Phosphatidylinositol transfer protein (PITPα) stimulates in vitro intra-Golgi transport

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Abstract Using a cell-free assay designed to reconstitute cis-to-medial intra-Golgi vesicular transport, we identified at least four crude activities in bovine brain cytosol that stimulate this assay. We have purified one of these activities to near homogeneity and have identified this M_r 36 kDa protein to be the α isoform of phosphatidylinositol transfer protein (PITPα) by N-terminal peptide sequencing, immunoreactivity with PITP-specific antisera, and the ability of recombinant PITPα to stimulate in vitro intra-Golgi transport. From these data, we conclude that in vitro Golgi transport is facilitated by PITPα.

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Key words: Golgi apparatus; Phosphatidylinositol transfer protein; Vesicular transport; Phospholipid; Secretion

1. Introduction

All eukaryotic cells maintain discrete membrane-bounded organelles which serve to compartmentalize cellular functions. The anterograde and retrograde transport of proteins between the organelles comprising the eukaryotic secretory pathway is mediated by small membrane-bounded vesicles [1–3], and at some steps, tubular or vesiculo-tubular carriers [4,5]. Transport vesicles bud from one compartment, and then target to and dock with the next compartment, where they fuse, thereby releasing their cargo of proteins and lipids. The number and variety of potential fusion partners for each transport intermediate creates the need for a precise docking and fusion mechanism to ensure the fidelity and efficiency of protein trafficking, and to maintain organelle integrity and identity. An important first step in understanding the phenomenon of protein transport and the mechanism of docking and fusion is to identify the full set of factors that mediate these processes and elucidate their individual roles in these events.

In an effort to biochemically dissect protein transport and to identify the factors involved, Rothman and colleagues developed an in vitro assay that reconstitutes protein traffic between the cis and medial cisternae of the Golgi apparatus [6,7], which enabled the biochemical purification of *N*-ethylmaleimide-sensitive fusion protein (NSF) [8], α, β, and γ isoforms of soluble NSF attachment protein (SNAP) [9], p115 [10], and recently, p16 [11]. This cis-to-medial in vitro Golgi transport assay has been suggested to reconstitute the entire vesicular transport cycle of budding, docking, and fusion [2,12], which is supported by the finding that functional vesicular intermediates can be isolated [13]. However, some data raise the possibility that the assay may reconstitute a partial reaction of transport comprised of only docking and fusion [14–16]. Nevertheless, because the cis-to-medial Golgi transport assay is dependent on NSF, the SNAPs, and p115, three bona fide transport factors, the transport events measured in the assay must be a specific and regulated process reflecting, at the very least, the later stages of the vesicular transport cycle.

To purify additional protein trafficking components, we modified the in vitro cis-to-medial Golgi transport assay to include functionally saturating levels of p115, NSF, and SNAPs. The modified in vitro Golgi transport assay has only a low level of activity in the absence of cytosol, indicating that at least one additional factor is required for full activity, as had been previously suggested [10]. Indeed, using this in vitro transport assay to test cytosolic fractions for activity, we identified multiple crude activities in bovine brain cytosol. We have biochemically purified one of these proteins to near homogeneity. By multiple criteria, including size, N-terminal peptide sequence analysis, immunoreactivity, and studies with a recombinant protein, we have identified this protein as the α isoform of phosphatidylinositol transfer protein (PITPα).

2. Materials and methods

2.1. Materials

Lec1 cell line (CRL 1735, ATCC, Rockville, MD [17]), anti-rat PITPα polyclonal antibodies ([18]; a generous gift of J. Alb and V. Bankiatis), polyclonal secondary antibodies (Jackson ImmunoResearch Laboratories, West Grove, PA), ^{125}I-labeled goat anti-rabbit IgG antibody (NEN, Boston, MA), His_6-tagged α-SNAP, His_6-tagged γ-SNAP, and His_6-tagged NSF constructs ([19]; generously provided by S. Whiteheart), His_6-tagged human PITPα construct ([20]; a kind donation by P. Swigart and S. Cockcroft), ^{3}H-UDP-*N*-acetylglucosamine (American Radiolabelled Chemicals, St. Louis, MO), L-α-phosphatidylcholine (Avanti Polar Lipids, Alabaster, AL). All other reagents were purchased from ACROS (Fisher Scientific, Pittsburgh, PA), Boehringer Mannheim (Indianapolis, IN), or Sigma Chemical Company (St. Louis, MO).

2.2. General methods

Protein concentrations were assayed using the Bradford method (Bio-Rad Laboratories, Hercules, CA) using BSA as a standard. All chromatography, centrifugation, and concentration steps were per-

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Abbreviations: BBC50, 50% ammonium sulfate precipitate of bovine brain cytosol; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; GlcNAc, *N*-acetylglucosamine; KPi, potassium phosphate; NSF, *N*-ethylmaleimide-sensitive fusion protein; PC, phosphatidylcholine; PCoA, palmitoyl coenzyme A; PI, phosphatidylinositol; PIP_2, phosphatidylinositol-4,5-bisphosphate; PITP, phosphatidylinositol transfer protein; PLC, phospholipase C; PVDF, polyvinylidene difluoride; SNAP, soluble NSF attachment protein; TGN, trans-Golgi network; VSV, vesicular stomatitis virus; Bd, bound; FT, flow-through; Ld, load

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formed at 4°C. All material was clarified by centrifugation at 10 000×*g* for 10 min prior to chromatography. All samples were dialyzed into transport assay buffer (25 mM HEPES-KOH, pH 7.4, 100 mM KOAc, 5 mM $Mg(OAc)_2$, 1 mM EDTA, 1 mM DTT) prior to analysis in the in vitro Golgi transport assay.

2.3. In vitro Golgi transport assay

The transport assay was performed as previously described [10], with the following modifications: the 'donor' Golgi membranes were prepared from VSV-infected Lec1 cells [21]; the 'donor' and 'acceptor' Golgi membranes were not salt-extracted, so they should retain peripherally bound membrane proteins; reactions were incubated at 35°C for 90 min; and functionally saturating levels of His_6-NSF (325 ng), His_6-α-SNAP (200 ng), His_6-γ-SNAP (200 ng), and p115 purified from bovine brain cytosol (100 ng) [10,19] were included in each assay, along with 0.075 μCi ^{3}H-UDP-GlcNAc (60 Ci/mmol), 3 μl (about 1.5 μg) 'donor' membranes, and 1 μl (about 0.5 μg) 'acceptor' membranes. For each assay, background was determined by omitting cytosol, and the complete, maximal signal was determined with 2 μl (128 μg) of a 50% ammonium sulfate precipitate of bovine brain cytosol (BBC50).

2.4. Electrophoresis, immunoblotting, and peptide sequencing

Proteins were separated by SDS-12% PAGE [22] and visualized by Coomassie staining. Immunoblotting was performed according to standard procedures [23]. Blots were developed using chemiluminescence (Amersham Life Science, Arlington Heights, IL). Antibody dilutions were as follows: rabbit anti-rat PITPα (recognizes both α and β isoforms), 1:20 000; chicken anti-rat PITPα (α-isoform-specific), 1:3000; HRP-conjugated goat anti-rabbit IgG, 1:2000; HRP-conjugated rabbit anti-chicken IgG, 1:5000; ^{125}I-labeled goat anti-rabbit IgG, 1:500. For the quantitative anti-PITP immunoblot used in Table 1, an ^{125}I-labeled goat anti-rabbit IgG secondary antibody was used for quantitation by PhosphorImaging (Molecular Dynamics, ImageQuant software) using the purified bovine PITPα as a standard. For our calculations, we assumed the bovine PITPα was 100% pure, which is a slight overestimate. For peptide sequencing, the 36 kDa protein was electrophoretically transferred to PVDF membrane (DuPont/NEN), and the readily visible, unstained band was excised from the blot and submitted to the Princeton University Synthesizing/Sequencing Facility for N-terminal peptide sequence determination using Edman degradation chemistry.

2.5. Preparation of bovine p115 and recombinant proteins

p115 was purified from bovine brain cytosol as previously described [10]. His_6-α- and His_6-γ-SNAPs as well as His_6-NSF were purified as described [19]. His_6-PITPα was purified, and bacterial phospholipids were exchanged for L-α-phosphatidylcholine as described [20]. His_6-PITPα was separated from unbound phospholipid by Superose 12 size exclusion chromatography (Pharmacia Biotech, Piscataway, NJ).

2.6. Purification of Q1 p36K/bovine PITPα

2.6.1. Preparation of bovine brain cytosol. A 50% ammonium sulfate precipitate of bovine brain cytosol was prepared as previously described (BBC50 [24]), and 17 700 mg of this material, derived from 10 cow brains, was used as the starting material for the purification.

2.6.2. DEAE anion exchange and Cibacron blue 3GA dye affinity chromatography. BBC50 (450 ml) was dialyzed into buffer A (25 mM Tris-HCl, pH 8.5, 10 mM KCl, 10% glycerol (v/v), 1 mM EDTA, 5 mM $MgCl_2$, 1 mM DTT), diluted with buffer A to a final concentration of 10 mg/ml (1780 ml final volume), and loaded at 2 ml/min onto a 900 ml DEAE Sepharose Fast Flow column connected in series to a 200 ml Cibacron blue 3GA column equilibrated in the same buffer. After a 2 l wash with buffer A, the two columns were disconnected from each other, and the material that bound to the Cibacron blue column was eluted with 600 ml of buffer B (25 mM Tris-HCl, pH 8.5, 500 mM KCl, 10% glycerol (v/v), 1 mM EDTA, 5 mM $MgCl_2$, 1 mM DTT) and termed Blue Bound pool. The 430 ml Blue Bound pool had 2.87 mg protein/ml.

2.6.3. Phenyl Sepharose chromatography. Solid KCl (62 g) was added to the Blue Bound pool to raise the final conductivity to that of phenyl buffer B (25 mM Tris-HCl, pH 8.5, 2.5 M KCl, 10% glycerol (v/v), 1 mM EDTA, 5 mM $MgCl_2$, 1 mM DTT), and then loaded at 2 ml/min onto a 60 ml phenyl Sepharose column equilibrated in phenyl buffer B. After a 220 ml wash in the same buffer, the column was eluted with 460 ml of phenyl buffer A (25 mM Tris-HCl, pH 8.5, 10% glycerol (v/v), 1 mM EDTA, 5 mM $MgCl_2$, 1 mM DTT) yielding the 365 ml Phenyl Bound pool, which had 1.12 mg protein/ml.

2.6.4. Mono Q anion exchange chromatography. The Phenyl Bound pool was dialyzed against Mono Q buffer A (25 mM Tris-HCl, pH 9.0, 10% (v/v) glycerol, 1 mM EDTA, 5 mM $MgCl_2$, 1 mM DTT) for 4 h to reduce the salt concentration. This partially dialyzed Phenyl Bound pool was then concentrated to 100 ml using a stirred-cell ultrafiltration unit (Amicon, Beverly, MA) and dialyzed again against Mono Q buffer A for 12–15 h. To avoid over-loading the column, the dialyzed Phenyl Bound pool was divided in half, and each half was loaded at 1 ml/min onto an 8 ml Mono Q HR10/10 column (Pharmacia) equilibrated in Mono Q buffer A. For each column run, after a 25 ml wash with Mono Q buffer A, the bound material was eluted with a biphasic linear KCl gradient (0–250 mM KCl in 52 ml, 250–500 mM KCl in 20 ml) in the same buffer. Assay of the fractions revealed two main peaks of activity. The first peak eluted at 50–100 mM KCl, and the second peak eluted at 110–150 mM KCl. The fractions from the first peak of activity from both column runs were pooled to generate the 14.4 ml Mono Q1 pool, which had 2.27 mg protein/ml.

2.6.5. Superose 12 size exclusion chromatography. The Mono Q1 pool was concentrated to 3 ml in a centrifugal ultrafiltration unit (Ultrafree-4 Biomax-10, Millipore, Bedford, MA), loaded at 0.3 ml/min onto a 100 ml prep grade Superose 12 size exclusion column (Pharmacia) equilibrated in 25 mM Tris-HCl, pH 7.4, 10 mM KCl, 1 mM DTT, 10% (v/v) glycerol, and eluted with the same buffer. The broad peak of activity eluted at approximately 64 ml, corresponding to an M_r of approximately 30 kDa. The active fractions were pooled, yielding the 11 ml S12 pool, which had 0.39 mg protein/ml.

2.6.6. Hydroxylapatite chromatography. The S12 pool was adjusted to a final concentration of 1 mM potassium phosphate (KPi) by the addition of 1 M KPi, pH 7.4, and then loaded at 0.5 ml/min onto a 2 ml Bio-scale ceramic hydroxylapatite column (Bio-Rad) equilibrated in 25 mM Tris-HCl, pH 7.4, 10 mM KCl, 1 mM DTT, 10% (v/v) glycerol, 1 mM KPi, pH 7.4. The bound material was eluted with a biphasic linear KPi gradient (1–100 mM KPi in 12 ml, 100–500 mM KPi in 4 ml) in the same buffer. Assay of gradient fractions revealed a peak of activity that eluted between 45 and 65 mM KPi. These active fractions were pooled to generate the 1.5 ml HAP pool, which had 0.57 mg protein/ml.

2.6.7. Superdex 75 size exclusion chromatography. Approximately

Table 1
Summary of Q1 p36K/PITPα purification

Purification step	mg protein	Protein yield (%)	mg PITP[a]	PITP yield[a] (%)	Specific activity (cpm/μg)
BBC50	17 700	100	36.3	100	103
Blue Bd	1 230	6.9	10.2	28	99
Phenyl Bd	409	2.3	11.4	31	427
Mono Q	32.7	0.18	4.3	12	231
Superose 12	4.3	0.024	2.1	5.8	370
Hydroxylapatite	0.85	0.0048	0.66	1.8	260
Superdex 75[b]	0.30	0.0017	0.30	0.8	320

[a]We used quantitative immunoblotting and PhosphorImager analysis to determine the PITPα concentration and % yield of PITPα at each purification step.
[b]In the purification we only used half of the hydroxylapatite pool for subsequent purification on Superdex 75. Thus, the numbers in this row represent the theoretical yield of the purification had we not halved the preparation prior to Superdex 75 size exclusion chromatography.

half of the hydroxylapatite pool (0.78 ml) was applied at 0.2 ml/min to a 24 ml Superdex 75 Pharmacia column equilibrated in 20 mM HEPES-KOH, pH 7.4, 200 mM KOAc, 5 mM $Mg(OAc)_2$, 1 mM DTT, 1 mM EDTA and eluted with the same buffer. The active fractions were pooled, yielding 1 ml of a highly purified preparation of Q1 p36K/PITPα, which was 0.15 mg/ml.

3. Results

In the absence of crude cytosol, the modified cis-to-medial Golgi transport assay (using non-salt-extracted membranes and assayed in the presence of NSF, α- and γ-SNAPs, and p115) yielded a low level of activity (Fig. 1A). However, when a 50% ammonium sulfate precipitate of bovine brain cytosol (BBC50) was titrated into the assay, the activity increased nearly seven-fold. This result indicated that the modified Golgi transport assay requires supplementation with at least one cytosolic factor necessary for full activation. To purify the factor(s) required for full stimulation, we biochemically fractionated BBC50 and assayed the fractions for their ability to stimulate the modified intra-Golgi transport assay.

As a first step, BBC50 was fractionated by DEAE anion exchange chromatography, and the DEAE load (Ld), flow-through (FT), and bound (Bd) pools were assayed for transport activity. Both the FT and Bd material possessed in vitro Golgi transport activity (Fig. 1B). The specific activities of the Ld (103 cpm/μg) and FT (97 cpm/μg) were similar, whereas that of the Bd (66 cpm/μg) was lower. The differing plateau levels of the FT and Bd, coupled with their differing migrations on a size exclusion column (data not shown), indicated that DEAE anion exchange chromatography separated BBC50 into two distinct activities. The purification of one of the DEAE Bd activities, which was shown to be a novel 13S protein complex, will be described elsewhere (Walter et al., submitted). The purification of the DEAE FT activity is presented below.

The DEAE FT was subjected to dye affinity and phenyl hydrophobic interaction chromatography steps, where it behaved as a single activity. However, the next step, Mono Q anion exchange chromatography, revealed the presence of multiple activities. The FT material of the Mono Q showed some activity, and elution of the material bound to the Mono Q column with a linear salt gradient revealed two peaks of activity: Q1 and Q2 (Fig. 1C). These two peaks, though reproducible, were never completely resolved from each other. A third peak was sometimes seen (asterisk in Fig. 1C), and based on its size exclusion profile, we attributed this peak to DEAE Bd material that had contaminated the DEAE FT (data not shown).

The Q1 activity was further purified by a combination of Superose 12 size exclusion, hydroxylapatite, and Superdex 75 size exclusion chromatography steps, resulting in an essentially pure preparation of a 36 kDa protein that comigrated with a single peak of activity (Fig. 2A,B). Interestingly, this protein had an apparent molecular weight by SDS-12% PAGE of 36 kDa, but an M_r of about 15 kDa by size exclusion chromatography (see below). When titrated into the Golgi transport assay, the purified 36 kDa protein (Q1 p36K) yielded a maximal two-fold stimulation (compared to seven-fold for BBC50) and a specific activity of 320 cpm/μg (Fig. 2C), only a three-fold enhancement over BBC50 (see Table 1 and Section 4). Further titration of Q1 p36K decreased transport activity (data not shown). A Coomassie-stained gel of fractions from the purification is shown in Fig. 2D. Table 1 summarizes the purification shown in Figs. 1 and 2.

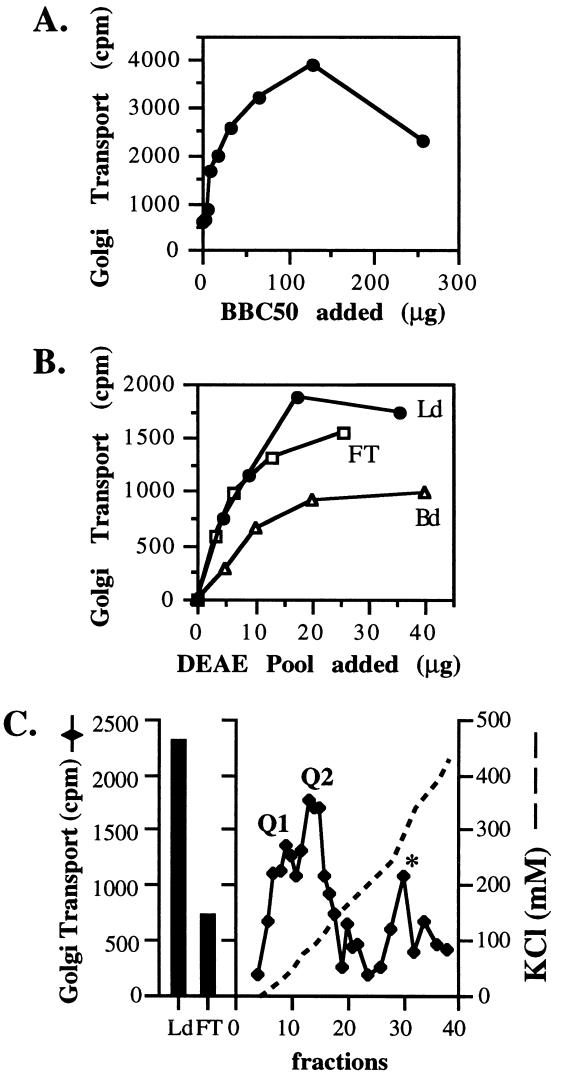

Fig. 1. Identification of three crude Golgi transport activities in bovine brain cytosol. A: BBC50 was titrated into the in vitro Golgi transport assay in the presence of p115, NSF, α-SNAP, and γ-SNAP. B: BBC50 was chromatographed on a DEAE anion exchange column, and the DEAE load (Ld; filled circles), flow-through (FT; open squares), and bound (Bd; open triangles) were titrated into the Golgi transport assay. The background (830 cpm) was subtracted, yielding the data shown. The assay maximum was 4243 cpm. C: After DEAE, Cibacron blue dye affinity, and phenyl chromatography steps, the Phenyl Bd pool was loaded onto a Mono Q column and eluted with KCl. The Mono Q Ld and FT pools (bars) and column fractions (diamonds) were assayed for Golgi transport activity. Three peaks of activity are evident: Q1, Q2, and a third peak (asterisk) that represents a contaminating activity. The assay maximum was 3289, and the background (927 cpm) was subtracted.

Determination of the N-terminal peptide sequence of the Q1 p36K protein and subsequent database searches yielded a nearly perfect match with the α isoform of the rat, mouse, human, and rabbit phosphatidylinositol transfer proteins, PITP (Fig. 3A), the only difference being a conserved glutamate-to-aspartate change, which probably represents a species difference [25]. PITP is a phospholipid binding protein with a

high affinity for phosphatidylinositol (PI), which can effect the net transfer of phospholipids between membranes in vitro (reviewed in [26]). Within a given species, e.g. rat, the α and β isoforms of PITP are approximately 77% identical [27,28], whereas the interspecific identity within isoforms is much higher, 98–99% [29]. Thus, given the high conservation of

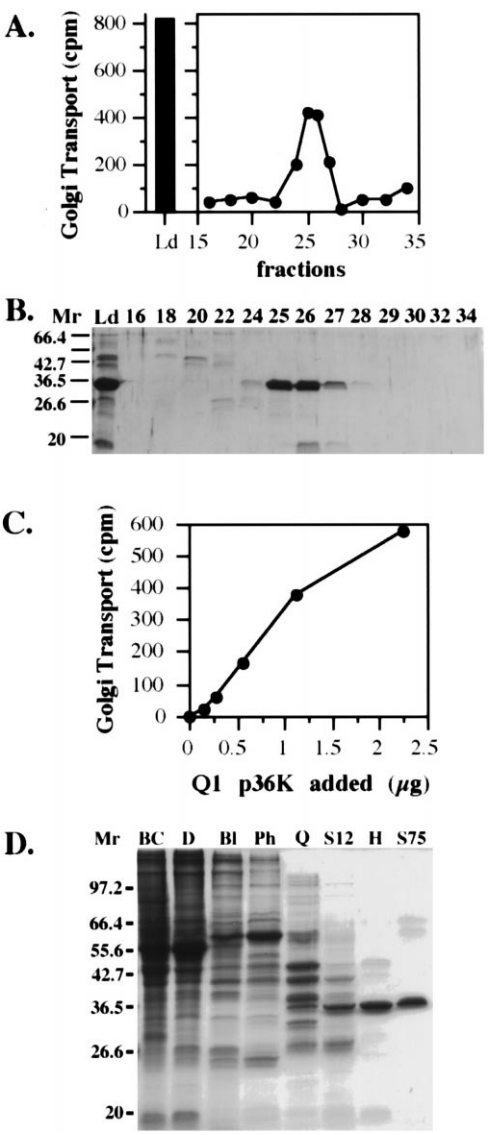

Fig. 2. Purification of the Q1 activity reveals it to be a 36 kDa protein. A: The hydroxylapatite pool was chromatographed on Superdex 75 size exclusion, and the Ld (bar) and column fractions (circles) were assayed for transport activity. A single peak of activity (fractions 25 and 26) was evident, with an M_r of about 15 kDa. The assay maximum was 3650, and the background (527 cpm) was subtracted, yielding the data shown. B: A Coomassie-stained gel of the Superdex 75 Ld and column fractions shown in A. The major 36 kDa band corresponds to the transport activity observed in A. C: Fractions 25 and 26 were pooled and titrated into the Golgi transport assay. The background (581 cpm) was subtracted. The assay maximum was 4124. D: A Coomassie-stained gel of column pools from the purification. The lanes, from left to right, contain 10 μg each of BBC50 (BC), DEAE FT (D), and Blue Bd (Bl); 6 μg of phenyl Bd (Ph); 3 μg of Mono Q1 (Q); 2 μg of Superose 12 (S12); and 1 μg each of hydroxylapatite (H) and Superdex 75 (S75) pools. In the last lane, the higher molecular weight bands are contaminants not present in the Superdex 75 fractions (see B), and the altered mobility of the 36 kDa protein was not reproducible.

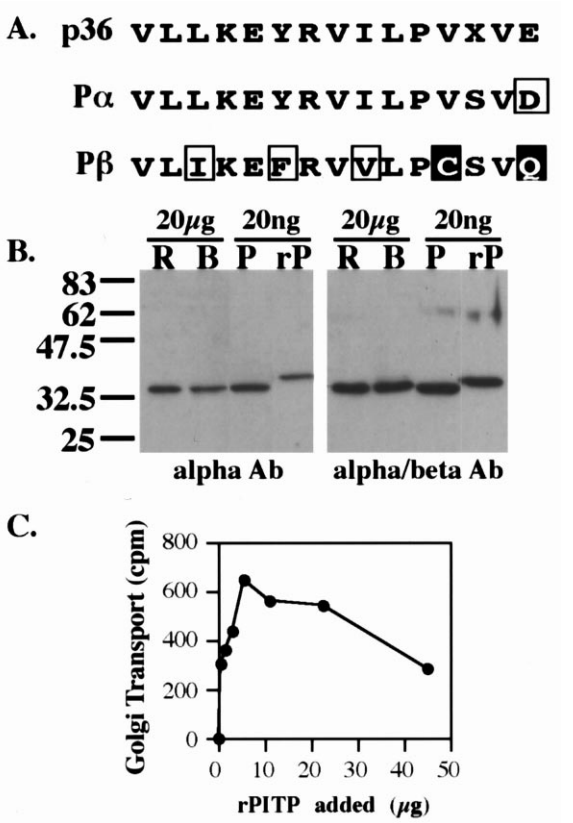

Fig. 3. Q1 p36K is the α isoform of phosphatidylinositol transfer protein (PITPα). A: The N-terminal peptide sequence of Q1 p36K is shown (p36) along with the N-terminal sequence of the α (Pα) and β (Pβ) isoforms of PITP from human, mouse, rabbit, and rat. The identity of the 13th residue of Q1 p36K was ambiguous (X) upon sequencing. Identical residues to p36 are indicated in open text, conserved residues are boxed, and unconserved residues are in reverse text. B: Duplicates of 20 μg each of rat brain cytosol (R) and BBC50 (B), along with 20 ng each of p36K/bovine PITPα (P) and recombinant human PITPα (rP), were resolved by SDS-12% PAGE and blotted to PVDF membrane. One half of the blot was probed with a polyclonal anti-PITPα antibody specific for the α isoform (left panel), while the other half was probed with a polyclonal anti-PITPα antibody that recognizes both the α and β isoforms (right panel). The slower migration of the recombinant protein is due to the His_6-tag. C: Recombinant human PITPα (rPITP) was prepared and titrated into the Golgi transport assay. The recombinant PITPα stimulated the assay, with a specific activity of 97 cpm/μg. The background (529 cpm) was subtracted. The assay maximum was 4605 cpm.

the mammalian PITP sequences, the PITPβ N-terminal sequence is clearly distinct from the PITPα and the Q1 p36K sequences.

Consistent with the peptide sequence data, immunoblotting analysis of Q1 p36K using an α-isoform-specific polyclonal antibody generated against rat PITPα indicated that Q1 p36K and PITPα are immunologically related (Fig. 3B, left panel). In addition, we analyzed the same samples using a polyclonal antibody that recognizes both the α and β isoforms of PITP. Analysis with this bi-specific antibody showed that the signal obtained from 20 ng of His_6-tagged recombinant PITPα (rP) was similar to the signal from 20 μg of both rat and bovine brain cytosols (R, B; Fig. 3B, right panel) indicating an approximate abundance of PITP in cytosol of 0.1%. A similar estimation using the α-specific antibodies yielded the same result (Fig. 3B, left panel), suggesting that the α isoform

may constitute the majority of PITP present in rat and bovine brain cytosols. This may be because PITPβ is associated with the Golgi in vivo [25] and therefore could have been discarded along with the cellular membrane fraction during our preparation of the cytosols.

Final confirmation of the identification of Q1 p36K as PITPα came from studies using a recombinant human His_6-tagged PITPα. When titrated into the in vitro intra-Golgi transport assay, purified recombinant human His_6-tagged PITPα also stimulated the assay, with a specific activity of 97 cpm/μg (Fig. 3C). Although the variation in activity between preparations of the bovine PITPα and recombinant PITPα complicated the determination of comparative specific activities, in general, the specific activity of the recombinant protein ranged from 26 to 97 cpm/μg whereas the specific activity of the bovine PITPα ranged from 107 to 345 cpm/μg (data not shown). Curiously, the discovery that Q1 p36K is PITPα served to explain its aberrantly slow migration on Superdex 75 size exclusion (Fig. 2A,B), as this anomalous behavior on size exclusion is typical of PITP [30].

To determine the concentration of PITPα at each step of the purification and to evaluate the purification of PITPα in comparison to bulk protein, we performed a quantitative immunoblotting analysis of the purification fractions using an ^{125}I-labeled secondary antibody (Table 1). Because the epitope recognized by the α-specific antibody is easily lost or degraded, potentially complicating quantitation (data not shown), we used the anti-rat PITP antibody that recognizes both the α and β isoforms for our analysis. Due to the low levels of PITPβ compared to PITPα in BBC50 (see Fig. 3B), cross-reactivity with the β isoform should be minimal. Using this method, we determined that PITPα is a fairly abundant protein in brain, representing about 0.2% of BBC50, which is in good agreement with our chemiluminescence-based determination of about 0.1% (Fig. 3B). The purification resulted in a 0.8% yield of PITPα compared to a 0.0017% yield of total protein, indicating a 500-fold enrichment of PITPα over total protein. The 0.0017% yield of PITP relative to total protein is comparable to other published PITP purifications [31,32].

4. Discussion

We have presented the purification of PITPα, a phospholipid binding protein, from bovine brain cytosol using an in vitro assay that reconstitutes cis-to-medial intra-Golgi transport. Of the two isoforms of PITP, α and β, only α was purified. However, because the α isoform is the predominant one in our cytosol preparations (see Fig. 3B) these results do not exclude the possibility that the β isoform is also capable of stimulating intra-Golgi transport. Indeed, in in vitro assays where both the α and β isoforms were tested, both isoforms were shown to be active [33–35].

Although PITPα is clearly active in the intra-Golgi transport assay, its specific activity is rather low, which might be accounted for by two factors. First, in addition to NSF, the SNAPs, and p115, at least four activities constitute the activity of BBC50, and all of these may be required for full activity. Thus, as the PITPα activity is purified away from the others, its specific activity and maximal level of stimulation may drop or stay level, rather than increase. For some steps of the purification, this seemed to be the case (see Table 1). Second, like others, we found PITPα to be a very labile protein [30]. Therefore, it is likely that we had partly inactivated the protein in the course of its purification, further contributing to an underestimation of the final specific activity. On the other hand, the concentration of bovine PITP required in the assay for maximal stimulation (80 μg/ml) is similar to that required for PC transfer [20] and the reconstitution of PLC signaling [33], though much higher than that required for PI transfer [34] or secretory vesicle formation [35].

Previous biochemical studies in mammalian cells have implicated a role in secretion for PITP in both the processes of vesicle formation from the TGN [35,36] and secretory vesicle priming, a post-docking event required for subsequent fusion at the plasma membrane [37]. Similarly, in yeast PITP mutants, the observed protein transport block through the Golgi apparatus may result from defects in both vesicle budding and fusion [38]. Taken together, the above genetic and biochemical data suggest that PITP plays a dual role in vesicular transport, affecting both the formation and consumption of vesicles. Interestingly, experiments using permeabilized mammalian cells have indicated that PITP plays yet a third role in mammalian cells as a necessary component of the phospholipase C (PLC)-mediated inositol-lipid signal transduction pathway [39–41].

Studies aimed at elucidating the mechanism of action of PITP in each of these seemingly disparate processes have yielded a singular theme: the activity of PITP stems from its ability to remodel/modulate the phospholipid composition of the membrane bilayer. Not only can PITP selectively exchange one phospholipid for another [34], but it can stimulate the local synthesis of phosphatidylinositol-4,5-bisphosphate (PIP_2) by presenting phosphatidylinositol (PI) to the phosphokinases involved in polyphosphoinositide synthesis [33,42–44]. Furthermore, in yeast, PITP can affect the diacyglycerol pool in Golgi membranes through the dual regulation of the biosynthesis of phosphatidylcholine (PC) and inositol-sphingolipids [45].

Regardless of whether the in vitro Golgi transport assay operates via a vesicular intermediate [13] or simply through membrane docking and fusion [14,16], PITPα may be acting by remodeling the phospholipid content of the membranes. By increasing the synthesis of PIP_2 and other polyphosphoinositides or modulating the abundance of a specific phospholipid pool, such as PC or diacyglycerol, PITP could alter the biophysical properties of the membrane or generate binding sites for the recruitment of proteins [46–48]. The identification of PITPα as a factor capable of stimulating in vitro intra-Golgi transport confirms the critical role that PITP plays in secretory transport and highlights the importance of phospholipids and the membrane environment for in vitro transport assays as well as for the in vivo biological processes they were designed to model. Future study of the role of PITP in Golgi transport and other secretory events may serve to clarify the role of phospholipids and membranes as active players in the process of vesicular transport.

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