Role of the γ Subunit Prenyl Moiety in G Protein βγ Complex Interaction with Phospholipase Cβ*

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The G protein βγ complex regulates a wide range of effectors, including the phospholipase Cβ isozymes (PLCβs). Prenyl modification of the γ subunit is necessary for this activity. Evidence presented here supports a direct interaction between the G protein γ subunit prenyl group and PLCβ isozymes. A geranylgeranylated peptide corresponding to the C-terminal region of the γ subunit type, γ2, strongly inhibits stimulation of PLCβ2 and PLCβ3 activity by the βγ complex. This effect is specific because the same peptide has no effect on stimulation of PLCβ by an α subunit type, αq. Prenylation of the γ peptide is required for its inhibitory effect. When interaction of prenylated γ subunit peptide to fluorophore-tagged PLCβ2 was examined by fluorescence spectroscopy, prenylated but not unprenylated peptide increased PLCβ2 fluorescence emission energy, indicating direct binding of the prenyl moiety to PLCβ. In addition, fluorescence resonance energy transfer was detected between fluorophore-tagged PLCβ and wild type βγ complex but not an unprenylated mutant βγ complex. We conclude that a major function of the γ subunit prenyl group is to facilitate direct protein-protein interaction between the βγ complex and an effector, phospholipase Cβ.

Prenylation with isoprenoids is a common post-translational modification of proteins. Isoprenoids are a diverse family of lipid compounds made up of a repeating five-carbon structure called the isoprene unit. Protein prenylation generally consists of the attachment of either of two isoprenoids: the 20-carbon geranylgeranyl group or the 15-carbon farnesyl group (1). Heterotrimeric G proteins (αβγ) are a class of prenylated proteins that mediate the majority of neuroendocrine signaling pathways in mammals. Upon G protein activation by a cell surface receptor, both the α subunit and the βγ complex of a G protein can regulate downstream effectors (2). The γ subunit of Gβγ is modified with either a geranylgeranylated group or a farnesyl group (3). The isoprenoid is added to a conserved cysteine residue at position −4 from the C-terminal end of the protein, in a consensus motif for prenylation called the CAAX box. The last amino acid in the motif (X) determines whether the cysteine is geranylgeranylated or farnesylated. Most γ subunits are geranylgeranylated, but γ1, γε, and γ11 are farnesylated (3).

The role of prenylation of the γ subunit in G protein function is still unclear. As with several other proteins, it has been established that the prenyl group on the G protein βγ complex plays a role in anchoring the protein to lipid membranes; βγ complexes mutated at the cysteine residue in the CAAX box of the γ subunit no longer associate with the plasma membrane and are located in the cytosol (4, 5). It is thought that the hydrophobic prenyl moiety associates with lipid membranes through lipid-lipid interactions, thus acting as a membrane anchor for proteins. In addition to lipid-lipid interactions, there have been suggestions from studies on small GTP-binding proteins that the prenyl moiety may also interact with proteins and stabilize protein-protein interactions (1).

It is not yet known whether the prenyl moiety of the G protein γ subunit is involved in direct interactions with proteins, although prenylation has been shown to be necessary for βγ interaction with receptors and effectors in a number of systems. The γ subunit prenyl group has been shown to be a requirement for receptor activation of a G protein (6). Prenylated peptides specific to the C-terminal region of the γ subunits have been shown to interact with receptors (7, 8). Prenylation of these peptides was a requirement for this activity. Similar to this requirement in the case of receptors, prenylation of Gβγ has been shown to be a requirement for effector interaction also. Gβγ containing a mutant unprenylated γ subunit does not activate PLCβ2 either in vivo or in vitro (9, 10). Unprenylated βγ complex also does not stimulate adenylyl cyclase type II or inhibit adenylyl cyclase type I (11). However, in all of these studies, the receptors or effectors were either integral membrane proteins (receptor and adenylyl cyclases) or membrane-associated proteins that required lipids as substrates (PLCβ isozymes). It has thus been unclear whether the prenyl group requirement facilitated βγ complex-membrane interaction or βγ complex interaction with receptor/effector protein.

Previous results have implied that the γ subunit prenyl moiety interacts directly with proteins. For instance, γ subunit peptides modified with isoprenoids of varying chain lengths differed in their ability to stabilize activated rhodopsin (12).
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However, there was no direct correlation between overall hydrophobicity with the efficacy of the modified peptides in the receptor stabilization assays. Farnesylated peptides were more active than geranylated (C-10) or geranylgeranylated peptides. These results indicated that the function of the prenyl moiety was more likely stabilization of protein interactions rather than membrane binding. The altered activity of mutant G\( \beta \gamma \) with prenyl moieties switched from farnesyl to geranyl or vice versa also implied that the prenyl moiety may be involved in protein interaction (13). Overall, the question of whether the prenyl group plays this important role of stabilizing G protein-receptor or G protein-effector interactions in addition to stabilizing contact with the membrane has remained unresolved. The recent solution of a crystal structure of prenylated Cdc42, a Rho family member, bound to RhoGDI provided the first direct evidence for specific contact between the prenylated Cdc42, a Rho family member, bound to RhoGDI and the geranylgeranyl group of Cdc42 occupying a hydrophobic pocket in RhoGDI (14).

To examine whether the \( \gamma \) subunit prenyl group is directly involved in interaction with a G protein effector, we synthesized prenylated peptides specific to \( \gamma \) subunits. These peptides were then tested for their ability to compete with G\( \beta \gamma \) in activation assays of PLC\( \beta 2 \) and PLC\( \beta 3 \). PLC\( \beta \) enzymes are a family of proteins that hydrolyze phosphatidylinositol 4,5-bisphosphate (PIP\( _2 \)), releasing the second messengers, diacylglycerol and inositol 1,4,5-trisphosphate (15). They are regulated by both protein \( \alpha \) subunits (of the G\( \alpha \) class) and the \( \beta \gamma \) complex (16). The results from the peptide inhibition assays suggest that the prenylated \( \gamma \) peptides compete with the \( \beta \gamma \) complex for a site on PLC\( \beta \). A fluorescence-based binding assay confirmed direct interaction of the prenylated \( \gamma \) peptide with PLC\( \beta \). To examine the role of the \( \gamma \) prenyl moiety within the context of the whole \( \beta \gamma \) complex, we then compared a mutant unprenylated \( \beta \gamma \) complex with prenylated \( \beta \gamma \) complex in FRET based assays, which measured direct binding to PLC\( \beta \) isozymes in a highly quantitative fashion. The results from these experiments provide strong evidence that the \( \gamma \) subunit prenyl group directly facilitates interaction of G\( \beta \gamma \) with PLC\( \beta \).

EXPERIMENTAL PROCEDURES

Materials

PIP\( _2 \) and phosphatidylethanolamine were obtained from Avanti Polar Lipids. [\(^{3} \)H]PIP\( _2 \) was from PerkinElmer Life Sciences. Nickel-nitrilotriacetic acid resin was from Qiagen. All other reagents were from Sigma.

Construction of Recombinant Baculoviruses

Details of the construction of baculoviruses expressing His-PLC\( \beta 2 \), His-PLC\( \beta 3 \), G protein His-\( \alpha i 2 \) subunit, \( \beta 4 \) subunit, \( \gamma 2 \) subunit, and mutant (C66S) unprenylated \( \gamma 2 \) subunit have been published (11, 17–21).

Purification of G protein Subunits and PLC\( \beta \) Isozymes

The final preparations of all proteins were over 90% pure. Purification of \( \beta \gamma \) subunits was performed essentially as described before (21).

Purification of Mutant \( \beta i2 \) C66S (Used in the PLC\( \beta 2 \) Binding Assay)—Purification of mutant unprenylated \( \beta i2 \) was performed using a modification of the procedure described in Ref. 22. SF9 cells co-infected with baculoviruses expressing \( \beta \) and \( \gamma 2 \)C66S were lysed, and the soluble fraction was subjected to sequential chromatography over Q-Sepharose and hydroxylapatite.

Purification of \( \omega q \)—\( \omega q \) was purified as described before (17). \( \omega q \) was expressed in SF9 cells using a recombinant baculovirus. To ensure stability of the Go subunit, SF9 cells were also co-infected with baculoviruses expressing \( \beta \) and \( \gamma 2 \)C66S were lysed, and the soluble fraction was subjected to sequential chromatographic steps as described before.

Purification of PLC\( \beta 2 \) and PLC\( \beta 3 \)—PLC\( \beta 2 \) and PLC\( \beta 3 \) were purified as described previously (19, 20). Briefly, SF9 cells were infected with baculoviruses expressing histidine-tagged PLC\( \beta 2 \) or histidine-tagged PLC\( \beta 3 \). PLC\( \beta \) proteins were purified from cell extracts by nickel chromatography.

Peptide Synthesis and Chemical Prenylation

Peptides were synthesized, chemically geranylated or farnesylated, and purified as described (23). The geranylgeranyl-bromide was obtained from American Radiolabeled Chemicals (St. Louis, MO). Farnesyl was obtained from Aldrich. Briefly, peptide (2 \( \mu \)mol) and prenyl bromide (4 \( \mu \)mol) were mixed in a solution of butanol:methanol:water (1:1:1) previously purged under nitrogen atmosphere. Butyl hydroxytoluene was provided as an antioxidant. The reaction was started with 0.5 M sodium carbonate. The reaction was allowed to proceed at room temperature under nitrogen atmosphere, in the dark with continuous agitation for 18 and 24 h. The reaction was stopped with acetic acid, and the samples were frozen at ~85 °C.

Prenylated peptides are purified by reverse chromatography on a PepRPC fast protein liquid chromatography column HR 10/16 (Amersham Pharmacia Biotech) using a linear (0–100%) gradient of acetonitrile in water containing 0.1% trifluoroacetic acid. The prenylated compounds elute at a position of the gradient corresponding to approximately 50–60% acetonitrile content. Farnesylated peptides elute earlier than their geranylated counterparts. Peptides were usually converted to prenyl peptide with a 30–50% yield, which after purification and other operations resulted in a yield of 15–25% neat prenylated peptide. Prenyl peptides were stored in butanol:methanol:water (1:1:1, volume) or dimethylsulfoxide at ~85 °C. The molecular masses of the prenylated peptide was checked by mass spectrometry. The concentration of the peptide was determined by amino acid analysis. The integrity of the modified peptides in stocks was checked regularly by chromatography in a PepRPC column by fast protein liquid chromatography.

PLC\( \beta \) Assays

\( \beta \gamma \) stimulation of phospholipase C\( \beta \) was performed as described previously (19).

Peptide Inhibition of \( \beta \gamma \) Complex Stimulated PLC\( \beta 2 \) Activity—Purified peptides were stored in a 1:1:1 solution of butanol:methanol:water at ~80 °C. Appropriate amounts were added to tubes and vacuum dried to remove the organic solvents. Peptides were then solubilized by sonication in presonicated lipid substrate (50 \( \mu \)M PIP\( _2 \), 200 \( \mu \)M phosphatidylethanolamine, and [\(^{3} \)H]PIP\( _2 \), ~8000 cpm/assay). 120 pmol PLC\( \beta 2 \) and 100 nm final concentration of \( \beta i2-\gamma 2 \) peptide were then added to the lipid vesicle/peptide mix. The reactions were started by addition of CaCl\( _2 \) and incubated for 15 min at 30 °C. The reactions were stopped by addition of 10% trichloroacetic acid, and bovine serum albumin was added to precipitate proteins and lipids. Inositol 1,4,5-trisphosphate remained in the supernatant, and [\(^{3} \)H]inositol 1,4,5-trisphosphate release was quantitated by scintillation counting. Peptide inhibition of \( \beta \gamma \) complex-stimulated PLC\( \beta 3 \) activity was performed as above with 600 pmol PLC\( \beta 3 \) and 10 nm final concentration \( \beta \gamma 2 \) per assay.

\( \omega q \) Activation (PLC\( \beta 3 \))—\( \omega q \) activation of PLC\( \beta 3 \) was performed essentially as described (17) except that \( \omega q \) was preactivated by incubation with 10 nm NaF and 30 \( \mu \)M AlCl\( _3 \). Peptide effect on \( \omega q \) stimulation of PLC\( \beta 3 \) was tested as above; 5 \( \mu \)M \( \omega 2-\gamma 2 \) peptide was used for these assays.

Measurement of Peptide-PLC\( \beta \) and \( \beta \gamma \) Complex-PLC\( \beta \) Associations Using Fluorescence Spectroscopy

Association between PLC\( \beta 2 \) and G\( \beta \gamma 2 \) or peptide was quantified by fluorescence (24, 25). All studies were done in the presence of extruded
lipids (diameter, 100 nm) composed of either 1-palmitoyl-2-oleoyl-sn-glycero-3-[phosphoserine] (POPS) or a 2:1 mixture of 1-palmitoyl-2-oleoyl-sn-glycero-3-[phosphocholine] and POPS. The inclusion of lipid was solely to promote solubility of Gβγ and of the prenylated peptide. Gβγ complexes (wild type or mutant) were reconstituted into membranes by adding concentrated lipid solution to detergent solubilized Gβγ complex and then removing the detergent by dialysis. Fluorescence studies were carried out by labeling PLCβ and Gβγ with an amine-reactive coumarin or DABCYL (Molecular Probes, Eugene, OR) by adding a 3-4-fold excess of probe to the proteins at pH 8.0, incubating for 30 min, and removing unreacted probe by dialysis (25). PLCβ and Gβγ activity were measured independently as unlabeled and unlabeled proteins to ensure that labeling did not affect activity.

Titrations were carried out by placing 120 μl of sample in a 3-mm-path length cell and adding small (0.5–2 μl) amounts of the titrating solution. The peptide was dried down with nitrogen and resuspended in the reaction mix or added directly in solution depending on the concentration. Spectra were taken on an ISS spectrofluorometer using an excitation wavelength of 340 for coumarin fluorescence and scanning from 380 to 500, and protein association was analyzed as described below.

Membrane binding of unprenylated Gβγ was measured by the 35% decrease in intrinsic fluorescence, exciting at 280 nm, and scanning from 290–400, because lipid bilayers composed of POPS were added to a 120 μl protein solution (24). Association was highly dependent on membrane surface charge and ionic conditions. The partition coefficient PLCβ/H9252 efficacy as PLCβ/H9252/H9253 the decrease in intrinsic fluorescence, exciting at 280 nm, and scanning the PLCβ maybe interacting on a quasi-two dimensional membrane surface. Used dissociation constant and do not take into account that the proteins transfer acceptor (DABCYL-PLCβ) studies were carried out by labeling PLCβ was solely to promote solubility of Gαq. Glycero-3-[phosphoserine] (POPS) or a 2:1 mixture of 1-palmitoyl-2-oleoyl-sn-glycerol (100 nm) composed of either 1-palmitoyl-2-oleoyl-sn-glycerol-3-phosphocholine), 20% of G2-gg) or a farnesyl group previous data suggesting that it is the prenyl moiety and not the geranylgeranyl. The geranylgeranyl group is the isoprenoid present on the C terminus formed a thioether linkage with the isoprenoid moieties (described under “Experimental Procedures”). C15, farnesyl; C20, geranylgeranyl.

We examined the ability of prenylated peptides specific to Gβγ intermediate and the last cysteine synthesized a geranylgeranylated 2 peptide in which the prenyl group is 4-fold excess of probe to the proteins at pH 8.0, incubating for 30 min, and removing unreacted probe by dialysis (25). PLCβ and Gβγ activity were measured independently as unlabeled and unlabeled proteins to ensure that labeling did not affect activity.

RESULTS

We examined the ability of prenylated peptides specified to the C terminus of the γ2 subunit to inhibit γy activation of PLCβ2 and PLCβ3. If a PLCβ isozyme makes direct contact with the prenyl group of the γ subunit during interaction with the Gβγ complex, then prenylated peptides from the γ subunit should block such interaction. The sequence of the γ subunit peptides and their prenyl modifications are shown in Fig. 1. The γ2 peptide corresponds to the C-terminal 12 amino acids of the mature, fully processed γ2 subunit. This peptide was either left unmodified or chemically prenylated at the last cysteine with either a geranylgeranylated group γ2-gg) or a farnesyl group (γ2-far). The geranylgeranylated group is the isoprenoid present on native γ2 subunits (26). We used the β4γ2 complex in these assays. β4γ2 stimulates both PLCβ2 and PLCβ3 with the same efficacy as β1γ2 under identical conditions (21). Basal activities of these PLCβ isozymes were 10–20% of Gβγ-stimulated activity under the conditions used. When tested, the γ2-gg peptide completely inhibits Gβγ stimulation of both PLCβ2 and PLCβ3, reducing PLCβ2 activity to basal levels. (Fig. 2). The γ2-gg peptide did not have any significant effect on basal activity of PLCβ2 (data not shown). The γ2-gg peptide inhibition of γy stimulation could be due to the hydrophobic prenyl group causing a general disturbance of lipid vesicles in the assay, preventing the PLCβ isozymes from accessing their lipid substrate or from a direct action upon PLCβ isozymes. To resolve this issue, we examined the effect of the γ2-gg peptide on Gαq stimulation of phospholipase Cβ3. The G protein α subunit type, Gαq, is known to strongly stimulate PLCβ3 and is thought to do so by a mechanism different from that of Gβγ stimulation (27). α-q binding and βγ-binding regions on PLCβ2 have been mapped and are distinct from another (28, 29). The γ2-gg peptide does not significantly affect αq stimulation of PLCβ3, even at a concentration of peptide that completely inhibits γy stimulation of PLCβ3 (5 μM) (Fig. 3). This indicates that the effect of the peptide is not the result of either direct inhibition of PLCβ activity or nonspecific disruption of enzyme access to substrate.

We next examined the relative contributions of both the geranylgeranyl group and the amino acid portion of the γ2-gg peptide in mediating this inhibitory effect. Unprenylated γ2 peptide had very little effect on γy complex stimulation of either PLCβ2 or PLCβ3, even at a concentration of 20 μM (Fig. 4A). At this concentration γ2-gg inhibits Gβγ-stimulated PLCβ activity almost completely (Fig. 2). Thus, the prenyl moiety is essential for γ2-gg inhibition of Gβγ action on PLCβ. To determine whether or not the prenyl moiety alone is sufficient for inhibition of γy stimulation of PLCβ, we initially worked with a prenylated cysteine compound. However, problems with solubility of the prenylcysteine prevented further study with this compound. Therefore, to determine the importance of the amino acid sequence in γ2-gg inhibition of Gβγ activity, we synthesized a geranylgeranylated γ2 peptide in which the amino acid sequence of the last 12 residues of the γ2 subunit was randomized (γ2scrg-gg). This scrambled peptide inhibited Gβγ stimulation of PLCβ2 and PLCβ3 as well as the wild type γ2-gg peptide (Fig. 4B). Thus, the amino acid sequence of the γ2-gg peptide is not important for its inhibitory effect on Gβγ stimulation of PLCβ isozymes. This result is consistent with previous data suggesting that it is the prenyl moiety and not the amino acid sequence of γ subunits that is the prime determinant on γ subunits for Gβγ interaction with PLCβ (13).

Inhibition of γy stimulation of PLCβ isozymes by geranylgeranylated peptides suggests that PLCβ isozymes may directly contact the prenyl moiety on the G protein γ subunit during activation by the γy complex. A fluorescence-based
binding method was used to determine direct interaction of the γ2-gg peptide with PLCβ2. PLCβ2 was labeled with the fluorescent probe coumarin, whose emission energy and intensity were found to be highly sensitive to the addition of prenylated γ2-gg but not its unprenylated counterpart. Specifically, the addition of unlabeled γ2-gg peptide produced a 2-fold increase in the emission intensity and a significant shift (330 cm⁻¹) of emission energy from coumarin-labeled PLCβ2 (Fig. 5). These shifts allowed us to monitor the association between the peptide and PLCβ2 without the need to attach a fluorescent label on the peptide that could affect its interaction with PLCβ2. The addition of unprenylated γ2 peptide resulted in no change in emission energy (data not shown).

To verify that the changes in fluorescence reflected a true interaction of peptide with protein, the association of γ2-gg with PLCβ2 was measured at two concentrations of PLCβ2. Upon an increase of PLCβ2 concentration from 5 to 30 nM, the titration curve shifted in the expected direction to the right (Fig. 5). Curves were fit to a bimolecular association constant, giving values of $K_d$ ($1.8 \pm 0.9$ and $5.7 \pm 3.1$ μM) that were not significantly different from each other.

To directly assess the importance of the isoprenoid group in βγ interaction with PLCβ isoforms, we performed a direct
binding assay with PLCβ3 and prenylated or unprenylated βγ complexes. Wild type γ2 protein was co-expressed with β1 protein, and the complex was purified as described under “Experimental Procedures.” Unprenylated βγ complex was synthesized by mutating the γ2 subunit C-terminal cysteine at position 68 to serine. This mutation prevents normal prenylation of the γ subunit without affecting its ability to bind to the β subunit (6). Mutant unprenylated γ2 protein was co-expressed with β1 protein using the baculovirus insect cell expression system, and the resulting βγ complex was purified as described under “Experimental Procedures.” Unprenylated β1γ2 was completely inactive in PLCβ3 assays, even at concentrations at which wild type βγ2 stimulation of PLCβ3 saturates (Fig. 6) (as mentioned before βγ2 has similar potency compared with β1γ2 in activating PLCβ3). We then examined whether prenylation is necessary for direct binding of β1γ2 to PLCβ.

Direct binding between PLCβ and Gβγ was assayed using FRET assays. Wild type and unprenylated β1γ2 complex were labeled with the fluorescent donor probe, coumarin. PLCβ2 was labeled with the acceptor probe, DABCYL. We have previously measured the affinities between PLCβ2 and Gβγ on membrane surfaces by fluorescence resonance energy transfer and found the membrane concentration to be a critical determinant in the magnitude of their interaction energies (24). Although prenylation of the βγ complex has been shown to increase the affinity of the complex for cell membranes (5), we found that is possible to conduct binding studies under conditions of lipid concentrations (200 μM POPS) (24) where both prenylated and unprenylated complexes are both completely bound to lipid vesicles. Preliminary studies showed that binding of unprenylated βγ complex to negatively charged lipid bilayers composed of POPS was very strong (Kd = −50 μM) and in the same range as previously determined for prenylated Gβγ (24). In Fig. 7, a comparison of the binding of DABCYL-PLCβ2 to coumarin-labeled wild type or unprenylated Gβγ as measured by FRET is shown. These results show clearly that the affinity of prenylated βγ for PLCβ is significantly higher compared with the unprenylated βγ complex.

The results from these FRET based assays indicate that the γ prenyl moiety is essential for direct interaction between the βγ complex and PLCβ isozymes. The results also suggest that there exists a site on PLCβ isozymes that binds the G protein γ subunit geranylgeranyl moiety. To test whether such a site specifically recognizes the geranylgeranyl group, we substituted the geranylgeranyl moiety in the γ2 peptide with a farnesyl group (γ2-far). γ2-far is consistently less effective than γ2-gg in inhibiting βγ activation of PLCβ3 (Fig. 8), indicating that if a site on the PLCβ enzymes binds the prenyl moiety, it can discriminate between geranylgeranyl and farnesyl iso prenoids.

**DISCUSSION**

What is the nature of the prenyl group involvement in interaction between βγ and PLCβ isozymes? As confirmed here (Fig. 6), it is known that prenylation of the βγ complex is required for
functional regulation of PLCβ (9, 10). Because PLCβ acts on lipid substrates, it is possible that the requirement for prenylation is due to the targeting of the βγ complex to membranes. However, it is also possible that the γ subunit prenyl moiety interacts directly with PLCβ and thus stabilizes protein-protein interactions between the βγ complex and PLCβ. The results presented here favor this latter possibility.

Inhibition of βγ stimulation of PLCβ activity by prenylated peptides suggests that the prenylated peptides compete with βγ for a native prenyl-binding site on PLCβ isozymes. The effect of these prenylated peptides is specific; the same peptides have no effect on either basal or, more importantly, ωq-stimulated PLCβ3 activity. The differing efficacies of geranylgeranylated and farnesylated peptides on PLCβ activity is consistent with previous experiments using whole proteins (13) and suggest that such a prenyl-binding site can discriminate between different types of isoprenoids. Furthermore, the ~3-fold difference between the efficacy of the farnesylated and geranylgeranylated peptides (IC50 values of ~5 and ~1.8 μM) is strikingly similar to the 3-fold difference in the KD of farnesyI and geranylgeranyl for rhoGDI (4.8 and 1.6 μM as determined in a fluorescence based assay) (14). Although the potential site on PLCβ can discriminate between different isoprenoids, our results demonstrate that it does not selectively bind a particular isoprenoid to the exclusion of a related molecule. Lipid-protein interactions are predominantly hydrophobic (14, 30). It is likely that the chemistry of these interactions will not allow for the kind of specificity seen in protein-protein interactions.

Experiments that examined the effect of γ subunit-specific peptides on fluorescence emission from fluorophore-tagged PLCβ provide direct evidence for interaction between the geranylgeranyl moiety and the PLCβ molecule. In this assay, only the γ2-gg peptide induced a significant increase in emission energy from PLCβ2. Unprenylated γ2 peptide had no effect on fluorescence emission from PLCβ. Because increases in emission energy are directly related to complex formation between the molecules in the assay (24), the KD for γ2-gg binding to PLCβ2 could be determined (1.8 ± 0.9 to 5.7 ± 3.1 μM). The differential binding of prenylated and unprenylated γ2 peptide in this assay reflects similar differences in the assays that measured inhibition of Gβγ-stimulated PLCβ activity by the peptides. Because the unprenylated γ2-gg peptide has no effect on fluorescence emission from coumarin-tagged PLCβ2, these results indicate that inhibition of a site on PLCβ with the prenyl moiety is at the basis of γ2-gg peptide binding to PLCβ.

The results from experiments using the whole βγ complex, wild type and mutant, provide further evidence for the interaction of the prenyl moiety with PLCβ. A strong FRET signal is detected from DABCYL-tagged PLCβ2 in the presence of coumarin-tagged prenylated βγ complex. The FRET response to increasing concentrations of PLCβ indicate highly efficient complex formation complex formation; KD for complex formation between prenylated βγ and PLCβ was 0.9 nM. Complex formation between unprenylated Gβγ and PLCβ was significantly weakened (estimated KD = 239 nM). The affinity of prenylated Gβγ is thus about 200-fold stronger for PLCβ compared with unprenylated mutant Gβγ. Unprenylated Gβγ complex consistently interacted with weaker affinity for PLCβ even under assay conditions where all of the unprenylated βγ complex was localized along with PLCβ to lipid surfaces (Fig. 7). Thus, differential interaction of prenylated and unprenylated βγ complexes with PLCβ is not a consequence of their differential interaction with membranes. Together these results from fluorescence spectroscopy directly support a role for the prenyl modification in stabilizing βγ complex-PLCβ interaction.

In summary, the results presented here demonstrate that the γ subunit prenyl moiety directly facilitates interaction of the G protein βγ complex with an effector, PLCβ. We predict that PLCβ has a site that specifically binds the prenyl group. The interaction of the G protein γ subunit prenyl moiety with PLCβ may serve as a general model for isoprenoid-protein interactions.

Acknowledgment—We thank Dr. Y. Hou (Gautam Laboratory) for pure βγ2 complex.

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J. Biol. Chem. 2001, 276:41797-41802.
doi: 10.1074/jbc.M107661200 originally published online September 6, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M107661200

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