Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 10054–10058, November 1991
Biochemistry

Amino acids 367–376 of the G_s α subunit induce membrane association when fused to soluble amino-terminal deleted G_{i1} α subunit

(GTP-binding proteins/signal transduction/fusion proteins/*in vitro* transcription/*in vitro* translation)

LAURENT JOURNOT*, COLETTE PANTALONI*, MARIE-ALIX POUL*, HONORÉ MAZARGUIL†, JOËL BOCKAERT*, AND YVES AUDIGIER*‡

*Centre National de la Recherche Scientifique-Institut National de la Santé et de la Recherche Médicale de Pharmacologie-Endocrinologie, rue de la Cardonille, F-34094 Montpellier Cedex 05, France; and †Laboratoire de Pharmacologie et Toxicologie Fondamentales, 205, route de Narbonne, F-31077 Toulouse Cedex, France

Communicated by Günter Blobel, August 2, 1991 (received for review March 28, 1991)

ABSTRACT **Signal transduction GTP-binding proteins are tightly associated with plasma membrane. In the resting state, the anchorage of the α subunit could be indirect by means of the other $\beta\gamma$ subunits or polydisperse multimers. In the activated state, although the α subunit is dissociated from other subunits, it is not released from the membrane and therefore is likely to contain information necessary to remain associated with the plasma membrane. Previous proteolytic experiments suggested that, in contrast to other G proteins α subunits, the C-terminal domain of $G_s\alpha$ (the G protein involved in adenylate cyclase stimulation) is essential for membrane association of the activated form. To better define the crucial residues involved in membrane attachment, we constructed chimeras between a soluble core and various parts of the $G_s\alpha$ C-terminal domain. We first deleted codons 2–6 of $G_{i1}\alpha$ (the inhibitory G protein of the i1 subtype) to generate a soluble GTP-binding protein, ΔN-$G_{i1}\alpha$. We then replaced the last 14 C-terminal codons of ΔN-$G_{i1}\alpha$ by different domains of the $G_s\alpha$ C terminus and looked for the membrane association of chimeric proteins after *in vitro* transcription, *in vitro* translation, and interaction with S49*cyc*$^-$ membranes (obtained from a mutant cell line that does not express $G_s\alpha$). Our results showed that addition of amino acids 367–376 of $G_s\alpha$ is sufficient to promote membrane association of the soluble N-terminal deleted $G_{i1}\alpha$.**

After solubilization in cholate or lubrol, signal transduction GTP-binding proteins share a heterotrimeric structure of the $\alpha\beta\gamma$ type. *In vitro* experimental evidence suggests that during the course of the transduction process the α subunit undergoes a cycle of association/dissociation with the β and γ subunits, effector(s), and receptor(s) (1–5). This cycle is concomitant with replacement of bound GDP by GTP and subsequent GTP hydrolysis. In the GDP-bound resting state, the α subunit is associated at least with $\beta\gamma$ subunits. In the GTP-bound activated state, the G protein α subunit ($G\alpha$) is supposed to be free of $\beta\gamma$ and can interact with the effector. After solubilization of brain synaptoneurosomes with octyl glucoside, the G proteins are extracted as large complexes containing $G\alpha$ subunits together with cytoskeletal proteins. These complexes dissociate upon addition of nonhydrolyzable analogs of GTP such as guanosine 5′-[γ-S]thiotriphosphate (GTP[γ-S]) (6).

From our point of view it was especially interesting to determine how and why most of the G protein α subunits are not released from the membrane after activation by GTP. Whatever the structural model retained for the resting state, the α subunit exists as a monomeric entity in the activated state and, therefore, the anchorage of the α subunit cannot proceed indirectly by means of $\beta\gamma$ subunits or polydisperse multimers.

As far as $G_i\alpha$ and $G_o\alpha$ subunit membrane association is concerned (where G_i is inhibitory G protein and G_o is a G protein of unknown function abundant in brain), the domains responsible for interaction with $\beta\gamma$ and for membrane association of the activated GTP-bound form are colocalized in the N-terminal domain of the protein. Neer *et al.* (7) showed that proteolytic removal of a short N-terminal domain of purified $G_o\alpha$ abolishes interaction with $\beta\gamma$. $G_i\alpha$ and $G_o\alpha$ have been shown to be myristoylated on Gly^2 after cotranslational removal of initiating methionine (8), and it has been proposed that this myristoyl group is essential for membrane anchorage. This hypothesis was further confirmed by showing that suppression of myristoylation, either by mutation of Gly^2 or by substitution of myristate by a less hydrophobic analog, abolished membrane interaction upon transfection of COS cells (9, 10). Eide *et al.* (11) proposed that the N-terminal domains of activated $G_{i1}\alpha$, $G_{i2}\alpha$, and $G_o\alpha$ are responsible for the interaction of these proteins with the plasma membrane since trypsinization of bovine brain or neutrophil membranes after treatment with GTP[γ-S] cleaves a 2-kDa N-terminal fragment and induces membrane dissociation of the large remaining C-terminal fragment.

With regard to the transducin α subunit ($G_t\alpha$), V8 *Staphylococcus aureus* proteolysis experiments showed that the domain of interaction with $\beta\gamma$ is also localized in the N-terminal region of the protein (12). However, the problem of the anchorage of the activated form does not arise, since in the retina the activated GTP-bound form of $G_t\alpha$ is released from the membrane. Interestingly, several investigators (9, 10) showed that $G_t\alpha$ is myristoylated and membrane attached upon transfection into COS cells, suggesting that the $G_t\alpha$ N-terminal domain has the same potential properties as the corresponding domain of $G_i\alpha$ or $G_o\alpha$.

The other well-characterized G protein α subunit is $G_s\alpha$ (where G_s is adenylate cyclase stimulatory G protein), for which no myristoylation has been reported (8), raising the possibility that another mode of anchorage could be involved. In the resting state, we showed that the domain of interaction with $\beta\gamma$ is located within the first 29 N-terminal amino acids (13) since genetic deletion of codons 2–29 impairs direct interaction with $\beta\gamma$, as visualized on sucrose gradients. After activation by GTP[γ-S], we showed that proteolytic removal

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.

Abbreviations: GTP[γ-S], guanosine 5′-[γ-S]thiotriphosphate; G protein, guanine nucleotide-binding protein; $G\alpha$, G protein α subunit; G_s, adenylate cyclase stimulatory G protein; G_{i1}, inhibitory G protein of the i1 subtype; G_o, G protein of unknown function abundant in the brain; G_t, transducin.

‡To whom reprint requests should be addressed.

of a short N-terminal domain does not impair membrane association of the large proteolytic fragment, whereas removal of a short C-terminal domain does (14). Therefore, in contrast to other G proteins, the domains responsible for interaction with $\beta\gamma$ and for membrane association after activation are not colocalized in $G_s\alpha$ since the first is N-terminal, whereas the second is C-terminal.

To show that no domain of $G_s\alpha$ other than the C terminus is involved and to define the exact amino acids implicated, we constructed chimeras between a soluble core (amino acids 7–340 of $G_{i1}\alpha$, representing >90% of the total protein) and various parts of the $G_s\alpha$ C terminus. After *in vitro* transcription and *in vitro* translation of the fusion cDNAs, we looked for the interaction of the fusion proteins with plasma membranes after activation by GTP[γ-S].

MATERIALS AND METHODS

Plasmids. The entire coding sequence of $G_{i1}\alpha$ (a generous gift from R. R. Reed and D. T. Jones, Johns Hopkins University, Baltimore) was subcloned into pIBI 31 (International Biotechnologies). The blunted *Bsp*MI/*Stu* I fragment of pIBI $G_s\alpha$ (15) was subcloned into pIBI $G_{i1}\alpha$ digested with *Aat* II and *Stu* I and blunt-ended. The resulting plasmid, named pIBI $G_{i1s367-394}\alpha$, contains the 340 first codons of $G_{i1}\alpha$ and the 28 last ones of $G_s\alpha$ fused in frame. The plasmid pIBI $G_{i1}\alpha$ was digested with *Eco*47III and *Stu* I, and the resulting 1.74-kilobase-pair (kbp) fragment was purified. The same plasmid was digested with *Nco* I and *Stu* I and blunt-ended, and the resulting 3.11-kbp fragment was purified. The two purified fragments were ligated and transformed into strain HB 101. The resulting plasmid, named pIBI ΔN-$G_{i1}\alpha$, contains the coding sequence for $G_{i1}\alpha$ except codons 2–6. The *Hin*dIII/*Bgl* II fragments of plasmids pIBI ΔN-$G_{i1}\alpha$ and pIBI $G_{i1s367-394}\alpha$ were exchanged. The resulting plasmid, named pIBI ΔN-$G_{i1s367-394}\alpha$, contains the initiation ATG plus codons 7–340 of $G_{i1}\alpha$ plus the 28 last codons of $G_s\alpha$ (codons 367–394). The plasmid pIBI $G_s\alpha$ was digested with *Afl* II, partially digested with *Sph* I, and blunt-ended, and the resulting 4.24-kbp fragment was purified and ligated into HB 101. The resulting plasmid, named pIBI-$G_s\alpha\Delta$386–394, contains the entire coding sequence of $G_s\alpha$ except the last 9 codons. The plasmid pIBI ΔN-$G_{i1s367-385}\alpha$ was constructed using the same method as for pIBI ΔN-$G_{i1s367-394}\alpha$ but with pIBI $G_s\alpha\Delta$386–394 instead of pIBI $G_s\alpha$. The plasmids pIBI ΔN-$G_{i1s367-380}\alpha$ and pIBI ΔN-$G_{i1s367-376}\alpha$ were constructed by polymerase chain reaction (PCR) amplification of appropriate fragments and subsequent cloning into *Bst*BI/*Afl* II-digested pIBI ΔN-$G_{i1s367-394}\alpha$. The plasmid pIBI ΔN-$G_{i1s381-394}\alpha$ was constructed by *Mae* III partial digestion of the *Bsp*MI/*Stu* I fragment of pIBI $G_s\alpha$ and subcloning into *Aat* II/*Stu* I-digested pIBI ΔN-$G_{i1}\alpha$. The resulting plasmid contains the initiation ATG plus codons 7–340 of $G_{i1}\alpha$ plus glycine arising from in-frame fusion plus codons 381–394 of $G_s\alpha$. All of the sequences were checked by dideoxy sequencing on both strands of double-stranded plasmids with T7 DNA polymerase as indicated by the manufacturer (Promega).

***In Vitro* Myristoylation.** Translation of the *in vitro* transcribed RNA was performed as described (15), except that unlabeled methionine was used and reticulocyte lysate was preincubated with [^{3}H]myristic acid [final concentration, 2 μM; specific activity, 47.5 Ci/mmol (1 Ci = 37 GBq)]. The translation medium (100 μl) was then immunoprecipitated as described (14) by antibodies directed against the last 10 C-terminal amino acids of either $G_s\alpha$ or $G_{i1}\alpha$. The immunoprecipitated material was then analyzed by SDS/PAGE and the gel was autoradiographed for 10 days using Kodak X-Omat XAR-5 film.

Interaction of Translation Products with S49*cyc*$^-$ Membranes (Reconstitution). Translation medium (10 μl) was incubated with membranes (10 μl × 2 mg/ml) from S49*cyc*$^-$ cells for 30 min at 37°C. After centrifugation (10,000 × *g*/10 min), supernatant S1 was discarded and the membrane pellet was resuspended in TC buffer (20 mM Hepes, pH 8.0/2 mM $MgCl_2$/1 mM EDTA). $MgCl_2$ and GTP[γ-S] were added at final concentrations of 10 mM and 100 μM, respectively. Membranes were further incubated for 10 min at 37°C and again centrifugated as before. Supernatant S2 and pellet P2 were loaded on polyacrylamide gels as were aliquots of translation medium and S1.

Other Methods. *In vitro* transcription and translation, preparation of S49*cyc*$^-$ membranes, trypsin proteolysis, immunoprecipitations, and SDS/PAGE were carried out exactly as described (14, 15).

RESULTS

Characterization of *in Vitro* Translated $G_s\alpha$, $G_{i1}\alpha$, ΔN-$G_{i1}\alpha$, and ΔN-$G_{i1s367-394}\alpha$. Translation of $G_s\alpha$ gave rise to a major translation product of expected molecular mass (46 kDa) plus lower bands corresponding to illegitimate initiation at internal AUG codons as previously shown (15) (Fig. 1*A*). The major translation products corresponding to $G_{i1}\alpha$, ΔN-$G_{i1}\alpha$, and ΔN-$G_{i1s367-394}\alpha$ have the expected size on the basis of amino acid number, 354, 349, and 363, respectively (Fig. 1*A*); furthermore, they displayed much less internal initiation than $G_s\alpha$, probably because the $G_{i1}\alpha$ region around the initiation AUG codon has not been modified for subcloning.

As expected, $G_s\alpha$ and ΔN-$G_{i1s367-394}\alpha$ were immunoprecipitated only by an antiserum raised against the last 10 C-terminal amino acids of $G_s\alpha$, whereas $G_{i1}\alpha$ and ΔN-$G_{i1}\alpha$ were immunoprecipitated only by an antiserum raised against the last 10 C-terminal amino acids of $G_{i1}\alpha$ (Fig. 1*B*). This result confirmed the expected sequence of the C-terminal region of each protein. Furthermore, the coding 3′ region of each clone was sequenced by the dideoxy sequencing method on both strands of the double-stranded plasmid with T7 DNA polymerase (unpublished data).

Several laboratories have shown that the conformational change induced by GTP on the α subunit of transducin (16), G_i and G_o (17), or G_s (18) could be visualized by differential sensitivity to trypsin degradation in the presence or absence of GTP[γ-S]. Accordingly, we also found that protected fragments are generated in the presence of trypsin plus GTP[γ-S], whereas all of the proteins are fully digested with trypsin alone (Fig. 1*C*).

These results suggested that the deletion within the N-terminal region of $G_{i1}\alpha$ did not affect the overall conformation of the core of the polypeptide, which remained a "GTP-binding protein." Similarly, replacement of the C-terminal region of $G_{i1}\alpha$ by the last 28 amino acids of the $G_s\alpha$ C terminus did not alter the ability of the chimeric protein to bind GTP[γ-S]. Therefore, the mutated proteins were suitable for studying anchorage of the GTP[γ-S]-activated form.

Deichate *et al.* (19) have shown that pp60$^{v\text{-}src}$ is actually myristoylated when translated in reticulocyte lysate. We now extend that result to $G_{i1}\alpha$ since a ^{3}H-labeled band of expected molecular mass is immunoprecipitated when $G_{i1}\alpha$ RNA is translated in the presence of [^{3}H]myristic acid (Fig. 1*D*). As expected from the results of Jones *et al.* (9) and Mumby *et al.* (10), deletion of codons 2–6, which contain the glycine to be myristoylated, is accompanied by disappearance of ^{3}H labeling in the ΔN polypeptides (Fig. 1*D*).

Interaction of $G_s\alpha$, $G_{i1}\alpha$, ΔN-$G_{i1}\alpha$, and ΔN-$G_{i1s367-394}\alpha$ with S49*cyc*$^-$ Membranes. The membrane association of the *in vitro* translated polypeptides was determined after reconstitution of S49*cyc*$^-$ membranes and GTP[γ-S] treatment. Under these conditions, about 40% of the total *in vitro* translated $G_s\alpha$ interacted with the membrane fraction P2 (Fig. 2, Table 1). This proportion is relatively small, but we earlier showed that

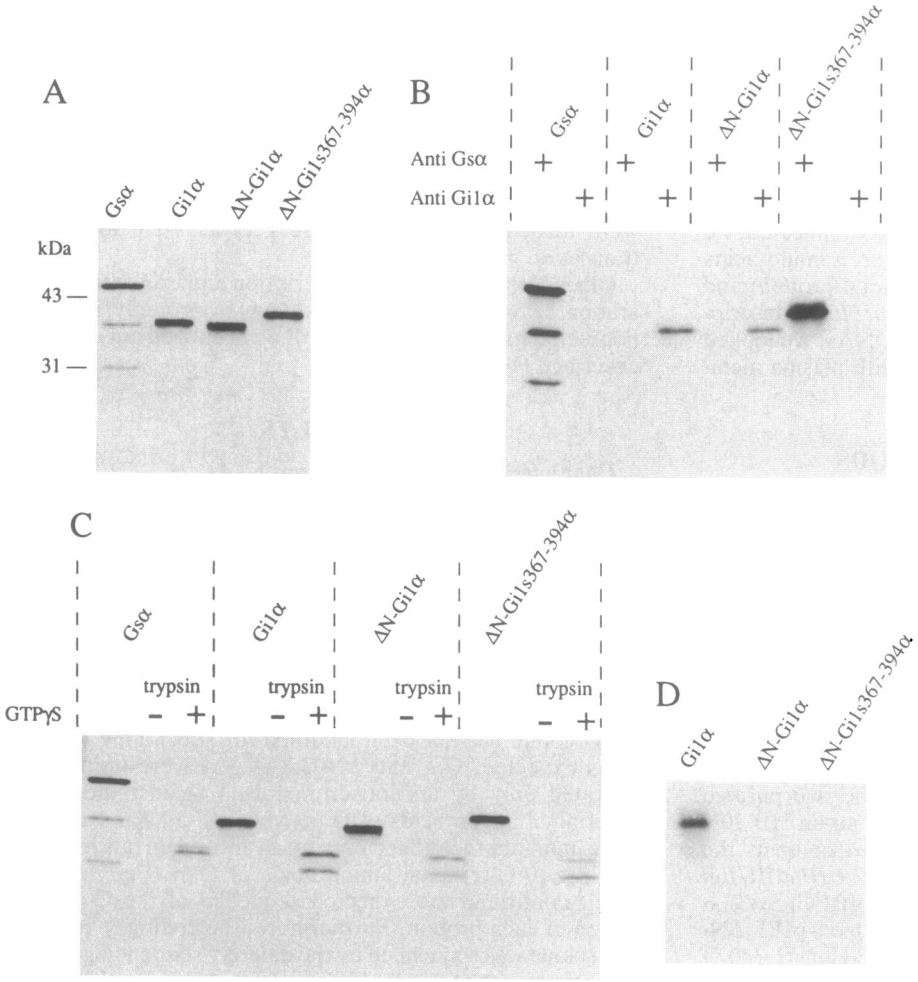

FIG. 1. Characterization of *in vitro* translated $G_s\alpha$, $G_{i1}\alpha$, $\Delta N\text{-}G_{i1}\alpha$, and $\Delta N\text{-}G_{i1s367-394}\alpha$. (*A*) Autoradiogram of 2 μl of ^{35}S-labeled *in vitro* translation media. (*B*) Autoradiogram of ^{35}S-labeled polypeptides after immunoprecipitation by antisera raised against the C-terminal decapeptide of either $G_s\alpha$ or $G_{i1}\alpha$. Each translation medium (5 μl) was immunoprecipitated with each antiserum (5 μl) and analyzed by 12% SDS/PAGE. (*C*) Autoradiogram of ^{35}S-labeled translation products after no treatment or incubation with trypsin (100 μg/ml for $G_s\alpha$ and 20 μg/ml for other polypeptides) for 30 min at 30°C in the absence or the presence of 100 μM GTP[γ-S] and 10 mM $MgCl_2$ for the same period of time. (*D*) Autoradiogram of ^3H-labeled polypeptides after analysis by 12% SDS/PAGE. Translation was performed with unlabeled methionine and [^3H]myristic acid, and 100 μl of the translation media were immunoprecipitated with antisera raised against the C-terminal decapeptide of either $G_{i1}\alpha$ ($G_{i1}\alpha$ and $\Delta N\text{-}G_{i1}\alpha$) or $G_s\alpha$ ($\Delta N\text{-}G_{i1s367-394}\alpha$).

the membrane-bound $G_s\alpha$ was able to functionally reconstitute the coupling between the β-adrenergic receptor and the adenylate cyclase (14, 15). Under the same conditions, >80% of $G_{i1}\alpha$ was associated with the pellet fraction. Deletion of amino acids 2–6 of $G_{i1}\alpha$ completely abolished membrane association, whereas replacement of the C terminus of $\Delta N\text{-}G_{i1}\alpha$ by the C terminus of $G_s\alpha$ restored, almost completely, membrane attachment of the fusion protein (Fig. 2, Table 1).

Consequently, amino acids 367–394 of $G_s\alpha$ contain information sufficient to trigger membrane anchorage that is not present in amino acids 341–354 of $G_{i1}\alpha$.

Interaction of $\Delta N\text{-}G_{i1s367-394}\alpha$, $\Delta N\text{-}G_{i1s367-385}\alpha$, $\Delta N\text{-}G_{i1s367-380}\alpha$, $\Delta N\text{-}G_{i1s367-376}\alpha$, and $\Delta N\text{-}G_{i1s381-394}\alpha$ with S49*cyc*$^-$ Membranes. To better localize the crucial residues for membrane association, we constructed other chimeras containing various sequences of $G_s\alpha$ C terminus (Fig. 3). Before analyzing their membrane association, we demonstrated that all chimeras still bound GTP[γ-S] and could therefore undergo the conformational change (unpublished data).

Whereas $\Delta N\text{-}G_{i1s367-380}\alpha$ was able to interact with plasma membrane, although less efficiently, $\Delta N\text{-}G_{i1s381-394}\alpha$ was not anchored, indicating that the domain responsible for mem-

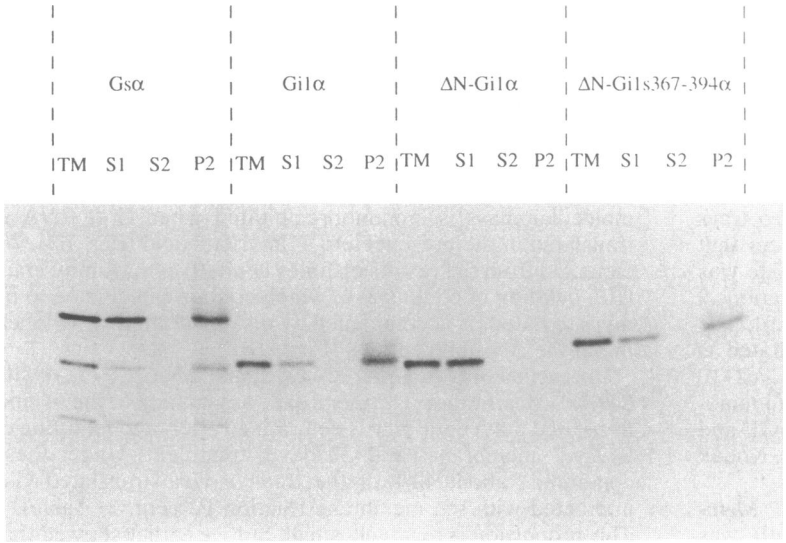

FIG. 2. Interaction of $G_s\alpha$, $G_{i1}\alpha$, $\Delta N\text{-}G_{i1}\alpha$, and $\Delta N\text{-}G_{i1s367-394}\alpha$ with S49*cyc*$^-$ plasma membrane. The proteins were *in vitro* translated in the presence of [^{35}S]methionine. The translation medium (10 μl) was mixed with S49*cyc*$^-$ membranes (10 μl × 2 mg/ml) and incubated at 37°C for 30 min. The medium was then centrifugated and the supernatant (S1) was discarded. The membrane pellet was resuspended in 10 μl of buffer containing 10 mM $MgCl_2$ and 100 μM GTP[γ-S] and centrifugated again. The supernatant (S2) and the pellet (P2) as well as aliquots of S1 (4 μl) and of the translation medium (2 μl, TM) were analyzed by 12% SDS/PAGE and autoradiographed.

Table 1. Quantitative analysis of the membrane association of the different chimeras

Polypeptide	% membrane bound (relative to $G_s\alpha$)
$G_s\alpha$	100 ± 13
$\Delta N\text{-}G_{i1}\alpha$	0
$\Delta N\text{-}G_{i1s367-394}\alpha$	86 ± 7
$\Delta N\text{-}G_{i1s367-385}\alpha$	41 ± 5
$\Delta N\text{-}G_{i1s367-380}\alpha$	35 ± 2
$\Delta N\text{-}G_{i1s367-376}\alpha$	68 ± 3
$\Delta N\text{-}G_{i1s381-394}\alpha$	7 ± 1

For each α chain, the ^{35}S-labeled proteins from translation medium or chimera-reconstituted membranes were separated by SDS/PAGE on 12% acrylamide gels. Autoradiograms of the dried gels were subjected to densitometry. The percentage of membrane-bound α chain was calculated as the percentage of the membrane-associated amount versus the total amount of *in vitro* translated protein. The corresponding value for $G_s\alpha$ was equal to 39% ± 5%, and the amount of membrane-associated chimera was expressed relative to this number. Values represent the mean ± SEM determined in three separate experiments.

brane association is located in the N-terminal half of the sequence 367–394 (Fig. 4, Table 1). Furthermore, $\Delta N\text{-}G_{i1s367-376}\alpha$ was still associated with the membrane fraction after activation by GTP[γ-S] and the amount of membrane-bound protein was not very different from that of $\Delta N\text{-}G_{i1s367-394}\alpha$ (Table 1). Consequently, amino acids 367–376 contain a determinant that confers to $G_s\alpha$ its ability to be anchored to the plasma membrane.

DISCUSSION

The validity of our *in vitro* reconstitution system has already been demonstrated by restoration of coupling between β-adrenergic receptor and adenylate cyclase and by showing that anchorage of reconstituted $G_s\alpha$ is indistinguishable from that of native $G_s\alpha$ (15). Moreover, we previously showed that, in contrast to $G_{i1}\alpha$, $G_{i2}\alpha$, and $G_o\alpha$ (10), the N-terminal domain of $G_s\alpha$ is not involved in membrane anchorage of the activated form since proteolytic removal of 2 kDa or genetic deletion of 28 amino acids on the N-terminal side of $G_s\alpha$ did not impair its membrane association (13, 14). On the other hand, V8 protease removal of a short C-terminal domain completely abolished membrane association of the large proteolytic fragment (14).

In the present study we used the same model to test various chimeric proteins in which a soluble core was fused to different domains of the $G_s\alpha$ C terminus. We wanted to conserve a G protein conformation because the information about membrane association may need a GTP-binding context to be expressed. In accord with the role of the N terminus in the anchorage of G_i and G_o α subunits (9, 10), we produced a soluble GTP-binding protein by deletion of codons 2–6 within $G_{i1}\alpha$: the resulting protein, $\Delta N\text{-}G_{i1}\alpha$, was not membrane associated, was not myristoylated, and was protected from trypsin degradation in the presence of GTP[γ-S].

Replacement of the last 14 codons of $\Delta N\text{-}G_{i1}\alpha$ (codons 341–354 of $G_{i1}\alpha$) by the last 28 ones of $G_s\alpha$ (367–394) restored membrane association, suggesting that the $G_s\alpha$ C-terminal domain contains information about membrane association that is not present in the corresponding C-terminal domain of $G_{i1}\alpha$. The construction of other chimeras clearly demonstrated that the last 14 residues of $G_s\alpha$ (381–394) were not able to induce membrane association, whereas all of the chimeras containing residues 367–376 of $G_s\alpha$ were interacting with the membrane. The relative differences (ranging from 86% to 35%) of membrane association found for the various chimeras containing this sequence may reflect the involvement of additional factors, such as slight changes in the overall conformation (Table 1). Nevertheless, amino acids 367–376 are sufficient to promote membrane association of the fusion protein.

These results confirm the involvement of the $G_s\alpha$ C-terminal domain in membrane association that was inferred from proteolysis experiments with V8 protease (14) and locate more precisely the crucial residues for membrane anchorage—i.e., the sequence Val-Asp-Thr-Glu-Asn-Ile-Arg-Arg-Val-Phe. Alignment of the sequence with various α subunits that are myristoylated reveals that the glutamate (Glu^{370}) and the arginine doublet (Arg^{373}-Arg^{374}) correspond to divergent residues. Interestingly, two new members of the Gα family (20)—$G_q\alpha$, implicated in phospholipase C activation (21), and $G_{11}\alpha$—share with $G_s\alpha$ the sequence Thr-Glu-Asn-Ile-Arg. Since they do not contain a glycine at the second position and consequently are unlikely to be myristoylated, it is tempting to speculate that they may be membrane associated by means of this peptide sequence, which is homologous to the one we determined for $G_s\alpha$.

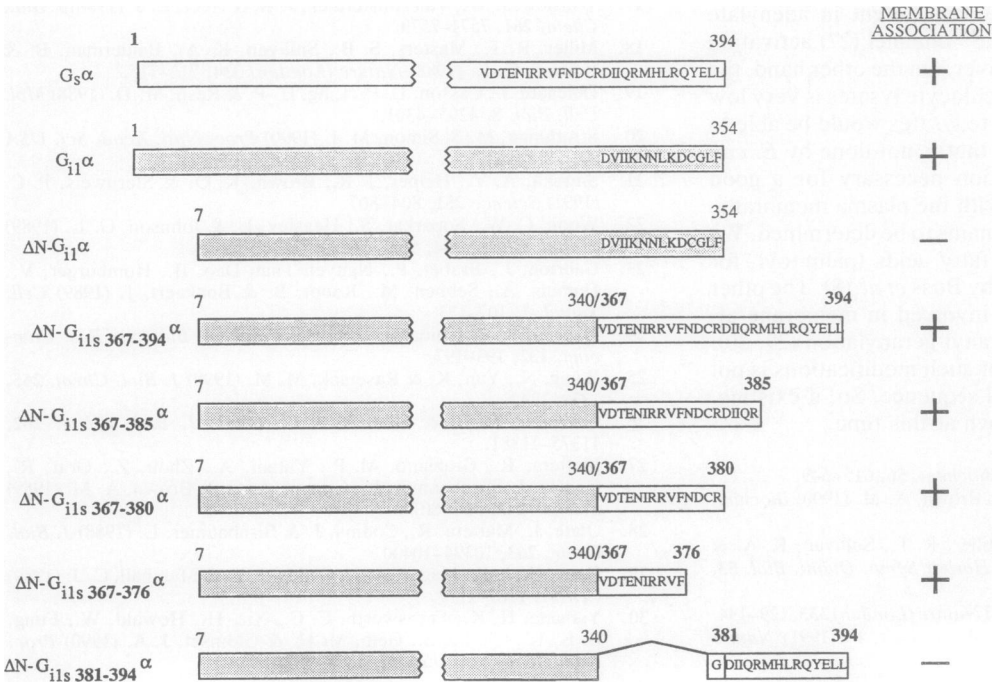

FIG. 3. Schematic drawing and membrane association of the chimeras. Open boxes and bold numbers represent $G_s\alpha$ sequences; dashed boxes and other numbers represent $G_{i1}\alpha$ sequences. In the case of $\Delta N\text{-}G_{i1s381-394}\alpha$, a glycine arises from in-frame fusion between $\Delta N\text{-}G_{i1}\alpha$ and $G_s\alpha$ sequences (see text). A gap has been introduced in the representation of that chimera to respect sequence alignment.

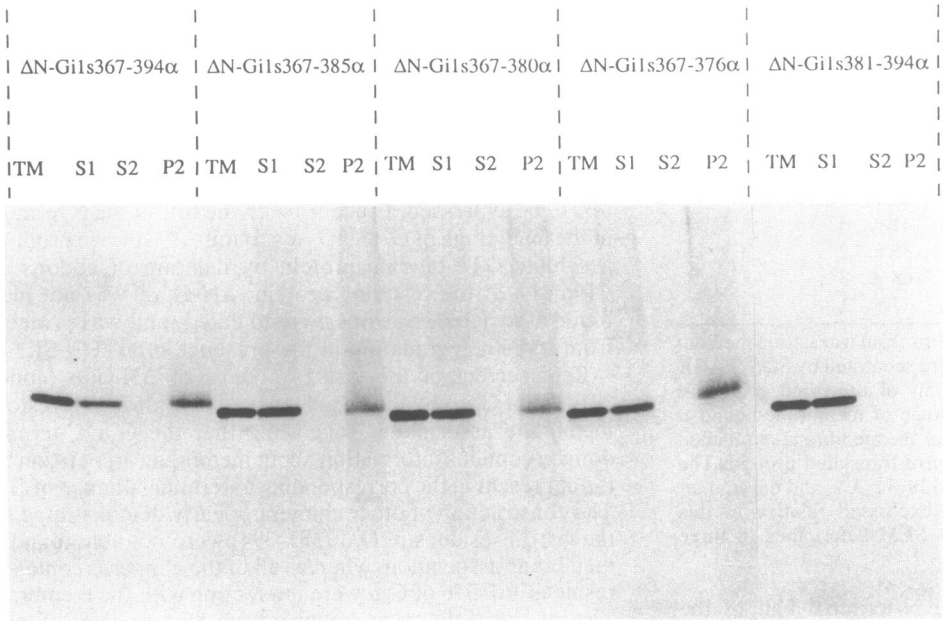

FIG. 4. Interaction of $\Delta N\text{-}G_{i1s367-394}\alpha$, $\Delta N\text{-}G_{i1s367-385}\alpha$, $\Delta N\text{-}G_{i1s367-380}\alpha$, $\Delta N\text{-}G_{i1s367-376}\alpha$, and $\Delta N\text{-}G_{i1s381-394}\alpha$ with S49*cyc*$^-$ plasma membranes. The experiment was carried out as described in the legend to Fig. 2.

Recently, a $G_s\alpha$ chimera has been constructed by replacement of the last 38 C-terminal amino acids of $G_s\alpha$ by the last 36 C-terminal residues of $G_{i2}\alpha$ (22). Since this chimeric protein does not contain residues 367–376 of $G_s\alpha$, it is noteworthy that, after stable transfection of CHO cells, the corresponding transcript is normally expressed but the polypeptide is not detectable in the membrane fraction (22).

As far as $G_s\alpha$ membrane anchorage is concerned, the results presented here focus on a short peptide sequence that is not strongly hydrophobic. Consequently, this sequence is either sufficient for interaction with a membrane component or undergoes a posttranslational modification that confers the ability to interact with the membrane. The membrane component could be the effector—i.e., adenylate cyclase itself. But other proteins can be involved since $G_o\alpha$ (23) and $G_s\alpha$ (24, 25) have been proposed to interact with some proteins of the cytoskeleton in the neighborhood of the plasma membrane.

A posttranslational modification has already been suggested to explain the finding that $G_s\alpha$ synthesized in *Escherichia coli* is about 50–100 times less potent in adenylate cyclase (26) or skeletal muscle Ca^{2+} channel (27) activation than the one purified from rabbit liver. On the other hand, the amount of $G_s\alpha$ synthesized in reticulocyte lysates is very low but is fully active (28). Reticulocyte lysates would be able to carry out a step in $G_s\alpha$ synthesis that is not done by *E. coli* and that could be the modification necessary for a good interaction of the activated $G_s\alpha$ with the plasma membrane. The nature of this modification remains to be determined. We only know that no myristoyl or fatty acids (palmitoyl, for instance) have been found on $G_s\alpha$ by Buss *et al.* (8). The other well-characterized modifications involved in membrane attachment are farnesylation or geranyl-geranylation (29, 30). But the CAAX motif necessary for such modifications is not present within the $G_s\alpha$ C-terminal sequence. So, if existing, the modification of $G_s\alpha$ is unknown at this time.

1. Gilman, A. G. (1987) *Annu. Rev. Biochem.* **56,** 615–649.
2. Birnbaumer, L., Abramowitz, J. & Brown, A. M. (1990) *Biochim. Biophys. Acta* **1031,** 163–224.
3. Bourne, H. R., Masters, S. B., Miller, R. T., Sullivan, K. A. & Heideman, W. (1988) *Cold Spring Harbor Symp. Quant. Biol.* **53,** 221–228.
4. Neer, E. J. & Clapham, D. E. (1988) *Nature (London)* **333,** 129–134.
5. Bourne, H. R., Sanders, D. A. & McCormick, F. (1991) *Nature (London)* **349,** 117–127.
6. Nakamura, S.-I. & Rodbell, M. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 6413–6417.
7. Neer, E. J., Pulsifer, L. & Wolf, L. G. (1988) *J. Biol. Chem.* **263,** 8996–9000.
8. Buss, J. E., Mumby, S. M., Casey, P. J., Gilman, A. G. & Sefton, B. M. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 7493–7497.
9. Jones, T. L. Z., Simonds, W. F., Merendino, J. J., Jr., Brann, M. R. & Spiegel, A. M. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 568–572.
10. Mumby, S. M., Heuckeroth, R. O., Gordon, J. I. & Gilman, A. G. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 728–732.
11. Eide, B., Gierschik, P., Milligan, G., Mullaney, I., Unson, C., Goldsmith, P. & Spiegel, A. (1987) *Biochem. Biophys. Res. Commun.* **148,** 1398–1405.
12. Navon, S. E. & Fung, K. K. (1987) *J. Biol. Chem.* **262,** 15746–15751.
13. Journot, L., Pantaloni, C., Bockaert, J. & Audigier, Y. (1991) *J. Biol. Chem.* **266,** 9009–9015.
14. Audigier, Y., Journot, L., Pantaloni, C. & Bockaert, J. (1990) *J. Cell Biol.* **111,** 1427–1435.
15. Journot, L., Bockaert, J. & Audigier, Y. (1989) *FEBS Lett.* **251,** 230–236.
16. Fung, B. K. K. & Nash, C. R. (1983) *J. Biol. Chem.* **258,** 10503–10510.
17. Winslow, J. W., Van Amsterdam, J. R. & Neer, E. J. (1986) *J. Biol. Chem.* **261,** 7571–7579.
18. Miller, R. T., Masters, S. B., Sullivan, K. A., Beiderman, B. & Bourne, H. R. (1988) *Nature (London)* **334,** 712–715.
19. Deichate, I., Casson, L. P., Ling, H.-P. & Resh, M. D. (1988) *Mol. Cell. Biol.* **8,** 4295–4301.
20. Strathman, M. & Simon, M. I. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 9113–9117.
21. Smrcka, A. V., Helper, J. R., Brown, K. O. & Sternweis, P. C. (1991) *Science* **251,** 804–807.
22. Woon, C. W., Soparkar, S., Heasley, L. & Johnson, G. L. (1989) *J. Biol. Chem.* **264,** 5687–5693.
23. Gabrion, J., Brabet, P., Nguyen Than Dao, B., Homburger, V., Dumuis, A., Sebben, M., Rouot, B. & Bockaert, J. (1989) *Cell. Signal.* **1,** 107–123.
24. Higashi, K. & Ishibashi, S. (1985) *Biochem. Biophys. Res. Commun.* **132,** 193–197.
25. Wang, N., Yan, K. & Rasenick, M. M. (1990) *J. Biol. Chem.* **265,** 1239–1242.
26. Graziano, M. P. & Gilman, A. G. (1987) *J. Biol. Chem.* **262,** 11375–11381.
27. 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.
28. Olate, J., Mattera, R., Codina, J. & Birnbaumer, L. (1988) *J. Biol. Chem.* **263,** 10394–10400.
29. Hancock, J. F., Magee, A. I., Childs, J. E. & Marshall, C. J. (1989) *Cell* **57,** 1167–1177.
30. Yamane, H. K., Farnsworth, C. C., Xie, H., Howald, W., Fung, B. K. K., Clarke, S., Gelb, M. H. & Glomset, J. A. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 5868–5872.