Rab Geranylgeranyl Transferase

A MULTISUBUNIT ENZYME THAT PRENYLATES GTP-BINDING PROTEINS TERMINATING IN CYS-X-CYS OR CYSCYS*

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Rab proteins are membrane-bound prenylated GTP-binding proteins required for the targeted movement of membrane vesicles from one organelle to another. In the current paper we have purified and characterized an enzyme that attaches geranylgeranyl residues to Rab proteins that bear the COOH-terminal sequence Cys-X-Cys (such as Rab3A) and Cys-Cys (such as Rab1A). This enzyme is designated Rab geranylgeranyl transferase (Rab GG transferase). At high salt concentrations, Rab GG transferase from rat brain cytosol separates into two components, designated A and B, both of which are required for activity. We purified Component B to apparent homogeneity and found that it contains two peptides of 60 and 38 kDa. The purified Rab GG transferase did not attach geranylgeranyl to p21<sup>Ha-ras-CVLL</sup>, which is prenylated by a GG transferase of the CAAX type that resembles the CAAX farnesyltransferase. Rab GG transferase was strongly inhibited by Zn<sup>2+</sup>, a cation that is absolutely required by farnesyltransferase. The Rab GG transferase was also inhibited by NaCl concentrations in excess of 100 mM. Together with previous data, the current findings indicate that mammalian cells possess at least three protein prenyltransferases (CAAX farnesyltransferase, CAAX GG transferase, and Rab GG transferase) that are specific for different classes of low molecular weight GTP-binding proteins and other proteins.

Rab proteins are small GTP-binding proteins with molecular masses of 21–25 kDa which are attached extrinsically to membranous organelles in animal and yeast cells (for review see Refs. 1 and 2). In yeast, mutations in one Rab protein, designated YPT1, block the movement of membrane vesicles within the Golgi apparatus. The mammalian counterpart of Rab1A, is attached to synaptic vesicles in neurons and dissociates reversibly upon calcium-triggered exocytosis (4). These findings have engendered the notion that Rab proteins play some role in the targeted movement of vesicles from one membranous structure to another (1, 2).

Adherence to membranes requires that Rab proteins possess prenyl groups attached by thioether bonds to cysteines near COOH termini (for review see Refs. 1 and 2). Most of the known Rab proteins terminate in either of two sequences: Cys-X-Cys (where X is alanine, serine, or glycine) or Cys-Cys. The synaptic vesicle protein Rab5A (also known as <i>sgn25A</i>) contains geranylgeranyl groups on each of the cysteines in its Cys-X-Cys sequence (5, 6). Rab1A contains at least one geranylgeranyl residue attached to its Cys-Cys sequence (6). Prenylation of Rab proteins is the initial step required for membrane attachment (7). Additional protein modifications and binding to specific receptors may also be required (1, 2).

In addition to Rab proteins, other small membrane-bound GTP-binding proteins, such as the p21<sup>ras</sup> proteins, are prenylated, as are other extrinsic membrane proteins, such as nuclear lamins and the γ-subunit of heterotrimeric G proteins (1, 2). Several protein prenyltransferases catalyze the attachment of either 20-carbon geranylgeranyl or 15-carbon farnesyl groups to cysteine residues in these proteins.

The best characterized prenyltransferase, protein farnesyltransferase, attaches farnesyl to cysteines at the fourth position from the COOH terminus of all known p21<sup>ras</sup> proteins as well as several other proteins (8–10). This enzyme recognizes peptides as short as 4 amino acids in length that correspond to the CAAX box consensus sequence, where the A residues are usually (but not always) aliphatic and the COOH-terminal X residue is methionine, serine, glutamine, or cysteine (11). Peptides terminating in leucine are recognized poorly, if at all, by this enzyme (11–13). Protein farnesyltransferase has been purified to homogeneity from rat brain (8), and cDNAs for its two subunits, designated α and β, have been isolated (14–16). The β-subunit contains Zn<sup>2+</sup>, which is necessary for binding the p21<sup>ras</sup> protein substrate (17). The function of the α-subunit is unknown, but it may participate in binding farnesyl pyrophosphate.

Other protein prenyltransferases attach geranylgeranyl groups to cysteine, and none has yet been purified to homogeneity. One of these enzymes, designated CAAX GG transferase<sup>1</sup> (12) or GG transferase-I (13), resembles the CAAX farnesyltransferase in prenylating peptides in which cysteine is fourth from the COOH terminus. This GG transferase prefers CAAX sequences that terminate in leucine (12, 13, 18, 19). Substrates include the γ-subunit of heterotrimeric G proteins from brain (COOH-terminal sequence CAL1) (20, 21) and rap1A/Krev1, a low molecular weight GTP-binding protein whose COOH-terminal sequence is CVLL (12, 19, 22). Remarkably, this enzyme contains an α-subunit that appears identical to the α-subunit of the farnesyltransferase (12, 13).

The abbreviations used are: GG transferase, geranylgeranyl transferase; GGPF, geranylgeranyl pyrophosphate; DTT, dithiothreitol; SDS, sodium dodecyl sulfate.

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Moore et al. (13) recently demonstrated a second GG transferase that attaches geranylgeranyl to Rab proteins that terminate in Cys-Cys. One known substrate is YPT1 from *Saccharomyces cerevisiae*. Other potential substrates that terminate in Cys-Cys include Rab1A, Rab1B, and Rab2 (1, 2, 6). This enzyme differs from the two CAAX prenyltransferases in that it does not recognize short peptides (13). Horiuchi et al. (23) demonstrated a cytosolic GG transferase in bovine brain that prenylates Rab3A, which terminates in Cys-Ala-Cys. The enzyme was detected in crude extracts, but it rapidly lost activity during purification, and no molecular characterization was reported. It is not yet clear whether this GG transferase, which prenylates Cys-X-Cys sequences, is different from the enzyme that recognizes Cys-Cys sequences.

In the current experiments we have purified a Rab GG transferase from rat brain. We found that the loss of activity during purification is attributable to the separation of two components, designated A and B, both of which are required for activity. We have purified the B component (consisting of two peptides of 60 and 38 kDa) to apparent homogeneity and have found that it participates in the attachment of geranylgeranyl to both classes of Rab proteins, namely those that terminate in Cys-X-Cys (Rab3A) and Cys-Cys (Rab1A). We have designated this enzyme Rab GG transferase.

**EXPERIMENTAL PROCEDURES**

**Materials**

[1-3H]GGPP (8 Ci/mmol) was purchased from Dr. R. Kennedy Keller (University of South Florida, Tampa, FL). Unlabeled GGPP was generously provided by Dr. Dale Postler (University of Utah, Salt Lake City). Unlabeled farnesyl pyrophosphate was synthesized by Dr. J. R. Falck (University of Texas Southwestern Medical Center at Dallas).

**Expression and Purification of Recombinant Protein Substrates**

Recombinant bovine Rab3A was expressed in *Escherichia coli* and purified as described previously (24). After purification, the column fractions were subjected to electrophoresis on a 12.5% SDS-polyacrylamide gel, stained with Coomassie Brilliant Blue, estimated to be more than 90% pure, divided into multiple aliquots, and stored at -70 °C. Recombinant canine Rab1A cDNA was kindly provided by Dr. Channing Der (La Jolla Cancer Research Foundation, La Jolla, CA) (Ref. 6). The plasmid was introduced into BL21DE3 E. coli strain, and the expressed protein was purified essentially as described for p21<sup>W*W</sup> (12) except that only the DEAE-chromatography step was performed. Rab1A was estimated to be more than 90% pure by SDS-gel electrophoresis, divided into multiple aliquots, and stored at -70 °C. Recombinant human p21<sup>W*W</sup> and p19<sup>W*W</sup> were expressed and purified as described previously (12).

**Assay for Rab GG Transferase Activity**

Rab GG transferase activity was determined by measuring the amount of [3H]geranylgeranyl transferred from [3H]GGPP to Rab3A protein. The standard reaction mixture contained the following concentrations of components in a final volume of 50 μl: 50 mM sodium Hepes (pH 7.2), 5 mM MgCl<sub>2</sub>, 0.3 mM Nonidet P-40, 25 μM EDTA, 1 mM DTT, 0.54 μM recombinant Rab3A, and 0.5 μM [3H]GGPP (17,600 dpm/pmol). After incubation for 10 min at 37 °C, the reaction mixtures were processed by trichloroacetic acid precipitation followed by filtration on glass-fiber filters as previously described for assay of protein farnesyltransferase activity (8).

**Assays for Protein Farnesyltransferase and CAAX GG Transferase Activities**

These enzymatic activities were determined as described previously (8, 12).

**Purification of Component B of Rab GG Transferase**

All steps were carried out at 4 °C. Steps 3-6 were performed with a fast protein liquid chromatography system (Pharmacia LKB Biotechnology Inc.).

Steps 1 and 2: Homogenization and Ammonium Sulfate Fractionation—Brains from 50 male Sprague-Dawley rats (100-150 g) were homogenized as described previously for the purification of protein farnesyltransferase (8). The brain homogenate was centrifuged at 60,000 x g for 70 min. The supernatant fraction was treated with 50 mM ammonium sulfate, and the material precipitating between 30 and 50% ammonium sulfate was dissolved and dialyzed overnight against buffer containing 20 mM Tris-chloride (pH 7.5), 1 mM DTT, and 20 μM ZnCl<sub>2</sub> (Buffer 1).

Step 3: Ion Exchange Chromatography—Approximately one-third of the dialyzed 30-50% ammonium sulfate fraction of protein) was chromatographed on a Mono Q 10/10 column (Pharmacia) as described in the legend to Fig. 1. The active fractions from three consecutive Mono Q runs were pooled (24 ml) and dialyzed overnight against 6 liters of buffer containing 20 mM sodium Hepes (pH 7.2), 10 mM NaCl, 0.1 mM Nonidet P-40, and 1 mM DTT (Buffer 2).

Step 4: Gel Filtration in 10 mM NaCl—The dialyzed Mono Q fraction was concentrated approximately 4-fold in CF25 Centrifiio cones (Amicon) One-half of the fraction (~3 ml) was loaded onto a Superdex 200 26/60 column (Pharmacia) that had been equilibrated in Buffer 2. The flow rate was 2 ml/min, and the material eluting between 100 and 260 ml was collected in 4-ml fractions (Fractions 1-25 in Fig. 2). The gel filtration step was then repeated with the other half of the Mono Q fraction.

Step 5: Hydrophobic Interaction Chromatography—The active fractions from both Superdex gel filtration runs in Step 4 were pooled (48 ml) and concentrated approximately 5-fold in CF25 Centrifiio cones. The concentration of ammonium sulfate was adjusted to 0.6 M by the addition of 0.79 g of solid ammonium sulfate, and the material was loaded onto a phenyl-Superose 5/5 column (Pharmacia) that had been equilibrated in Buffer 3. The flow rate was 0.5 ml/min, and the material eluting between 50 and 90 ml was collected in 1-ml fractions (fractions 1-34 in Fig. 5). The fractions containing Rab GG transferase activity were pooled (usual final volume of 4-5 ml). This material, which is hereafter called purified Component B, was divided into multiple aliquots and stored at -70 °C. The protein content of this material was measured by Method B (see below).

Partial Purification of Component A of Rab GG Transferase

Rat brain extract was processed through Steps 1-4 as described above. The sample eluted from Step 4 was adjusted to 0.25 M NaCl, and 48 ml was applied to a 5-ml Red A Dyematrix column (Amicon) that had been equilibrated at 4 °C with buffer containing 20 mM sodium Hepes (pH 7.2), 0.5 mM Nonidet P-40, and 1 mM DTT (Buffer 5). The flow rate was 0.5 ml/min, and the material eluting between 50 and 90 ml was collected in 1-ml fractions (fractions 1-34 in Fig. 5). The fractions containing Rab GG transferase activity were pooled (usual final volume of 4-5 ml). This material, which is hereafter called partially purified Component A, was eluted with 30 ml of 0.2 M NaCl in the same buffer and dialyzed overnight against 6 liters of Buffer 2. The dialyzed fraction was concentrated 10-fold on CF25 Centrifiio cones, divided into multiple aliquots, and stored at -70 °C. This material is hereafter called purified Component A.

**Other Methods**

SDS-polyacrylamide gel electrophoresis was carried out as described by Laemmli (25), and the gels were calibrated with low range SDS-polyacrylamide gel electrophoresis standards (Bio-Rad). Gel filtration columns were calibrated with a gel filtration calibration kit (Pharmacia) for the Superdex 200 26/60 column and with a gel filtration standard (Bio-Rad) for the Superdex 200 16/60 column. The protein concentration of all samples (except purified Component B) was determined with protein assay, a modification of the method of Bradford (26). The amount of protein in the protein concentrated purified component B was estimated from Coomassie Blue R-250 staining and densitometric scanning of a 10% SDS-polyacrylamide gel in which known amounts (0.5-2 μg) of low molecular weight range Bio-Rad.
For the first step in the purification of Rab GG transferase, we subjected a 30–50% ammonium sulfate fraction of rat brain cytosol to ion exchange chromatography on a Mono Q column. Fractions were assayed for Rab GG transferase activity by trichloroacetic acid precipitation following incubation with [3H]GGPP and recombinant Rab3A isolated from E. coli. We also assayed the fractions of CAAX GG transferase activity with a chimeric p21H·mut substrate containing CVLL at the COOH terminus and for farnesyltransferase activity with p21H·mut. Fig. 1 shows that the two GG transferases were clearly separated on the column, with the farnesyltransferase eluting between them.

The Mono Q fractions containing Rab GG transferase activity were pooled and applied to a Superdex 200 gel filtration column in low ionic strength buffer (10 mM NaCl). Fig. 2 shows that under these conditions Rab GG transferase activity eluted at an apparent molecular weight of ~330,000, preceding the farnesyltransferase which eluted at an apparent molecular weight of ~230,000.

When we attempted to purify the Rab GG transferase further by hydrophobic chromatography, the activity was resolved into two components that required mixing to restore activity. In one such experiment, shown in Fig. 3, fractions containing Rab GG transferase activity from a Mono Q column similar to the one in Fig. 1 were applied to a phenyl-Superose column in 0.5 mM ammonium sulfate, 1 mM DTT, and 0.1 mM Nonidet-P40. Although the starting material contained abundant Rab GG transferase activity, virtually no activity was found in any of the five column fractions (panel designated none in Fig. 3). We then performed a systematic mixing experiment in which aliquots of each fraction were added to assays employing each of the other fractions. We observed a peak of activity that appeared to be maximal when Fractions 2 and 3 were mixed (Fig. 3).

**RESULTS**

**FIG. 2. Chromatography of Rab GG transferase on Superdex 200 in 10 mM NaCl.** The fractions containing Rab GG transferase activity from three consecutive Mono Q runs were pooled and concentrated as described under “Experimental Procedures.” The sample was applied in a volume of 3 ml (16.5 mg of protein) to a Superdex 200 26/60 column equilibrated in Buffer 2 and eluted as described under “Experimental Procedures.” An aliquot of each fraction was assayed for Rab GG transferase activity (5 μl) and farnesyltransferase activity (2 μl) ( ). The units of activity represent the total nmol of prenyl groups transferred per 10-min assay calculated from the岗 fraction. The dashed lines (---) denote the protein content of each fraction. The column was calibrated with thyroglobulin (670 kDa), ferritin (440 kDa), catalase (232 kDa), and aldolase (158 kDa); the arrows denote the positions of elution of the markers.

**FIG. 3. Separation of Rab GG transferase activity into non-active components by phenyl-Superose chromatography.** Partially purified Rab GG transferase (~10 mg; Mono Q fraction, Step 3) in 10 ml of buffer containing 50 mM sodium Hepes (pH 7.2), 0.5 mM ammonium sulfate, 1 mM DTT, and 0.1 mM Nonidet-P40 was loaded onto a phenyl-Superose 5/5 column (Pharmacia) presaturated in the same buffer containing 0.5 mM ammonium sulfate. The column was run at a flow rate of 1 ml/min, and the flow-through was collected (Fraction 1). A step to 0.38 M ammonium sulfate was performed with a buffer containing 10 mM sodium Hepes (pH 7.2), 1 mM DTT, and 0.1 mM Nonidet-P40, and Fraction 2 was collected. Three other consecutive steps to 0.28 M ammonium sulfate (Fraction 3), 0.05 M ammonium sulfate (Fraction 4), and no ammonium sulfate (Fraction 5) were performed. The volume of each fraction (Fractions 1–5) was 10 ml. These fractions and an aliquot (0.5 ml) of the starting material (SM) were dialyzed overnight at 4 °C against Buffer 2. An aliquot (10 μl) of each of these dialyzed fractions was then used to measure Rab GG transferase activity in the standard assay incubated for 30 min at 37 °C in the absence or presence of an aliquot (10 μl) of the indicated added fraction.
and low salt Superdex chromatography steps, which failed to resolve the two components. We then applied the mixture to a red A Dyematrex column in 0.25 M NaCl. After washing, Component A was eluted with 2 M NaCl. This Component A material had minimal Rab GG transferase activity on its own, but it did restore the activity of Component B (see below).

To continue the purification of Component B, the fractions from the low salt Superdex 200 column of Fig. 2 which contained Rab GG transferase activity were pooled and applied to a phenyl-Superose column in 0.6 M (NH₄)₂SO₄, which was eluted with a linear gradient containing decreasing ammonium sulfate concentrations (Fig. 4). Fractions were assayed for Rab GG transferase activity either alone (open circles) or in the presence of added Component A (closed circles). In the absence of Component A, the fractions had no transferase activity. In the presence of Component A, we observed a peak of activity emerging at the midpoint of the gradient.

The next step took advantage of the fact that Component B had been separated from Component A, and therefore Component B should have a lower apparent molecular weight on gel filtration. Accordingly, the fractions from the phenyl-Superose column with Component B activity were pooled and reapplied to a Superdex 200 gel filtration column, this time in the presence of high salt (0.5 M NaCl). The peak of protein eluted ahead of the 158-kDa marker (Fig. 5). The activity of Rab GG transferase Component B, assayed in the presence of added Component A, was well separated from the protein peak, eluting at a position corresponding roughly to an apparent molecular weight of 90,000.

Table I summarizes the results of one complete purification procedure for Component B of the Rab GG transferase using the steps outlined above. For consistency, we assayed all of the fractions throughout the purification in the presence of excess Component A. The results suggested that Component B had been purified approximately 17,000-fold relative to its activity in crude cytosol, with an overall yield of approximately 17%. With this procedure, we were able to isolate ~16 µg of purified protein from 1.6 g of rat brain cytosol protein. These figures should be considered as gross approximations since the assays for Component B activity in crude cytosol are not accurate and since the protein concentration of the final purified material was not measured chemically but was estimated from the intensity of the bands observed on Coomassie-stained SDS-polyacrylamide electrophoresis gels (see below).

SDS-polyacrylamide gel electrophoresis of the fractions that emerged from the final high salt Superdex 200 column revealed two peptides with molecular masses of 60 and 38 kDa that co-eluted with the Rab GG transferase Component B activity (Fig. 6, Panel A). When the pooled active fractions from this Superdex 200 column were subjected to further analytical chromatography on Mono Q, both peptides again eluted coincident with Component B activity, and no other bands were visible in the peak fraction (Fig. 6, Panel B). In multiple preparations of enzyme purified by the above procedure, we always observed both the 60- and 38-kDa proteins in an approximate 1:1 ratio as indicated by Coomassie Blue staining of the gels. Moreover, the molecular weight of the final Component B preparation, as estimated by gel filtration in high salt, was ~90,000 (Fig. 5). We therefore believe that Component B may be a 1:1 heterodimer of 60- and 38-kDa subunits.

Fig. 7 shows that purified Component B required Component A for Rab GG transferase activity. The partially purified preparation of Component A had slight Rab GG transferase activity on its own, but this was stimulated 20-fold with the addition of 64 ng of Component B (Fig. 7A). Similarly, Component B had little detectable activity unless supplemented with Component A (Fig. 7B). The two components appeared to saturate each other when present in a ratio of 64 ng of Component B to 2.6 µg of Component A. The inequality of this ratio reflects the impurity of the Component A preparation.

We used the mixture of purified Component B and partially purified Component A to characterize the enzyme reaction
TABLE I
Purification of Rab GG transferase component B

| Step | Fraction | Protein | Specific activity (pmol/min/mg) | Total activity (pmol/min) | Purification (fold) | Recovery (%) |
|------|----------|---------|--------------------------------|--------------------------|-------------------|--------------|
| 1    | Cytosol  | 1,595   | 1.7                            | 2,760                    | 1                 | 100          |
| 2    | 30-50% ammonium sulfate | 397 | 9.1                          | 3,630                    | 5                 | 131          |
| 3    | Mono Q   | 33      | 97                            | 3,200                    | 57                | 115          |
| 4    | Superdex 200, 10 mM NaCl | 9.6 | 172                          | 1,650                    | 101               | 60           |
| 5    | Phenyl-Superose | 0.67 | 1,015                        | 680                      | 25                |              |
| 6    | Superdex 200, 0.5 M NaCl | 0.016 | 30,000                       | 480                      | 17,650            | 17           |

*Protein concentration was estimated by Method B as described under “Experimental Procedures.”

FIG. 6. Purified Component B of Rab GG transferase contains two proteins with apparent molecular weights of 60,000 and 38,000. Panel A, aliquots (25 μl) of the indicated fractions obtained from the Superdex 200 chromatography in 0.5 M NaCl described in Fig. 5 were subjected to electrophoresis (30 mA, 45 min, 24 °C) on a 10% SDS-polyacrylamide minigel, and the protein bands were detected by silver staining. Panel B, the pooled active fraction obtained after Superdex 200 chromatography in 0.5 M NaCl (~16 μg of protein in a volume of 5 ml) was diluted 4-fold to adjust the NaCl concentration to 0.125 M and then applied to a Mono Q 5/5 column (Pharmacia) equilibrated in buffer containing 20 mM sodium Hepes (pH 7.2), 0.1 mM Nonidet P-40, and 1 mM DTT (Fractions 1–10). The column was run at a flow rate of 1 ml/min, and 2-ml fractions were collected. The column was washed with 5 ml of the same buffer (Fractions 11–13), followed by 5 ml of the same buffer containing 0.23 M NaCl (Fractions 13–15). Component B of Rab GG transferase was eluted with a 24-ml gradient from 0.23 to 0.35 M NaCl in the same buffer (Fractions 16–27). An aliquot (2 μl) of the fractions eluting between approximately 0.28 and 0.30 M NaCl was assayed (Fractions 21–24), and another aliquot (25 μl) of the same fractions was subjected to electrophoresis (30 mA, 45 min, 24 °C) on a 10% SDS-gel electrophoresis minigel, and the protein bands were detected by silver staining. For each fraction, the activity of Rab GG transferase Component B is shown at the top. The gels were calibrated with the indicated protein molecular weight standards. Arrows denote the presence of the 60- and 38-kDa proteins that correlate with the activity of Component B.

FIG. 7. Rab GG transferase activity: requirement for two components. Each reaction mixture contained 0.5 μM [3H]GGPP, 0.54 μM Rab3A, and the indicated concentrations of partially purified Component A and purified Component B. Duplicate reactions were carried out for 10 min at 37 °C, and the trichloroacetic acid-precipitable radioactivity was measured as described under “Experimental Procedures.”

FIG. 8. Substrate saturation curves for Rab GG transferase. Panel A, each reaction mixture contained 0.54 μM Rab3A and the indicated concentration of [3H]GGPP (17,600 dpm/pmol) in the presence (○) or absence (©) of 1.25 μg of partially purified Component A plus 32 ng of purified Component B. Panel B, each reaction mixture contained 1.25 μg of partially purified Component A, 32 ng of purified Component B, 0.5 μM [3H]GGPP (17,600 dpm/pmol), and the indicated concentration of Rab3A. For both panels, duplicate samples were incubated for 10 min at 37 °C, and trichloroacetic acid-precipitable radioactivity was measured as described under “Experimental Procedures.” The inset in Panel B shows the migration on a 12.5% SDS-polyacrylamide gel of the product of a reaction containing 14 μg of partially purified Rab GG transferase (Mono Q fraction, Step 3) and the standard reaction components in the presence (+) or absence (–) of 5 mM MgCl2. The gel was treated with Entensify solution (Du Pont), dried, and exposed to XAR film for 2 days at ~70 °C. The migration of 14C-methylated markers loaded onto an adjacent lane is shown.

The concentrations of GGPP (Panel A) and Rab3A (Panel B) giving half-maximal reaction velocity were both approximately 0.2 μM. The [3H]geranylgeranyl from [3H]GGPP was indeed transferred to the Rab3A protein, as verified by SDS-polyacrylamide gel electrophoresis and autoradiography (inset to Fig. 8). The transfer of [3H]geranylgeranyl from [3H]GGPP was competitively inhibited by unlabeled GGPP, with 50% inhibition occurring at ~0.2 μM (Fig. 9). Farnesyl pyrophosphate at concentrations as high as 10 μM failed to inhibit (Fig. 9).

The purified Rab GG transferase showed an absolute requirement for MgCl2 with a half-maximal concentration of about 0.5 mM (Fig. 10A). In the presence of saturating concentrations of MgCl2, the addition of ZnCl2 was inhibitory with 100% inhibition achieved at 0.2 mM (Fig. 10B). The enzyme activity was very sensitive to the salt concentration in the assay (Fig. 11). The activity was inhibited by 50% at 100 mM concentrations of NaCl, NaOAc, or KCl, and was abolished at 300 mM concentrations of these salts.

In addition to Rab3A, which terminates in Cys-Ala-Cys, the purified Rab GG transferase prenylated Rab1A, which terminates in Cys-Sys (Fig. 12). The concentrations of the two protein substrates giving half-maximal reaction velocities kinetically (Fig. 8).
**Purification of Rab Geranylgeranyl Transferase**

**FIG. 9.** Inhibition of Rab GG transferase by prenyl pyrophosphates. Each reaction mixture contained 1.25 μg of partially purified Component A, 32 ng of purified Component B, 0.5 μM [3H]GGPP, 0.54 μM Rab3A, and the indicated concentration of unlabeled GGPP (●) or farnesyl pyrophosphate (FPP) (○). Duplicate incubations were carried out for 10 min at 37 °C, and trichloroacetic acid-precipitable radioactivity was measured as described under “Experimental Procedures.”

**FIG. 10.** Divalent cation requirement for Rab GG transferase. Panel A, each reaction mixture contained 1.25 μg of partially purified Component A, 32 ng of purified Component B, 0.5 μM [3H]GGPP, 0.54 μM Rab3A, and the indicated concentration of MgCl₂ in the absence of ZnCl₂. Panel B, each reaction mixture contained 1.25 μg of partially purified Component A, 32 ng of purified Component B, 0.5 μM [3H]GGPP, 0.54 μM Rab3A, 5 mM MgCl₂, and the indicated concentration of ZnCl₂. For both panels, incubations were carried out in duplicate for 10 min at 37 °C, and trichloroacetic acid-precipitable radioactivity was measured as described under “Experimental Procedures.”

**FIG. 11.** Inhibition of Rab GG transferase by salts. Each reaction mixture contained 1.25 μg of partially purified Component A, 32 ng of purified Component B, 0.5 μM [3H]GGPP, 0.54 μM Rab3A, and the indicated concentration of NaCl (●), NaOAc (○), or KCl (△). Incubations were carried out in duplicate for 10 min at 37 °C, and trichloroacetic acid-precipitable radioactivity was measured as described under “Experimental Procedures.”

**FIG. 12.** Purified Rab GG transferase recognizes Rab3A and Rab1A. Each reaction mixture contained 16 μg of partially purified Component A, 48 ng of purified Component B, 0.5 μM [3H]GGPP, and the indicated amounts of Rab3A (●), Rab1A (○), or p21H-s-CVLL (A). Incubations were carried out in duplicate for 10 min at 37 °C, and trichloroacetic acid-precipitable radioactivity was measured as described under “Experimental Procedures.”

**FIG. 13.** Geranylgeranylation of Rab3A (Panel A) and Rab1A (Panel B): requirement for Components A and B. Each reaction mixture contained either 0.54 μM Rab3A (Panel A) or 0.54 μM Rab1A (Panel B), 0.5 μM [3H]GGPP (17,600 dpm/pmol) in the presence (●) or absence (○) of 1.25 μg of partially purified Component A plus the indicated amounts of purified Component B. For both panels, duplicate samples were incubated for 10 min at 37 °C, and trichloroacetic acid-precipitable radioactivity was measured as described under “Experimental Procedures.”

**DISCUSSION**

The current results indicate that Rab GG transferase is a complex enzyme with at least two components, designated A and B. Purified preparations of Component B contain two peptides of approximately 38 and 60 kDa. We have been unable to resolve these two peptides with retention of Component B activity, and we therefore believe provisionally that both are subunits of this component. Component A was purified only partially, and its subunit composition is unknown.

Component B has an apparent molecular weight of approximately 90,000 on gel filtration in high salt (Fig. 5), raising the possibility that it is a 1:1 heterodimer of the 60- and 38-kDa subunits. When the intact Rab GG transferase was subjected to gel filtration on low salt prior to resolution of the
two components, all of the activity emerged at a position corresponding to a molecular weight of ~330,000 (Fig. 2). The peak fractions possessed both Components A and B. Assay of other column fractions in the presence of added Components A or B failed to generate additional activity, suggesting that the 330-kDa peak fraction contained essentially all of the Components A and B that were present in the crude extract. These data suggest that the native GG transferase is a macromolecular complex of about 330 kDa that contains Components A and B.

The distinction between Components A and B was first appreciated when we lost nearly all Rab GG transferase activity during hydrophobic chromatography and were able to restore activity by mixing fractions from the column (Fig. 3). Subsequent experiments showed that the separation of the two components was not the result of exposure to the hydrophobic resin but rather that it occurred whenever the salt concentration was raised above about 200 mM. We took advantage of this observation to separate the two components by absorption to a Red A Dymatrex column followed by elution with 2 M NaCl, which eluted Component A essentially free of Component B.

The activity of the purified enzyme depended on Component A as well as Component B. Moreover, the activity was sensitive to the ionic strength of the assay mixture, with 50% inhibition at a salt concentration of 100 mM. This is close to the salt concentration necessary to separate Components A and B and raises the possibility that the two components must be physically associated for geranylgeranyl transfer to occur.

We were surprised to find that the purified Rab GG transferase used RablaA as well as Rab3A as an acceptor of geranylgeranyl groups. Transfer of geranylgeranyl to RablaA required Component A as well as Component B (Fig. 13), and the reaction was inhibited by Zn2+ and sodium chloride in the same fashion as was transfer to Rab3A (data not shown). Inasmuch as our preparation of Component B appears to be free of Component B.

The mechanism by which the Rab GG transferase recognizes two different COOH-terminal sequences is not yet known. In experiments not shown, we found that the enzyme was not inhibited by peptides as long as 13 residues corresponding to the COOH-terminal sequence of Rab3A. Thus, the enzyme must recognize additional sequences that are remote from the COOH terminus. It is possible that the enzyme recognizes sequences that are similar in the RablaA and Rab3A proteins and may be common to all members of the Rab family. In this respect the Rab GG transferase differs markedly from the two CAAAX prenyltransferases whose sub-