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Biochemistry

Brain G protein γ subunits contain an all-*trans*-geranylgeranylcysteine methyl ester at their carboxyl termini

(prenylation/carboxyl methylation/signal transduction)

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ABSTRACT **We have shown previously that guanine nucleotide-binding protein (G protein) $\beta\gamma$ complexes purified from bovine brain membranes are methyl esterified on a C-terminal cysteine residue of the γ polypeptide. In the present study, ^{3}H-methylated $G_{\beta\gamma}$ complexes cleaved to their constituent amino acids by exhaustive proteolysis were shown to contain radiolabeled material that coeluted with geranylgeranylcysteine methyl ester on reversed-phase HPLC and two TLC systems. Further treatment by performic acid oxidation yielded radiolabeled material that coeluted with L-cysteic acid methyl ester, verifying that the prenyl modification occurs on a C-terminal cysteine residue. Analysis by gas chromatography-coupled mass spectrometry of material released from purified $G_{\beta\gamma}$ by treatment with Raney nickel positively identified the covalently bound lipid as an all-*trans*-geranylgeranyl (C_{20}) isoprenoid moiety. To delineate the distribution of this modification among γ subunits, purified $G_{\beta\gamma}$ complexes were separated into 5-kDa (γ_5) and 6-kDa (γ_6) forms of the γ polypeptide by reversed-phase HPLC. Gas chromatography-coupled mass spectrometry analyses of Raney nickel-treated purified γ_5 and γ_6 subunits showed that both polypeptides were modified by geranylgeranylation. These results demonstrate that at least two forms of brain γ subunit are posttranslationally modified by geranylgeranylation and carboxyl methylation. These modifications may be important for targeting $G_{\beta\gamma}$ complexes to membranes.**

Guanine nucleotide-binding regulatory proteins (G proteins) transduce a variety of extracellular signals across the cell membrane into changes in the levels of intracellular second messengers (for review, see ref. 1). Members of this protein family are all heterotrimers consisting of α, β, and γ subunits. In most biological systems, the GTP-binding α subunit is known to serve as the transducer of signals between receptors and effectors. In contrast, the role of the $\beta\gamma$ complex is less well understood. The $\beta\gamma$ complex has been demonstrated to facilitate the interaction of the α subunit with receptors (2, 3) and to mediate the binding of α subunits to membranes (4). $\beta\gamma$ complexes released during G protein activation have also been suggested to suppress the activity of α subunits by shifting their equilibrium toward inactive heterotrimeric complexes (5, 6). More recently, $\beta\gamma$ complexes have been implicated in the direct activation of phospholipase A_2 (7, 8) and regulation of mating factor signal transduction in yeast (9).

The deduced amino acid sequences of three known G protein γ subunits (9–12) all possess a C-terminal Cys-Xaa-Xaa-Xaa motif. This motif is postulated to be a signal for prenylation of the cysteine sulfhydryl group (13, 14), followed by proteolytic removal of the three terminal residues and carboxyl methylation of the resulting terminal cysteine (15). We had shown (16) that at least one γ subunit of brain G proteins is methyl esterified on a C-terminal cysteine residue and postulated that this cysteine residue might also be modified by lipidation. Here, we provide conclusive evidence that brain G protein $\beta\gamma$ complexes contain at least two γ subunits that are modified by an all-*trans*-geranylgeranyl moiety on a carboxyl methylated terminal cysteine residue. Such a group was recently shown to be the predominant isoprenoid covalently linked to proteins in mammalian cells (17, 18).

MATERIALS AND METHODS

Preparation of Brain $G_{\beta\gamma}$ and ^{3}H-Methylated $G_{\beta\gamma}$. Bovine brain $G_{\beta\gamma}$ complexes were purified as described (16), concentrated to >30 mg/ml, and used immediately or stored frozen at −20°C. The final purified $G_{\beta\gamma}$ was >95% pure as determined by SDS/PAGE. Protein concentration was determined by the method of Bradford (19), with γ globulin (Bio-Rad) used as a standard.

Purified $G_{\beta\gamma}$ was carboxyl methylated by using stripped bovine brain membranes as a source of carboxyl methyltransferase and [^{3}H]*S*-adenosyl-L-methionine (DuPont/NEN) as substrate (16), followed by extraction of ^{3}H-methylated $G_{\beta\gamma}$ with 1% CHAPS (Pierce). The preparation was used directly for analysis of geranylgeranylcysteine [^{3}H]methyl ester (GGC[^{3}H]ME).

Purification of 5- and 6-kDa Forms of γ Subunit. Purified $G_{\beta\gamma}$ (6 mg) was applied to 4.1-mm × 22-cm Aquapore RP-300 reversed-phase HPLC column (Pierce) equilibrated with 0.1% (wt/vol) trifluoroacetic acid in water (solvent A) and eluted with increased concentrations of solvent B (80% acetonitrile/20% water/0.1% trifluoroacetic acid) (vol/vol/wt) according to the following schedule: 0% B for 5 min, 0–38% over 5 min, 38–50% over 5 min, 50–62% over 10 min, 62–75% over 15 min, and 75–88% over 5 min, at a flow rate of 1 ml/min. Fractions of 0.5 ml were collected and assayed for the presence of the 5-kDa and 6-kDa forms of the γ subunit on SDS/polyacrylamide gels (16.5% acrylamide/6% bisacrylamide, with glycerol) using the tricine buffer system (20). The two forms of γ subunit were pooled separately, and their concentrations were determined by quantitative amino acid analysis based on the deduced composition of γ_6 (11, 12).

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Abbreviations: G protein, guanine nucleotide-binding regulatory protein; $G_{\beta\gamma}$, G_β, and G_γ, $\beta\gamma$ complex, β subunit, and γ subunit of brain G proteins, respectively; GGCME, geranylgeranylcysteine methyl ester; GGC[^{3}H]ME, geranylgeranylcysteine [^{3}H]methyl ester; γ_5 and γ_6, brain G protein γ subunits migrating at 5 and 6 kDa on SDS/PAGE, respectively; CHAPS, 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate; T_γ, the γ subunit of the retinal G protein transducin; GC/MS, gas chromatography-coupled mass spectrometry; t_R, retention time.

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Synthesis of *S*-(All-*trans*-geranylgeranyl)-L-Cysteine Methyl Ester (GGCME) and C_{30} Isoprenoids. All-*trans*-geranylgeraniol was obtained from R. M. Coates (University of Illinois) and was converted to the bromide by treatment with PBr_3 (17). To the residue of crude bromide from 30 mg of the prenol was added 3 ml of 1-butanol/methanol/water (1:1:1), 23 mg of guanidine carbonate, and 32 mg of L-cysteine methyl ester. The mixture was stirred at room temperature for 3–4 hr. An equal volume of water was added, and the mixture was extracted twice with equal volumes of 1-butanol. The extract was concentrated, and the residue was purified by flash chromatography on silica gel (21) by using chloroform/pentane (2:1) as solvent. TLC on silica gel with 1-butanol/acetic acid/water (4:1:5, upper phase) showed a single spot with an R_f value of 0.66 after staining with ninhydrin spray. Analysis reported for 300 MHz NMR (chloroform): δ 1.59, s, 9 H; δ 1.65, s, 6 H; δ 1.77–2.22, m, 12 H; δ 2.69, dd, 1 H; δ 2.89, dd, 1 H; δ 3.20, dd, 2 H; δ 3.66, m, 1 H; δ 3.74, s, 3 H; δ 5.10, m, 2 H; δ 5.22, t, 1 H.

The C_{30} hexaene was derived from all-*trans*-farnesylfarnesol (from S. Inoue, Yokohama National University) as described (17) and hydrogenated over Pt to give the saturated material (17).

Exhaustive Proteolysis of ^{3}H-Methylated $G_{\beta\gamma}$. A sample of extracted ^{3}H-methylated $G_{\beta\gamma}$ (5 μl containing 1.25 μg of protein; 26,600 cpm) was mixed with 460 μl of 0.1 M Bistris acetate buffer at pH 7.0, 1.5 μl of GGCME standard in methanol ($\approx$1 nmol), 20 μl of 1 M $CaCl_2$, and 20 μl of 1 M $MnCl_2$. This preparation was initially digested with *Staphylococcus aureus* protease V8 (164 μl containing 0.37 unit; Sigma type XVII-B) for 30 min at 37°C. An aliquot of leucine aminopeptidase (100 μl containing 2.5 units; Sigma type VI) was added, and the incubation was continued for 90 min at 37°C. Finally, a second aliquot of leucine aminopeptidase (100 μl) was added, and the incubation was continued for 60 min.

Gas Chromatography-Coupled Mass Spectrometry (GC/MS) Analysis. Purified $G_{\beta\gamma}$ (200 μg) was dialyzed against 8 M guanidine hydrochloride, treated with 50 mg of Raney nickel (Aldrich), and analyzed by GC/MS in the scanning mode as described (14).

Quantitation of the C_{20} isoprenoid released from $G_{\beta\gamma}$, γ_5, and γ_6 was done as follows: $G_{\beta\gamma}$ (150 μg) and HPLC-purified γ_5 and γ_6 (6 μg and 15 μg, respectively) were aliquoted into siliconized, pentane-rinsed glass ampules and dried in a Speed-Vac concentrator (Savant). Formic acid/ethanol (1:4, vol/vol), 400 μl, was added, and the solution was extracted three times with 1-ml aliquots of pentane. The solutions were readjusted to 400 μl with formic acid/ethanol and hydrogenated over Pt (Adams catalyst) for 3 hr, whereupon 50 mg of Raney nickel was added to each ampule. Ampules were flame sealed and placed in a Reacti-Therm heating/stirring module (Pierce) at 100°C and stirred for 15 hr, after which ampules were cooled, opened, and 400 μl of water was added. The aqueous phase was extracted with 1 ml of pentane containing coprostane (20 ng/ml) and eicosane (10 ng/ml) as internal standards. The samples were re-extracted with 0.5 ml of pentane, and the combined extracts were concentrated.

GC/MS analysis was performed using instrumentation as described (14). Analyses were performed using a 30-m DB5W column (J & W Scientific) with He as the carrier gas [5 psi (1 psi = 6.9 kPa) head pressure]. After splitless injection onto the column at 30°C, the oven temperature was rapidly raised to 80°C and programmed as follows: 4°C/min to 220°C (0–45 min) and then 2°C/min to 280°C (45–60 min). To obtain maximum sensitivity and accuracy in quantitation, the mass spectrometer was operated in the selected ion monitoring mode at 70 eV (1 eV = 1.602×10^{-19} J) ionization energy. The following masses were monitored during the indicated time windows: 0–45 min using m/z 163, 169, 183, 197 and 45–60 min using m/z 163, 169, 183, 217. These ions, m/z 169, 183, and 197, are abundant high-mass ions common to all saturated isoprenoids. The ion m/z 217 is an abundant and unique ion from the fragmentation of the coprostane internal standard. Quantitation of phytane (and other saturated isoprenoids) was achieved by fitting the ion ratios, m/z $183_{\text{isoprenoid}}$:m/z $217_{\text{coprostane}}$, to a standard curve of these ratios prepared the day of analysis.

RESULTS

Analysis of GGC[^{3}H]ME in Enzymatic Digests of [^{3}H]-Methylated $G_{\beta\gamma}$. We have shown previously that the γ subunit of bovine brain $G_{\beta\gamma}$ complexes is a substrate for an enzymatic C-terminal methyltransfer reaction that results in the formation of a cysteine α-methyl ester derivative (16). To determine whether this cysteine residue is also lipidated at the sulfhydryl group, we treated [^{3}H]methylated $G_{\beta\gamma}$ with *S. aureus* V8 protease and microsomal leucine aminopeptidase to cleave these proteins into their amino acid constituents. This digest was mixed with farnesyl cysteine methyl ester and GGCME standards and fractionated by reversed-phase HPLC (Fig. 1). We observed two major peaks of ^{3}H radioactivity. One peak eluted at 3–4 min in the position expected for [^{3}H]methanol and a second peak comigrated with the GGCME standard at 28–29 min and was well resolved from the farnesyl cysteine methyl ester standard at 25 min. Identification of the radioactive material present in fractions 28–29 as GGC[^{3}H]ME was further substantiated by silica TLC in solvents of acetonitrile or 1-butanol/acetic acid/water (4:1:5) (vol/vol/vol); radioactivity in [^{3}H]methyl esters comigrated with ninhydrin reactivity determined as described (ref. 22; data not shown).

The yield of GGC[^{3}H]ME was 30% of the total radioactivity initially present as methyl esters in the undigested $\beta\gamma$ complex and accounted for essentially all of the radioactivity as methyl esters in the digested sample. The bulk of the remaining radioactivity was found as [^{3}H]methanol and probably represents the product of methyl ester hydrolysis.

To verify the localization of the isoprenoid moiety to the carboxyl methylated terminal cysteine, we oxidized the HPLC-purified GGC[^{3}H]ME peak material with performic acid to remove the lipid and separated the products by chromatography on sulfonated polystyrene amino acid analysis resin (16, 22, 23). The results, shown in Fig. 2, demonstrate that the oxidized material coelutes with a standard of cysteic acid methyl ester, the expected product of oxidative removal of the lipid moiety.

GC/MS Analysis of Raney Nickel-Treated $G_{\beta\gamma}$. Having established that a ^{3}H-methylated C-terminal cysteine from a G protein γ subunit coelutes with GGCME by HPLC and TLC, we proceeded to identify the modifying lipid moiety by GC/MS analysis. Purified $G_{\beta\gamma}$ was treated with Raney nickel, which cleaves thioether linkages, and the hydrophobic cleavage products were extracted with pentane. Analysis of a portion of the pentane extract yielded a peak that cochromatographed with authentic all-*trans*-2,6,10,14-tetramethyl-2,6,10,14-hexadecatetraene and possessed an identical fragmentation pattern (Fig. 3 *A* and *B*). Retention time analysis unequivocally demonstrated that the released hydrocarbon had the all-trans configuration (17). Upon hydrogenation, the remaining extract yielded a peak with a retention time and spectrum identical to those of phytane (Fig. 3 *C* and *D*). Quantitation of bound C_{20} isoprenoid by hydrogenation of the sample before Raney nickel treatment and GC/MS analysis in the selected ion monitoring mode showed a recovery of 0.36 nmol of C_{20} isoprenoid/nmol of $G_{\beta\gamma}$. No C_{15}, C_{25}, or C_{30} isoprenoids, apart from trace amounts of squalane, were detected.

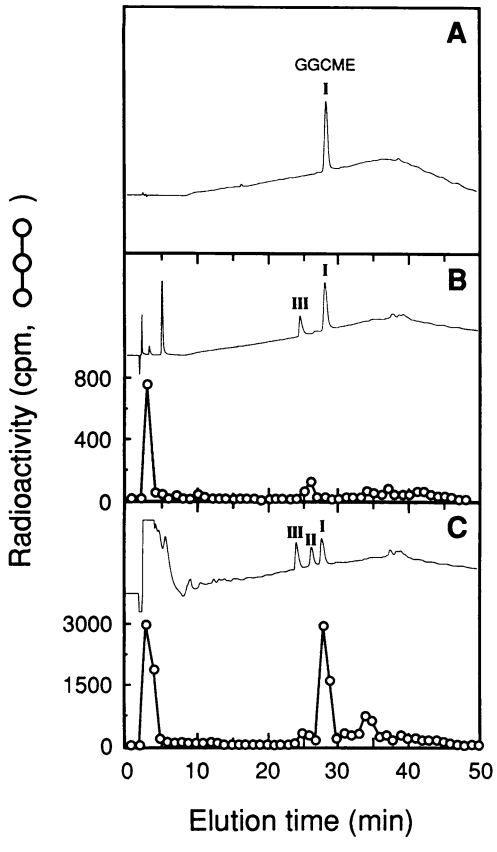

FIG. 1. HPLC separation of exhaustively digested ^{3}H-methylated $G_{\beta\gamma}$. ^{3}H-methylated $G_{\beta\gamma}$ digests were applied to a C_{18} reversed-phase column (Alltech Econosphere; 4.6 mm × 25 cm) equilibrated in 0.1% trifluoroacetic acid in water (vol/vol, solvent A). Elution was performed at 1 ml/min with a gradient over 50 min of 0–100% solvent B (90% acetonitrile/0.1% trifluoroacetic acid/9.9% water (vol/vol/vol). The UV absorbance of the effluent was monitored at 214 nm. Fractions (1 ml) were collected, and aliquots (500 μl) were counted in 10 ml of scintillation cocktail (Amersham, ACS II). (*A*) UV trace of authentic GGCME. (*B*) Control experiment where ^{3}H-methylated $G_{\beta\gamma}$ (1 μg protein; 10,640 cpm) was mixed with 0.15 nmol of GGCME standard and chromatographed as above. (*C*) Proteolytic digest of ^{3}H-methylated $G_{\beta\gamma}$ (2.5 μg of protein, 18,637 cpm) was mixed with 1 nmol of GGCME standard and chromatographed as above. UV peaks are seen at the position of the GGCME standard (I, 28.8 min), the hydrolysis product of the standard, geranylgeranyl cysteine (II, 27.3 min), and a background peak from the ^{3}H-methylated $G_{\beta\gamma}$ sample itself (III, 25.3 min). In this chromatography system, farnesyl cysteine methyl ester elutes at 25 min and farnesyl cysteine at 22 min. GC/MS analysis of selected HPLC fractions of a $G_{\beta\gamma}$ digest (50 μg) confirmed the presence of C_{20} isoprenoid in regions corresponding to GGCME and geranylgeranyl cysteine, whereas analysis of other regions and of a control digest showed no detectable C_{20} present.

Reversed-Phase Purification and Identification of G protein γ Subunits. Previous studies (12, 24) have shown that the γ subunits of bovine brain G proteins are composed of, at least, two forms that differ in their mobility on SDS/PAGE. To determine which of the γ subunits were modified by prenylation, the γ polypeptides of $G_{\beta\gamma}$ were first purified by reversed-phase HPLC. Analysis of HPLC fractions by SDS/PAGE showed that fractions 47–51 contained predominantly a protein band at 5 kDa, whereas fractions 52–60 contained predominantly a band at 6 kDa. G_β subunits eluted as a broad peak in fractions 48–80. Fractions 47–51 and 52–60 were pooled and rechromatographed twice under the same conditions, which effectively removed any contaminating G_β subunits. From 6 mg of $G_{\beta\gamma}$, the yield of γ_5 and γ_6 (Fig. 4*A*) was 30 μg and 130 μg, respectively.

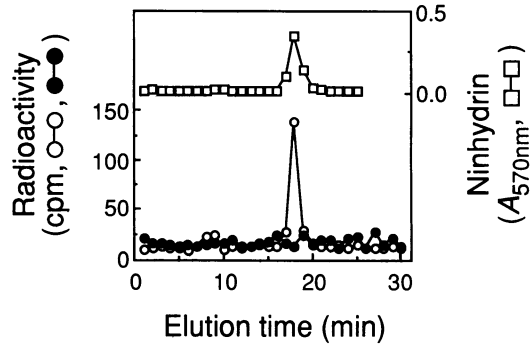

FIG. 2. Analysis of oxidized ^{3}H-methylated $G_{\beta\gamma}$ for L-cysteic acid methyl ester. A sample of ^{3}H-methylated material from the C_{18} column (fractions 28–29) was lyophilized and treated with performic acid for 1 hr at 0°C as described (22). After lyophilization, the oxidized sample was dissolved in 200 μl of sodium citrate buffer, pH 2.20 (0.2 M in Na^+), and mixed with a standard of L-cysteic acid methyl ester (2 μmol). The mixture was applied to a column of Beckman AA-15 sulfonated polystyrene amino acid analysis resin (0.9 ×54 cm) equilibrated at 57°C in sodium citrate buffer, pH 3.25 (0.2 M in Na^+). Fractions (1 min, 1 ml) were collected, and a 500-μl aliquot of each fraction was counted (○). An additional 500-μl aliquot of each fraction was mixed with 200 μl of 2 M NaOH, incubated for 1 hr at room temperature to hydrolyze methyl ester linkages, and lyophilized. After neutralization with glacial acetic acid (50 μl), the entire sample was counted (●). The elution of the cysteic acid methyl ester standard was determined by ninhydrin assay (□).

Previous results verified the identity of the purified protein as $G_{\beta\gamma}$ by coimmunoprecipitation with the α subunit of transducin bound to the monoclonal antibody TF16 (16). Analysis of the immunoprecipitate by SDS/PAGE using a tricine buffer system (20) revealed the presence of both the 5-kDa and 6-kDa forms of G_γ (data not shown). Identification of the 5- and 6-kDa bands as brain G protein γ subunits was further verified by immune reactivity with an antibody directed against the C-terminal 14 residues of a brain G_γ (refs. 11, 12; Fig. 4*B*). The lower reactivity of the 5-kDa band suggested that it contains a similar, but not identical, C-terminal sequence, whereas the γ subunit of transducin (T_γ) was unreactive. Amino acid analyses of the purified γ_5 and γ_6 preparations indicated that the amino acid compositions of these preparations were very similar to each other and to the predicted composition of the cloned γ subunit (refs. 11, 12); data not shown). Both γ_5 and γ_6 were carboxyl methylated when purified $G_{\beta\gamma}$ was reconstituted with detergent-stripped bovine brain membranes and [^{3}H]*S*-adenosyl-L-methionine (Fig. 4*C*).

GC/MS Analysis of Purified γ_5 and γ_6. When purified γ_5, γ_6, and G_β subunits were hydrogenated over Pt and treated with Raney nickel, γ_5 and γ_6 were found to contain 0.50 and 0.68 nmol of phytane/nmol of γ polypeptide, respectively (Fig. 5 *B* and *C*). These values are based on phytane quantitated against a standard curve and γ polypeptide concentrations determined by quantitative amino acid analysis. G_β yielded only residual levels of phytane (Fig. 5*D*). Corresponding fractions of a blank reversed-phase HPLC gradient showed no detectable levels of phytane (data not shown). Unlabeled peaks (Fig. 5 *B*, *C*, and *D*) were seen in all samples analyzed.

DISCUSSION

In this study, we present evidence that two forms of the γ subunit of brain G proteins are posttranslationally modified by an all-trans geranylgeranyl isoprenoid moiety on the thiol group of a carboxyl methylated terminal cysteine residue. The identification of a C-terminal -Cys-Xaa-Xaa-Xaa-encoded protein modified by geranylgeranylation, with pre-

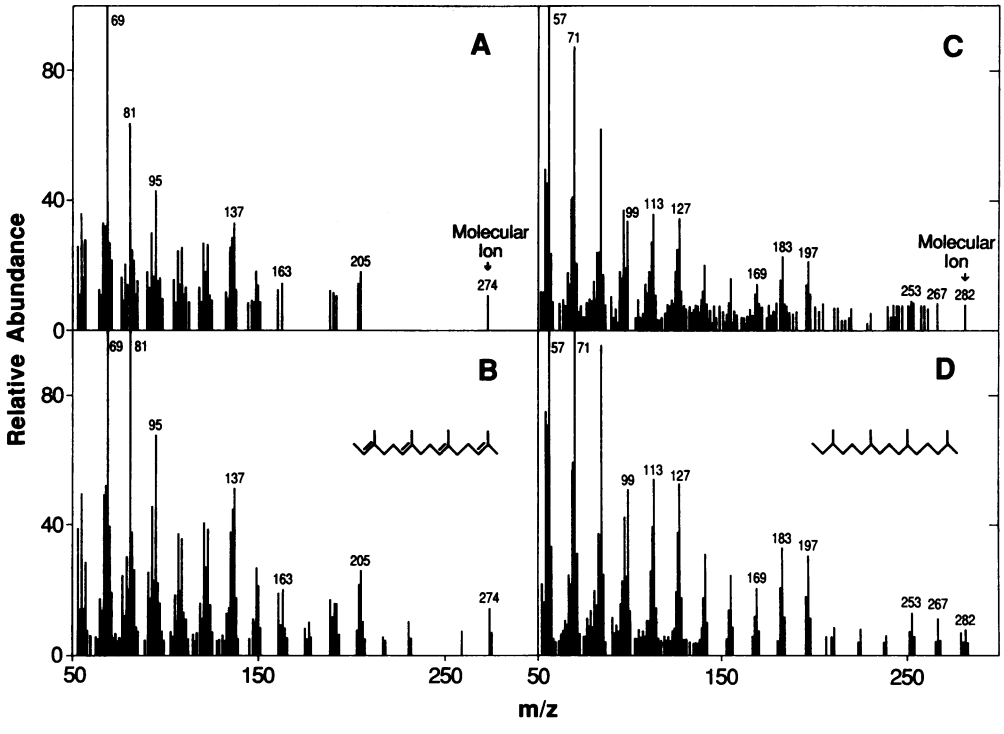

FIG. 3. Enhanced electron ionization spectra of C_{20} isoprenoids released from $G_{\beta\gamma}$ after Raney nickel treatment. $G_{\beta\gamma}$ was treated with Raney nickel as described. The released material was extracted into pentane and analyzed by GC/MS before or after hydrogenation in the scan mode of data acquisition. The retention times and spectra were then compared to those of an authentic standard, 2,6,10,14-tetramethyl-2,6,10,14-hexadecatetraene, and its saturated isomer, phytane. (*A*) Spectrum of nonhydrogenated GC peak from $G_{\beta\gamma}$ [retention time (t_R) = 42:32 (min:sec)]. (*B*) Spectrum of 2,6,10,14-tetramethyl-2,6,10,14-hexadecatetraene (t_R = 42:33). (*C*) Spectrum of the hydrogenated GC peak from $G_{\beta\gamma}$ (t_R = 37:01). (*D*) Spectrum of phytane (t_R = 37:02). The spectra shown have been enhanced by a standard method to highlight ions of low abundance (14).

vious reports of farnesylation of other -Cys-Xaa-Xaa-Xaa-encoded proteins (refs. 14, 27; J. B. Stimmel, R. J. Deschenes, C. Volker, S.C., and J. Stock, unpublished results), raises several questions. Is lipidation by geranylgeranyl and farnesyl groups mediated by a single enzyme or distinct enzymes for each chain length? Is the -Cys-Xaa-Xaa-Xaa motif sufficient for conferring specificity or does another region on the substrate protein dictate this selectivity? Alternatively, are prenyltransferases for C_{15} and C_{20} isoprenoids localized to specific cellular compartments, and, hence, is selectivity exerted in this manner? Several proteins, most notably the α subunits of G proteins, possess a signature -Cys-Xaa-Xaa-Xaa motif, yet the sulfhydryl group of this cysteine residue appears to be unmodified because it is available for pertussis toxin-catalyzed ADP-ribosylation (28). How are these proteins "protected" from prenylation? The answers to these questions await characterization of the enzyme(s) responsible for these lipid modifications.

Another question raised by the discovery of geranylgeranylated and farnesylated proteins relates to possible differences in function between the two isoprenoid modifications. Differences in chain length immediately suggest differences in lipophilicity. Modification by the longer chain geranylgeranyl group suggests greater lipophilicity and is consistent with the tight membrane binding behavior of brain $G_{\beta\gamma}$ complexes (4). Lipidation of the yeast mating factor peptides (29–31) and ras proteins (ref. 27; J. B. Stimmel *et al.*, unpublished results) by the shorter chain farnesyl group, meanwhile, are consistent with the lower avidity of these proteins for membranes.

Another possible function of prenylation is to direct the interaction of a prenylated protein with a specific receptor or effector protein or to enhance the effectiveness of such an interaction. In this regard, the yeast mating factor peptides are known to act through receptors on cells of the opposite mating type (32, 33), whereas a putative receptor has been identified for nuclear lamin B (34, 35). Additionally, GTPase-activating

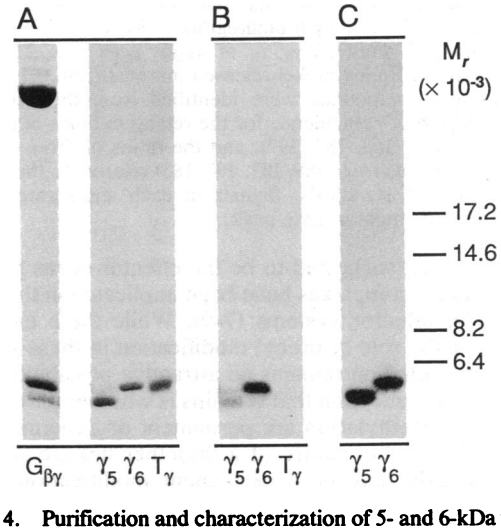

FIG. 4. Purification and characterization of 5- and 6-kDa forms of γ subunit. (*A*) Coomassie blue-stained SDS/polyacrylamide gel of purified $G_{\beta\gamma}$ (15 μg), γ_5, γ_6, and T_γ (2 μg each). (*B*) Immunologic blot of purified γ_5, γ_6, and T_γ (0.25 μg each) using an anti-G_γ peptide antibody produced as described below. (*C*) Fluorogram of ^{3}H-methylated γ_5 and ^{3}H-methylated γ_6 purified from ^{3}H-methylated $G_{\beta\gamma}$. The peptide PASENPFREKKFFC (in one-letter code) corresponding to residues 55–68 of a deduced brain G protein γ subunit sequence (11, 12) was synthesized at the University of California at Los Angeles peptide synthesis facility by the Merrifield solid-phase method, and anti-peptide antibodies were produced as described (25). Immunologic blotting was performed as described by Towbin *et al.* (26). Fluorography of gels was performed using the fluorographic image enhancer Amplify (Amersham). Transducin γ subunits were purified from transducin $\beta\gamma$ complexes as described by Hurley *et al.* (10).

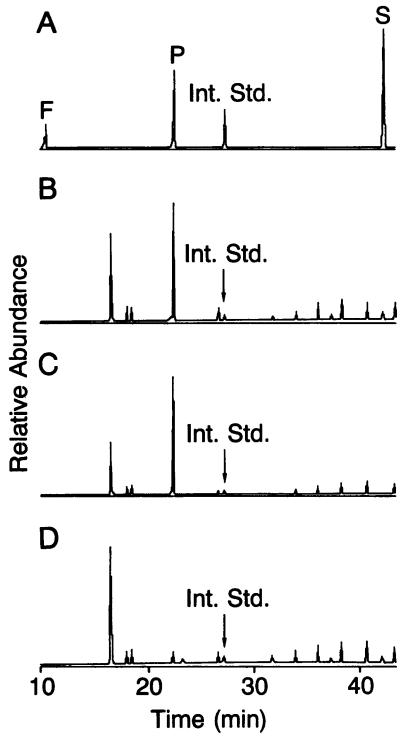

FIG. 5. Selected ion current chromatograms for m/z 183 of Raney nickel-released material from purified γ_5 and γ_6, G_β polypeptides, and isoprenoid standards. After HPLC purification, individual protein fractions were dried, resolubilized in formic acid/ethanol (1:4), and preextracted. The samples were then hydrogenated and treated with Raney nickel. The released material was extracted into pentane and analyzed by GC/MS in the selected ion monitoring mode. (*A*) Chromatogram of the saturated isoprenoid standards: F (farnesane; t_R = 10:56), P (phytane; (t) = 23:04), S (squalane; t_R = 42:57), and an internal standard (Int. Std.), eicosane (t_R = 27:53). (*B*) Chromatogram of Raney nickel-released material from 1.2 nmol of γ_5; t_R of major peak was 23:03. (*C*) Chromatogram of Raney nickel-released material from 2.5 nmol of γ_6; t_R of major peak was 23:01. (*D*) Chromatogram of Raney nickel-released material from 0.7 nmol of G_β. Isoprenoid compounds were identified from their retention times, the degree of coincidence for the retention times of all three reporter ions (m/z 169, 183, 197), and the ratios of three intramolecular reporter ions (m/z 169/183, 197/183) relative to these same parameters in the standards. Signals in each chromatogram are normalized to the most intense peak.

protein has been suggested to be the effector of ras proteins (36, 37), and $\beta\gamma$ complexes have been implicated in the direct activation of effector systems (7–9). While there exists no evidence for the role of prenyl modification in these interactions, such a function remains an attractive possibility.

An important question that remains is whether prenylation and carboxyl methylation are permanent or dynamic modifications. The stable nature of a thioether linkage suggests that prenylation may be a permanent modification, while carboxyl methylation of the terminal cysteine residue may confer the added dimension of an "on/off" signal. Whichever is the case, G protein γ subunits represent a well characterized system for further study of the mechanism and function of these posttranslational modifications.

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