Gβγ Affinity for Bovine Rhodopsin Is Determined by the Carboxyl-terminal Sequences of the γ Subunit*

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Two native βγ dimers, β1γ1 and β1γ2, display very different affinities for receptors. Since these γ subunits differ in both primary structure and isoprenoid modification, we examined the relative contributions of each to Gβγ affinity for binding to receptors. We constructed baculoviruses encoding γ1 and γ2 subunits with altered CAAX (where A is an aliphatic amino acid) motifs to direct alternate or no prenylation of the γ chains and a set of γ1 and γ2 chimeras with the γ2 CAAX motif at the carboxyl terminus. All the γ constructs coexpressed with β1 in SF9 cells yielded β1γ1 dimers, which were purified to near homogeneity, and their affinities for receptors and Gaα were quantitatively determined. Whereas alteration of the isoprenoid of γ1 from farnesyl to geranylgeranylation and of γ2 from geranylgeranylation to farnesyl had no impact on the affinities of β1γ1 for Gaα, the non-prenylated β1γ2 dimer had significantly diminished affinity. Altered prenylation resulted in a <2-fold decrease in affinity of the β1γ2 dimer for rhodopsin and a <3-fold change for the β1γ1 dimer. In each case with identical isoprenylation, the β1γ2 dimer displayed significantly greater affinity for rhodopsin compared with the β1γ1 dimer. Furthermore, dimers containing chimeric Gγ γ chains with identical geranylgeranylation modification displayed rhodopsin affinities largely determined by the carboxy-terminal one-third of the protein. These results indicate that isoprenoid modification of the Gγ subunit is essential for binding to both Ga and receptors. The isoprenoid type influences the binding affinity for receptors, but not for Gaα. Finally, the primary structure of the Gγ subunit provides a major contribution to receptor binding of Gβγ, with the carboxyl-terminal sequence conferring receptor selectivity.

Heterotrimeric G proteins transduce a wide variety of extracellular signals recognized by seven-transmembrane receptors, initiating signaling through a diverse array of intracellular effectors (1). G proteins are composed of three polypeptides: a GTP-binding α subunit and a dimer of β and γ subunits that functions as a monomer. Ligand-activated G protein-coupled receptors catalyze the exchange of GTP for GDP tightly bound to the inactive Gaα subunit, resulting in dissociation of the GTP-activated α subunit from both its cognate Gβγ dimer and the receptor. The GTP-activated α subunit and the dissociated Gβγ dimer in turn regulate intracellular effectors (1,2). At least 20 different α subunits, 5 β subunits, and 12 γ subunits (3) have been identified to date. Such a diversity of structures is believed to contribute importantly to the specificity of signaling through these pathways.

It is well established that the selective interaction between receptors and the Gaα subunits provides a major determinant of signaling specificity. There are numerous examples of the selectivity of receptors for Gaα subunits. For example, the β-adrenergic receptor couples primarily to members of the Gaα family (4–6), whereas the α2A-adrenergic receptor couples to members of the Gaα family (7–10). G protein-coupled receptors can even display remarkable selectivity among closely related Gaα structures. The bombesin receptor subtypes selectively couple with different subtypes of Gaα (11). A growing body of evidence also points to the contribution of βγ subunits in determining receptor-G protein coupling selectivity. The role of βγ diversity in the specificity of G protein signaling is supported by both in vivo and in vitro studies. Antisense RNA constructs for Gaα, Gβ, and Gγ selectively disrupt receptor signaling in rat pituitary GH3 cells (12,13). These studies have demonstrated a specific requirement of β5 and γ5 subunits for muscarinic receptor signaling, whereas β1 and γ1 subunits mediate somatostatin receptor signaling. Also, ribozyme-mediated suppression of γ7 subunit expression has led to a specific attenuation of β-adrenergic receptor signaling in HEK293 cells (14). Moreover, in vitro reconstitution with dimers of differing βγ composition demonstrates that both the β subunit (15–17) and the γ subunit (16,18,19) provide coupling specificity for various receptors. We initially interpreted the synergy and βγ selectivity of rhodopsin-catalyzed GTP binding to Gaα to mean that rhodopsin made independent binding contacts with the α and βγ subunits (20). Direct interaction of transducin βγ with rhodopsin has been demonstrated separately with fluorescence spectroscopy (21) and with surface plasmon resonance measurements (22). A receptor contact site on the β subunit was identified in cross-linking studies in which a synthetic peptide derived from the third intracellular loop of the α2A-adrenergic receptor could be cross-linked to the carboxyl-terminal region of the β subunit (23). A series of post-translational modifications are required for the functions of G proteins. In the case of Gγ subunits, these modifications include the thioether linkage of an isoprenoid

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** The abbreviations used are: G proteins, guanine nucleotide-binding regulatory proteins; FPLC, fast protein liquid chromatography; MOFS, 4-morpholinoethanesulfonic acid; CHAPS, 3-(3-cholamidopropyl)dimethylammonio)-1-propanesulfonate; Tricine, N-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl] glycine; HPLC, high pressure liquid chromatography; GTPγS, guanosine 5′-O-(3-thiotriphosphate).
group to the conserved cysteine side chain within a carboxy-terminal CAAX motif (where C is cysteine, A is an aliphatic amino acid, and X is any amino acid), proteolytic cleavage of the CAAX tripeptide, and methylation of the resulting carboxyl terminal. The terminating residue in the CAAX motif appears to determine the identity of the isoprenoid. G protein subunits \( \gamma \) and \( \gamma \) with serine at this position are modified with a 15-carbon farnesyl, whereas the other \( \gamma \) subunits terminating in leucine are modified with a 20-carbon geranylgeranyl. The lipid modification of the \( \gamma \) subunits has been shown to be responsible for attaching G proteins to membranes (24, 25). There is also ample evidence suggesting the involvement of the prenyl groups in protein-protein interactions (26–29). Two \( \beta \gamma \) dimers, \( \beta_1 \gamma_1 \) and \( \beta_1 \gamma_2 \), display a >10-fold difference in their affinity for bovine rhodopsin (20, 30), but no apparent difference in interaction with retinal Go. Since these two \( \gamma \) subunits differ in both primary amino acid sequence and isoprenoid modification, we designed this study to examine the quantitative contributions of isoprenoid modification and protein structure to the \( \beta \gamma \) dimer affinity for rhodopsin. Our data indicate that although the lipid modification is essential for a competent \( \beta \gamma \) dimer, the type of prenyl group on the \( \gamma \) subunits influences the binding affinity for rhodopsin, but not for Go. In addition, the primary structure of the \( \gamma \) subunit provides a major contribution to rhodopsin binding of \( \beta \gamma \) dimers, with the carboxy-terminal sequences conferring receptor selectivity.

EXPERIMENTAL PROCEDURES

Construction of \( \gamma \) Prenylation Mutants and Chimeras—Mutant and chimeric \( \gamma \) cDNA clones were made by PCR amplification. Bovine \( \gamma_1 \) and \( \gamma_2 \) were the original templates. A recombinant baculovirus encoding \( \gamma_2 \)-C\(_{19251}\) (NIDCR, National Institutes of Health). Plasmids encoding chimeric \( \gamma_2 \)-C\(_{19252}\) and \( \gamma_2 \)-C\(_{19253}\) (Virginia), and recombinant baculoviruses encoding \( \gamma_1 \)-C\(_{19254}\) were gifts from Dr. Nick Ryba (NIDCR, National Institutes of Health). Plasmids encoding chimeric \( \gamma_1 \)-C\(_{19255}\) and \( \gamma_1 \)-C\(_{19256}\) as previously reported (32) were used as PCR templates for generating the current chimeric \( \gamma \) DNA constructs, in which all the 3'-reverse primers contained nucleotides encoding CAIL prior to the stop codon. The resulting chimeric \( \gamma \) structures therefore all contain a carboxyl-terminal sequence that is known to direct geranylgeranyl transfer. The PCR products encoding the chimeric \( \gamma \) sequences were cloned into the transfer vector pBatPARK\(_8\) (CLONTECH) for production of recombinant baculoviruses. Insect cell culture, transfection, plaque purification, and virus amplification were carried out according to the manufacturer’s recommended procedures (CLONTECH).

Purification of G Protein Subunits—G proteins were isolated from bovine retina and baculovirus-infected S9F cells expressing recombinant \( \beta \gamma \) dimers. Bovine retinal transducin was isolated from rod outer segment discs prepared by discontinuous gradient sedimentation (33). Go, \( \gamma_1 \), and \( \gamma_2 \) were purified using previously published procedures (20, 34, 35). S9F cell-expressed \( \beta \gamma \) dimers were purified as described for \( \beta_1 \gamma_2 \) (30) with minor modifications. S9F cells were co-infected with \( \beta \gamma \)-encoding and the appropriate \( \gamma \)-encoding baculoviruses at multiplicities of infection of 1 for the \( \beta \) virus and 3 for the \( \gamma \) virus. The cells were harvested 60 hours after infection; the cells were lysed; and a P2 membrane fraction was isolated. All the dimers were purified from S9F cell membranes, except \( \beta_1 \gamma_2 \)-A, which was purified from the cytosolic fraction. After preparative chromatography over the staining intensity of the \( \beta \) chain calibrated with different amounts of \( \beta_1 \gamma_1 \) on a Coomassie Blue-stained gel by densitometry, with all the samples and \( \beta_1 \gamma_1 \) standards within linear range.

Reconstitution Assays—The activities of the \( \beta \gamma \) dimers were quantified by rhodopsin-catalyzed GTPS binding (20). \( \beta_1 \gamma_1 \) was determined by the Amido Black binding assay (36) using bovine serum albumin as a standard. The protein concentrations of all other \( \beta \gamma \) dimers were determined from the guanine nucleotide binding of each receptor.

RESULTS

The goal of this study was to delineate the relative contributions of isoprenoid modification and protein structure of the \( \gamma \) subunit to the interactions of the \( \beta \gamma \) dimer with Go subunits and receptors. We have examined these questions using the bovine G protein and rhodopsin because affinity differences between \( \beta_1 \gamma_1 \) and \( \beta_1 \gamma_2 \) dimers are well characterized for this receptor, and these two dimers differ in both the primary structure and isoprenoid modification of the \( \gamma \) chain. Data from several laboratories have suggested that the isoprenoid modification provides the major determinant of differences between these two dimers, so we initially tested the effects of altering the prenylation. To examine this question, we tested three the electrospray ion source of an LCQ ion trap mass spectrometer. Reversed-phase HPLC was performed at a flow rate of 10 \( \mu \)l/min using a 0.3 × 100-mm Betabasic 4 column (Keystone Scientific Inc., Bellefonte, PA). Solution A was \( \text{H}_2\text{O}/\text{acetonitrile} (19:1) \) and 0.1% formic acid; Solution B was acetonitrile/1-propanol (4:1) and 0.1% formic acid. The column was equilibrated at 80% Solution A and 20% Solution B, and the chromatogram was developed using a linear gradient to 20% Solution A and 80% Solution B in 20 min. Full scan mass spectra over the m/z 300–3000 range were acquired continuously for the duration of the chromatography.

\( \beta \gamma \) dimers were prepared for mass spectrometry by acetone precipitation. Samples (60–201 pmol in 20 \( \mu \)l) in 8 mM CHAPS were mixed with 9 volumes of ice-cold acetone in a Vortex mixer and incubated for 20 min on ice, and precipitates were collected by sedimentation at 12,000 g for 15 min at 4 °C. After aspirating the supernatant liquid, pellets were allowed to air-dry and were suspended in 25 \( \mu \)l of Solution A, and 5 \( \mu \)l of the sample was injected for liquid chromatography/mass spectrometry analysis.

Protein Concentration Determination—Go concentration was determined by rhodopsin-catalyzed GTPS binding (20). \( \beta_1 \gamma_1 \) was determined by the Amido Black binding assay (36) using bovine serum albumin as a standard. The protein concentrations of all other \( \beta \gamma \) dimers were determined from the guanine nucleotide binding of each receptor.

Reconstitution Assays—The activities of the \( \beta \gamma \) dimers were quantified by rhodopsin-catalyzed GTPS binding to Go, and pertussis toxin-catalyzed ADP-ribosylation of Go as described previously (20). For both assays, the detergent CHAPS was adjusted to a final concentration of 1.6 mM. Urea-washed rod outer segment disc membranes, the source of rhodopsin, were prepared as described (37). Initial rate estimates for the catalyzed GTPS binding to Go, and pertussis toxin-catalyzed ADP-ribosylation of Go were determined by single time point reactions, which consumed <20% of the Go substrate.
As predicted, the \( \beta \gamma \) subunits with altered isoprenylation.

Samples of purified dimers containing prenylation mutant and wild-type \( \gamma \) chains were acetone-precipitated and analyzed by liquid chromatography/mass spectrometry as described under “Experimental Procedures.” The sample amounts were as follows: retinal \( \beta \gamma \), 40 pmol; \( \beta \gamma \), 40 pmol; wild-type \( \gamma \), 19 pmol; \( \beta \gamma \), 40 pmol. The spectra are labeled for the masses corresponding to \( \gamma \) chains modified with farnesyl (C\(_{15}\)) and geranylgeranyl (C\(_{20}\)) or non-prenylated with and without methyl (CH\(_3\)).

To confirm the nature of the post-translational modifications, we analyzed purified \( \beta \gamma \) dimers using electrospray ionization mass spectrometry. Fig. 2 shows the deconvoluted mass spectra of \( \gamma \) constructs and a mutant \( \beta \) chain for rhodopsin increased almost 3-fold vs. wild-type (Fig. 2, A and B). The relative abundance of farnesylated vs. geranylgeranylated \( \gamma \) subunits is summarized in Table I as well.

To assess the effects of altering prenylation of \( \gamma \) on \( \beta \gamma \) interactions with receptors, we tested \( \beta \gamma \)-dependent, rhodopsin-catalyzed GTP\( \gamma \)S binding to Gs, in the presence of different \( \beta \gamma \) samples. Fig. 3 shows results from one such experiment. The \( K_\text{d} \) values obtained are summarized in Table II. The \( \beta \gamma \) subunit showed a >12-fold lower affinity compared with the \( \beta \gamma \) subunit for rhodopsin (\( K_\text{d} = 227 \text{ vs.} 18 \text{ nM} \)). When \( \gamma \) prenylation was altered from farnesyl to geranylgeranyl, the affinity of \( \beta \gamma \) for rhodopsin increased almost 3-fold (Fig. 3A), but still did not equal that of \( \beta \gamma \) for rhodopsin (\( K_\text{d} = 78 \text{ vs.} 18 \text{ nM} \)). When \( \gamma \) prenylation was altered from geranylgeranyl to farnesyl, the affinity of \( \beta \gamma \) for rhodopsin decreased.

**Fig. 2.** Electrospray ionization mass spectra of recombinant \( \beta \gamma \) subunits with altered isoprenylation.

Panel A shows the deconvoluted mass spectra of recombinant \( \beta \gamma \) dimers resulting from such co-infections purified to near homogeneity and separated on a Tricine gel stained with Coomassie Blue. The electrophoretic mobility differences among the \( \gamma \) chains are noticeable in this gel system. None of the samples contained any detectable G\( \gamma \) and geranylgeranyl (C\(_{20}\)) or non-prenylated with and without methyl (CH\(_3\)).

**Table I**

| Molecular masses of \( \gamma \) subunits with altered isoprenylation sequences | Calculated mass | Farnesyl | Geranylgeranyl |
|---|---|---|---|
| \( \beta \gamma \) | 8331 | 8332 | 100 | ND |
| \( \beta \gamma \) | 8399 | 8401 | ND | 100 |
| \( \beta \gamma \) | 7749 | 7750 | 11 | 89 |
| \( \beta \gamma \) | 7681 | 7682 | 66 | 34 |
| \( \beta \gamma \) | 7463 | 7463 | ND | ND |

* The calculated masses are based upon the amino acid sequence deduced from the DNA sequence plus the expected prenyl modifications (farnesyl for \( \gamma \) and \( \gamma \)-CVIS; geranylgeranyl for \( \gamma \)-CVIL and \( \gamma \)); endoproteolysis, carboxyl methylation, removal of the amino-terminal methionine, and amino-terminal acetylation (for \( \gamma \)-CVIL, and \( \gamma \)-A).

**Fractional isoprenoid modification was calculated from the integrated peaks of the farnesylated and geranylgeranylated species in the deconvoluted mass spectra.**

* ND, not determined.
Rhodopsin Selectivity for Gγ

Among βγ subunit forms were due to differing affinities for interaction with Gaq, we analyzed the βγ dependence of pertussis toxin-catalyzed ADP-ribosylation of Gaq. Fig. 4 shows the saturation of ADP-ribosylation of Gaq by increasing concentrations of different forms of βγ dimers. In contrast with the rhodopsin-catalyzed GTPγS binding to Gaq, the pertussis toxin-catalyzed ADP-ribosylation of Gaq displayed no distinction between β1γ1 and β1γ2 with either form of prenylation (Table II). The non-prenylated γ2 dimer (β1γ2-A) displayed a measurable enhancement of the ADP-ribosylation of Gaq, but with an affinity for Gaq that was 30-fold lower than that for the prenylated β1γ1 or β1γ2 dimer. These data suggest that for the βγ interaction with the Gaq subunit, the isoprenoid modification of the γ subunit, not the exact identity of the prenyl group, is important.

Since the difference in prenyl group did not account for the majority of the affinity difference between β1γ1 and β1γ2 for rhodopsin, we investigated the contribution of primary structures. Fig. 5 depicts the set of chimeras we employed containing heterologous sequence at approximately one-third intervals based upon regions of sequence identity between the γ1 and γ2 proteins. These constructs were based upon the previously published chimeras (32), except that all of the chimeric constructs were altered to code for CAIL at the carboxyl terminus so that they would all have the same geranylgeranyl modification. Co-infection of Sf9 cells with baculoviruses encoding these chimeric proteins. These constructs were based upon regions of sequence identity between the rhodopsin subunit, not with the parent structure β1γ1-CVIS and β1γ2 purified to near homogeneity and separated on

![Graph](image_url)

**Fig. 3.** Saturation of rhodopsin-catalyzed GTPγS binding to Gaq by βγ subunits with altered isoprenylation. The indicated concentrations of β1γ1 (■), β1γ1-CVIS (▲), and β1γ2-A (○) in A and β1γ2 (▲) and β1γ2-CVIS (○) in B were incubated in reactions containing 500 nM Gaq and 30 nM rhodopsin as described under “Experimental Procedures.” The curves drawn are the best fit for a single site binding model using GraphPAD Prism.

| Activity assessment of βγ dimers with altered isoprenylation | Kβγ (nM) |
|-------------------------------------------------------------|----------|
| β1γ2            | 25 ± 2 (4)  |
| β1γ2-CVIS       | 28 ± 3 (4)  |
| β1γ2-A          | ≥900      |
| β1γ1            | 29 ± 4 (4)  |
| β1γ1-CVIS       | 27 ± 2 (4)  |

*Mean ± S.E. for (n) determinations.

*This was determined by saturation of pertussis toxin-catalyzed ADP-ribosylation of Gaq.

*This was determined by saturation of rhodopsin-catalyzed GTPγS binding to Gaq.

*ND, not determined.
A used as junctions between chimeric segments.

B indicate junctions between chimeric segments.

These differences could be sorted based upon the identity of the

domain in the pertussis toxin-catalyzed ADP-ribosylation assay (Fig. 8 and Table IV). These data imply that the affinity differences found for dimers with distinct γ subunit primary structures are due to differences in rhodopsin-βγ interaction rather than differences in βγ-Gαi interaction.

**DISCUSSION**

With the emerging evidence suggesting that in addition to the Gα subunit, both the β and γ subunits of G proteins also affect receptor-G protein interactions (15–19), it has become an increasingly accepted notion that βγ dimers contribute to the selectivity of the receptor interaction. This study examined quantitatively the role of the prenyl modification and amino acid sequence of the Gγ subunit in determining signaling specificity. We confirmed that the apparent affinity for rhodopsin was >12 times higher for β1γ2 than for β1γ1 for the wild-type structures, whereas the two βγ dimers displayed no difference in affinity for retinal Gαi. Although one study using rhodopsin to catalyze GDP/GTP exchange on the Gαi subunit showed that the β1γ1 dimer supported a faster rate of exchange than did β1γ2 or β2γ1 (38), others have found a much lower apparent affinity for β1γ1 than for other βγ dimers (20, 27, 30, 39). The direct measurement of rhodopsin-G protein interactions using surface plasmon resonance also revealed a higher affinity of β1γ2 for rhodopsin compared with β1γ1 and showed that this difference was due to a lower dissociation rate for β1γ2 (22). We have capitalized upon this significant quantitative difference in the binding interaction with rhodopsin to assess the independent contributions of the γ subunit primary structure and post-translational modification to this difference. Our results clearly implicate the primary structure of the γ chain as a major determinant of the affinity of βγ dimers for rhodopsin. The set of γ chimeras with uniform geranylgeranylation modification further pinpointed the carboxyl-terminal sequence of the γ subunit as the region responsible for receptor selectivity. Under-scoring the importance of the amino acid sequence of the γ subunit in receptor-G protein interactions, a recent study showed that β1γ1 supported only a very low level of coupling to the α2A-adrenergic receptors, whereas the β1γ11 dimer produced a high level of coupling (16). Since both the γ1 and γ11 subtypes are farnesylated, the difference in coupling level observed for these dimers must also be attributed to differences in the amino acid sequences.

Consistent with previous findings on 5-hydroxytryptamine type 1A receptors (40), adenosine receptors (28), and bombesin receptors (11), we found that bovine rhodopsin prefers Gaβγ complexes containing the geranylgeranylated γ subunit. Our results obtained with prenylation mutants confirmed the findings of several laboratories that dimers containing geranylgeranylated γ subunits have higher affinities for receptors than those with farnesylated γ chains (11, 27, 28, 40). In our study, given the same amino acid sequence of the γ subunit, the

**Fig. 5. Construction of chimeric γ subunits.** A, the amino acid sequences of the γ1 and γ2 subunits served as parent structures for the chimeric γ subunits as previously described (32). Numbers on the right indicate the amino acid residue positions. Before C-terminal processing, γ1 contains 74 residues, and γ2 contains 71 residues. In the current constructs, the C-terminal CAX motif in γ1 has been changed from its native CVIS to CAIL so that all the resulting chimeric γ subunits contain the same C-terminal CAIL as γ2 that directs geranylgeranylation. **Boldface residues** (QLK and EDPL) correspond to sequence motifs used as junctions between chimeric segments. B, shown is a schematic overview of the γ1/γ2 chimeras. Open bars indicate polypeptide sequence derived from γ1, and closed bars indicate that derived from γ2. Vertical lines indicate junctions between chimeric segments.

**Fig. 6. SDS-PAGE of purified Gβγ subunits with chimeric γ chains.** Purified recombinant βγ subunits of defined subtypes were electrophoresed on a Tricine-16% acrylamide gel and stained with Coomassie Blue. The positions of the β1 and various γ subunits are indicated on the left.

a Tricine-16% acrylamide gel stained with Coomassie Blue. As found for the prenylation mutant γ chains, this gel system clearly resolves the chimeric γ chains. HPLC electrospray ionization mass spectrometry confirmed that the isoprenoid modification of the γ chains was predominantly the geranylgeranyl directed by the CAIL sequence. Geranylgeranylation-modified chain accounted for >74% of the mass of each purified γ subunit (Table III).

Fig. 7 shows the saturation of rhodopsin-catalyzed GTPγS binding to Gαi for the purified β1γ chimeras. They displayed clear differences in both affinity and maximum catalytic rate, as seen for the dimers containing the parent γ1 or γ2 protein. These differences could be sorted based upon the identity of the sequence for the carboxyl-terminal one-third of the chimera. As summarized in Table IV, β1γ112 and β1γ112 had similar high affinity for rhodopsin compared with β1γ1 (Kd = 24, 23, and 18 nM, respectively). The other two chimeric dimers, β1γ221 and β2γ111, had lower apparent affinity (Kd = 39 and 68 nM, respectively), with the value for the latter close to the value for β1γ111 (i.e. β1γ1-CVIL, 78 nM). This result suggests that the primary structure in the carboxyl-terminal region of the γ subunit determines the affinity of the interaction with rhodopsin. The amino acid sequence in the middle and amino-terminal regions of the γ2 subunit also contributed to the high affinity interaction, although to a lesser degree.

As expected from the similar affinities of wild-type β1γ1 and β1γ2, the β1γ chimeras, β1γ1-CVIL, and β1γ1 showed essentially identical affinity for Gαi in the pertussis toxin-catalyzed ADP-ribosylation assay (Fig. 8 and Table IV). These data imply that the affinity differences found for dimers with distinct γ subunit primary structures are due to differences in rhodopsin-βγ interaction rather than differences in βγ-Gαi interaction.

Rhodopsin Selectivity for Gγ

**Table IV**

| Subunit | Kd (nM) | Kd (nM) |
|---------|---------|---------|
| β1γ1    | 24      | 24      |
| β1γ2    | 23      | 23      |
| β1γ111  | 39      | 39      |
| β2γ111  | 68      | 68      |
| β1γ221  | 22      | 22      |
| β2γ111  | 78      | 78      |

Consistent with previous findings on 5-hydroxytryptamine type 1A receptors (40), adenosine receptors (28), and bombesin receptors (11), we found that bovine rhodopsin prefers Gaβγ complexes containing the geranylgeranylated γ subunit. Our results obtained with prenylation mutants confirmed the findings of several laboratories that dimers containing geranylgeranylated γ subunits have higher affinities for receptors than those with farnesylated γ chains (11, 27, 28, 40). In our study, given the same amino acid sequence of the γ subunit, the
positions clearly do not originate from distinct G/H9251 difference (27, 28), we found that identically isoprenylated affinity for G/H9251 since all of the dimers displayed essentially identical apparent interaction with other G/H9251 chains. Moreover, the effect of the prenyl type was more pro-

The differences that we observed among the βγ dimers in rhodopsin interaction are also not likely to originate from the different purities of the samples. We have previously shown that substances interfering with the quantitative assay of βγ dimers in cholate extracts from Y1 adrenal cortical tumor cells were completely removed by chromatography over Ultrogel AcA44 (43). Similarly, we found that chromatography over DEAE-Sephaeil was sufficient to produce partially purified recombinant β1γ2 dimers expressed in Sf9 cells using baculoviral vectors that showed quantitatively identical apparent rhodopsin affinity as homogenous preparations of the dimer. All βγ dimers utilized in the present study were purified by both DEAE-Sephaeil and Ultrogel AcA44 chromatography with additional FPLC size-exclusion chromatography to assure hydrodynamic βγ dimers with defined detergent concentration, and they were assessed to contain no measurable contaminat-

**TABLE III**

| Molecular masses of chimeric γ subunits |
|----------------------------------------|
| Calculated mass | Observed mass | Farnesyl | Geranyleranyl |
| β1γ11 | 8399 | 8401 | ND |
| β1γ21 | 7885 | 7886 | ND |
| β1γ22 | 7598 | 7599 | ND |
| β1γ112 | 8550 | 8552 | 11 |
| β1γ222 | 8265 | 26 |
| β1γ222 | 7749 | 11 |

a The calculated masses are based upon the DNA sequence plus geranyleranyl, endoproteolysis, carboxy methylation, removal of the amino-terminal methionine, and amino-terminal acetylation (γ/H9251, γ/H9252, and γ/H9253).

Fractional isoprenoid modification was calculated from the integrated peaks of the farnesylated and geranyleranlated species in the deconvoluted mass spectra.

*β1γ11 is the β1-CVIL protein.

**Fig. 7. Saturation of rhodopsin-catalyzed GTPγS binding to Goa by βγ subunits with chimeric γ chains.** The indicated concentrations of β1γ12 (■), β1γ112 (▲), β1γ212 (▼), and β1γ221 (●) were incubated in reactions containing 500 nM Goa and 30 nM rhodopsin as described under “Experimental Procedures.” The curves drawn are the best fit for a single site binding model using GraphPad Prism.

βγ dimer was more effective in coupling the Goa subunit to rhodopsin when the γ subunit was modified with geranyleranyl compared with farnesyl. Furthermore, a prenylation-deficient construct was found to be incompetent to participate in these protein-protein interactions. Although this suggested that the affinity differences found between retinal β1γ1 and other βγ dimers could be attributed solely to the isoprenylation difference (27, 28), we found that identically isoprenylated β1γ1 and β1γ2 retained significant affinity differences for bovine rhodopsin for both geranyleranlated and farnesylated γ chains. Moreover, the effect of the prenyl type was more profound with the γ1 primary structure than with the γ2 structure, which implies the importance of the amino acid sequence of the γγ subunit in conferring a high affinity rhodopsin interaction.

The apparent affinity differences that we measured for bovine rhodopsin for this set of βγ dimers with varying γ compositions clearly do not originate from distinct Goa interaction since all of the dimers displayed essentially identical apparent affinity for Goa, as others have also noted for the retinal G protein subunits (20, 41). This may not be the case for βγ dimers interacting with other Goa proteins. A significant difference in apparent affinity for Goa, but not for Goa, or Goa, has been reported for βγ dimers of differing compositions (42). Whereas ADP-ribosylation by pertussis toxin did not reveal a difference among brain βγ dimers for Goa, inhibition of GDP release did (41). Since the primary contacts between Goa and Gβγ occur within the amino-terminal sequences of α subunits, and these are distinct for the various Go species, it is likely that α subunit selectivity may contribute importantly to differences among βγ dimers in receptor signaling. This will be important to clarify in future studies.

Similar to the previously reported results from Sf9 expression systems (27, 31, 44), we found heterogeneity in isoprenoid modification of several of the Gγ subunit constructs. There is no doubt that in the presence of a high concentration of virally encoded βγ substrate, both farnesyl- and geranyleranlatedtransfases in Sf9 cells misprenylate a fraction of the γ protein. Whether such misprenylation is caused by excessive substrate, as suggested by Lindorfer et al. (31), or prenyltransferase specificity is also encoded by primary structures other than the CAXX motif, as suggested by Kalman et al. (44), remains to be tested. Neither our nor the previous data have directly assessed the prenyltransferase activities; rather, we have measured the long-term accumulation of recombinant protein products. Our data appear to suggest the lack of a consensus

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2 D. E. Wildman and J. K. Northup, unpublished data.
sequence upstream of the CAAX motif that directs farnesylation or geranylgeranylation since the proportion of prenylation did not vary systematically in our γ1γ2 dimers with the presence of γ1 or γ2 sequence. In addition to the lipid modification, Gγ subunits are also methylated at the carboxyl-terminal cysteine after the proteolytic cleavage of the AAX tripeptide. The importance of the γ subunit carboxyl methylation to the βγ interaction with Go subunits has been reported (45, 46); its effect on βγ interaction with receptors has yet to be fully examined. Whatever the effect is, it should not complicate the interpretation of our data for the following reasons. First, our mass spectra showed that except for retinal β1γ1, which showed >50% methylation, all the S9-expressed β1γ dimers were predominantly methylated (>90%). Second, we did not observe any difference among all the tested β1γ dimers in their interaction with Goa, including retinal β1γ1. Moreover, fully methylated β1γ1 purified from heterologously expressed S9 cells showed low receptor coupling with adenose receptors (28, 31), just as we have observed for retinal β1γ1 with rhodopsin.

The data presented in this study demonstrate that the prenyl group on the γ subunit is necessary for βγ interaction with the α subunit and rhodopsin. However, the type of prenyl group affects the binding only with rhodopsin, not with Goa. The primary structure of the γ subunit, particularly the carboxyl-terminal sequence, is of equal or greater importance for receptor-G protein interaction.

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