphatidylserine (PamOlePtdSer), 1-palmitoyl-2-oleoyl-3-*sn*-phosphatidylethanolamine (PamOlePtdEtn), 1-palmitoyl-2-oleoyl-3-*sn*-phosphatidic acid (PamOlePtdOH), phosphatidylinositol (PtdIns, from bovine liver), cholesterol, and sphingomyelin (bovine brain) were obtained from Avanti Polar Lipids (Alabaster, AL). Purity (≥ 99%) was monitored by thin layer chromatography. Lactosyl ceramide (LacCer; Gal(β 1→4)GlcCer) isolated from bovine buttermilk (Calbiochem, San Diego, CA) and agglutinin (*Ricinus communis*, average molecular weight of 120,000, Sigma) were used as supplied. Ins(1)*P* was obtained from Alexis Biochemicals (San Diego, CA) or A. G. Scientific, Inc. (San Diego, CA) as the potassium or ammonium salt. Its limited availability shaped the experimental design. Recombinant mouse PITP-α carrying phosphatidylglycerol was prepared from inclusion bodies after expression of the cDNA in Escherichia coli (20) and stored at −80°C.

Methods

The activity of PITP-α was monitored as its ability to transfer radiolabeled phospholipids ([^{3}H]-phosphatidylinositol or [^{14}C]-phosphatidylcholine) from large donor to small unilamellar acceptor liposomes using an agglutination protocol for vesicle separation developed by Kasper and Helmkamp (21). Lipids in organic solvent were dried to a film under N_2, placed in vacuo (< 5 mtorr) for at least 1 h and hydrated by vortex at 4°C in SET buffer (0.25 M sucrose, 1 mM EDTA, and 10 mM Tris, pH 7.4). Donor large unilamellar vesicles (LUV) were prepared by extrusion (200 nm pore size) as described (11). The plasma inner membrane model was modeled after the composition reported for erythrocytes (22). LUV compositions are listed in the figure legends. [^{3}H]-PtdIns was used in the PtdIns transfer assay, while [^{14}C]-PtdCho was used in the PtdCho transfer assay.

Small, unilamellar acceptor vesicles (SUVs) were prepared by sonication of the lipid dispersion at 4°C for 15 min under N_2 using a probe-type sonicator (Heat System W-225, Farmingdale, NY) at a continuous power of 40 W. The sonicated solutions were centrifuged at 3,000 g (10 min) to remove titanium particles and any residual multilamellar structures. Unless otherwise noted, the SUV composition in mol% was as follows: PamOlePtdCho (98), PamOlePtdOH (2), CholOle (< 0.05). [^{14}C]-CholOle was used in the PtdIns transfer assay, while [^{3}H]-CholOle was used in the PtdCho transfer assay to quantitate the recovery of the acceptor vesicles.

The donor (225 μM) and acceptor (225 μM) vesicles were incubated with PITP-α (56 nM) and the compounds indicated for 15 min at 37°C unless otherwise noted. The samples were put on ice and agglutinin (66–396 μg ml^{-1} in SET buffer) was added to aggregate the donor vesicles. The samples were centrifuged at 500 g (2 min) and 10,000 g (12 min). Aliquots of the supernatant containing the acceptor vesicles with the transferred labeled phospholipid and the labeled CholOle marker of acceptor vesicle concentration were reserved for dual window liquid scintillation analysis performed as described (11). Less than 5% of the acceptor vesicles were coprecipitated with the donor vesicles based on the recovery of the CholOle label. All transfer rates were corrected for this loss. Contamination of the supernatant by donor vesicles was less than 1% as monitored in parallel experiments with donor vesicles doped with labeled CholOle. All transfer assays included a blank lacking PITP-α, but containing all other additives, to quantitate and correct for spontaneous transfer (less than 1%). Rat liver microsomes were isolated by differential centrifugation, and PtdIns was radiolabeled by head-group exchange of inositol using *myo*-[2-^{3}H]inositol as described previously (11). The transfer assay applied to donor microsomal membranes and acceptor SUVs was as described previously except that the acceptor vesicles were composed of PamOlePtdCho (98 mol%), PtdOH (2 mol%), and CholOle (< 0.05 mol%) (11). The inhibitory anionic vesicles used in selected experiments did not function as acceptor membranes based on a lack of significant transfer in the absence of normal acceptor membranes. Phospholipid transfer was expressed as mol phospholipid transferred per minute per mol PITP-α, as the percentage of the labeled PtdIns pool transferred in 15 min (microsomes), or normalized to the activity in the absence of additives as 100%.

RESULTS

Effect of Ins(1)P on PITP-α-Mediated Transfer

The effect of Ins(1)*P* on PITP-α-mediated phospholipid transfer is shown in Fig. 1 for three membrane systems. The transfer data shown in Panels A and B employed lightly charged donor membranes containing low equimolar levels (2.5 mol%) of PtdIns and PtdCho. The known preference of PITP-α for PtdIns was apparent from the greater activity toward PtdIns. Ins(1)*P* accelerated PtdIns and PtdCho transfer in the low millimolar range with similar efficacies. PITP-α-mediated transfer of PtdCho was nearly doubled by the addition Ins(1)*P*, whereas PtdIns transfer was enhanced by about a third over the range of concentrations examined. By contrast, *myo*-inositol exerted no influence on either transfer activity.

The transfer data summarized in Panels C and D employed cholesterol-rich donor membranes that were modeled after the composition of an inner plasma membrane leaflet (22).

The Ins(1)*P*-induced increase in PITP-α-mediated activity toward PtdIns and PtdCho on a percentage basis was similar to that shown in the upper panels [at 5.9 mM Ins(1)*P* the increase in PtdIns transfer from Panels A and C were 22.1% and 15.4%, respectively, while the increase in PtdCho transfer from Panels B and D were 56.7% and 51.6%, respectively].

Panel E summarizes data obtained with rat hepatic microsomal donor membranes. Transfer is plotted as the percentage of the labeled microsomal PtdIns pool transferred in 15 min. PITP-α-mediated PtdIns transfer was increased substantially by Ins(1)*P* compared to the situation with no additives.

Surface Charge and Ins(1)P Action

Anionic vesicles added to the transfer assay avidly bind PITP-α and thereby sequester it from the membranes involved in the transfer assay. An inhibition of phospholipid transfer results (23,24). In this paradigm, PITP-α-mediated transfer served to report on changes in the amount of unsequestered PITP-α available to the assay and thereby changes in the binding of PITP-α to the anionic vesicles. To test whether Ins(1)*P* altered the degree of sequestration of PITP-α by anionic vesicles, vesicles composed of 100 mol% phosphatidic acid or 20 mol% phosphatidic acid were titrated into the transfer assay described in Fig. 1A in the presence and absence of Ins(1)*P* as shown in Fig. 2A. (These anionic vesicles contained no transferable lipids and were otherwise inert in the transfer process based on their inability to act as acceptor membranes when used in the absence of normal acceptor membranes.) Transfer was normalized to the rate obtained in the absence of additives [Ins(1)*P* or anionic vesicles] being 100%. The presence of anionic vesicles caused a concentration dependent decline in PtdIns transfer that was less pronounced in the presence of Ins(1)*P*. This effect is easily visualized in the format shown in Fig. 2B, which isolates the influence of Ins(1)*P* and reveals an enhancement of several-fold in the PITP-α-mediated PtdIns transfer activity, indicating that the added anionic vesicles were less able to bind and sequester PITP-α in the presence of Ins(1)*P*.

To show that the influence of surface charge on the action of Ins(1)*P* effect also was manifest directly on the substrate membranes, the content of anionic PtdSer in the

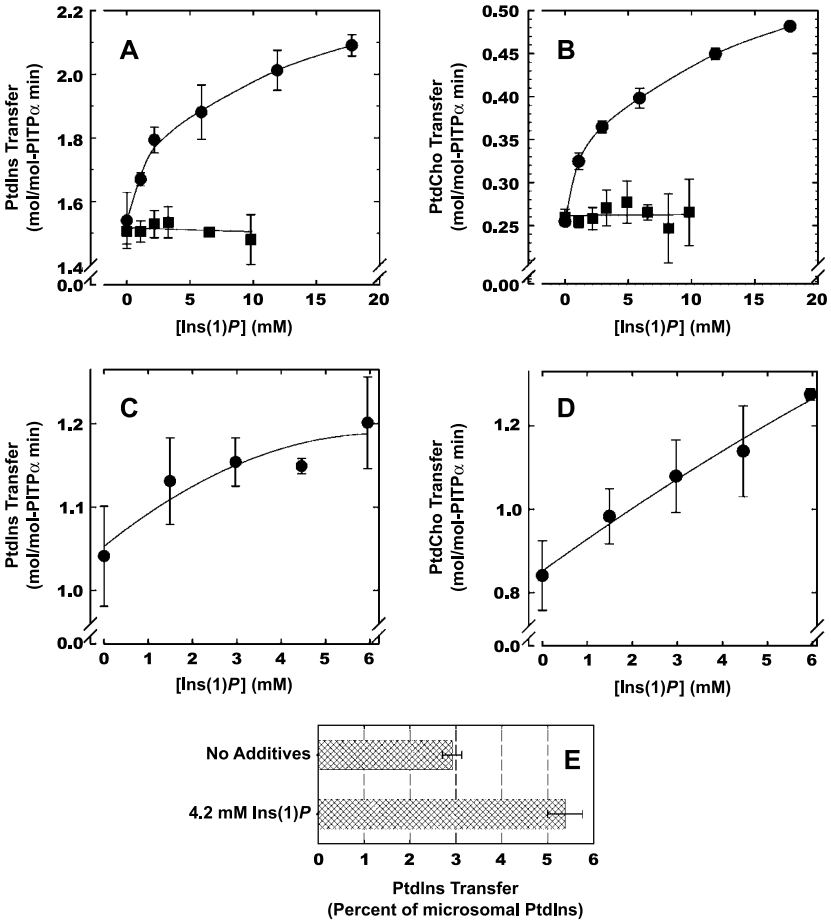

Figure 1. Influence of Ins(1)*P* on PITP-α-mediated phospholipid transfer from three membranes. (A, B) Effects of Ins(1)*P* (●) and *myo*-inositol (■) on PtdIns transfer and PtdCho transfer from a simple membrane system (donor LUV (mol%): PamOlePtdCho (2.5), PtdIns (2.5), PamOlePtdSer (2.5), PamOlePtdEtn (82.5), and LacCer (10); acceptor SUV: PamOlePtdCho (98), PamOlePtdOH (2), CholOle (< 0.05); N $\geq$ 3, mean ± SD). (C, D) Effect of Ins(1)*P* on PtdIns and PtdCho transfer from a liposomal model of the bulk inner plasma membrane leaflet [(PamOlePtdCho (6.8), PtdIns (1.8), PamOlePtdSer (10.8), PamOlePtdEtn (21), sphingomyelin (4.5), cholesterol (45), and LacCer (10) in mol%; acceptor SUV as above; N = 3, mean ± SD]. (E) Effect of Ins(1)*P* on PITP-α-mediated PtdIns transfer from rat hepatic microsomal donor membranes expressed as the percentage of the total labeled PtdIns transferred in 15 min (N = 3, acceptor SUV as above).

donor membranes was increased in the absence of anionic vesicles. Shown in Fig. 2C is the effect of 4.2 mM Ins(1)*P* on PtdIns transfer from highly charged donor membranes containing 40 and 50 mol% PtdSer. The transfer activity from the highly charged donor membranes was substantially lower than was observed with donors containing 2.5 mol% PtdSer in the absence of anionic vesicles (Fig. 1A). Such a decrease in transfer is

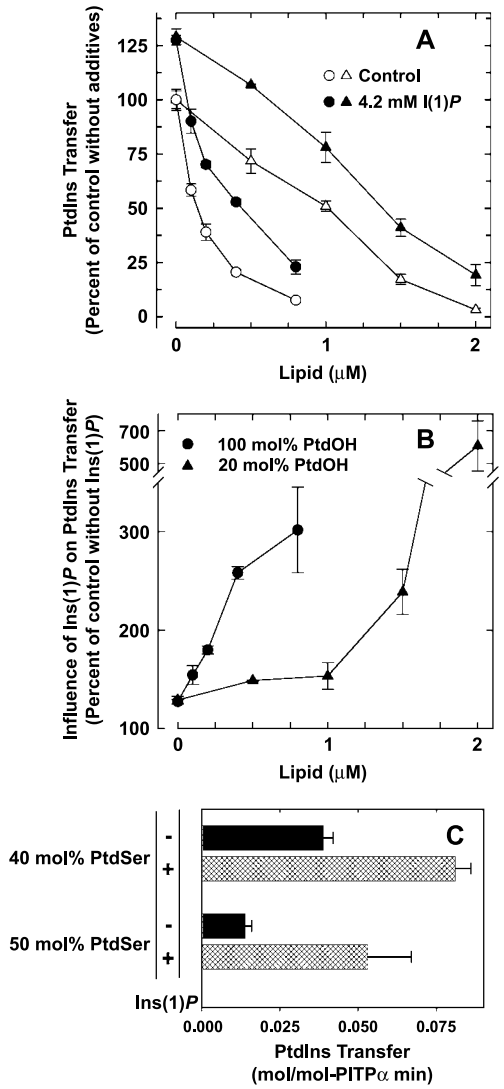

Figure 2. Surface charge potentiates the action of Ins(1)*P*. The influence of Ins(1)*P* on the ability of anionic vesicles to sequester PITP-α and inhibit transfer. PtdIns transfer is a percentage referenced to the transfer obtained in the absence of additives as 100%. (A) Influence of anionic vesicles on PITP-α-mediated PtdIns transfer. Vesicles composed of 100 mol% PtdOH (●, ○) or 20 mol% PtdOH (▲, △; 80 mol% PtdEtn), at the lipid concentrations indicated, were added to the transfer assay described in Fig. 1A in the presence (shaded symbols) or absence (open symbols) of 4.2 mM Ins(1)*P* (N = 3, mean ± SD). (B) The data in A is replotted to isolate the influence of Ins(1)*P* on PtdIns transfer for 100 mol% (●) or 20 mol% (▲) PtdOH. The increase in PtdIns transfer elicited by Ins(1)*P* is shown as a ratio of the transfer in the presence of Ins(1)*P* divided by the transfer in the absence of Ins(1)*P* times 100. (C) In the absence of anionic vesicles, the ability of 4.2 mM Ins(1)*P* to enhance PITP-α-mediated transfer from donor membranes enriched to 40 mol% and 50 mol% PamOlePtdSer, as indicated, is shown. PtdSer replaced the PtdEtn in the donor membranes above. Other conditions were as above (N = 3, mean ± SD).

consistent with strengthened electrostatic interactions toward highly charged membrane surfaces such that the membrane off-rates become too slowed to support the degree of transfer observed from less-charged membrane surfaces. Similar to the situation observed above with anionic vesicles, 4.2 mM Ins(1)*P* reduced the effects of donor membrane charge on the transfer rate and elicited a 2–4-fold increase in PtdIns transfer from the highly charged membranes.

Effect of PtdEtn Content on Ins(1)P Action

The membrane PtdEtn content has been implicated in facilitating PITP-α binding (19). The influence of PtdEtn content on the action of Ins(1)*P* in lightly-charged membranes is shown in Fig. 3 with the raw data inset (the sharp increase in PtdIns transfer shown is due in part to reduced competition with the PtdCho ligand as it is replaced by PtdEtn). The presence of PtdEtn progressively enhanced the effect of Ins(1)*P* on PITP-α′s PtdIns transfer activity.

Membrane Recognition and Ins(1)P Action

To address whether the binding site for Ins(1)*P* might have originated as a site for the recognition of membrane PtdIns, a transfer assay was adopted wherein two populations of donor membranes, alternately radiolabeled with PtdCho but differing in PtdIns content, were assayed for activity toward PITP-α in the presence of Ins(1)*P*. The

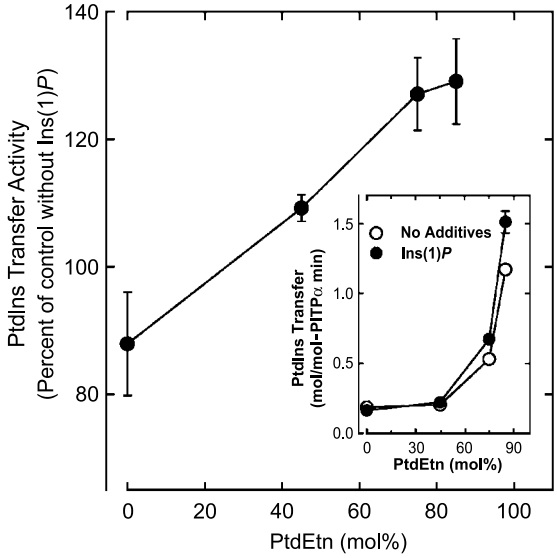

Figure 3. PtdEtn content potentiates the action of Ins(1)*P*. The influence of 4.1 mM Ins(1)*P* on PITP-α-mediated PtdIns transfer activity is shown for varied donor membrane PtdEtn content [donor membrane, PamOlePtdEtn (0–85), PamOlePtdCho (85–0), PtdIns (2.5), PamOlePtdSer (2.5), LacCer (10)]. Other conditions as in Fig. 1A. The raw data showing the combined effects of Ins(1)*P* and PtdEtn are inset (N = 3, mean ± SD).

results are shown in Fig. 4A. The lower trace represents the PtdCho transfer obtained in the presence of 10 mol% PtdIns, whereas the upper trace represents the PtdCho transfer obtained in the presence of 10 mol% PtdSer in the absence of PtdIns.

The Ins(1)*P* dose response is examined in Panel B. The enhancement of transfer observed at 17.8 mM Ins(1)*P* is taken as a maximum of 100% for purposes of normalization. Transfer is displayed as a percentage of that maximal enhancement. Shown are the data from Panel A, along with the PtdIns transfer data from Fig. 1A

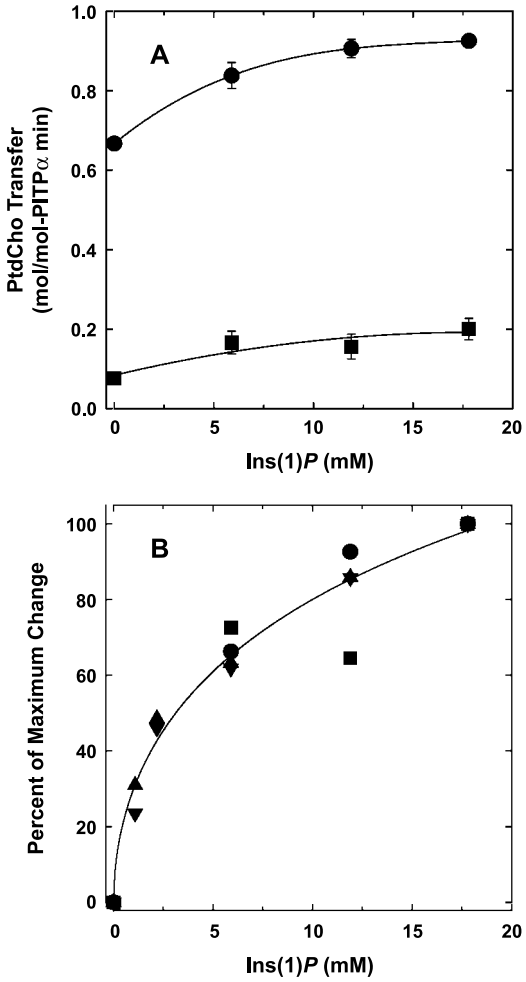

Figure 4. Role of membrane composition in Ins(1)*P* action. (A) Results from a dual donor transfer paradigm are shown wherein half the donor membranes contained 10 mol% PtdSer (●) and half contained 10 mol% PtdIns [■, balance: PamOlePtdEtn (77.5), PamOlePtdCho (2.5), LacCer (10)]. Donor membranes were alternately radiolabeled with PtdCho and transfer was monitored in the presence of Ins(1)*P* (N = 3, mean ± SD). (B) The action of Ins(1)*P* taken from Panel A (●, ■ as above) and from Panels A (▼) and B (▲) of Fig. 1 are displayed to shown the response as a percentage of the change observed at 17.8 mM Ins(1)*P*.

and the PtdCho transfer data from Fig. 1B. Although the absolute increase in transfer and the percentage increase in transfer varied among the different membrane substrates examined, the data when normalized as a fraction of the enhancement at 17.8 mM followed a smooth curve independent of membrane composition or transferred phospholipid.

DISCUSSION

Ins(1)*P* acted in the low mM range to alter PITP-α-mediated PtdIns and PtdCho transfer in vitro. This ability was not shared by *myo*-inositol (Fig. 1) or D-myo-inositol 1,4,5-trisphosphate (19). This effect was demonstrated in Fig. 1 for transfer from 1) compositionally simple lightly charged lipid membranes, 2) cholesterol-rich and moderately charged lipid membranes modeled after a plasma membrane inner leaflet, and 3) hepatic microsomal membranes. Dose-response curves performed in several membrane systems revealed that while the changes in transfer rates varied among the membranes in terms of both percentile change and absolute change, when expressed as a fractional response of the maximum observed at 17.8 mM Ins(1)*P* the curves coincided (Fig. 4B). The constancy of the normalized response in the face of a varying maximal response was consistent with an Ins(1)*P* binding site that was unaffected by the membrane composition.

The transfer activity of PITP-α is dependent on two factors: membrane binding and phospholipid exchange at the membrane surface. To investigate altered PITP-α binding as a potential mediator of the Ins(1)*P* response, strongly inhibitory anionic vesicles were added to the transfer assay. These anionic vesicles bind and sequester PITP-α, but are otherwise inert in the transfer process. In the presence of Ins(1)*P*, the ability of anionic vesicles to inhibit PtdIns transfer was reduced. Conversely, in the presence of anionic vesicles, the ability of Ins(1)*P* to enhance PITP-α-mediated PtdIns transfer was increased. The PtdIns transfer activity elicited by Ins(1)*P* (4.2 mM) increased to as much as 600% in the presence of anionic vesicles. This result indicates that Ins(1)*P* diminishes the ability of anionic vesicles to bind and sequester PITP-α.

Donor membrane charge potentiated the ability of Ins(1)*P* to accelerate PtdIns transfer. Inclusion of 40 or 50 mol% PtdSer in donor membranes increased the Ins(1)*P* enhancement from about a quarter to several-fold. By analogy to the effects observed with anionic vesicles, a reduction in the PITP-α membrane-binding strength is implicated as a factor facilitating the enhancement. The PITP-α activity is about 60-fold smaller in the PtdSer-rich membranes than the lightly charged membranes of otherwise identical composition (compare Figs. 2C and 1A). Likewise, the decrease in transfer observed between the 40 and 50 mol% PtdSer-containing membranes was characteristic of the membrane surface charge (19). Dense surface charge strengthens PITP-α membrane binding to the extent that the residence time at the membrane surface is too long to support optimal transfer. It appears that Ins(1)*P* reduces the binding strength and the residence time at the membrane surface thereby to increase the off-rate and facilitate transfer. Possibly, this action is partly a consequence of the anionic charge possessed by Ins(1)*P* such that the charge balance of PITP-α is altered and its electrostatic attraction to anionic membrane surfaces is reduced.

Membrane PtdEtn content is implicated as a factor facilitating PITP-α binding (19). High PtdEtn content proved synergistic in the Ins(1)*P* effect in lightly charged

membranes, consistent with an action of Ins(1)*P* that decreases the membrane binding strength of PITP-α. (It should be noted that there is an optimal binding for transfer. If PITP-α never associates with membranes, there is no transfer. Conversely, if PITP-α never dissociates from membranes, there is no transfer. The weak, possibly inhibitory, effects of Ins(1)*P* shown in Fig. 3 in the absence of PtdEtn are consistent with the expectation that decreases in PITP-α binding will be inhibitory in the weak binding regime.) Ethanolamine glycerophospholipids (as well as PtdSer mentioned above) are abundant in the inner monolayer of the plasma membrane where PITP-α delivers PtdIns for the polyphosphoinositide signal transduction cascade (25).

The Ins(1)*P* binding site does not appear to overlap the binding site for the PtdIns headgroup. No competitive inhibition with PtdIns transfer was observed at the highest Ins(1)*P* concentrations employed. To address whether there existed a PtdIns site distinct from the site involved in PtdIns transfer that might provide, for instance, a membrane PtdIns recognition site and a target for Ins(1)*P*, a dual donor paradigm was initiated with PtdIns-enriched and PtdIns-null donor membranes with PtdCho transfer monitored. We reasoned that the influence of Ins(1)*P* would be lessened in the presence of PtdIns-enriched membranes as these sites, if present, would be filled in the absence of Ins(1)*P*. Any binding effects would be compensated by having both donor membranes present, but alternately labeled, in the same assay. A greatly diminished PtdCho transfer rate from PtdIns-enriched membranes presumably is due to competition from PtdIns for the ligand transfer site and is not otherwise noteworthy. When the results for the PtdIns-enriched and PtdIns-null membranes were normalized in terms of the fractional change of the maximum, and plotted with the PtdIns and PtdCho transfer data from the lightly charged membrane systems shown in Fig. 1, the traces coincided. This result does not support the concept of an Ins(1)*P*-sensitive membrane PtdIns recognition site. However, previous reports (10,24,26), though mixed, have suggested and our unpublished studies have confirmed that 100 mol% PtdIns vesicles impart exceptionally weak inhibition in the inhibitory anionic vesicle paradigm discussed above. Whether this is a consequence related to the transferability of PtdIns or due to interactions with PtdIns distinct from its role as a transferred ligand is unknown. Regardless, our data are not supportive of PtdIns-specific membrane recognition as involved in Ins(1)*P* action.

Cellular Ins(1)*P* concentrations normally are in the mid-micromolar range and below the lowest concentrations used in this study [e.g., Ref. (27)]. Overexpression of PITP-α in fibroblast cells increased Ins(1)*P* levels five-fold (13). Ins(1)*P* concentrations relevant to this study are found following lithium treatment (27,28). The ability of therapeutic concentrations of lithium to uncompetitively inhibit inositol monophosphatase has focused attention on inositol signaling (15) and inositol phosphate accumulation (14) as possible mediators of lithium's psychotherapeutic efficacy and spurred efforts to develop inositol monophosphatase inhibitors that were more specific and less toxic than lithium (29).

Our interest is in factors that influence PITP-α activity and membrane targeting, particularly those that may relate to the effects of ethanol in inositol signal transduction (11,30). Ethanol and other anesthetics depress rat brain Ins(1)*P* levels in the presence and absence of lithium (28,31,32). Comorbid alcohol abuse presents a poor prognosis for lithium therapy (33), but whether the opposing effects on Ins(1)*P* levels contribute to the poor clinical outcome is conjecture.

A screen (19) of polar lipid metabolites for their influence on PITP-α′s phospholipid transfer activity identified two metabolites as exerting notable influence: L-α-glycerylphosphorylcholine as an inhibitory factor (19) and Ins(1)*P* as a stimulatory factor. This study focused on the effects of Ins(1)*P* and demonstrated that its effects on PITP-α′s phospholipid transfer function were most pronounced with more anionic membranes and thereby raised the possibility that Ins(1)*P* might provide an avenue for therapeutic intervention.

ABBREVIATIONS

CholOle	cholesteryl oleate
Ins(1)*P*	D-myo-inositol 1-phosphate
LacCer	lactosyl ceramide
LUV	large unilamellar vesicle
Ole	oleoyl
Pam	palmitoyl
PITP-α	phosphatidylinositol transfer protein α
PtdCho	phosphatidylcholine
PtdEtn	phosphatidylethanolamine
PtdIns	phosphatidylinositol
PtdOH	phosphatidic acid
PtdSer	phosphatidylserine
SUV	small unilamellar vesicle

ACKNOWLEDGMENTS

This work was supported by NIH grants AA00293, AA07186, and AA07463.

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Received February 2004
Revised May 2004