In Vitro Assembly and Disassembly of Coatomer*

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Coatomer, a complex of seven proteins, is the major component of the non-clathrin (COP I) membrane coat. We report here the first system to reversibly disassemble and reassemble this complex in vitro. Coatomer disassembles at high salt concentrations and reassembles when returned to a more physiological buffer. Using this system, we show that α-, β-, and ε-COP interact directly and that γ-COP interacts with ζ-COP. A partial complex comprising α-, β-, and ε-COP, obtained after coatomer disassembly, can bind to membranes in vitro. This binding is, at least in part, mediated by interactions with cytoplasmic KXXX motifs of proteins normally retained in or retrieved to the endoplasmic reticulum. Using coatomer disassembly and epitope-specific antibodies, we also demonstrate that the N- and C-terminal domains of β-COP are buried within the native coatomer complex. These results provide the first insights into how the coatomer is structured.

Coat proteins (COPs)1 play a key role in regulating intracellular membrane traffic (Kreis and Pepperkok, 1994). They are required to form transport vesicles, and they also prevent uncoupled membrane fusion (Orci et al., 1993; Ostermann et al., 1993; Barlowe et al., 1994; Elazar et al., 1994). Three types of coated vesicles have so far been described: clathrin-coated vesicles bud from the plasma membrane and from the trans-Golgi network (Pearse and Robinson, 1990), non-clathrin-coated or COP I-coated vesicles are involved in transport between the intermediate compartment and the Golgi complex (Pepperkok et al., 1993) and in intra-Golgi transport (Ostermann et al., 1993), and COP II-coated vesicles participate in endoplasmic reticulum (ER) to Golgi transport (Barlowe et al., 1994).

The non-clathrin (COP I) coat consists of two major components, coatomer and ADP-ribosylation factor (ARF) (Serafini et al., 1991; Waters et al., 1991). Coatomer and ARF exist separately in the cytosol but co-assemble to form coats. Coatomer is a complex of seven subunits, termed α(-160 kDa), β(107 kDa), β(102 kDa), γ(-100 kDa), δ(-60 kDa), ε(36 kDa), and ζ(20 kDa) COP (Waters et al., 1991). ARF is a small GTPase that is required for membrane association of coatomer (Donaldson et al., 1992b; Palmer et al., 1993). ARF binding to membranes is dependent upon the activity of a brefeldin A-sensitive, membrane-associated, guanine nucleotide exchange factor, which converts ARF-GDP to ARF-GTP (Donaldson et al., 1992b; Helms and Rothman, 1992). ARF-GTP on the membranes stimulates coatomer binding, coat formation, and probably vesicle budding. At a later stage, GTP hydrolysis by ARF triggers coat disassembly and allows vesicle fusion to occur (Tanigawa et al., 1993).

Coatomer and ARF were originally identified using an in vitro intra-Golgi transport assay (Malhotra et al., 1989; Serafini et al., 1991). Good evidence now exists that these components function in transport to the Golgi complex in vivo. For example, mutations in the γ-COP homologue of yeast coatomer, Sec12p, block transport between the ER and the Golgi complex (Hosobuchi et al., 1992). Microinjection of antibodies against β-COP into living mammalian cells blocks transport from the ER to the Golgi complex (Pepperkok et al., 1993). Interestingly, microinjected antibodies against β-COP also inhibit brefeldin A (BFA)-induced retrograde transport from the Golgi complex to the ER, suggesting a role for coatomer in retrograde traffic. Indeed, it has recently been shown that coatomer is essential in yeast for retrieval from the Golgi complex of ER proteins bearing the KXXX signal (Letourneur et al., 1994).

The molecular weights of the coatomer subunits are similar to those of clathrin and the adaptors (Malhotra et al., 1989); β-COP has weak homology to β-adaptin (Duden et al., 1991), and more recently the cloning of ζ-COP has shown that it has homology to the AP17 and AP20 adaptor subunits (Kuge et al., 1993), suggesting there may be similarities in structure and function between the two types of coat. In addition, the binding of coatomer and the AP1 adaptor to the Golgi complex and trans-Golgi network, respectively, is sensitive to brefeldin A (Robinson and Kreis, 1992) and requires ARF (Stamnes and Rothman, 1993), suggesting that similar mechanisms of coat assembly may occur. Interestingly, β'-COP contains five WD40 repeats, which are also found in trimeric G protein β-subunits (Stenbeck et al., 1993; Harrison-Lavoie et al., 1993). The recent finding that yeast α-COP also contains WD-40 repeats in its N terminus (Letourneur et al., 1994) suggests that these repeats might mediate interaction between the β'- and ζ-COP subunits.

We wanted to look at the intersubunit associations between individual COPs within the coatomer complex. To study interactions between the COPs, an in vitro system was established to reversibly disassemble and reassemble coatomer. Using this system, we show that α-, β-, and ε-COP interact directly and that ζ-COP interacts with γ-COP. We also demonstrate that a partial complex comprising α-, β-, and ε-COP, obtained after coatomer disassembly, can bind to membranes in vitro. In addition, using anti-peptide antibodies we show that the N-
and C-terminal domains of β-COP are buried within the coatomer complex.

MATERIALS AND METHODS

Antibodies—Antibodies against synthetic peptides of β-COP have been prepared and affinity purified as described (Pepperkok et al., 1993). Anti-β-COP antibodies were raised in rabbits against the peptide KTDLNDEDILDLD. A murine monoclonal antibody against β-COP (M3A5) was also used (Allan and Kreis, 1986). An anti-peptide antibody raised against γ-COP was kindly donated by Drs. C. Harter and F. T. Windhorst. Fab fragments were prepared according to the instructions of the manufacturer using papain immobilized on beads (Pierce).

Cell Culture—Vero cells (African green monkey kidney cells, ATCC CCL 81) were maintained as described earlier (Kreis and Lodish, 1986).

Metabolic Labeling of Vero Cells and Preparation of Cytosol—Cells were incubated in labeling medium (prepared by mixing 9 parts of methionine/cysteine-free minimal essential medium with 1 part of normal minimal essential medium) containing 30 μCi/ml [35S]methionine/cysteine (Pro-Mix, Amersham) for 18–22 h at 37°C. To prepare cytosol, cells were washed three times with ice-cold homogenization buffer, and the dishes were drained of excess liquid.

Homogenization buffer was either HB-N (0.15 M NaCl, 20 mM Tris, pH 7.5, 2 mM EDTA) if the cytosol was to be used immediately for immunoprecipitation, or HB-P (0.1 M KPO4, pH 6.7, 5 mM MgCl2) if the cytosol was to be used in experiments requiring dialysis. Cells from four 10-cm dishes were minced in 60 ml of homogenization buffer containing protease inhibitors (1 mM phenylmethylsulfonyl fluoride, 0.5 mM iodoacetamide, 2 μg/ml pepstatin A, and 0.5 μg/ml leupeptin) and passed 20 times through a 25-gauge needle. The homogenate was centrifuged at 15,000 rpm for 15 min at 4°C in a microcentrifuge. The supernatant was collected and centrifuged again at 100,000 × g for 1 h at 4°C, and the resulting high speed supernatant was used for experiments.

Immunoprecipitation—Cytosol, which had been dialyzed or frozen/thawed was first centrifuged at 15,000 rpm in a microcentrifuge for 10 min at 4°C to remove aggregates. Triton X-100 was added from a 10% stock to a final concentration of 0.5% final concentration of Protein A-Sepharose (Pharmacia, 50% slurry in immunoprecipitation buffer) was added, and the mixture was incubated for 2 h at 4°C on a rotating wheel. The beads were removed by centrifugation, and specific antibodies were added to the supernatant. After incubation overnight at 4°C, 20 μl of protein A-Sepharose was added. For immunoprecipitations with Fab fragments, 20 μg of goat anti-rabbit IgG was also added here. For immunoprecipitations with M3A5, 20 μg of sheep anti-mouse IgG was added. After 2 h at 4°C, the beads were collected by centrifugation and washed five times with 1 ml of immunoprecipitation (IP) buffer and then once with 1 ml phosphate-buffered saline. IP buffer was IP-N (0.15 M NaCl, 20 mM Tris, pH 7.5, 2 mM EDTA, 0.5% Triton X-100) for immortal Fab fragments from cytosols prepared using HB-N, IP-P (0.1 M KPO4, pH 6.7, 5 mM MgCl2, 0.5% Triton X-100) for cytosols prepared using HB-P and cytosol dialyzed against RB-P, or IP-T (0.5 M Tris, pH 7.5, 2 mM EDTA, 0.5% Triton X-100) for cytosols dialyzed against DB-T. Proteins were eluted from the beads by boiling in sample buffer and were subjected to SDS-PAGE analysis. Disassembly of Immunoprecipitated Coatomer—Coatomer was immunoprecipitated from [35S]methionine-labeled Vero cytosol with 2 μg of anti-EAGE polyclonal antibodies as described above. After the final wash in immunoprecipitation buffer, the beads were resuspended in 1 ml of IP-N buffer, DB-TT (0.5 M Tris, pH 7.5, 2 mM EDTA, 1 mM dithiothreitol, 0.5% Triton X-100), DB-MT (0.25 M MgCl2, 20 mM Tris, pH 7.5, 1 mM dithiothreitol, 0.5% Triton X-100), or DB-NT (1.0 M NaCl, 20 mM Tris, pH 7.5, 2 mM EDTA, 1 mM dithiothreitol, 0.5% Triton X-100) and incubated for 1 h at 4°C on a rotating wheel. The beads were collected by centrifugation and washed twice with 1 ml of the same buffer and then once with 1 ml of phosphate-buffered saline. Proteins were eluted from the beads in sample buffer and subjected to SDS-PAGE analysis. In Vitro Membrane Binding Assay—To measure binding of coatomer, incubations (120 μl) were carried out for 10 min at 37°C in the presence of 0.2 μM sucrose, 25 mM Hepes-KOH, pH 7.0, 25 mM KCl, 2.5 mM MgCl2, 40 μM ATP regenerating system (50 μM ATP, 2 mM creatine phosphate, 12.5 μM creatine kinase), 34 μg of GST-WBP1, and 160 μg of non-dialyzed (3.3 mg/ml) or dialyzed (3.0 mg/ml) rat liver cytosol were added as indicated in the figure legends. Reactions were layered on top of 200 μl of 15% sucrose (in 25 mM Hepes-KOH, pH 7.0, 25 mM KCl) and centrifuged at 4°C in a microcentrifuge for 30 min at 15,000 rpm.

In Vivo Membrane Binding Assay—To measure binding of coatomer, incubations (120 μl) were carried out for 10 min at 37°C in the presence of 0.2 μM sucrose, 25 mM Hepes-KOH, pH 7.0, 25 mM KCl, 2.5 mM MgCl2, 125 μM ATP regenerating system (50 μM ATP, 2 mM creatine phosphate, 12.5 μM creatine kinase), 34 μg of GST-WBP1, and 160 μg of non-dialyzed (3.3 mg/ml) or dialyzed (3.0 mg/ml) rat liver cytosol were added as indicated in the figure legends. Reactions were layered on top of 200 μl of 15% sucrose (in 25 mM Hepes-KOH, pH 7.0, 25 mM KCl) and centrifuged at 4°C in a microcentrifuge for 30 min at 15,000 rpm.

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were precipitated with 10% trichloroacetic acid and processed for SDS-PAGE. To immunoprecipitate membrane-bound proteins, the membrane pellet was solubilized in IP-N for 15 min on ice and then centrifuged for 10 min at 15,000 rpm. Proteins were then immunoprecipitated as described above.

In Vitro Binding to Dilysine Motifs—The GST-WBP1 and GST-WBP1-SS fusion proteins were purified from bacteria as described previously (Cosson and Letourneur, 1994). Incubations (240 μl) were carried out for 2 h at 4 °C in the presence of 40 μl of disassembled COPs (prepared as for the membrane binding experiments), 50 μM Hepes, pH 7.0, 90 mM KCl, 0.5% Triton X-100, and 30 μg of GST-WBP1 or GST-WBP1-SS immobilized on 20 μl of glutathione-Sepharose beads (Pharmacia). The beads were pelleted by centrifugation, and the proteins in the supernatant were precipitated with 10% trichloroacetic acid and processed for SDS-PAGE. The beads were washed 4 × 1 ml with 50 mM Hepes, pH 7.0, 90 mM KCl, 0.5% Triton X-100 and then 1 × 1 ml phosphate-buffered saline. Bound proteins were eluted by boiling in SDS-sample buffer and analyzed by SDS-PAGE.

Electrophoresis and Immunoblotting—Reduced proteins were separated on SDS-polyacrylamide gels according to Laemmli (1970). For the analysis of 35S-labeled proteins, gels were stained with Coomassie Blue, destained, washed in water for 30 min, incubated in 1 mL saline for 20 min, and vacuum dried. Fluorograms were taken on x-ray film (X-Omat-AR films, Eastman Kodak) at −70 °C. Quantitation of radioabeled proteins was performed with a Scan et Plus (Hewlett Packard) using the Deskscan and NIH Image programs on the Macintosh. For immunoblotting, proteins separated by SDS-PAGE were transferred onto nitrocellulose filters. Filters were then incubated with primary antibodies followed by peroxidase-conjugated secondary antibodies (Cappel). Peroxidase labeling was detected by ECL (Amersham).

RESULTS

Coatomer Disassembles at High Salt Concentrations—To study disassembly of coatomer in vitro, the complex was immunoprecipitated from 35S-labeled Vero cytosol and then subjected to various treatments. Immunoprecipitation with rabbit polyclonal antibodies against the EAGE epitope of β-COP under native conditions gave a pattern characteristic of coatomer with bands corresponding to α-, β-, γ-, δ-, ε-, and ζ-COP (Fig. 1A). If the immune complex was incubated in buffer containing 0.5 M Tris, 0.25 M MgCl2, or 1 mM NaCl, only one major band at ~100 kDa remained bound to the beads (Fig. 1A). Note that with 0.5 M Tris there was a small amount of δ-COP remaining bound to the beads. This was reproducible between experiments and represented 15% of the amount of δ-COP present before incubation in 0.5 M Tris. Quantitation of the other COPs revealed that in each case less than 2% of the amount originally bound was present after treatment with Tris. Since β-, γ-, and ζ-COP migrate at ~100 kDa on a normal SDS-polyacrylamide gel, immunoblotting was performed to identify which of these COPs remained on the beads. After incubation with 0.5 M Tris, 0.25 M MgCl2, or 1 mM NaCl, only β-COP was present (Fig. 1B). β-COP therefore dissociates from the other coatomer subunits when incubated in high salt concentrations. The finding that ~15% of δ-COP remained on the beads in 0.5 M Tris while the other subunits were completely dissociated suggests that β-COP can interact directly with δ-COP.

We next analyzed whether the other COPs had dissociated from each other after incubation in high salt concentrations. Immunoprecipitated coatomer was incubated with 0.5 M Tris, 0.25 M MgCl2, or 1 mM NaCl; the beads were removed by centrifugation, and the COPs in the supernatant were analyzed by sucrose gradient centrifugation and gel filtration chromatography. After incubation with 0.5 M Tris or 1 mM NaCl, α-COP comigrated with e-COP and a 100-kDa COP on a sucrose gradient (Fig. 2A). The three proteins peaked at fraction 6. There was another peak of 100-kDa COPs at fraction 4, and δ-COP peaked at fraction 3. In buffer containing 0.5 M Tris, ζ-COP comigrated with a 100-kDa COP at fraction 4 and had a second peak at fraction 2, whereas in buffer containing 1 mM NaCl there was only one peak at fraction 2. 0.25 M MgCl2 gave identical results to 1 mM NaCl (data not shown). After gel filtration chromatography in buffer containing 0.5 M Tris, there was a peak of 100-kDa COPs at fractions 10 and 11 (Fig. 2B). δ-COP peaked at fraction 12 while ζ-COP migrated as two species, one at fractions 10 and 11 and one at fraction 16. There were faint bands for α- and e-COP between fractions 3 and 8. Both proteins were much less abundant than the other COPs, however, suggesting that they are degraded or stick to the column under these conditions. Gel filtration in buffer containing 1 mM NaCl (0.25 M MgCl2, not shown) gave similar results to 0.5 M Tris except that ζ-COP migrated as one peak at fraction 17, and strong bands were present for α-COP, e-COP, and a 100-kDa COP at fractions 6 and 7.

To identify the 100-kDa COPs, proteins were separated on a 7% gel and immunoblotted with antibodies to γ- and β'-COP; note that β-COP remained attached to the beads (see Figs. 1 and 2B). γ-COP migrated as one species peaking at fraction 4 on a sucrose gradient (Fig. 2C) and fraction 12 on a gel filtration column (Fig. 2D). β'-COP migrated as two species. The major species was at fraction 6 on sucrose gradient and fraction 7 on a gel filtration column, and the other (minor) species was at fraction 4 on a sucrose gradient and fraction 11 on a gel filtration column (Fig. 2, C and D). β'-COP is therefore the 100-kDa subunit that cofractionates with α- and e-COP. It was also possible to co-immunoprecipitate α-, β', and e-COP in buffer containing 0.5 M Tris using an antibody to β'-COP,
confirming that these three proteins interact (data not shown).

Thus, incubation with 0.5 m Tris dissociates the coatomer into smaller units, consisting of an α, βγ, and ε-COP complex, a γ- and δ-COP complex, monomeric βδ-COP and δ-COP, and some monomeric ε-COP and βδ-COP. Incubation with 0.25 m MgCl2 or 1 m NaCl dissociates coatomer into an α, βγ, and ε-COP complex and monomeric βδ-COP, γ-COP, δ-COP, ε-COP, and some monomeric βδ-COP. These results show that α, βγ, and ε-COP interact directly in the coatomer complex and that γ-COP interacts with ε-COP.

Membrane Binding of Disassembled Coatomer—We used an in vitro assay (Donaldson et al., 1991, 1992a; Palmer et al., 1993) to study the membrane binding activity of disassembled coatomer subunits. Coatomer that had been immunoprecipitated from 35S-labeled Vero cytosol was incubated with 0.5 m Tris, and the beads were removed by centrifugation. The eluted COPs were then incubated with Golgi-enriched membranes in the presence of a 10–80-kDa fraction of rat liver cytosol (as a source of ARF). α-COP, ε-COP, and a 100-kDa COP bound to the membranes (Fig. 3A). Note that δ-COP and ε-COP did not bind. The 100-kDa COP was identified as βγ-COP since an antibody raised against this subunit co-immunoprecipitated α, βγ, and ε-COP from the membranes. This also showed that these three proteins were in a complex with each other in the membranes. In addition, separation of proteins on a 7% gel to resolve βγ from γ-COP (βδ-COP was not present in the assay since it is not eluted from the antibody with 0.5 m Tris) showed that only βδ-COP, and not γ-COP, was present in the membrane pellet (Fig. 3B). A partial coatomer complex comprising α, βγ, and ε-COP can therefore bind to membranes.
Interactions between Coatomer Subunits

Coatomer and then redialyzed against a more physiological buffer to see if reassembly occurred. Coatomer could be immunoprecipitated from non-dialyzed cytosol under native conditions with the anti-peptide antibodies EAGE (β-COP) or KTDI (β'-COP) (Fig. 4A). After dialysis against buffer containing 0.5 M Tris, only β- or β'-COP was precipitated, showing that both of these proteins had completely dissociated from the other COPs. The complete dissociation of β'-COP was unexpected, since we had already demonstrated that it binds e-COP and e-COP after disassembly of immunoprecipitated coatomer with 0.5 M Tris (see Fig. 2). This difference was not due to the longer time used for dialysis since incubation of immunoprecipitated coatomer in buffer containing 0.5 M Tris for 24 h gave the same result as shorter incubations (data not shown). Perhaps coatomer bound to the EAGE antibody adopts a different conformation to coatomer in cytosol, and this limits the extent of disassembly. Alternatively, cytosolic factors might be required for complete disassembly of coatomer. If cytosol that had first been dialyzed against 0.5 M Tris was then redialyzed against 0.1 M potassium phosphate (RB-P), both EAGE and KTDI again immunoprecipitated the entire coatomer complex showing that reassembly had occurred. Densitometry confirmed that the stoichiometry of subunits was the same for native and for reassembled coatomer (Table I).

Sucrose gradient centrifugation confirmed that disassembly with 0.5 M Tris is reversible. β-COP peaked at fraction 11 in non-dialyzed Vero cytosol, giving a sedimentation coefficient of ~13 S as previously reported (Duden et al., 1991) (Fig. 4B). After dialysis against buffer containing 0.5 M Tris, β-COP shifted to a lower density and peaked at fraction 5, corresponding to a sedimentation coefficient of ~6 S. Redialysis against 0.1 M potassium phosphate resulted in a β-COP distribution similar to that of non-dialyzed cytosol, showing that it had fully integrated into reassembled coatomer. Identical results were obtained with rat liver cytosol (data not shown).

Reassembled Coatomer Is Functional—The in vitro membrane binding assay was used to study the membrane binding activity of reassembled coatomer and to compare it to that of native coatomer. Incubation of non-dialyzed rat liver cytosol with Golgi-enriched membranes resulted in membrane binding of coatomer (Fig. 5). This binding was increased 2 to 3 fold by GTPγS in agreement with previously reported results (Donaldson et al., 1991; Palmer et al., 1993). Rat liver cytosol, which had previously been dialyzed against buffer containing 0.5 M Tris and then 0.1 M potassium phosphate to disassemble and reassemble coatomer, gave a similar amount of binding to untreated cytosol. This was enhanced 2-fold by GTPγS. Thus, reassembled coatomer is competent to bind membranes in a GTP-dependent manner, as described for native coatomer.

The N and C Termini of β-COP Are Sequestered within the Coatomer Complex—To identify domains of β-COP required for interactions with the other coatomer subunits, we took advantage of the large number of anti-peptide antibodies raised against β-COP and the finding that coatomer can be disassembled using relatively mild conditions. 35S-Labeled Vero cytosol was subjected to immunoprecipitation under native conditions with eight antibodies raised against β-COP (see Fig. 7) (we initially used 24 different antibodies, but 16 of these did not immunoprecipitate under native conditions or in buffer containing 0.5 M Tris (data not shown)). Of the eight antibodies, seven failed to precipitate any specific proteins and only one, EAGE, immunoprecipitated coatomer (Fig. 6A). If the cytosol was first dialyzed against buffer...
containing 0.5 M Tris, all eight of the antibodies now immunoprecipitated β-COP. Note that, as expected, only β-COP and none of the other COPs was precipitated under these conditions since β-COP is dissociated from the other COPs. Similar results were obtained when the experiment was repeated with Fab fragments (Fig. 6B). The other coatomer subunits therefore appear to mask the epitopes of seven of these antibodies, and it is only when they are removed that the epitopes can be recognized. These epitopes may form interaction sites for binding to the other coatomer subunits.

FIG. 4. Disassembly of coatomer is reversible. Panel A, 35S-labeled Vero cytosol was dialyzed against buffer containing 0.5 M Tris, pH 7.5 (DB-T), and then half of the dialysate was redialyzed against 0.1 M KPO₄, pH 6.7, 5 mM MgCl₂ (RB-P). Non-dialyzed, Tris-dialyzed, and potassium phosphate-dialyzed samples were immunoprecipitated with antibodies to β-COP (1, EAGE) or β'-COP (2, KTDI) as indicated and analyzed by SDS-PAGE on a 10% gel. Proteins were detected by fluorography. Molecular mass standards are in kilodaltons. Panel B, Vero cytosol was dialyzed as described above and fractionated by sucrose gradient centrifugation. Proteins were subjected to SDS-PAGE on a 10% gel. β-COP was detected by immunoblotting with M3A5 antibodies at 1:1,000 dilution using ECL. The positions of marker proteins are indicated (A, BSA, 4.3 S; C, catalase, 11.1 S; T, thyroglobulin, 16.5 S).

TABLE I

Stoichiometry of COPs in native and reassembled coatomer

Coatomer was immunoprecipitated using EAGE antibodies from non-dialyzed cytosol (native) or cytosol that had been dialyzed against buffer containing 0.5 M Tris, pH 7.5, and then redialyzed against 0.1 M potassium phosphate (reassembled) (see Fig. 4A). The amount of each subunit was measured by densitometry as described under “Materials and Methods” and is expressed in arbitrary units relative to the amount of α-COP.

| Subunit | Amount (arbitrary units) |
|---------|--------------------------|
| Native  | Reassembled              |
| α-COP   | 1.0                      |
| β, β', γ-COP | 5.1                    |
| δ-COP   | 0.80                     |
| ε-COP   | 0.50                     |
| ζ-COP   | 0.13                     |

FIG. 5. Reassembled coatomer can bind to membranes. 14 µg of Golgi-enriched membranes were incubated for 10 min at 37 °C in the presence or absence of 160 µg of non-dialyzed rat liver cytosol or 160 µg of cytosol that had been dialyzed against buffer containing 0.5 M Tris, pH 7.5 (DB-T), and then redialyzed against 0.1 M potassium phosphate (RB-P). 25 µM GTPγS was added as indicated. Panel A, membrane-bound β-COP was detected by immunoblotting with M3A5 at 1:1000 dilution. Panel B, quantitation of the immunoblot shown in A.

FIG. 6. Immunoprecipitation with eight anti-β-COP antibodies. Non-dialyzed (top portion) or 0.5 M Tris (DB-T)-dialyzed (bottom portion) 35S-labeled Vero cytosol was immunoprecipitated with different anti-β-COP antibodies as indicated. Proteins were fractionated by SDS-PAGE using a 10% gel and detected by fluorography. Immunoprecipitations were carried out with whole IgG5 (A) or Fab fragments in conjunction with a linker antibody (B). Molecular mass standards are in kilodaltons.

DISCUSSION

We have developed an in vitro system that allows reversible disassembly of coatomer. Under defined conditions α-, β'-, and ε-COP and γ- and ζ-COP remain associated, showing that these subunits interact directly in the coatomer complex. In addition, β-COP can interact directly with δ-COP since a significant fraction of the δ-COP present in immunoprecipitated coatomer remains associated with β-COP after treatment with 0.5 M Tris, while the other subunits are completely released. This interaction is weaker than that between γ- and ζ-COP. The interaction between γ- and ζ-COP is, in turn, weaker than that between α-, β'-, and ε-COP since γ- and ζ-COP dissociate in buffer contain-
Interactions between Coatomer Subunits

The epitope of antibody 110-12 is shown. 110-12 did not work by immunoprecipitation (this study) but did recognize the protein (data not shown). The antibodies marked with an filled arrow head (EAGE) recognizes β-COP in coatomer and after disassembly of the complex.

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