The phosphoinositide binding selectivity of Golgi coatomer COPI polypeptides was examined using photoaffinity analogs of the soluble inositol polyphosphates Ins(1,4,5)P_3, Ins(1,3,4,5)P_4, and InsP_6, and of the polyphosphoinositides PtdIns(3,4,5)P_3, PtdIns(4,5)P_2, and PtdIns(3,4,5)P_3. Highly selective Ins(1,3,4,5)P_4-photoaffinity labeling of one of the alpha-COP subunits was observed with a benzoyldihydrocinnamide (BZDC)-containing probe, [^3H]BZDC-Ins(1,3,4,5)P_4. A more highly phosphorylated probe, [^3H]BZDC-InsP_6 probe labeled six of the seven subunits, with only beta, delta, and e-COP showing competitive displacement by excess InsP_6. Importantly, [^3H]BZDC-triester-PtdIns(3,4,5)P_3, the lipid with the same phosphorylation pattern as Ins(1,3,4,5)P_4, showed specific, PtdIns(3,4,5)P_3-photoaffinity labeling of only alpha-COP. Labeling by the PtdIns(4,5)P_2 and PtdIns(3,4,5)P_3 photoaffinity probes was less intense and showed no discrimination based on PtdInsP_2, ligand. Thus, both the D-3 and D-5 phosphates are critical for the alpha-COP-PtdIns(3,4,5)P_3 interaction, suggesting an important role for this phosphoinositide in vesicular trafficking.

Coatomer, a complex of seven polypeptides, is the major component of the non-clathrin (COPI) membrane coat (1). These coat proteins play a key role in regulating intracellular membrane traffic (2, 3). The coat complex consists of seven subunits: alpha-COP, 170 kDa; beta-COP, 110 kDa; beta'-COP, 110 kDa; gamma-COP, 98 kDa; delta-COP, 58 kDa; epsilon-COP, 36 kDa; and zeta-COP, 20 kDa. An eighth essential component is a small, N-terminal myristoylated GTP-binding protein ADP-ribosylation factor (ARF) (4), which is required for coatomer assembly on the donor membrane leading to the formation of coated vesicles (5, 6). ARF associates with PtdIns(4,5)P_2-rich membranes and initiates budding by providing a binding site for coatomer (7, 8).

This paper is available online at http://www.jbc.org.
able GTP analogs and Alfβ-COP is homologous to β subunits of heterotrimeric G-proteins. γ-COP binds to diylsine motifs of membrane proteins (28) and is related to Sec 21, a secretory mutant of the yeast S. cerevisiae (29). A single point mutation in ε-COP has been shown to result in temperature-sensitive lethal defects in membrane transport (30). γ-COP has sequence homology to AP-17 and AP-20 subunits (31). The B complex (α-COP, β'-COP, and ε-COP) has been found to interact directly and bind to membranes (32), while γ-COP and ε-COP form a second subcomplex (33).

Direct binding studies using purified coatomer isolated from bovine liver cytosol show that coatomer specifically binds both Ins(1,3,4,5)P4 and InsP6 with affinities of 0.1 and 0.2 nM, respectively (34). The degree of phosphorylation of the inositol polyphosphates (InsPn) has been proposed to dictate the order of binding to coatomer (InsPn > InsPn > InsPn) (35). Since dissociation of the COPII polypeptides can only be accomplished under conditions that would not permit measurement of InsPn binding, the affinities of individual COPII subunits for InsPn are unknown. Moreover, binding of coatomer complexes to phosphatidylinositol polyphosphates (PtdInsPn) remains unreported. To address the InsPn and PtdInsPn binding specificities of individual COPII subunits, and to obtain evidence to support the roles of these high affinity interactions in vesicular trafficking, we employed a photoaffinity labeling approach with benzophenone-containing InsPn and PtdInsPn analogs (36). The benzophenone photophore allows handling in ambient light, activation at wavelengths >320 nm, and covalent labeling of active site residues in hydrophobic regions of proteins with high efficiency (37, 38). Photoaffinity analogs of soluble inositol polyphosphates Ins(1,4,5)P3, Ins(1,3,4,5)P4, and InsP6 were prepared for Ins(1,4,5)P3, Ins(1,3,4,5)P4, and InsP6 binding to coatomer (39, 40) and the lipid-containing polyphosphoinositides PtdIns(3,4,5)P3 (41), PtdIns(4,5)P2 (41), and PtdIns(3,4)P2 (42) are used herein to determine the polyphosphoinositide selectivity for the COPII subunits in Golgi coatomer (39).

**EXPERIMENTAL PROCEDURES**

**Materials**—The specific activities of the [3H]BZDC-PtdInsPn and InsPn derivatives used were 42.5 Ci/mmol. α-myosin-InsPn, PtdIns(3,4,5)P3, PtdIns(4,5)P2, and PtdIns(3,4)P2 were synthesized from methyl-α-glucopyranoside as described previously (41–43). PtdIns(3)P was prepared similarly. All phosphoinositides had sn-1,2-dipalmitoyl acyl chains in the diacylglycerol moiety. InsPn was obtained from Sigma as N9-tetra(isopropyl)phosphordiamidite (37, 38, 41). This, and other phospholipids, were prepared for Ins(1,4,5)P3 (41), PtdIns(4,5)P2 (41), and PtdIns(3,4)P2 (42) were used herein to determine the polyphosphoinositide selectivity for the COPII subunits in Golgi coatomer (39).

**RESULTS**

**Synthesis of Photoaffinity Labels**—Five photoaffinity labels (Fig. 1) were used to study the coatomer subunit specificity and selectivity for different InsPn and PtdInsPn probes. Each InsPn and PtdInsPn photoaffinity probe was prepared from the corresponding 1- or 2-O-(ω-aminooalkyl)-InsPn or -PtdInsPn derivative with the heterobifunctional reagent [3H]BZDC-NHS ester (38) and had the same nominal specific activity of 42.5 Ci/mmol. This ensured that levels of radioactivity in each probe corresponded to equivalent concentrations, thereby allowing direct comparison of relative efficiencies of photoattachment.

**Photoaffinity Labeling of Golgi Coatomer**—Selectivity of the probes for coatomer subunits was determined by photoaffinity labeling experiments employing [3H]BZDC-InsPn or [3H]BZDC-PtdInsPn probes. Specific binding was determined by competitive displacement of photocouvalent modification in the presence of a 1000-fold excess of unlabeled PtdInsPn. The specificity could be approached by the difference between the total binding (no competitor) and binding in the presence of the competing ligand. Since coatomer has been demonstrated to bind Ins(1,3,4,5)P4 and InsP6 with subnanomolar affinities (0.1 and 0.2 nM, respectively (34)), we initially employed the [3H]BZDC-InsPn and [3H]BZDC-PtdInsPn probes for photoaffinity labeling of the COPII subunits.

Fig. 2 shows the photolabeling of coatomer polypeptides with the P-1-tethered [3H]BZDC-Ins(1,3,4,5)P4 probe. This probe exhibited specific labeling of the 170-kDa subunit α-COP. La-
beling was competitively displaced by a 1000-fold excess (0.28 mM) of Ins(1,3,4,5)P₄ and InsP₆. Thus, both unlabeled Ins(1,3,4,5)P₄ and InsP₆ appeared to compete for the same binding site as [3H]BZDC-Ins(1,3,4,5)P₄.

Coatomer has also been shown to have high affinity binding interactions with InsP₆. To study the polypeptides involved in this binding, we employed the P-2-tethered [3H]BZDC-InsP₆ probe. Fig. 3 shows the labeling obtained with this probe.

[3H]BZDC-InsP₆ exhibited intense labeling of most proteins in this partially purified preparation. Interestingly, addition of competitors InsP₆ and Ins(1,3,4,5)P₄ (0.28 mM) competitively displaced the labeling from b-COP, b₂-COP, d-COP, and e-COP, indicating specific labeling of these subunits. However, labeling of the α-COP and γ-COP resisted competitive displacement, suggesting that the soluble probe might not be sufficient to displace a combined electrostatic-hydrophobic interaction afforded by the photoaffinity analog.

Evidence from our laboratories using photoaffinity labeling (39, 40) has implicated highly selective binding of PtdInsPₙ derivatives to a number of proteins important in the budding and fusion of lipid bilayers, as well as those known to be recruited to polyphosphoinositide-rich membranes. Thus, coatom-mer was labeled with a series of [3H]BZDC-triester-PtdInsPₙ (n = 2 or 3) probes (40) that have diacylglycerol moieties for membrane anchoring, the correct phosphorylation pattern on the β-my/o-inositol ring, and a photoactivatable group that can covalently modify proteins recruited to the membrane surface. Fig. 4 shows that each of the PtdInsPₙ probes, [3H]BZDC-triester-PtdIns(3,4,5)P₃, -PtdIns(3,4)P₂, and -PtdIns(4,5)P₂ (Fig. 1), labeled only α-COP with high subunit and PtdInsPₙ selectivity.

Four main points should be emphasized in interpreting the results of the PtdInsPₙ photoaffinity labeling. First, the labeling profile obtained with the [3H]BZDC-triester-linked bis- and trisphosphoinositides was similar to that observed with the [3H]BZDC-InsP₆ probe. Only the α-COP subunit was labeled significantly with each of these probes. This is consistent with the previous observation that centaurin-α, a PtdIns(3,4,5)P₃-binding protein, was also selectively labeled by this soluble Ins(1,3,4,5)P₄ photoaffinity probe (47). The three-carbon linker with the appended benzoyldihydrocinnamoyl amide group appears to act as a 2-desoxy-acylglycerol analog of PtdIns(3,4,5)P₃ (40).

Second, the labeling intensities indicated that the relative
affinities of the three triester polyphosphoinositide probes for α-COP are in the order [3H]BZDC-triester-PtdIns(3,4,5)P3 > PtdIns(3,4)P2 > PtdIns(4,5)P2. These data further support the hypothesis that this site is selective for binding to the phosphatidylinositol 3,4,5-trisphosphate.

Third, a 1000-fold molar excess of either PtdIns(3,4,5)P3 or PtdIns(3,4)P2 completely displaced labeling of α-COP by [3H]BZDC-triester-PtdIns(3,4,5)P3, but the same excess of PtdIns(4,5)P2 showed only limited displacement. The observation that PtdIns(3,4,5)P3 as well as both PtdIns(4,5)P2 and PtdIns(3,4)P2 completely displaced the covalent modification of α-COP by either of the [3H]BZDC-triester-PtdIns(3,4,5)P3 or -PtdIns(4,5)P2 probes suggests a lack of head group discrimination for the labeling by the two bisphosphoinositide triester probes. This further supports the hypothesis that α-COP has a PtdIns(3,4,5)P3-specific binding site.

Fourth, a titration of the competitive displacement of the photoaffinity labeling of the 170-kDa α-COP band by three D-3 phosphoinositides was performed (Fig. 5), using the pure synthetic di-C16 phosphoinositides (40). The monophosphate PtdIns(3)P showed less than 50% displacement at 1000-fold molar excess relative to the [3H]BZDC-triester-PtdIns(3,4,5)P3 probe, while PtdIns(3,4)P2 showed displacement only at the 1000-fold level. In contrast, even a modest 10-fold excess of PtdIns(3,4,5)P3 showed >50% displacement of the labeling. Because the photoaffinity experiment is intrinsically a nonequilibrium process, “all-or-nothing” displacement is often observed for low affinity competitors.

To verify that the labeled 170-kDa band was indeed α-COP and not an unrelated protein of molecular weight similar to α-COP, immunoprecipitation experiments were performed (Fig. 6). Panel B, quantitation of the photoaffinity-labeling data in panel A using NIH IMAGE 1.59. Intensity of the photolabeled bands is expressed as a percentage of binding relative to [3H]BZDC-triester-PtdIns(3,4,5)P3 binding. Blanks indicate that no labeling was observed.
10 mM GTP was found to have no effect on this binding. These and gel filtration, the complex of PtdIns(3,4)P2 at 10-, 100-, 500-, and 1000-fold excess; lanes g–l dissociated using a high salt buffer (33). After buffer exchange and following the immunoprecipitation reaction. Lanes a and b, with 1 and 2 μl of anti-β-COP antisera, respectively. Lanes c and d, with 3 and 4 μl of anti-α-COP antisera, respectively. Bottom, fluorogram corresponding to the 10% SDS-PAGE shown above. Partially purified protein was photoaffinity labeled with [3H]BZDC-triester-PtdIns(3,4,5)P3 as described earlier. Immunoprecipitation was performed employing the indicated antibodies. Coatomer dissociation (IP buffer with 1 M NaCl) was then performed as a wash step.

in the high salt washes of the anti-β-COP-precipitated proteins. Interestingly, the anti-α-COP antibody used was developed against the C terminus of the α-subunit, strongly suggesting that the PtdIns(3,4,5)P3-binding site on α-COP is not contained within the epitope for antibody recognition on this subunit.

To assess the role of other coatomer subunits in α-COP-PtdIns(3,4,5)P3 interaction, coatomer subunits were partially dissociated using a high salt buffer (33). After buffer exchange and gel filtration, the complex of α-COP, β-COP, and ε-COP (B complex, about 316 kDa) was photoaffinity labeled with the [3H]BZDC-triester-PtdIns(3,4,5)P3 probe. Surprisingly, no labeling was observed on this heterotrimeric complex (data not shown), which has been reported to bind to membranes (32, 33).

To examine the possibility that PtdIns(3,4,5)P3 binding might be involved in the ARF-mediated recruitment of coatomer, two experiments were performed. Brefeldin A (BFA), which has been shown to decrease β-COP binding to membranes (48), had no effect on the [3H]BZDC-triester-PtdIns(3,4,5)P3 photoaffinity labeling of α-COP (Fig. 7A). This fungal metabolite has been shown to prevent the assembly of coatomer onto the membrane by inhibiting the GTP-dependent interaction of ARF with the Golgi membrane (49). Addition of up to 200 μM BFA did not affect the covalent modification of α-COP by the PtdIns(3,4,5)P3 photoaffinity probe. Similarly, addition of up to 10 mM GTP was found to have no effect on this binding. These results further support a specific interaction between PtdIns(3,4,5)P3 and α-COP.

Finally, the effects of salts on this interaction were investigated (Fig. 7B), since the high affinity InsP6-coatomer interaction has been reported to exhibit salt dependence (34). Addition of up to 500 mM CaCl2 had no effect on the labeling of α-COP by [3H]BZDC-triester-PtdIns(3,4,5)P3. However, presence of greater than 300 mM KCl inhibited labeling. Interestingly, addition of both 5 mM GTP and 150 mM KCl was also found to inhibit the labeling.

**DISCUSSION**

Benzophenone-containing photoaffinity labels (37) have proven to be extremely useful as tools for identification of new PtdInsPα- and InsPα-binding proteins (47), characterization of...
their ligand-binding sites, and verification of their PtdIns$_3^{(3,4,5)}$ and InsP$_6$ selectivity (36). The advantages of benzophenone over the classical arylazide photochemistry include improved chemically stability of ligands and adducts, stability in ambient light, low background from nonspecific labeling, and the efficient C-H insertion of the triplet diradicaloid intermediate formed by irradiation at 360 nm (39). Herein we report an application of this photochemical technique to study the subunit specificity of these benzophenone-tethered InsP$_6$ and PtdIns$_3^{(3,4,5)}$ probes with Golgi coatomer COP-I polypeptides.

Initially, photoaffinity labeling studies on bovine coatomer employed soluble Ins$_1(3,4,5)P_3$ and Ins$_6$ photoprobos, since it had been reported that these ligands bound to coatomer with subnanomolar affinities (34). We found that $[^3H]$BZDC-Ins$_1(3,4,5)P_4$ exhibited exquisite selectivity for labeling of the $\alpha$-COP subunit, with complete displacement by the soluble ligand Ins$_1(3,4,5)P_4$. Interestingly, $[^3H]$BZDC-Ins$_1(3,4,5)P_4$ exhibited a much more complex labeling profile. Virtually all COP subunits were photovalently modified, but only $\beta$-COP, $\beta'$-COP, and $\epsilon$-COP showed Ins$_6^\gamma$ and Ins$_1(3,4,5)P_4^\gamma$-competible labeling. The failure of these two ligands to displace labeling of the $\alpha$-COP and $\gamma$-COP subunits can be attributed to a dual hydrophobic-electrostatic interaction of the phosphoinositide-like photoprobe with the protein that could not be disrupted by the electrostatic component only.

The photoaffinity labeling studies on bovine coatomer employed soluble Ins$_1(3,4,5)P_3$ and Ins$_6$ photoprobe, since it had been reported that these ligands bound to coatomer with subnanomolar affinities (34). We found that $[^3H]$BZDC-Ins$_1(3,4,5)P_4$ exhibited exquisite selectivity for labeling of the $\alpha$-COP subunit, with complete displacement by the soluble ligand Ins$_1(3,4,5)P_4$. Interestingly, $[^3H]$BZDC-Ins$_1(3,4,5)P_4$ exhibited a much more complex labeling profile. Virtually all COP subunits were photovalently modified, but only $\beta$-COP, $\beta'$-COP, and $\epsilon$-COP showed Ins$_6^\gamma$ and Ins$_1(3,4,5)P_4^\gamma$-competible labeling. The failure of these two ligands to displace labeling of the $\alpha$-COP and $\gamma$-COP subunits can be attributed to a dual hydrophobic-electrostatic interaction of the phosphoinositide-like photoprobe with the protein that could not be disrupted by the electrostatic component only.

The probes employed in this study (Fig. 1) are more hydrophobic than the endogenous ligands Ins$_1(3,4,5)P_3$ and Ins$_6$ due to the presence of the photoactivatable BZDC moiety and the aminopropyl phosphate ester. Indeed, BZDC-Ins$_1(3,4,5)P_4$ is a reasonable structural surrogate for the inositol phospholipid PtdIns$_3^{(3,4,5)}P_3$ (40), as previously observed for the PtdIns$_3^{(3,4,5)}P_3$-binding protein centaurin-3 (47). Analogously, $[^3H]$BZDC-Ins$_1(4,5)P_3$ has been used as a probe to study the PtdIns$_3^{(4,5)}P_3$-binding sites of the pleckstrin homology domain of recombinant phospholipase C$_{S\gamma}$ isozyme (50) and of recombinant human profilin I. 3

Coatomer has been shown to be similar to AP-2 and cardiac AP-3 in that all three proteins formed K$^+$ channels when incorporated into planar lipid bilayers (34, 51), and each exhibited high affinity binding to certain Ins$P_s$. PtdIns$_3^{(3,4,5)}P_3$ has been shown to be a high affinity ligand for AP-2 (52) and AP-3 (53). Phosphorylated phosphatidylinositol moieties cooperate with membrane proteins in the recruitment of cytosolic proteins for certain vesicle coats (50). It has been postulated that the binding of a coat protein to the head group of a phospholipid may orient the coat protein and facilitate side-to-side association through homophilic-heterophilic interaction with other proteins to generate the coat (54). To test this hypothesis, we examined PtdIns$_3^{(3,4,5)}$-coatomer interactions using photoaffinity labeling.

Photoaffinity labeling with the $[^3H]$BZDC-triestester-PtdIns$_3^{(3,4,5)}P_n$ ($n = 2$ and $3$) probes was highly specific, in analogy to that observed with $[^3H]$BZDC-Ins$_1(3,4,5)P_3$. Thus, $\alpha$-COP was labeled exclusively, and the rank order of labeling intensities was $[^3H]$BZDC-triestester-PtdIns$_3^{(3,4,5)}P_3 > ^3H$BZDC-triestester-PtdIns$_3^{(3,4,5)}P_2 > ^3H$BZDC-triestester-PtdIns$_3^{(3,4,5)}P_1$. Similarly, the concentration dependence of displacement of the labeling of $\alpha$-COP by $[^3H]$BZDC-triestester-PtdIns$_3^{(3,4,5)}P_3$ showed that among the D-3 phosphoinositides, only PtdIns$_3^{(3,4,5)}P_3$ showed full competitive displacement below the 1000-fold molar excess, with the monophosphate PtdIns$_3^{(3)}P$ and the bisphosphate PtdIns$_3^{(4,5)}P_2$ showing substantially lower affinity. The photoaffinity-labeled 170-kDa protein was uniquely immuno precipitated by antibodies against $\alpha$-COP but not by those raised against $\beta$-COP, verifying the identity of the labeled protein as $\alpha$-COP and not a co-migrating protein. Importantly, the rigorous high salt washes employed prior to electrophoresis of the immunoprecipitated protein ensured that only $\alpha$-COP was present in the 170-kDa band.

A human phosphatidylinositol (PI)-specific 3-kinase activity has been implicated in non-clathrin-mediated Golgi membrane traffic (55, 56). This PI-3 kinase complex has been related to the yeast Vps34p-Vps15p protein sorting. Our data thus reflect the potential role of Golgi coatomer as a ligand for PtdIns$_3^{(3,4,5)}P$s and emphasize the potential role of a PI-3 kinase on its recruitment to membranes. Coatomer bound to the products (PtdIns$_3^{(3,4,5)}P_3$ and PtdIns$_3^{(3,4,5)}P_2$) of a PI-3 kinase with higher affinity than a potential substrate PtdIns$_3^{(4,5)}P_2$. Also, the substrate PtdIns$_3^{(4,5)}P_2$ was unable to displace the binding of the product PtdIns$_3^{(3,4,5)}P_3$.

The phosphoinositide products of PI-3 kinase have pivotal roles in regulation of protein trafficking, cell survival, cell growth, actin rearrangement, and cell adhesion (57). Indeed, the actions of a variety of proteins implicated in membrane trafficking and in exo- and endocytosis are modulated by interactions with PtdIns$_3^{(3,4,5)}P$s (54). For example, PtdIns$_3^{(3,4,5)}P_3$ binds specifically and saturably to soluble AP-2, and this binding inhibits the clathrin binding and assembly activities of this heterotetrameric protein (52). Similarly, the brain-derived assembly protein AP-3 (a.k.a. AP-180) also showed preferential binding to and functional regulation by PtdIns$_3^{(3,4,5)}P_3$ (53). In the synaptic vesicle cycle, synaptotagmin I acts as a bimodal calcium-regulated switch, binding with high affinity to PtdIns$_3^{(3,4,5)}P_2$-containing liposomes at $[Ca^{2+}]_{1}$ below 1 $\mu$M but preferentially to PtdIns$_3^{(4,5)}P_2$-containing liposomes at calcium concentrations above 10 $\mu$M. In addition, phospholipase D is activated by polyphosphoinositides (13) and has been shown to mediate ARF-dependent formation of Golgi-coated vesicles (14). Kistakis and co-workers (14) have demonstrated that purified coatomer binds selectively to artificial lipid vesicles that contain phosphatidic acid and PtdIns$_3^{(4,5)}P_2$.

We investigated the effects of salt concentration on the photoaffinity labeling of the COPII polypeptides, since binding of Ins$_6^\gamma$ to coatomer was previously reported to be highest at pH 8.9 with 140 mM KCl (34) and decreased with increased salt concentrations. In corroboration of these results, no photoaffinity labeling was observed at pH below 7.5 (data not shown). Moreover, no labeling was observed above 300 mM KCl, while up to 500 mM CaCl$_2$ had no apparent effect on labeling. High (millimolar) GTP concentrations were reported to block the K$^+$ channel activity on coatomer (34) but had little effect on its Ins$_6^\gamma$ binding. The results herein reflect on a similar behavior for the interaction of PtdIns$_6^{(3,4,5)}P$s with $\alpha$-COP. In addition, the inability of BFA or GTP to interfere with the PtdIns$_6^{(3,4,5)}$-COP interaction suggests that separate, non-allosterically regulated binding sites are involved. Thus, the PtdIns$_3^{(3,4,5)}P_3$-$\alpha$-COP interaction appears to be independent of ARF binding and the coatomer recruitment process.

The inability of the chromatographically isolated B complex of $\alpha$-COP, $\beta$-COP, and $\epsilon$-COP to complex with PtdIns$_3^{(3,4,5)}P_3$ suggests that the PtdIns$_3^{(3,4,5)}P_2$-COP interaction may involve a more complex set of protein-protein interactions. Thus, conformational changes due to subunit interactions may be required to permit PtdIns$_6^{(3,4,5)}P$s binding to $\alpha$-COP. Alternatively, the observed failure of the B complex to undergo photoaffinity labeling could be an artifact of a non-reversible effect resulting from the buffer conditions required for subunit dissociation. However, the physiological significance of this dissociated B complex is not clear, despite reports of its binding to membranes (33).

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A. Chaudhary, J. Chen, Q.-M. Gu, W. Witke, D. Kwiatkowski, and G. D. Prestwich, submitted for publication.
In conclusion, the data presented offer the first evidence for a specific interaction of one, and only one, polypeptide subunit of Golgi coatomer, α-COP, with the polyporphosphoinositide PtdIns(3,4,5)P$_3$. Moreover, these data also demonstrate the specificity of interactions of the soluble inositol polyphosphates Ins(1,3,4,5)P$_4$ and InsP$_6$ with individual coatomer polypeptides. This result offers a new perspective on the potential role of PI 3-kinase in non-clathrin-mediated Golgi membrane trafficking. Moreover, while the 3-phosphate on the inositol ring plays a critical role in defining this interaction, the 5-phosphate is also required for maximal binding activity. In addition, the specific interaction of one, and only one, polypeptide subunit of Golgi coatomer, α-COP, with the polyporphosphoinositide PtdIns(3,4,5)P$_3$. Moreover, these data also demonstrate the specificity of interactions of the soluble inositol polyphosphates Ins(1,3,4,5)P$_4$ and InsP$_6$ with individual coatomer polypeptides. This result offers a new perspective on the potential role of PI 3-kinase in non-clathrin-mediated Golgi membrane trafficking. Finally, the results reported herein represent an application of a new class of PtdIns(3,4,5)P$_3$α-COP probes that sample the interface between the charged phosphoinositide head group and the lipid bilayer. Additional examples of the uses of these [3H]BZDC triester-PtdInsP$_6$ probes for characterization of other protein targets will be presented in due course.

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Anu Chaudhary, Qu-Ming Gu, Oliver Thum, Adam A. Profit, Ying Qi, Loice Jeyakumar, Sidney Fleischer and Glenn D. Prestwich

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