Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 3675–3679, April 1993
Biochemistry

Lipid modifications of G proteins: α subunits are palmitoylated

MAURINE E. LINDER*, PAMELA MIDDLETON, JOHN R. HEPLER, RONALD TAUSSIG, ALFRED G. GILMAN, AND SUSANNE M. MUMBY

Department of Pharmacology, University of Texas Southwestern Medical Center, Dallas, TX 75235

Contributed by Alfred G. Gilman, January 14, 1993

ABSTRACT **A small number of membrane-associated proteins are reversibly and covalently modified with palmitic acid. Palmitoylation of G-protein α and β subunits was assessed by metabolic labeling of subunits expressed in simian COS cells or insect Sf9 cells. The fatty acid was incorporated into all of the α subunits examined (α_s, α_o, α_{i1}, α_{i2}, α_{i3}, α_z, and α_q), including those that are also myristoylated (α_o, α_i, and α_z). Palmitate was released by treatment with base, suggesting attachment to the protein through a thioester or ester bond. Limited tryptic digestion of activated α_o and α_s resulted in release of the amino-terminal portions of the proteins and radioactive palmitate. These data are consistent with palmitoylation of the proteins near their amino termini, most likely on Cys-3. Reversible acylation of G-protein α subunits may provide an additional mechanism for regulation of signal transduction.**

Covalent lipid modifications are found on both the α and γ subunits of heterotrimeric, signal-transducing, guanine nucleotide-binding proteins (G proteins) (for review, see ref. 1). The γ subunits are prenylated and carboxyl-methylated at their carboxyl termini. These modifications facilitate association of the βγ subunit complex with membranes and are indispensable for interactions of βγ with α subunits and effector molecules (2). Members of the α_i subfamily of α subunits are myristoylated at their amino termini. Myristoylation increases the affinity of α_o for βγ and also plays a role in membrane localization of α_o and α_i.

The α subunit of G_s (α_s) (an activator of adenylyl cyclase) is not myristoylated (3). However, when synthesized in *Escherichia coli*, α_s has reduced affinities for βγ, adenylyl cyclase, and Ca^{2+} channels (4–6). Hypothetically, the differences between native and recombinant α_s are due to the lack of unknown posttranslational modifications of the recombinant protein (4). Furthermore, the structural features of α_s necessary for association with membranes have not been fully characterized (7–9). Other α subunits, including members of the G_q family (activators of phospholipase C-β), lack the requisite glycine residue at position 2 (10) and are also presumably not myristoylated.

Some membrane-associated proteins, including certain forms of $p21^{ras}$ and receptors such as rhodopsin, are palmitoylated (11). Palmitate is almost always linked to cysteine residues through a thioester bond. The function of protein-bound palmitate is poorly defined. In an attempt to identify posttranslational modifications of α_s and α_q, we examined α subunits for incorporation of radioactive palmitate. We report here that tritiated palmitate is incorporated into α_s and α_q and, in addition, into α subunits that are also myristoylated (α_o, α_i, and α_z).

MATERIALS AND METHODS

Construction of Plasmids. cDNAs encoding α_s and α_{i1} (in vector NpT7-5; ref. 4) were digested with *Eco*RI and *Hin*dIII for ligation into the baculovirus expression vector pVL1392 (2). A plasmid encoding α_s with six histidine residues added at the amino terminus ($His_6\alpha_s$) was prepared by using complementary oligonucleotides (coding-strand sequence: 5′-AATTCTAAGGAGGTTTAACCATGGCACATCACCATCACCATCACGC-3′). Vector NpT7-5/$G_{s\alpha-s}$ was digested with *Eco*RI and *Nco* I and ligated with the annealed oligonucleotides. An *Eco*RI–*Hin*dIII fragment encoding $His_6\alpha_s$ was also cloned into pVL1392.

Transfection of Mammalian Cells for Biosynthetic Labeling. Nearly confluent COS-M6 cells were transfected by lipofection with cDNAs encoding G-protein α subunits (3). Radiolabeling of proteins was achieved with [^{35}S]methionine (25 μCi/ml, 710 Ci/mmol; 1 Ci = 37 GBq) [9,10-^{3}H]myristic acid (0.4 mCi/ml, 39 Ci/mmol), or [9,10-^{3}H]palmitic acid (0.5–1.9 mCi/ml, 60 Ci/mmol) (12). Cells were then lysed with RIPA buffer [100 mM NaCl/50 mM sodium phosphate, pH 7.2/1% (wt/vol) sodium deoxycholate/1% (vol/vol) Triton X-100/0.5% (wt/vol) SDS/1 mM dithiothreitol with protease inhibitors (L-1-tosylamido-2 phenylethyl ketone, 16 μg/ml; 7-amino-1-chloro-3-tosylamido-2-heptanone, 16 μg/ml; phenylmethanesulfonyl fluoride, 16 μg/ml; leupeptin, 3.2 μg/ml; soybean trypsin inhibitor, 3.2 μg/ml; aprotinin, 2 μg/ml)].

Insect Cell Culture and Radiolabeling. Baculoviruses encoding G-protein α_q, β_1, β_2, and γ_2 subunits have been described, as have methods for culture and infection of Sf9 (fall-armyworm ovarian) cells (ref. 2; J.R.H., unpublished data). Baculoviruses encoding α_s, $His_6\alpha_s$, and α_{i1} were generated as described by Iñiguez-Lluhi *et al.* (2). Baculoviruses expressing α_o and α_{i2} were kindly provided by J. Garrison (University of Virginia) (13). After 40–48 hr of infection, cells were incubated in Grace's medium containing 10% fetal bovine serum, nonessential amino acids, sodium pyruvate, and either [9,10-^{3}H]palmitic acid (1 mCi/ml) for 2 hr or [9,10-^{3}H]myristic acid (0.1–0.2 mCi/ml) for 8 hr. Labeling with [^{35}S]methionine (50 μCi/ml) in methionine-free Grace's medium was for 3 hr. Cells were suspended in 10 mM sodium phosphate (pH 7.4) containing 1 mM EDTA, 1 mM dithiothreitol, and protease inhibitors, flash-frozen, and fractionated into cytosol and membranes by three freeze/thaw cycles, followed by centrifugation at 125,000 × *g* at 4°C for 20 min. An equal volume of 2× RIPA buffer was added to the cytosolic fraction. Membranes were solubilized in RIPA buffer.

Antisera and Immunoprecipitations. Antibodies were produced in rabbits against synthetic peptides corresponding to G-protein sequences. Antibody 584 was used to precipitate α_s (14), P961 for α_z (14), WO82 for α_q (15), BO87 for α_{i1} and α_{i2},

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviation: GTP[γS], guanosine 5′-[γ-thio]triphosphate.

*Present address: Department of Cell Biology and Physiology, Washington University School of Medicine, St. Louis, MO 63110.

†However, simple interpretation of these data is hampered by the fact that myristoylation is a stable modification, whereas palmitoylation is dynamic. The specific activity of protein-bound myristate would reflect that of the precursor pool over the time course of the experiment. The specific activity of protein-bound palmitate might reflect that of the precursor pool only at the end of the experiment if turnover were sufficiently rapid.

C260 for α_{i3}, B600 for β, and A569 (14) or P960 (14) for α_o. Antisera BO87 and C260 were prepared against peptides corresponding to the carboxyl-terminal 10 amino acids of α_{i1}/α_{i2} or α_{i3}, respectively. Antisera were generated against peptides beginning with cysteine, followed by the carboxyl-terminal 16 amino acids of β (B600) or the carboxyl-terminal 11 amino acids of α_s (C267). All antibodies were affinity-purified (14).

Protein Purification. For partial purification of radiolabeled $His_6\alpha_s$, cells were suspended in 10 mM sodium phosphate (pH 7.4) containing 0.5 mM dithiothreitol, 0.2 mM EDTA, and protease inhibitors. After homogenization, membranes were suspended in 150 mM NaCl/20 mM sodium Hepes, pH 8/20 mM 2-mercaptoethanol with protease inhibitors (buffer B) and extracted with cholate (2). The detergent extract was diluted 5-fold into buffer B containing 0.1% polyoxyethylene-10 lauryl ether ($C_{12}E_{10}$) and applied to a Ni-agarose column (Qiagen, Chatsworth, CA). After the column was washed with buffer B plus 0.1% $C_{12}E_{10}$ and 500 mM NaCl, $His_6\alpha_s$ was eluted with a step gradient of 20, 50, 100, and 200 mM imidazole (pH 7.5) in buffer B plus 0.1% $C_{12}E_{10}$.

Cytosolic α_o from Sf9 cells labeled with [^{3}H]palmitate was partially purified using G-protein $\beta\gamma$-agarose (16). After elution, α_o was precipitated with trichloroacetic acid and dissolved in sample buffer for SDS/PAGE.

Chemical Analysis. Radioactive fatty acids liberated by hydrolysis of α_o, α_{i1}, and $His_6\alpha_s$ were analyzed as described (17), with some modifications. Protein in a polyacrylamide gel slice was first hydrolyzed with 1.5 M NaOH; fatty acids were extracted with chloroform/methanol. The gel slice in residual water and methanol was dried under N_2 and treated with 6 M HCl. Prior to extraction with chloroform/methanol, the solution containing the gel slice was adjusted with 10 M NaOH to pH 1–3. This prevented conversion of myristate to a more hydrophobic molecule, tentatively identified as myristate methyl ester. After addition of 40 μg of palmitic acid, extracted fatty acids were chromatographed on an Altex Ultrasphere octyl 5-μm column with acetonitrile/0.1% trifluoroacetic acid (80:20). Fatty acids were also analyzed by thin-layer chromatography on Whatman C_{18} reverse-phase or silica-gel plates with acetonitrile/acetic acid (90:10) or hexane/ethyl acetate/acetic acid (80:20:1), respectively, as the mobile phase.

Tryptic Digests. Membrane fractions of infected Sf9 cells labeled with [^{3}H]palmitate were extracted with sodium cholate (2). Aliquots of the extracts were incubated at 30°C for 45 min with guanosine 5′-[γ-thio]triphosphate (GTP[γS]) and $MgCl_2$, so that the final concentrations of components were 50 mM sodium Hepes (pH 8), 1 mM EDTA, 1 mM dithiothreitol, 50 mM NaCl, 0.33% (wt/vol) sodium cholate, 1 mM GTP[γS], and 10 mM $MgCl_2$. Trypsin (0.1 mg/ml) or water was added prior to incubation for 30 min on ice. Reactions were stopped with soybean trypsin inhibitor (0.2 mg/ml).

RESULTS

Radiolabeling of Transfected Mammalian Cells. In previous attempts to label endogenous α_s in mammalian cells with [^{3}H]palmitate, weak signals were sometimes seen after prolonged exposures of fluorograms (17). Because the concentration of α_s is very low, we expressed the protein transiently in COS cells for radiolabeling (3). Incorporation of [^{35}S]methionine into α_s indicated expression of the protein at reasonably high levels (Fig. 1*A*, lane i). COS cells expressing α_s were incubated with radioactive fatty acids, and label from [^{3}H]palmitate, but not [^{3}H]myristate, was incorporated into the protein (Fig. 1*A*, lanes g and h). The failure to incorporate label from [^{3}H]myristate into α_s is consistent with previous results (3). COS cells transfected with cDNAs encoding α_o, α_{i1}, α_{i2}, α_{i3}, or α_z were also incubated with radioactive fatty acids. All of these α subunits were labeled with [^{3}H]palmitate (Fig. 1*A*, lanes a and d; Fig. 1*B*, lanes n, o, and p), as well as [^{3}H]myristate (3) (Fig. 1*A*, lanes b and e). However, incorporation of label from [^{3}H]palmitate into these α subunits could result from conversion of palmitate to myristate *in vivo* (ref. 18; see below). Not all membrane proteins incorporated [^{3}H]palmitate under these conditions; endogenous G-protein β subunits were not labeled (Fig. 1*B*, lane q), nor were overexpressed β subunits (data not shown).

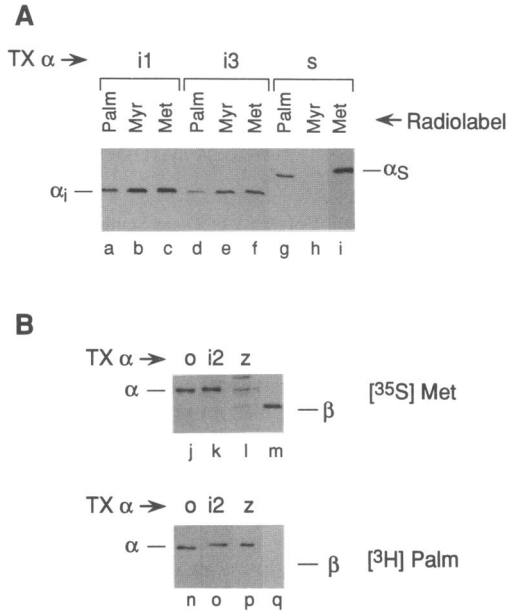

FIG. 1. Incorporation of radiolabeled methionine and fatty acids into G-protein α and β subunits immunoprecipitated from COS cells. Cells were transfected with a cDNA encoding the α subunit indicated above each panel (TX α). Cells were incubated with [^{35}S]methionine (Met), [^{3}H]myristic acid (Myr), or [^{3}H]palmitic acid (Palm), as indicated above each lane in *A* or to the right in *B*. Antibody selective for the expressed α subunit (or endogenous β subunit; lanes m and q) was used to immunoprecipitate the protein from whole-cell lysates in both *A* and *B*. Immunoprecipitates were resolved by SDS/PAGE and visualized by fluorography. Only those portions of the fluorograms corresponding to the regions of α and β subunits are shown; no other bands were visible except for the immunoprecipitate of [^{35}S]methionine-labeled α_z (*B*, lane 1). Lanes a–f, i, and l were exposed for 3 days; lanes g, h, j, and k for 9 days; lanes m, p, and q for 12 days; and lanes n and o for 30 days.

Radiolabeling of Baculovirus-Infected Sf9 Cells. The recombinant baculovirus/insect cell expression system was utilized to obtain quantities of labeled protein sufficient for more detailed analysis. When expressed in this system, G-protein α subunits are found in both the cytosolic and the membrane fraction (ref. 13; J.R.H., unpublished data). Concurrent expression of α_s with both β_1 and γ_2 subunits or α_q with both β_2 and γ_2 increases the amount of active α that can be extracted from Sf9 cell membranes (J.R.H. and M.E.L., unpublished data). Since palmitoylated proteins are usually associated with membranes, we coexpressed G-protein α, β, and γ subunits in Sf9 cells to increase the membrane-bound pool of α.

[^{35}S]Methionine-labeled α_s was found in both the cytosol and membranes of Sf9 cells (Fig. 2 *Top*, lanes 11 and 12). However, labeling with [^{3}H]palmitate was observed only in the membrane-bound pool of α_s (Fig. 2 *Middle*, lanes 11 and 12). As expected, there was no incorporation of [^{3}H]myristate (Fig. 2 *Bottom*, lanes 11 and 12). The same results were obtained with cells expressing α_q, β_2, and γ_2 (Fig. 2, lanes 9 and 10). β_1 subunits were not labeled with either [^{3}H]palmitate or [^{3}H]myristate (Fig. 2 *Middle* and *Bottom*, lanes 1 and 2).

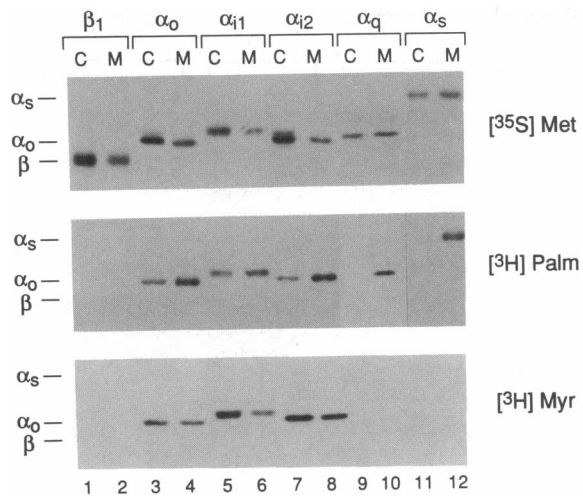

FIG. 2. Incorporation of radiolabel into α and β subunits immunoprecipitated from cytosolic fractions and membrane extracts of Sf9 cells expressing the indicated proteins. Subunits α_o, α_{i1}, α_{i2}, or α_s were expressed with β_1 and γ_2, while α_q was expressed with β_2 and γ_2; β_1 and γ_2 were expressed in the absence of α. Cells were incubated with [^{35}S]methionine (Met), [^{3}H]palmitate (Palm), or [^{3}H]myristate (Myr), as indicated. Extracts from crude membrane (M) and cytosolic (C) fractions were immunoprecipitated with the appropriate antibodies. Immunoprecipitates were resolved by SDS/PAGE and visualized by fluorography. Exposure times were 1 day (*Top*), 2 weeks (except lanes 9 and 10, 1 month) (*Middle*), and 2 weeks (*Bottom*). The positions of migration of α_s, α_o, and β_1 are indicated to the left of each panel.

When α_o, α_{i1}, or α_{i2} was expressed concurrently with β_1 and γ_2, radiolabel from [^{3}H]palmitate was observed in both the cytosolic and membrane fractions (Fig. 2 *Middle*, lanes 3–8), as was label from [^{3}H]myristate (Fig. 2 *Bottom*, lanes 3–8). Since only myristoylated α subunits were labeled with [^{3}H]palmitate in the cytosol, we hypothesized that the palmitate-derived label in cytosolic α_o or α_i was incorporated as [^{3}H]myristate rather than [^{3}H]palmitate.

Analysis of Labeled Fatty Acids in α Subunits. A recombinant baculovirus encoding α_s with a hexahistidine tag at the amino terminus ($His_6\alpha_s$) was constructed to facilitate isolation of the radiolabeled protein. $His_6\alpha_s$ expressed and purified from *E. coli* is indistinguishable from wild-type α_s produced in *E. coli* with respect to interactions with adenylyl cyclase and guanine nucleotides (M.E.L., unpublished data). After synthesis in Sf9 cells, both cytosolic and membrane-associated $His_6\alpha_s$ could stimulate adenylyl cyclase. [^{3}H]Palmitate was found only in the membrane-bound fraction, consistent with results obtained with wild-type α_s (data not shown). $His_6\alpha_s$ was partially purified from membranes of [^{3}H]palmitate-labeled cells by using Ni-agarose affinity resin, and the protein was resolved by SDS/PAGE. Fatty acid linked through an ester or thioester (but not amide) linkage is sensitive to cleavage by hydroxylamine. Treatment of the gels with hydroxylamine caused a reduction of the label associated with the protein. The loss of signal was comparable to that observed for [^{3}H]palmitate-labeled p21^{ras} (data not shown). Thus, the label incorporated into the protein appeared to be ester- or thioester-linked, consistent with modification by palmitate.

Fatty acids were hydrolyzed from the protein with base and subsequently with acid. Ester- or thioester-linked fatty acids are cleaved by base; amide-linked fatty acids (e.g., myristate) are resistant to base but are sensitive to acid. Free fatty acids were resolved by HPLC on a reverse-phase column. The bulk of the radioactivity cleaved from $His_6\alpha_s$ was alkali-sensitive and eluted from the column with the same retention time as palmitate (Fig. 3 *Left*). Identification

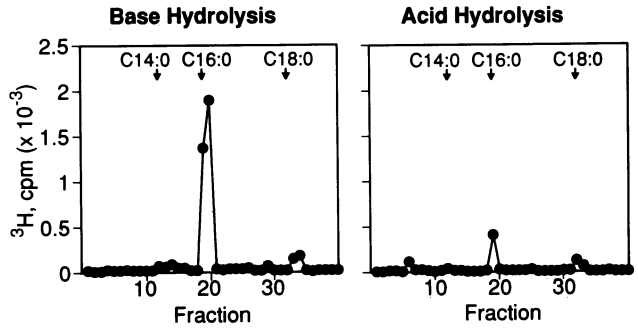

FIG. 3. Reverse-phase chromatography of fatty acids hydrolyzed from purified radiolabeled $His_6\alpha_s$. $His_6\alpha_s$ was coexpressed with β_1 and γ_2 subunits and was isolated from membranes of [^{3}H]palmitate-labeled Sf9 cells by Ni-agarose chromatography and SDS/PAGE. Fatty acids were cleaved from the protein first with base (*Left*) and then with acid (*Right*). Extracts of the hydrolysates were chromatographed over a C_8 reverse-phase column, and radioactive fatty acids were detected by scintillation counting. Positions of elution of standards [myristate (C14:0), palmitate (C16:0), and stearate (C18:0)] are indicated.

of the major radioactive product as palmitate was confirmed by reverse-phase thin layer chromatography on a C_{18} matrix (data not shown). Little additional radioactivity was released from the protein with acid (Fig. 3 *Right*).

Analyses of two independent preparations of α_o labeled by incubation of cells with [^{3}H]palmitate are shown in Fig. 4. In the first, the bulk of the radioactivity associated with the protein was in the membrane fraction and was alkali-sensitive palmitate (Fig. 4*A i*). Subsequent hydrolysis with acid released only small amounts of myristate and palmitate (Fig. 4*A ii*). There was very little radioactivity in the cytosolic pool of α_o (Fig. 4*A iii* and *iv*). In the second experiment, there was considerably more interconversion of label, and 23% of the radioactivity associated with membrane-bound α_o was palmitate (Fig. 4*B i*), whereas 60% was myristate (Fig. 4*B ii*). Importantly, labeled myristate, but not palmitate, was detected in the cytosolic pool of α_o (Fig. 4*B iii* and *iv*). Analysis of the total fatty acids of membranes from [^{3}H]palmitate-labeled Sf9 cells revealed that 26% of the radiolabel was myristate whereas 51% remained as palmitate after 4 hr of labeling (19). Since myristate is much less abundant in cells than palmitate, metabolic conversion of labeled palmitate results in a myristate pool of significant specific activity.

After incubation of cells with [^{3}H]myristate, analysis of fatty acids cleaved from α_o revealed only [^{3}H]myristate in both membrane and cytosolic fractions. Radioactive palmitate was detected in membrane-associated α_{i1} after incubation of cells with [^{3}H]palmitate. Some label from [^{3}H]palmitate was also incorporated as [^{3}H]myristate in both cytosolic and membrane-associated α_{i1} (data not shown), as in the second experiment described for α_o. Incorporation of radiolabel from [^{3}H]palmitate as [^{3}H]myristate has been reported for other myristoylated proteins [e.g., pp60^{src} (18)].

Location of Palmitate on α_s and α_o. We examined protein labeled with [^{3}H]palmitate after limited tryptic digestion. When GTP[γS] is bound, α_o is cleaved by trypsin to a 37-kDa fragment with loss of the amino terminus (20). Radiolabeled α_o is the major palmitoylated protein in cholate extracts of membranes from Sf9 cells expressing this protein (Fig. 5, lane 1). At least 80% of the radioactivity released from this protein by acid hydrolysis was [^{3}H]palmitate (HPLC analysis not shown). After incubation with GTP[γS] and digestion with trypsin, about 50% of the protein was detected as the 37-kDa fragment (Fig. 5, lanes 3 and 4); essentially no radioactivity was associated with this fragment (lane 2). The carboxyl terminus of α_o appeared to be intact, since protein was detected by an antibody (C260) that recognizes the last 10

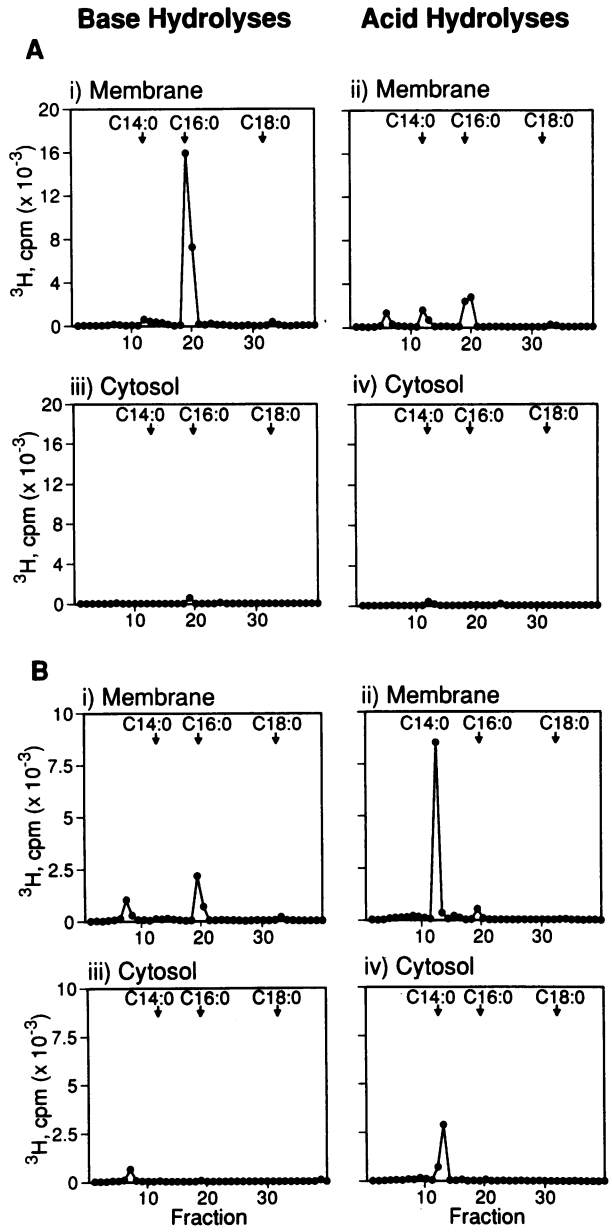

FIG. 4. HPLC analysis of radiolabeled α_o from two independent experiments. Sf9 cells expressing α_o, β_1, and γ_2 were labeled with [^{3}H]palmitate and fractionated into membranes (*i* and *ii*) and cytosol (*iii* and *iv*). α_o was isolated by immunoprecipitation (*A i–iv; B i* and *ii*) or $\beta\gamma$-agarose chromatography (*B iii* and *iv*), followed by SDS/PAGE. Fatty acids were hydrolyzed with base (*Left*) and then acid (*Right*) prior to chromatography on a C_8 reverse-phase column. Results were confirmed by thin-layer chromatography. The minor and variable component eluted in fraction 6 or 7 was not identified.

amino acids of α_o (Fig. 5, lane 6). The site(s) of palmitoylation of α_o appears to be located within the amino-terminal 2 kDa of the protein.

Two major tryptic fragments of α_s were detected with antibodies directed against amino acids 325–339 (antibody 584) (Fig. 5, lane 10) and amino acids 28–42 (A572) (data not shown). The carboxyl termini of both fragments were cleaved, since they did not react with an antibody (C267) directed against these residues (Fig. 5, lane 12). The smaller fragment, cleaved at both ends, was no longer radioactive, whereas the larger fragment was still labeled (Fig. 5, lane 8). Thus, α_s is palmitoylated within ≈30 amino acid residues of its amino terminus.

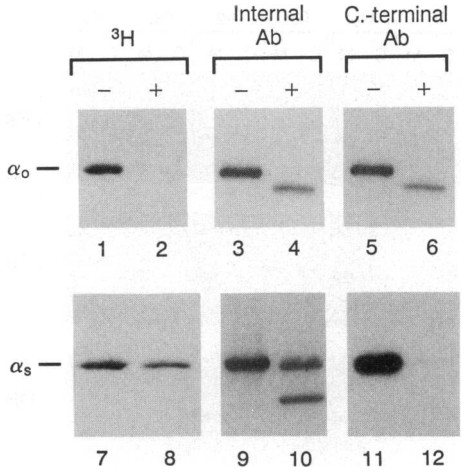

FIG. 5. Tryptic digestion of α_o and α_s labeled with [^{3}H]palmitate. Sf9 cells expressing α_o or α_s (with β_1 and γ_2) were labeled with [^{3}H]palmitate. Cholate extracts of membrane fractions were incubated with GTP[γS] and subjected to partial digestion with trypsin where indicated (+). The samples were divided and resolved by SDS/PAGE for subsequent fluorography (lanes 1, 2, 7, and 8) or immunoblotting with antibody (Ab) P960 (lanes 3 and 4), C260 (lanes 5 and 6), 584 (lanes 9 and 10), or C267 (lanes 11 and 12). Mobilities of intact α_o and α_s are indicated at left.

DISCUSSION

At least seven G-protein α subunits, including those that are normally myristoylated, can be labeled with [^{3}H]palmitate when expressed in COS or Sf9 cells. Incorporation of palmitate into α_o and α_s was verified by both HPLC and thin-layer chromatography. However, label from [^{3}H]palmitate can also be incorporated into members of the α_i subfamily as myristate.

Palmitate is attached to G-protein α subunits through an alkali-sensitive linkage, as is true for most palmitoylated proteins. Although identification of the exact nature of the modification will require mass spectroscopic analysis, it is most likely an ester or thioester. We have tentatively assigned the location of this modification to the amino-terminal region of α_o and α_s. If this is correct, a cysteine residue at position 3 of α_s and the members of the α_i subfamily (α_i, α_o, α_z) seems a likely candidate for modification. This cysteine is conserved in these α subunits and is the only cysteine within the first 65 residues of the amino terminus. Members of the α_q and α_{12}/α_{13} families have multiple cysteine residues near their amino termini. The α subunit of transducin does not contain such a cysteine residue, consistent with the findings of Neubert *et al.* (21) and Kokame *et al.* (22), who did not detect any palmitic acid in their mass spectroscopic analysis of transducin.

We have not assessed the stoichiometry of palmitoylation. However, the data of Fig. 4*B* suggest that it can be low, since, in this experiment, more label from [^{3}H]palmitate was incorporated into the membrane-bound pool of α_o as myristate than as palmitate.† Furthermore, palmitate was not detected in hydrolysates of α_o or α_i purified from bovine brain (17). Thus, α subunits purified by conventional means are likely to have substoichiometric amounts of palmitic acid. This could reflect the stoichiometry *in vivo* or loss during purification.

It appears that both myristate and palmitate are present on α_o, the α_i subunits, and α_z. Although we have no formal proof that the same protein molecule contains both fatty acids, there is precedent for two lipid modifications on a single protein. The insulin receptor is acylated with both myristate and palmitate (23). Palmitoylated forms of p21^{ras} are so modified a few amino acids upstream of the carboxyl-terminal cysteine residue that is farnesylated and methylated

(24). The close proximity of the lipid groups at the carboxyl terminus of $p21^{ras}$ may be analogous to the juxtaposition of myristate and palmitate groups at the amino terminus of the members of the α_i subfamily.

When expressed in Sf9 cells, G-protein α subunits accumulate in both cytosolic and membrane-bound pools. It is notable that palmitate is associated only with the latter. Radiolabel from [^{3}H]palmitate in cytosolic α_o was all incorporated as myristate. Thus, palmitoylation may play a role in mediating interactions of G-protein α subunits with membranes. Activation of α_s by receptors results in translocation of some fraction of the protein from the membrane to the cytosol (25–27). This redistribution could result from (or cause) deacylation of the protein. Association of protein with the membrane could be facilitated by acylation. Although palmitoylation enhances membrane binding of some proteins [including some forms of $p21^{ras}$ (24) and the neuronal growth-cone protein GAP-43 (28, 29)], other palmitoylated proteins (e.g., the transferrin receptor) are integral to the membrane; thus, palmitoylation is likely to have roles in addition to promotion of interactions of proteins with the lipid bilayer. Particularly when compared with myristoylation and prenylation, the dynamic potential of palmitoylation obviously speaks to the possibility of important regulatory cycles of acylation and deacylation.

We thank Steven Gutowski and Paul Sternweis for α_q antibodies; James Garrison, Jorge Iñiguez-Lluhi, and Wei-Jen Tang for G-protein recombinant baculovirus; Robert Munford for use of HPLC facilities; Laurie Camp and Sandra Hoffman for $p21^{ras}$ recombinant baculovirus; and Helen Aronovich, Linda Hannigan, and Pamela Sternweis for excellent technical assistance. This work was supported by Grant-in-Aid 91R-077 from the American Heart Association, Texas Affiliate, and by National Institutes of Health Grant GM34497, American Cancer Society Grant BE30N, The Perot Family Foundation, The Lucille P. Markey Charitable Trust, and The Raymond and Ellen Willie Chair of Molecular Neuropharmacology.

1. Spiegel, A. M., Backlund, P. S., Jr., Butrynski, J. E., Jones, T. L. Z. & Simonds, W. F. (1991) *Trends Biochem. Sci.* **16**, 338–341.
2. Iñiguez-Lluhi, J., Simon, M. I., Robishaw, J. D. & Gilman, A. G. (1992) *J. Biol. Chem.* **267**, 23409–23417.
3. Mumby, S. M., Heuckeroth, R. O., Gordon, J. I. & Gilman, A. G. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 728–732.
4. Graziano, M. P., Freissmuth, M. & Gilman, A. G. (1989) *J. Biol. Chem.* **264**, 409–418.
5. Mattera, R., Graziano, M. P., Yatani, A., Zhou, Z., Graf, R., Codina, J., Birnbaumer, L., Gilman, A. G. & Brown, A. M. (1989) *Science* **243**, 804–807.
6. Lee, E., Taussig, R. & Gilman, A. G. (1992) *J. Biol. Chem.* **267**, 1212–1218.
7. Audigier, Y., Journot, L., Pantaloni, C. & Bockaert, J. (1990) *J. Cell Biol.* **111**, 1427–1435.
8. Journot, L., Pantaloni, C., Poul, M.-A., Mazarguil, H., Bockaert, J. & Audigier, Y. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 10054–10058.
9. Juhnn, Y.-S., Jones, T. L. Z. & Spiegel, A. M. (1992) *J. Cell Biol.* **119**, 523–530.
10. Simon, M. I., Strathmann, M. P. & Gautam, N. (1991) *Science* **252**, 802–808.
11. James, G. & Olson, E. N. (1990) *Biochemistry* **29**, 2623–2634.
12. Mumby, S. M. & Buss, J. E. (1990) *Methods* **1**, 216–220.
13. Graber, S. G., Figler, R. A. & Garrison, J. C. (1992) *J. Biol. Chem.* **267**, 1271–1278.
14. Mumby, S. M. & Gilman, A. G. (1991) *Methods Enzymol.* **195**, 215–233.
15. Gutowski, S., Smrcka, A. V., Nowak, L., Wu, D., Simon, M. I. & Sternweis, P. C. (1991) *J. Biol. Chem.* **266**, 20519–20524.
16. Pang, I.-H. & Sternweis, P. C. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 7814–7818.
17. Buss, J. E., Mumby, S. M., Casey, P. J., Gilman, A. G. & Sefton, B. M. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 7493–7497.
18. Buss, J. E. & Sefton, B. M. (1985) *J. Virol.* **53**, 7–12.
19. Christgau, S., Aanstoot, H.-J., Schierbeck, H., Begley, K., Tullin, S., Hejnaes, K. & Baekkeskov, S. (1992) *J. Cell Biol.* **118**, 309–320.
20. Hurley, J. B., Simon, M. I., Teplow, D. B., Robishaw, J. D. & Gilman, A. G. (1984) *Science* **226**, 860–862.
21. Neubert, T. A., Johnson, R. S., Hurley, J. B. & Walsh, K. A. (1992) *J. Biol. Chem.* **267**, 18274–18277.
22. Kokame, K., Fukada, Y., Yoshizawa, T., Takao, T. & Shimonishi, Y. (1992) *Nature (London)* **359**, 749–752.
23. Hedo, J. A., Collier, E. & Watkinson, A. (1987) *J. Biol. Chem.* **262**, 954–957.
24. Hancock, J. F., Magee, A. I., Childs, J. E. & Marshall, C. J. (1989) *Cell* **57**, 1167–1177.
25. Ransnas, L. A. & Insel, P. A. (1988) *J. Biol. Chem.* **263**, 17239–17242.
26. Negishi, M., Hashimoto, H. & Ichikawa, A. (1992) *J. Biol. Chem.* **267**, 2364–2369.
27. Levis, M. J. & Bourne, H. R. (1992) *J. Cell Biol.* **119**, 1297–1307.
28. Skene, J. H. P. & Virag, I. (1989) *J. Cell Biol.* **108**, 613–624.
29. Zuber, M. X., Strittmatter, S. M. & Fishman, M. C. (1989) *Nature (London)* **341**, 345–348.