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Role of Isoprenoid Lipids on the Heterotrimeric G Protein γ Subunit in Determining Effector Activation*

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Chang-Seon Myung‡, Hiroshi Yasuda‡§, Wendy W. Liu‡¶, T. Kendall Harden‖, and James C. Garrison‡**

From the ‡Department of Pharmacology, University of Virginia Health Sciences Center, Charlottesville, Virginia 22908 and the ‖Department of Pharmacology, University of North Carolina School of Medicine, Chapel Hill, North Carolina 27599-7365

Post-translational prenylation of heterotrimeric G protein γ subunits is essential for high affinity α-βγ and α-βγ-receptor interactions, suggesting that the prenyl group is an important domain in the βγ dimer. To determine the role of the prenyl modification in the interaction of βγ dimers with effectors, the C*AAX* (where A indicates alipathic amino acid) motifs in the γ_1, γ_2, and γ_{11} subunits were altered to direct modification with different prenyl groups. Six recombinant βγ dimers were overexpressed in baculovirus-infected Sf9 insect cells, purified, and examined for their ability to stimulate three phospholipase C-β isozymes and type II adenylyl cyclase. The native $\beta_1\gamma_2$ dimer (γ subunit modified with geranylgeranyl) is more potent and effective in activating phospholipase C-β than either the $\beta_1\gamma_1$ (farnesyl) or the $\beta_1\gamma_{11}$ (farnesyl) dimers. However, farnesyl modification of the γ subunit in the $\beta_1\gamma_2$ dimer ($\beta_1\gamma_{2\text{-L71S}}$) caused a decrement in its ability to activate phospholipase C-β. In contrast, both the $\beta_1\gamma_{1\text{-S74L}}$ (geranylgeranyl) and the $\beta_1\gamma_{11\text{-S73L}}$ (geranylgeranyl) dimers were more active than the native forms. The $\beta_1\gamma_2$ dimer activates type II adenylyl cyclase about 12-fold; however, neither the $\beta_1\gamma_1$ nor the $\beta_1\gamma_{11}$ dimers activate the enzyme. As was the case with phospholipase C-β, the $\beta_1\gamma_{2\text{-L71S}}$ dimer was less able to activate adenylyl cyclase than the native $\beta_1\gamma_2$ dimer. Interestingly, neither the $\beta_1\gamma_{1\text{-S74L}}$ nor the $\beta_1\gamma_{11\text{-S73L}}$ dimers stimulated adenylyl cyclase. The results suggest that both the amino acid sequence of the γ subunit and its prenyl group play a role in determining the activity of the βγ-effector complex.

Heterotrimeric G proteins[1] are transducers of numerous extracellular signals from seven transmembrane receptors to intracellular effectors (1–4). G proteins are composed of α, β, and γ subunits and associate with the inner side of the plasma membrane. Receptor activation catalyzes the exchange of GDP for GTP on the α subunit, resulting in dissociation of the GTP-liganded α subunit from the βγ dimer (1). Both the GTP-bound form of the α subunit and the released βγ dimer regulate a variety of effectors, including PLC-β (5, 6) and adenylyl cyclases (7, 8). To date, 7 β subunits and 11 γ subunits have been identified in mammalian systems (9–15). Selective assembly of βγ heterodimers from these proteins may produce a large number of unique complexes that differ in their interactions with α subunits, receptors, and effectors. While the first four β subunits identified, β_1–β_4, are 85–90% identical in primary amino acid sequence (9), the γ subunits are much more divergent. For example, the γ_1 and γ_5 subunits are only 25% identical. Thus, the γ subunits may impart some specificity to the βγ signal.

The G protein γ subunits are subject to post-translational modification by the addition of isoprenoid lipids to an invariant cysteine residue in the C*AAX* motif at their C terminus (15–17). The last amino acid (*X*) of the C*AAX* motif is an important determinant for modification by one of two distinct isoprenoids, the 15-carbon farnesyl group or the 20-carbon geranylgeranyl group. Among the 11 known γ subunits, the γ_1, γ_8, and γ_{11} subunits are thought to be modified with the addition of a 15-carbon farnesyl group, whereas the other γ subunits contain a 20-carbon geranylgeranyl group (10). The γ_{11} subunit was recently identified as a widely expressed γ subunit that is 76% identical to the γ_1 subunit (10, 18). However, the function of βγ dimers containing the γ_{11} subunit has not been studied. Moreover, the significance of farnesyl *versus* geranylgeranyl modification of the γ subunit in βγ subunit-mediated activation of effectors has not been established.

Lipid modification is important for anchoring the βγ subunit to the membrane (17, 19, 20), but the prenyl group may also play a major role in determining functional interaction with other proteins (15, 17, 21). For instance, prenylation of the γ subunit is necessary for formation of an active transducin α-βγ complex (22, 23), for translocation of the β-adrenergic receptor kinase to the plasma membrane (24), for high affinity interactions with either α subunits or adenylyl cyclases (25), and for stimulation of PLC-β by the $\beta_1\gamma_1$ dimer (26). Since the γ subunits may have different primary amino acid sequences and different prenyl groups at their C terminus, investigators have attempted to determine whether the type of prenyl group is an

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§ Present address: Dept. of Medicine, University of Tokyo, Tokyo 112-8688, Japan.

¶ Present address: IDEXX Laboratories, Inc., One IDEXX Drive, Westbrook, ME 04092.

** To whom correspondence should be addressed: Dept. of Pharmacology, Box 448 Health Sciences Center, University of Virginia, Charlottesville, VA 22908. Tel.: 804-924-5618; Fax: 804-982-3878; E-mail: jcg8w@virginia.edu.

[1] The abbreviations used are: G proteins, guanine nucleotide-binding regulatory proteins; Sf9 cells, *Spodoptera frugiperda* cells (ATCC number CRL 1711); PLC-β, phosphatidylinositol-specific phospholipase C-β isoform; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate; BSA, bovine serum albumin; Genapol C-100, polyoxyethylene (10) dodecyl ether; PIP_2, phosphatidylinositol 4,5-bisphosphate; GTPγS, guanosine 5′-3-*O*-(thio)triphosphate; Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine; FTase, protein farnesyltransferase; GGTase-I, protein geranylgeranyltransferase; GGTase-II, Rab geranylgeranyltransferase.

important determinant of the activity of the βγ dimer. Some studies suggest that βγ dimers containing the farnesylated γ_1 subunit couple rhodopsin to the G_t α subunit more effectively (27, 28), while others show a small increase in the activity of βγ dimers containing a geranylgeranylated γ_1 subunit in receptor-coupling assays (29). We have determined that βγ dimers containing a geranylgeranyl group on the γ subunit couple the G_i α subunit to the A1 adenosine receptor more effectively than those containing a farnesyl group (30). Overall, these results suggest that the type of prenyl group on the γ subunit does play an important role in determining the activity of βγ dimers.

Most investigators have considered that the prenyl group is involved in tethering the βγ subunit to the membrane (17, 19, 20). However, the recent x-ray crystal structure of a phosducin-βγ complex, which was crystallized with an intact γ subunit containing a farnesyl group, shows that the isoprenoid group folds into a hydrophobic pocket formed by blades 6 and 7 of the β propeller (31). Interestingly, the conformation of the β subunit in this complex is different from the conformation of the free βγ dimer (32). Thus, it is possible that there is an active conformation of the βγ dimer, which occurs upon binding to effectors, and that the prenyl group participates in establishing this conformation. These observations provide a compelling reason to examine the ability of βγ dimers in which the γ subunit contains different isoprenoid lipids in regulating effectors.

We have reported previously that the prenyl group on the γ_1 and γ_2 subunits can be altered by mutation of the last amino acid (*X*) in the C*AAX* motif at their C terminus (30, 33). Expression of the mutant γ subunits in Sf9 cells with a β subunit allows formation of functional βγ dimers in which the lipid on the γ subunit has been changed from farnesyl to geranylgeranyl or vice versa (30, 33). Using this approach, we have purified six recombinant βγ dimers containing γ subunits with native or altered prenyl groups and verified that the altered C*AAX* sequences result in the expected post-translational modifications using mass spectrometry. We examined the effects of the $\beta_1\gamma_2$, $\beta_1\gamma_1$, and $\beta_1\gamma_{11}$ dimers and their prenyl mutants on the activity of PLC-β and type II adenylyl cyclase. The results indicate that both the primary amino acid sequence of the γ subunits and the type of prenyl group on the γ subunit are important determinants of the activation of PLC-β and type II adenylyl cyclase.

EXPERIMENTAL PROCEDURES

Construction of Recombinant Baculoviruses—The recombinant baculoviruses encoding the α_S, β_1, γ_1, γ_2 subunits (34, 35), and the γ_1 and γ_2 subunits with altered prenylation sequences in the C*AAX* motifs, $\gamma_{1\text{-}S74L}$ and $\gamma_{2\text{-}L71S}$, were prepared as described (33). A full-length cDNA encoding the human γ_{11} protein was identified in the expressed sequence tag data base and obtained from Research Genetics, Inc. (γ_{11}, GenBank™ accession number H00850). To minimize the length of the construct 5′ from the ATG start codon, the polymerase chain reaction was used to add a *Sma*I restriction site to the 5′ end of γ_{11}. For the γ_{11} cDNA, the primers used were: sense primer, 5′-TCCCGGGCGAAAATGCCTGCC-3′; antisense primer, 5′-CCACTTAGGATGCAGTTTCTCCC-3′. The polymerase chain reaction products were subcloned into the pCNTR shuttle vector, and the γ_{11} coding sequence excised from pCNTR with *Sma*I and *Xba*I was ligated into these sites in the baculovirus transfer vector, pVL1393. The cDNA encoding a γ_{11} with the altered prenylation sequence in the C*AAX* motif, $\gamma_{11\text{-}S73L}$, was constructed with the same strategy. Modification of the C-terminal C*AAX* motif of γ_{11} was performed using polymerase chain reaction to introduce a CVIS → CVIL into the *CAAX* sequence. To produce the $\gamma_{11\text{-}S73L}$ cDNA, the primers used were: sense primer, 5′-TCCCGGGCGAAAATGCCTGCC-3′; antisense primer, 5′-TTTATAAAATAACACAGCTGCC-3′. The polymerase chain reaction products were subcloned into the pCNTR shuttle vector, digested with *Sma*I and *Xba*I, and ligated into these sites in the pVL1393 transfer vector. The completed constructs of both γ_{11} and $\gamma_{11\text{-}S73L}$ in the pVL1393 transfer vector were sequenced to confirm the fidelity of the γ sequences. Recombinant baculoviruses were produced by co-transfecting each recombinant plasmid DNA with linear wild type BaculoGold® viral DNA (PharMingen) into Sf9 cells as described (36). The recombinant baculoviruses were purified by one round of plaque purification (37).

Expression and Purification of Recombinant G Protein βγ Subunits—G protein α and βγ subunits were overexpressed by infecting Sf9 insect cells with recombinant baculoviruses as described (34, 35). Sf9 cells were co-infected at a multiplicity of infection of 3 with the appropriate β and γ recombinant baculoviruses and harvested 48 h after infection. The βγ subunits were extracted from frozen cell pellets with 0.1% Genapol C-100 and purified on a DEAE column followed by affinity chromatography on a G_{i1}-α-agarose column as described (35). The G_s α subunit used in the adenylyl cyclase assays was prepared from a 0.1% (w/v) CHAPS extract of crude cell lysates as described (36).

Purification of Phospholipase C-β—Recombinant turkey PLC-β and human PLC-β1 and PLC-β2 were purified to homogeneity following overexpression in baculovirus-infected Sf9 insect cells using chromatography on Q-Sepharose FF, heparin-Sepharose CL-6B, HPHT hydroxylapatite, Sephacryl S-300, and FPLC Mono Q HR 5/5 columns as described previously (38, 39).

Analysis of the Post-translational Processing of γ Subunits by Mass Spectrometry—Full processing of the γ subunit requires the addition of either a farnesyl or a geranylgeranyl group to the C-terminal cysteine in the C*AAX* motif, the removal of the three C-terminal amino acids (*AAX*), and the addition of a carboxymethyl group to the C terminus (16, 33). To determine the extent of the post-translational lipid modification of the γ subunits, the six purified βγ dimers used in this study were analyzed by electrospray ionization mass spectrometry as described previously (33). The result of this analysis showed that 96% of the native γ_1 subunit contained the expected farnesyl group and had a completely processed C terminus. Similarly, 87% of the prenyl mutant, $\gamma_{1\text{-}S74L}$, contained the geranylgeranyl group and was fully processed at its C terminus. Ninety-one percent of the γ_2 subunit and 70% of the γ_{11} subunit were found to have the expected geranylgeranyl and farnesyl groups, respectively, and a completely processed C terminus. The prenyl mutants of these two γ subunits, $\gamma_{2\text{-}L71S}$ and $\gamma_{11\text{-}S73L}$, were fully processed with expected isoprenoid lipids to the extent of 95 and 87%, respectively. Therefore, all γ subunits used in this study contain the expected isoprenoid lipid at the C terminus and are capable of high-affinity interactions with α subunits, receptors and effectors.

Preparation of Large Unilamellar Vesicles—Phospholipid vesicles were prepared as described (40). Briefly, phospholipids were mixed at a molar ratio of 4:1 of phosphatidylethanolamine to PIP_2 with [*inositol*-2-^{3}H]PIP_2 in a buffer containing 50 mM HEPES, pH 8.0, 3 mM EGTA, 80 mM KCl, 1 mM dithiothreitol and the mixture dried under argon. The dried lipid was suspended with 3.0 ml of the above buffer to give final concentrations of 1 mM phosphatidylethanolamine, 250 μM PIP_2, and 800 cpm/μl [^{3}H]PIP_2 and hydrated in the dark at room temperature. Extruded large unilamellar vesicles were formed from multilamellar vesicles by vortexing the lipid solution followed by 10 extrusion cycles through a stack of two polycarbonate filters using a mini-extruder (Avanti Polar Lipids, Inc.) (41). After extrusion, the final lipid concentration (phosphatidylethanolamine and PIP_2) was about 1100 μM. Extruded large unilamellar vesicles were stored under argon at 4 °C until use.

Reconstitution of βγ Dimers to Large Unilamellar Vesicles by Gel Filtration—CHAPS-solubilized βγ subunits (ranging from 10 ng to 10 μg) were mixed with vesicles in a volume of 200 μl. The final CHAPS concentration in the mixture was held to 0.01%. After reconstituting the mixture on ice for 30 min, the vesicles were applied to a 2-ml gel filtration column prepared with Ultrogel AcA34 as described previously (40, 42). The column was pre-equilibrated at 4 °C with buffer containing 20 mM HEPES, pH 8.0, 2 mM $MgCl_2$, 100 mM NaCl, and 1 mM EDTA (450 μl/min). The vesicles reconstituted with βγ dimers were eluted at the void volume of the column in a total volume of about 900 μl. This protocol has been demonstrated to remove more than 98% of the detergent from the vesicles (42, 43) and to resolve the vesicles from the free βγ dimers (40). The concentration of lipid in each fraction was monitored by counting the incorporated [^{3}H]PIP_2, and the amount of βγ protein inserted into the vesicles was quantified by silver staining as described below. The second fraction from the AcA34 column contained vesicles with the least amount of free βγ dimer and were used in all experiments (40).

Measurement of Phospholipase C-β Activity—Reaction buffer containing 50 mM HEPES, pH 8.0, 17 mM NaCl, 67 mM KCl, 0.83 mM $MgCl_2$, 0.17 mM EDTA, 3 mM EGTA, 1 mM dithiothreitol, and 1 mg/ml BSA was added to 70 μl of gel-filtered vesicles (fraction 2) in a total volume of 80 μl. Controls were performed using column-reconstituted vesicles without βγ dimers. The reactions were initiated by adding 10

ng of PLC-β and 3 μM free Ca^{2+} and placing the mixture in a 30 °C water bath. After a 15-min incubation, the reactions were terminated by transferring each assay tube to a 4 °C ice bath and adding 200 μl of ice-cold 10% trichloroacetic acid followed by the addition of 100 μl of 10 mg/ml BSA (40). The assay mixtures were centrifuged to remove precipitated protein and intact PIP_2 and the [^{3}H]inositol 1,4,5-trisphosphate released into the supernatant quantitated by liquid scintillation spectrometry (44, 45).

Measurement of Adenylyl Cyclase Activity—Adenylyl cyclase activity was measured as described previously (36, 46). Briefly, Sf9 insect cells were infected with recombinant baculovirus encoding rat type II adenylyl cyclase (47) and harvested 48 h after infection. Sf9 membranes containing type II adenylyl cyclase (5 μg of protein/assay tube) were reconstituted with GTPγS-activated G_s α subunit (48) and varying concentrations of βγ dimers on ice for 30 min. The reaction was initiated by addition of the reconstituted membranes to the reaction buffer containing 25 mM HEPES, pH 8.0, 10 mM phosphocreatine, 10 units/ml of creatine phosphokinase, 0.4 mM 3-isobutyl-1-methylxanthine, 10 mM $MgCl_2$, 0.5 mM ATP, and 0.1 mg/ml BSA and incubated for 7 min at 30 °C. The assay was stopped by addition of 0.1 N HCl and cyclic AMP measured using an automated radioimmunoassay (49).

Electrophoresis—For quantitation of the βγ concentration in vesicles, the βγ dimers reconstituted into phospholipid vesicles were electrophoresed on a 12% acrylamide, sodium dodecyl sulfate-polyacrylamide gel and stained with silver according to the method of Bloom *et al.* (50). The βγ concentrations in the gel were estimated using the amount of stained β subunit protein as compared with ovalbumin standards run in the same gel. The stained proteins were quantitated using a Bio-Image scanning densitometer and the Whole Band® software (Bio-Image, Ann Arbor, MI) as described (36, 40). The accuracy of this procedure was verified by comparison with the BCA (Pierce) protein assay (40). To better display the mobility of the γ subunits, Tricine/SDS-polyacrylamide gels were run according to the procedure of Schagger and von Jagow (51). The separating gel contained 16.5% total acrylamide, 0.4% bisacrylamide, and 10% (v/v) glycerol. The stacking gel contained 4% total acrylamide and 0.1% bisacrylamide. Gels were run at constant voltage (~100 volts) at 10 °C for 4–5 h. Resolved proteins were stained with silver according to the method of Morrissey (52) with the modification that the dithiothreitol incubation was reduced to 15 min.

Calculation and Expression of Results—Experiments presented under "Results" are representative of three or more similar experiments. Data expressed as concentration-response curves were fit to sigmoid curves using the fitting routines in the GraphPad Prism™ software. Statistical differences between the curves were determined using all the individual data points from multiple experiments to calculate the F statistic as described (53).

Materials—All reagents used in the culture of Sf9 cells and for the expression and purification of G protein βγ subunits have been described previously in detail (34, 35). The baculovirus transfer vector, pVL1393, was purchased from Invitrogen; the BaculoGold® kit from PharMingen; 10% Genapol C-100 and phosphatidylinositol 4,5-bisphosphate from Calbiochem®; phosphatidylethanolamine (bovine heart) from Avanti Polar Lipids, Inc.; [*inositol*-2-^{3}H]phosphatidylinositol 4,5-bisphosphate from NEN Life Science Products; CHAPS from Roche Molecular Biochemicals; BSA (fatty acid-free) from Sigma; the pCNTR shuttle vector from 5 Prime → 3 Prime, Inc. (Boulder, CO); Q-Sepharose FF, heparin-Sepharose CL-6B, Sephacryl S-300, and FPLC Mono Q HR 5/5 columns from Amersham Pharmacia Biotech; and HPHT hydroxylapatite from Bio-Rad. All other reagents were of the highest purity available.

RESULTS

To determine the role of the prenyl modification of the γ subunit in the interaction of βγ dimers with effectors, the C*AAX* motifs in the γ_1, γ_2, and γ_{11} subunits were altered to direct the addition of different prenyl isoprenoids. Each γ subunit was co-expressed with the β_1 subunit in Sf9 insect cells, and the six βγ dimers, $\beta_1\gamma_1$, $\beta_1\gamma_{1\text{-S74L}}$, $\beta_1\gamma_2$, $\beta_1\gamma_{2\text{-L71S}}$, $\beta_1\gamma_{11}$, and $\beta_1\gamma_{11\text{-S73L}}$, were purified using α_{i1}-agarose affinity chromatography and tested for their ability to activate two effectors, PLC-β and type II adenylyl cyclase. All six γ subunits were analyzed by mass spectrometry to ensure that their C*AAX* motifs were appropriately processed. The results of the analysis showed that each γ subunit had the expected isoprenoid lipids at its C terminus with the extent of processing ranging from 70 to 95% (see "Experimental Procedures"). Therefore, all βγ dimers used in this study are capable of high-affinity interactions with α subunits, receptors, and effectors. The purity of these dimers is shown in Fig. 1*A*. The purity of the three recombinant PLC-β isoforms used in this study is shown in Fig. 1*B*.

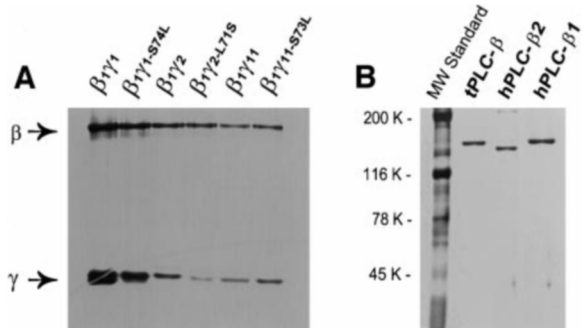

FIG. 1. **Sodium dodecyl sulfate-polyacrylamide electrophoresis of purified G protein βγ subunits and three PLC-β isoforms.** *A*, purified, recombinant βγ subunits of defined subtypes were electrophoresed on a 16% acrylamide, 10% (v/v) glycerol, sodium dodecyl sulfate-Tricine gel and stained with silver. The migration of the β and γ subunits are indicated on the left. *B*, three isoforms of purified, recombinant PLC-β were electrophoresed on a 8% acrylamide SDS-PAGE and stained with silver. The mobility of the molecular weight standards is indicated on the *left*.

Differential Effect of Three Different βγ Dimers on PLC-β and Adenylyl Cyclase Activity—We compared the ability of βγ dimers with γ subunits containing either a farnesyl or a geranylgeranyl moiety to stimulate PLC-β. An assay system was used in which purified βγ dimers were reconstituted into extruded phospholipid vesicles and the vesicles separated from mono-dispersed βγ dimers and the detergent used to solubilize G proteins on an Ultrogel AcA34 column (40). The data in Fig. 2*A* illustrate the ability of three βγ dimers to activate recombinant turkey PLC-β. Avian PLC-β was used initially, because it has a lower basal activity and higher βγ subunit-stimulated activity than either the mammalian PLC-β1 or PLC-β2 isozymes (39) (see also Fig. 3). The $\beta_1\gamma_2$ dimer activated turkey PLC-β about 10-fold with an estimated EC_{50} value of 0.7 nM (refer to Table II). The $\beta_1\gamma_{11}$ and $\beta_1\gamma_1$ subunits activated turkey PLC-β with estimated EC_{50} values of 4.1 and 1.9 nM, respectively. The V_{max} values were 50–60% of those observed with the $\beta_1\gamma_2$ dimer (refer to Table II). Therefore, the $\beta_1\gamma_2$ dimer is more potent and effective in activating PLC-β than either $\beta_1\gamma_1$ or $\beta_1\gamma_{11}$, and the potency of $\beta_1\gamma_{11}$ is less than that of $\beta_1\gamma_1$. The data in Fig. 2*B* compare the ability of the three βγ dimers to activate type II adenylyl cyclase. The $\beta_1\gamma_2$ dimer activated type II adenylyl cyclase about 12-fold with an estimated EC_{50} value of 14 nM. However, neither $\beta_1\gamma_1$ (54) nor $\beta_1\gamma_{11}$ effectively activated type II adenylyl cyclase. Since the γ subunits were combined with the same β_1 subunit, these results indicate that differences in the activation of either PLC-β or type II adenylyl cyclase are due to differences in the γ subunit. The major differences between the known γ subunits are their divergent amino acid sequences and their lipid modifications. Interestingly, the data in Fig. 2*A* show that βγ dimers containing the γ_2 subunit modified with a geranylgeranyl group are more potent and effective in activating both effectors than those containing either the γ_1 or the γ_{11} subunit modified with a farnesyl group. Therefore, to address the possibility that the identity of the prenyl group on the γ subunit is an important determinant of the dimer's activity, the C*AAX* motif in the γ_2 subunit was altered to direct the addition of the farnesyl group and the ability of the $\beta_1\gamma_{2\text{-L71S}}$ dimer to activate three PLC-β isoforms was examined.

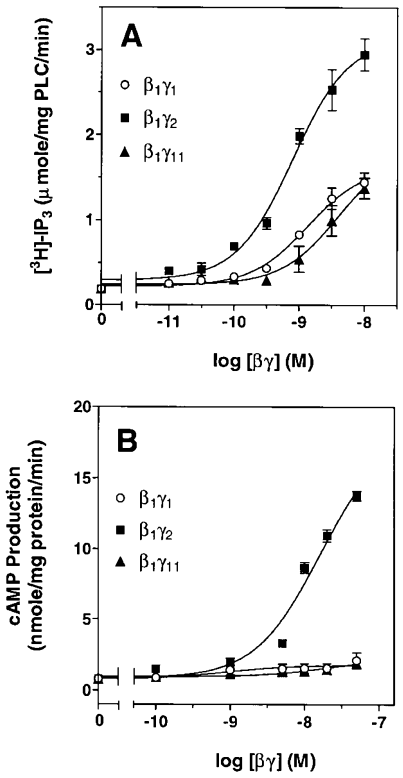

FIG. 2. **Differential abilities of three βγ dimers to regulate PLC-β and adenylyl cyclase.** *A*, the ability of the $\beta_1\gamma_1$ and $\beta_1\gamma_{11}$ dimers to activate phospholipase C-β as compared with that of the $\beta_1\gamma_2$ dimer. Extruded phospholipid vesicles containing phosphatidylinositol 4,5-bisphosphate were reconstituted with 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, or 10.0 μg of each βγ dimer on ice for 30 min. The mixture was applied to a 2-ml Ultrogel AcA34 column, fraction 2 was collected, and the ability of $\beta_1\gamma_1$ (*open circles*), $\beta_1\gamma_2$ (*closed squares*), and $\beta_1\gamma_{11}$ (*closed triangles*) in fraction 2 to stimulate turkey PLC-β activity was measured as described under "Experimental Procedures." The difference between the effect of $\beta_1\gamma_2$ and either $\beta_1\gamma_1$ or $\beta_1\gamma_{11}$ was statistically significant ($p < 0.0001$). *B*, comparison of the ability of three βγ dimers to stimulate type II adenylyl cyclase. Sf9 cells were infected with a recombinant baculovirus encoding type II adenylyl cyclase, membranes prepared, and the cyclase reaction performed with the indicated concentrations of three recombinant βγ dimers as described under "Experimental Procedures." The results are representative of six similar experiments, each performed in duplicate. Sigmoid curves were fit to the data and statistical differences between the curves determined as described under "Experimental Procedures." The difference between the effect of $\beta_1\gamma_2$ and either $\beta_1\gamma_1$ or $\beta_1\gamma_{11}$ was statistically significant ($p < 0.0001$).

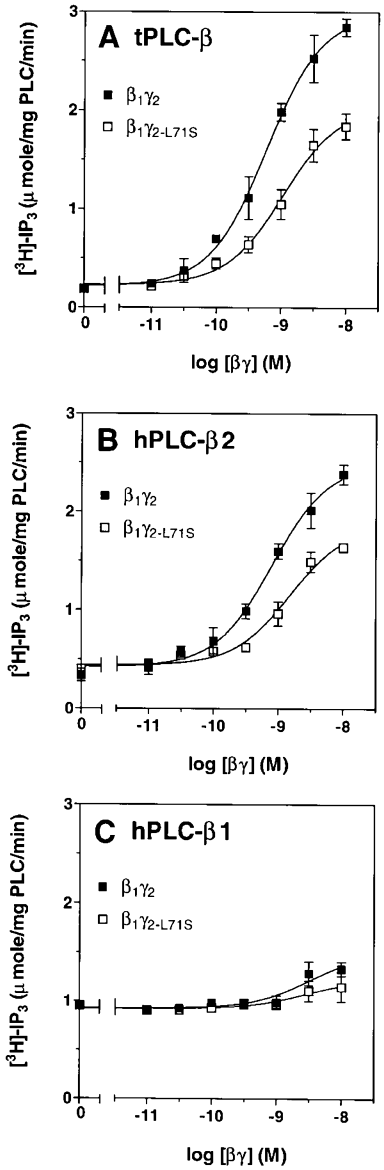

FIG. 3. **Comparison of the activity of the $\beta_1\gamma_2$ and $\beta_1\gamma_{2\text{-L71S}}$ dimers to activate three different isoforms of purified PLC-β.** *A*, the ability of $\beta_1\gamma_2$ (modified with geranylgeranyl; *closed squares*) and $\beta_1\gamma_{2\text{-L71S}}$ (modified with farnesyl; *open squares*) to stimulate recombinant turkey PLC-β (tPLC-β) was measured as described under "Experimental Procedures." The difference between the effect of $\beta_1\gamma_2$ and $\beta_1\gamma_{2\text{-L71S}}$ was statistically significant ($p < 0.001$). *B*, analogous experiments were performed with recombinant human PLC-β2 (*hPLC-β2*). The difference between the effect of $\beta_1\gamma_2$ and $\beta_1\gamma_{2\text{-L71S}}$ was statistically significant ($p < 0.001$). *C*, analogous experiments were performed with human PLC-β1 (*hPLC-β1*).

Effect of $\beta_1\gamma_2$ and $\beta_1\gamma_{2\text{-L71S}}$ on PLC-β Activity—The data in Fig. 3 show that the $\beta_1\gamma_2$ dimer stimulated PLC-β with a subnanomolar potency and that exchanging the prenyl group on the γ_2 subunit from geranylgeranyl to farnesyl ($\beta_1\gamma_{2\text{-L71S}}$) caused a diminution in the ability of the $\beta_1\gamma_2$ dimer to activate all three isoforms of PLC-β. The farnesyl-modified $\beta_1\gamma_{2\text{-L71S}}$ dimer was less potent and effective in activating turkey PLC-β than the native $\beta_1\gamma_2$ dimer (Fig. 3*A*). Fitting the data to sigmoid curves estimated that the maximal stimulation with the $\beta_1\gamma_{2\text{-L71S}}$ dimer decreased by 35% and that the EC_{50} value increased from 0.7 to 1.2 nM (refer to Table II). Similarly, the $\beta_1\gamma_{2\text{-L71S}}$ dimer was less potent and effective than the native $\beta_1\gamma_2$ dimer in the activation of human PLC-β2 (Fig. 3*B*). The data in Fig. 3*C* indicate that the βγ subunits produced little effect on the activity of PLC-β1 as demonstrated previously (5, 44, 45, 55, 56). Thus, βγ subunits stimulated activity in the order of tPLC-β ≥ PLC-β2 ⋙ PLC-β1. Overall, these results indicate that the prenyl group on the γ subunit is an important determinant of the interaction between PLC-β and βγ dimers.

Partition of Different Recombinant βγ Dimers into Phospholipid Vesicles—Since most studies of G protein activation of PLC-β have used the reconstitution of pre-aggregated lipid with α or βγ subunits to activate PLC-β, a potential problem might arise if βγ dimers containing different isoprenoid lipids did not partition equally into pre-aggregated lipid layers. This is especially important considering the different chain lengths of the lipids associated with the native γ_1, γ_2, or γ_{11} subunits. To determine the partitioning into vesicles of the six different βγ dimers used in this study, fractions 2 and 3 from the AcA34 column were collected and the amount of protein inserted into the vesicles was measured as described (40). Table I shows that both native $\beta_1\gamma_1$ and $\beta_1\gamma_{11}$ dimers containing farnesyl groups partitioned equally with the native $\beta_1\gamma_2$ dimer containing a geranylgeranyl group. About 27% of the βγ dimers added to the

TABLE I

Partition of different recombinant βγ dimers into phospholipid vesicles

Purified βγ subunits were mixed with phospholipid vesicles, reconstituted on ice for 30 min, applied to a 2-ml Ultrogel AcA34 column at 4 °C, and fractions 2 and 3 eluting at the void volume of the column used for measurement of the amount of dimer inserted into the vesicles. The amount of phospholipid eluted and βγ dimers bound to the vesicles were quantitated as described under "Experimental Procedures." Data are representative of three separate experiments.

βγ dimer	Added dimer	Dimer incorporated into vesicles		Total reconstituted, fractions 2 + 3
		Fraction 2	Fraction 3	
	ng	*ng*		*%*
$\beta_1\gamma_1$	3010	289 (9.6%)	551 (18.3%)	27.9
$\beta_1\gamma_{1\text{-S74L}}$	3009	280 (9.3%)	595 (19.8%)	29.1
$\beta_1\gamma_2$	3026	306 (10.1%)	503 (16.6%)	26.7
$\beta_1\gamma_{2\text{-L71S}}$	2990	286 (9.6%)	501 (16.8%)	26.4
$\beta_1\gamma_{11}$	2970	302 (10.2%)	560 (18.9%)	29.1
$\beta_1\gamma_{11\text{-S73L}}$	3006	278 (9.2%)	523 (17.4%)	26.6

reaction mixture were incorporated into the phospholipid vesicles isolated in fractions 2 and 3. Moreover, the βγ dimers with altered C*AAX* sequences partitioned equally with native βγ dimers. Therefore, the length of the prenyl chain does not significantly affect the concentration of βγ dimers reconstituted into phospholipid vesicles containing phosphatidylinositol 4,5-bisphosphate.

Effect of $\beta_1\gamma_1$, $\beta_1\gamma_{1\text{-S74L}}$, $\beta_1\gamma_{11}$, and $\beta_1\gamma_{11\text{-S73L}}$ on PLC-β Activity—It was important to determine whether switching the farnesyl group to geranylgeranyl improves the ability of either the $\beta_1\gamma_1$ or the $\beta_1\gamma_{11}$ dimer to activate PLC-β. Thus, the C*AAX* motifs in the γ_1 and γ_{11} subunits were altered to direct the addition of a geranylgeranyl group, and the ability of both the $\beta_1\gamma_{1\text{-S74L}}$ and $\beta_1\gamma_{11\text{-S73L}}$ dimers to activate either turkey PLC-β or human PLC-β2 was examined. The data in Fig. 4*A* show that the geranylgeranyl-modified $\beta_1\gamma_{1\text{-S74L}}$ dimer exhibited an increased potency and maximal activity compared with the native $\beta_1\gamma_1$ dimer. Similarly, the geranylgeranyl-modified $\beta_1\gamma_{11\text{-S73L}}$ dimer was more potent and effective in activating turkey PLC-β than the native $\beta_1\gamma_{11}$ dimer. The $\beta_1\gamma_{1\text{-S74L}}$ and $\beta_1\gamma_{11\text{-S73L}}$ dimers also exhibited increased capacity to stimulate human PLC-β2 activity (Fig. 5). These four βγ dimers ($\beta_1\gamma_1$, $\beta_1\gamma_{1\text{-S74L}}$, $\beta_1\gamma_{11}$, and $\beta_1\gamma_{11\text{-S73L}}$) showed little effect on the activity of PLC-β1 (data not shown, but see Fig. 3*C*). The data in Table II summarize the potency and efficacy of the six βγ dimers used in this study on the activation of these two PLC-β isoforms. The $\beta_1\gamma_{1\text{-S74L}}$ dimer exhibited an about 1.6-fold increase in maximal activity as compared with the native $\beta_1\gamma_1$ dimer and the EC_{50} value decreased from 1.9 to 1.1 nM. The estimated maximal activity of the $\beta_1\gamma_{11\text{-S73L}}$ dimer increased about 1.3-fold and the EC_{50} value decreased from 4.1 to 1.6 nM. Similar changes were observed in the ability of the dimers to activate human PLC-β2. Taken together, the data indicate that βγ dimers with a γ subunit containing a geranylgeranyl group interact with higher affinity with PLC-β than βγ dimers with a γ subunit containing a farnesyl group.

Effect of the Prenyl Group on the Activity of Type II Adenylyl Cyclase—To determine the role of the isoprenoid group in the interaction with an effector which is membrane-associated, the capacity of the six βγ dimers to activate type II adenylyl cyclase was also examined. The data in Fig. 6*A* indicate that switching the prenyl group on the γ_2 subunit from geranylgeranyl to farnesyl caused a significant decrement in the ability of the $\beta_1\gamma_2$ dimer to activate type II adenylyl cyclase. Our previous experiments showed that about the same amount of the $\beta_1\gamma_2$ and $\beta_1\gamma_{2\text{-L71S}}$ (about 25% of the βγ subunits added to a reconstitution mixture) are incorporated into Sf9 cell membrane, indicating regardless of the prenyl group on the γ subunit, the dimers partition equally into the Sf9 cell membrane (30). Thus, the result from Fig. 6*A* suggests that the type of prenyl group is important for the interaction of the βγ dimer with adenylyl

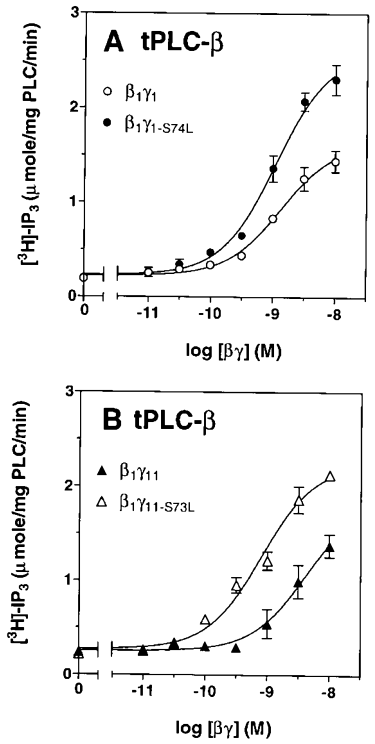

FIG. 4. **Comparison of the activity of native and altered βγ dimers to stimulate recombinant turkey PLC-β.** *A*, the ability of $\beta_1\gamma_1$ (modified with farnesyl; *open circles*) and $\beta_1\gamma_{1\text{-S74L}}$ (modified with geranylgeranyl; *closed circles*) dimers to stimulate recombinant turkey PLC-β (tPLC-β) was measured as described under "Experimental Procedures." The difference between the effect of $\beta_1\gamma_1$ and $\beta_1\gamma_{1\text{-S74L}}$ was statistically significant ($p < 0.001$). *B*, analogous experiments were performed with $\beta_1\gamma_{11}$ (modified with farnesyl; *closed triangles*) and $\beta_1\gamma_{11\text{-S73L}}$ (modified with geranylgeranyl; *open triangles*) dimers. The difference between the effect of $\beta_1\gamma_{11}$ and $\beta_1\gamma_{11\text{-S73L}}$ was statistically significant ($p < 0.001$).

cyclase. As expected, the $\beta_1\gamma_1$ dimer did not activate type II adenylyl cyclase (54). Interestingly, the $\beta_1\gamma_{11}$ dimer was completely ineffective on type II adenylyl cyclase. Surprisingly, neither the $\beta_1\gamma_{1\text{-S74L}}$ nor the $\beta_1\gamma_{11\text{-S73L}}$ dimer containing a geranylgeranyl group on the γ subunit were able to significantly stimulate type II adenylyl cyclase (Fig. 6, *B* and *C*). The data in Table III summarize the potency and efficacy of the six βγ dimers on the activation of type II adenylyl cyclase. The farnesyl-modified $\beta_1\gamma_{2\text{-L71S}}$ dimer was about 60–65% less active, and its EC_{50} value was increased from 14 to 76 nM. This change in potency and maximal activity is larger than that observed with PLC-β (see Fig. 3). Thus, βγ dimers with a γ subunit containing a geranylgeranyl group interact with higher affinity with type II adenylyl cyclase than those with a

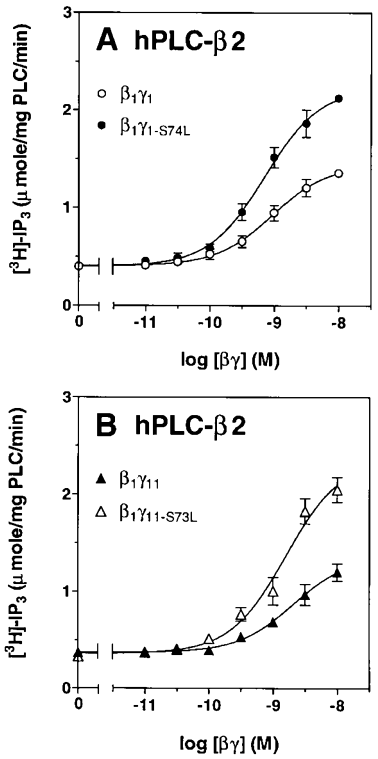

FIG. 5. **Comparison of the activity of native and altered βγ dimers to stimulate recombinant human PLC-β2.** *A*, phospholipid vesicles reconstituted with $\beta_1\gamma_1$ (modified with farnesyl; *open circles*) or $\beta_1\gamma_{1\text{-S74L}}$ (modified with geranylgeranyl; *closed circles*) dimer were incubated with recombinant human PLC-β2 (hPLC-β2) and PLC-β activity measured as described under "Experimental Procedures." The data were fit to sigmoid curves. The difference between the effect of $\beta_1\gamma_1$ and $\beta_1\gamma_{1\text{-S74L}}$ was statistically significant ($p < 0.001$). *B*, analogous experiments were performed with $\beta_1\gamma_{11}$ (modified with farnesyl; *closed triangles*) and $\beta_1\gamma_{11\text{-S73L}}$ (modified with geranylgeranyl; *open triangles*) dimers. The difference between the effect of $\beta_1\gamma_{11}$ and $\beta_1\gamma_{11\text{-S73L}}$ was statistically significant ($p < 0.001$).

TABLE II
Comparison of EC_{50} and V_{max} of six βγ dimers on the activation of two PLC-β isoforms

Phospholipid vesicles containing phosphatidylinositol 4,5-bisphosphate were reconstituted with different recombinant βγ dimers on ice for 30 min. After the incubation, the mixture was applied to a 2-ml Ultrogel AcA34 column at 4 °C and fraction 2 eluting at the void volume used for the measurement of PLC-β activity. Fraction 2 was incubated for 15 min at 30 °C in the presence of purified recombinant turkey PLC-β (tPLC-β) or human PLC-β2 (hPLC-β2) as described under "Experimental Procedures." The data are expressed as mean ± S.E. and the average of three determinations, each performed in duplicate. The EC_{50} (nM) and V_{max} (μmol/mg of PLC/min) values were estimated by fitting of the averaged data to sigmoid curves.

βγ dimer	tPLC-β		hPLC-β2	
	EC_{50}	V_{max}	EC_{50}	V_{max}
	nM	*μmol/mg PLC/min*	*nM*	*μmol/mg PLC/min*
$\beta_1\gamma_1$	1.9 ± 0.2	1.63 ± 0.08	2.7 ± 0.1	1.37 ± 0.06
$\beta_1\gamma_{1\text{-S74L}}$	1.1 ± 0.3	2.60 ± 0.04	1.7 ± 0.2	2.37 ± 0.07
$\beta_1\gamma_2$	0.7 ± 0.2	3.15 ± 0.05	1.0 ± 0.2	2.52 ± 0.03
$\beta_1\gamma_{2\text{-L71S}}$	1.2 ± 0.2	2.04 ± 0.11	1.6 ± 0.1	1.85 ± 0.11
$\beta_1\gamma_{11}$	4.1 ± 0.6	1.86 ± 0.10	4.0 ± 0.3	1.45 ± 0.17
$\beta_1\gamma_{11\text{-S73L}}$	1.6 ± 0.1	2.42 ± 0.08	2.2 ± 0.5	2.52 ± 0.13

γ subunit containing a farnesyl group. While these results are not surprising, the observation that neither the γ_1 or the γ_{11} subunit modified with a geranylgeranyl group have any activity on type II cyclase was unexpected. These observations suggest that the differences in amino acid sequence between γ_1 and γ_2 greatly affect the ability of the dimers to activate adenylyl cyclase.

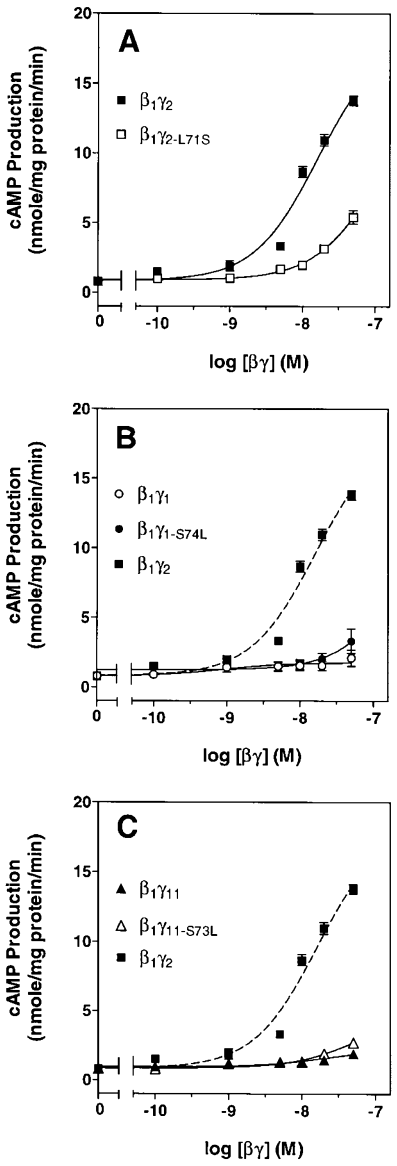

FIG. 6. **Comparison of the activity of native and altered βγ dimers to stimulate type II adenylyl cyclase.** *A*, Sf9 cells were infected with a recombinant baculovirus encoding the type II adenylyl cyclase, membranes prepared, and the cyclase reaction performed with the indicated concentrations of $\beta_1\gamma_2$ (modified with geranylgeranyl; *closed squares*) and $\beta_1\gamma_{2\text{-L71S}}$ (modified with farnesyl; *open squares*) as described under "Experimental Procedures." The difference between the effect of $\beta_1\gamma_2$ and $\beta_1\gamma_{2\text{-L71S}}$ was statistically significant ($p < 0.0001$). *B*, the cyclase reaction was performed with the indicated concentrations of $\beta_1\gamma_1$ (modified with farnesyl; *open circles*) and $\beta_1\gamma_{1\text{-S74L}}$ (modified with geranylgeranyl; *closed circles*) and compared with the effect of $\beta_1\gamma_2$ (*dotted lines*). The difference between the effect of $\beta_1\gamma_2$ and $\beta_1\gamma_1$ was statistically significant ($p < 0.0001$), but the difference between $\beta_1\gamma_1$ and $\beta_1\gamma_{1\text{-S74L}}$ was not statistically significant. *C*, analogous experiments were performed with $\beta_1\gamma_{11}$ (modified with farnesyl; *closed triangles*) and $\beta_1\gamma_{11\text{-S73L}}$ (modified with geranylgeranyl; *open triangles*) dimers. The difference between the effect of $\beta_1\gamma_2$ and $\beta_1\gamma_{11}$ was statistically significant ($p < 0.0001$), but the difference between $\beta_1\gamma_{11}$ and $\beta_1\gamma_{11\text{-S73L}}$ was not significant.

DISCUSSION

This study demonstrates that βγ dimers with γ subunits containing different isoprenoids have distinct abilities to activate PLC-β and type II adenylyl cyclase. The two major findings of this study are (*a*) the isoprenoid lipid added to the

TABLE III
Comparison of EC_{50} and V_{max} of six βγ dimers on the activation of type II adenylyl cyclase

Sf9 cells were infected with a recombinant baculovirus encoding the type II adenylyl cyclase, membranes prepared, and the cyclase reaction performed with the range of βγ dimer concentrations described under "Experimental Procedures." The data are expressed as mean ± S.E. and the average of three determinations, each performed in duplicate. The EC_{50} (nM) and V_{max} (nmol/mg of protein/min) values were estimated by fitting of the averaged data to sigmoid curves.

βγ dimer	Type II adenylyl cyclase	
	EC_{50}	V_{max}
	nM	*nmol/mg protein/min*
$\beta_1\gamma_1$	97.4 ± 7.2	1.67 ± 0.03
$\beta_1\gamma_{1\text{-S74L}}$	85.1 ± 10.6	3.04 ± 0.63
$\beta_1\gamma_2$	13.9 ± 1.1	18.34 ± 1.65
$\beta_1\gamma_{2\text{-L71S}}$	76.3 ± 10.5	7.17 ± 1.82
$\beta_1\gamma_{11}$	79.8 ± 8.9	2.85 ± 0.89
$\beta_1\gamma_{11\text{-S73L}}$	66.9 ± 6.2	4.67 ± 0.89

cysteine residue in the C*AAX* motif of the γ subunit plays a role in determining the activity of the βγ dimer at both effectors and (*b*) βγ dimers with a γ subunit containing a geranylgeranyl group are more potent and effective in activating either PLC-β or type II adenylyl cyclase than those with a γ subunit containing a farnesyl group. This study also presents the first demonstration of the functional activity of a βγ dimer containing the γ_{11} subunit. The $\beta_1\gamma_{11}$ dimer is about six times less potent and about 50% less effective in activating PLC-β than the $\beta_1\gamma_2$ dimer and has little or no effect on the type II adenylyl cyclase. Overall, the data presented here indicate that in addition to the primary amino acid sequence of the γ subunits, the prenyl group on the γ subunit is an important determinant of the activation of PLC-β and type II adenylyl cyclase.

The recent x-ray crystal structure of a complex of bovine retinal phosducin with the transducin βγ subunit in which the γ subunit contained a farnesyl group provides a conceptual background for interpreting the results obtained with PLC-β and adenylyl cyclase (31). Remarkably, the interaction between phosducin and the βγ dimer appears to result in a significant conformational change in the β subunit as compared with the conformation seen in the free βγ dimer (32). This event also appears to cause the insertion of the prenyl group into a hydrophobic pocket in the β subunit formed between blades 6 and 7 (31). Interestingly, this conformational change occurs on the outer strands of blades 6 and 7 of the β propeller and recent mutagenesis studies show that these regions are critical for the activation of PLC-β (57). If the hydrophobic pocket within the β subunit that binds the prenyl group is able to discriminate between the different length of the farnesyl and geranylgeranyl lipids, different conformational changes might be induced by the γ_1 and γ_2 subunits (or their prenyl mutants). This possibility might explain differences in the ability of the various βγ dimers to stimulate the activity of PLC-β and adenylyl cyclase. Overall, these observations suggest that insertion of a prenyl group into the hydrophobic pocket formed in the β subunit may be an important part of the mechanism by which the βγ dimers activate effectors when released from the α subunit.

The interaction of protein prenyltransferases with their isoprenoid substrates provides another example of a hydrophobic binding pocket determining isoprenoid specificity. Protein prenyltransferases are responsible for the addition of isoprenoid lipid to proteins and are classified by their lipid substrate: protein farnesyltransferase (FTase), protein geranylgeranyltransferase (GGTase-I), and the Rab geranylgeranyltransferase (GGTase-II) (58). G protein γ subunits are targets for either FTase or GGTase-I. These two prenyltransferases are heterodimers composed of the common 48-kDa α subunit and distinct β subunits: 46-kDa β subunit for FTase (β_F) and 43-kDa β subunit for GGTase-I (β_{GGI}) (59–62). Thus, the binding sites for different lipid substrates on these two prenyltransferases are thought to reside in their β subunits (58). The x-ray crystal structure determined by Park *et al.* (63) illustrates that farnesyl diphosphate occupies a hydrophobic pocket in the center of the β subunit barrel. Recently, it has been demonstrated that the isoprenoid moiety of farnesyl diphosphate binds in an extended conformation within the hydrophobic cavity of the β subunit of the FTase, and the diphosphate moiety binds to a positively charged cleft at the top of this cavity near the subunit interface (64). These findings suggest that the isoprenoid substrate specificity of the hydrophobic binding cavity is determined by the depth of the cavity and that the pocket acts as a ruler discriminating between isoprenoids of different length (64).

The three classes of PLC isozymes, PLC-β, PLC-γ, and PLC-δ, contain two highly conserved catalytic domains, X and Y, but only the PLC-β isoforms are regulated by G proteins (65). These isoforms are distinguished from the other families by an extended C terminus. Deletion of the C-terminal amino acids of PLC-β1 and PLC-β2 eliminated G_q α subunit-mediated PLC-β activation (66–68). Peptides corresponding to a portion of the C-terminal region of PLC-β1 inhibited activation by G_q α subunit *in vitro* (67), suggesting that the C-terminal region might be important for the α subunit-induced PLC-β activation. Since removal of the C-terminal region of PLC-β1 and PLC-β2 did not impair βγ subunit-induced enzyme activation, the remaining domains of the enzyme must provide the βγ subunit interaction sites. A PLC-β2 fragment corresponding to a region between the X and Y domains (Glu^{435} to Val^{641}) inhibited βγ subunit-mediated activation of PLC-β2 in transfected COS-7 cells (69). Peptides from this sequence expressed as a series of fusion proteins bound to purified βγ subunits *in vitro* (69). Overexpression of this region also blocked βγ subunit-induced activation of co-transfected PLC-β2 in COS cells (69). It has been reported recently that two overlapping PLC-β2 fragments corresponding to Asn^{564}–Lys^{583} and Glu^{574}–Lys^{593} of PLC-β2 inhibited activation of PLC-β2 by βγ subunits, inhibited βγ subunit-dependent ADP-ribosylation of G_{i1} α subunit by pertussis toxin, and inhibited βγ subunit-dependent activation of phosphoinositide 3-kinase (70). These observations suggest that the region between Glu^{574} and Lys^{583} in PLC-β2 may be the site that interacts with βγ subunits (70).

A number of studies have attempted to determine the sites at which the βγ dimer interacts with adenylyl cyclase. Type I adenylyl cyclase is inhibited by G_i α subunits and βγ subunits, whereas type II cyclase is activated by the βγ subunits in the presence of G_s α subunits (7, 71). In an attempt to identify the sites in adenylyl cyclase that interact with the βγ subunits, a chimeric soluble adenylyl cyclase was engineered by linking the type I adenylyl cyclase C1a domain (the N-terminal part of the first cytoplasmic domain) with the type II adenylyl cyclase C2 domain (the second cytoplasmic domain) (72, 73). The chimeric soluble enzyme was unresponsive to the G_i α subunit but was inhibited almost completely by the $\beta_1\gamma_2$ dimer, suggesting that the C1a domain is critical for βγ subunit-induced inhibition of type I adenylyl cyclase (72, 73). In contrast, a 27-amino acid peptide (956–982) derived from the C2 domain of type II adenylyl cyclase blocked the interaction of βγ dimers with several effectors, including PLC-β, type II adenylyl cyclase, and K^+ channels, but did not block βγ interaction with α subunits (74). This result suggests that βγ dimers can also interact with a region on the C2 domain of type II adenylyl cyclase (74).

Whereas the G protein β_1–β_4 subunits are highly conserved

in primary amino acid sequence, the γ subunits are much more divergent, suggesting that the functional specificity of different βγ dimer combinations may be due to the differences in γ subunits. The results presented here support this possibility. Combination of different γ subunits with the same β subunit resulted in βγ dimers exhibiting very different ability to activate PLC-β and adenylyl cyclase. The differences are due both to the primary amino acid sequences of the γ subunits and to their lipid modifications. For example, all βγ dimers containing a geranylgeranyl group on the γ subunit were more potent and effective in activating the two effectors than those containing a farnesyl group. Thus, the type of lipid at the C terminus of the γ subunit is important to the ability of βγ dimers to activate effectors. Other groups have also obtained data suggesting that the isoprenoid lipid is important in the interaction of βγ dimers with effectors. Matsuda *et al.* (29) have shown that the $\beta_1\gamma_{1\text{-}S74L}$ dimer containing a mixture of farnesyl and geranylgeranyl groups had a greater ability to inhibit Ca^{2+}/calmodulin-stimulated adenylyl cyclase and stimulate PLC-β than did the native farnesylated $\beta_1\gamma_1$ dimer. However, our data suggest that the amino acid sequence of the γ subunit is also an important determinant of the activity of the βγ dimer. For example, neither the $\beta_1\gamma_1$ nor the $\beta_1\gamma_{11}$ dimer are able to activate type II adenylyl cyclase. In contrast to the results obtained with PLC-β, switching the prenyl group in the $\beta_1\gamma_1$ and $\beta_1\gamma_{11}$ dimers to geranylgeranyl did not increase their ability to activate type II adenylyl cyclase. Overall, these results indicate that both the primary amino acid sequence and the prenyl group of the γ subunits are important determinants for the activation of effectors. For the activation of PLC-β, the type of prenyl group appears to be a major determinant of the activity of βγ dimer. In the case of type II adenylyl cyclase, the prenyl group also appears to be important, but the amino acid sequences of certain γ subunits, such as γ_1 and γ_{11}, appear to be incapable of activating the enzyme regardless of prenyl group at their C terminus.

Our previous data supports the possibility that charge differences in the γ subunit can have a large effect on the ability of βγ dimers to activate type II adenylyl cyclase. Phosphorylation of the γ_{12} subunit in the $\beta_1\gamma_{12}$ dimer significantly inhibits the ability of the dimer to stimulate type II adenylyl cyclase (46), and the phosphorylation site has been determined to be at Ser^1 in the N terminus of the molecule (11). This result suggests that introduction of negative charges at the N-terminal region of the γ subunit inhibits the interaction of the βγ dimer with the type II adenylyl cyclase. Interestingly, comparison of the N-terminal 20 amino acids of the γ_2 with the γ_1 or γ_{11} subunits shows that γ_1 and γ_{11} have six negatively charged amino acids whereas γ_2 has only one. Thus, the negatively charged N terminus of the γ_1 and γ_{11} subunits may explain the inability of dimers containing these γ subunits to activate type II adenylyl cyclase. Preliminary experiments with chimeric γ subunits in which the N-terminal 20 amino acids of γ_2 were replaced with the corresponding amino acids from γ_1 support this explanation. When expressed with the β_1 subunit, this dimer has a greatly reduced ability to stimulate type II adenylyl cyclase.[2] Thus, multiple domains of the γ subunit may be involved in the interaction with different effectors.

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[2] C.-S. Myung and J. C. Garrison, unpublished observation.

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