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The N terminus of phosducin is involved in binding of βγ subunits of G protein

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ABSTRACT **Phosducin is a soluble phosphoprotein found in retinal photoreceptor cells and in the pineal gland. It binds to the βγ subunits of guanine nucleotide-binding proteins (G proteins) (Gβγ) and may regulate G-protein function. In this study, the ability of specific regions of phosducin to bind Gβγ was characterized. A series of deletion mutants were made in bovine phosducin. They were tested in cotransfection assays for their ability to inhibit Gβγ-mediated phospholipase C β_2 isoform activation. Overexpression of the N-terminal half of phosducin showed inhibition, whereas overexpression of the C-terminal half did not. The first 63 amino acid residues were required for inhibition. A tryptophan-to-valine substitution at residue 29, which is part of a well conserved 11-amino acid sequence, severely impaired phosducin inhibitory function. Glutathione *S*-transferase–phosducin fusion proteins were expressed in *Escherichia coli* to study phosducin–Gβγ interaction *in vitro*. The N-terminal 63-amino acid fragment was able to bind to Gβγ. In contrast, the C-terminal half failed to bind to Gβγ. The substitution mutants showed little or no binding. Furthermore, direct measurements of interaction between Gβγ and fragments of phosducin, using surface plasmon resonance technology, confirmed the assignment of binding activity to the 63-amino acid fragment and the importance of the tryptophan residue.**

Guanine nucleotide-binding proteins (G proteins) mediate signal transduction across cellular membranes (1, 2). Activated cell surface receptors stimulate the binding of GTP to the α subunits of target G proteins and GTP binding promotes dissociation of G protein βγ subunits (Gβγ), allowing the α and βγ subunits to interact with a variety of effector molecules. Recent studies have revealed new and independent functions for the βγ subunits (3). In addition to regulation of phospholipase C activity (4, 5), Gβγ was also found to be involved in modulation of the activities of adenylyl cyclase (6), potassium channels (7–9), and kinases, including β-adrenergic receptor kinase (βARK) (10, 11), mitogen-activated protein kinase (12), and phosphatidylinositol-3-kinase (13). By interacting with cellular molecules other than the α subunits, Gβγ contributes to the complex regulation of signal transducing circuits.

Phosducin (Phd) is a 33-kDa soluble phosphoprotein identified in vertebrate photoreceptor cells (14, 15) and the pineal gland (16, 17). It forms complexes with the βγ subunits of transducin (14), which belong to the G-protein family. Binding of Phd to Gβγ is regulated by the phosphorylation state of Phd. Phd can be phosphorylated by protein kinase A (18), and upon phosphorylation the affinity for Gβγ decreases (19). Inside the cell, Phd may compete with other Gβγ targets for Gβγ binding, and thus it may be involved in regulation of many biological processes. It has been suggested that Phd, which is relatively abundant in the rod photoreceptor cells, regulates the amount of βγ subunits available to interact with the α subunits of transducin to form heterotrimers and thus can function as a negative regulator of the cGMP cascade in the visual system. Addition of Phd to photolysed rod outer segment membranes reduces the GTP hydrolysis activity of transducin and the subsequent activation of the cGMP phosphodiesterase (20). Phd also inhibits receptor phosphorylation by βARK; it presumably prevents βARK from translocating to the membranes by scavenging Gβγ. Phosphorylation of Phd attenuates this inhibition (21). Phd was also found to inhibit the intrinsic GTPase activity of several G proteins, including G_s, G_o, and G_i (19). In addition, direct binding of Phd to Gβγ could also lead to other Phd functions that have not yet been identified. It is therefore important to further investigate Phd–Gβγ interaction.

In this study, a series of specific deletion and amino acid substitution mutations were made in bovine Phd. Their ability to bind to Gβγ was tested by inhibition of Gβγ-mediated phospholipase C β_2 isoform (PLCβ_2) activation in the cotransfection assay and by direct *in vitro* binding assays. The Gβγ binding activity was localized to the first 63 amino acid residues of Phd.

MATERIALS AND METHODS

Plasmids for COS-7 Cell Transfection. All of the Phd deletion mutants were constructed by PCR with the plasmid pPDTSC (kindly provided by Rehwa Ho Lee, University of California, Los Angeles) as the template. A sequence encoding the 9-amino acid epitope tag YPYDVPDYA, derived from human influenza virus hemagglutinin (HA tag) (22), was introduced into the C terminus of each construct during PCR. PCR products were cloned between *Cla* I and *Xho* I sites of the pCIS vector (23) for transfection in COS-7 cells. A point mutation changing tryptophan to valine at position 29 was introduced into the wild-type cDNA by the double-strand mutagenesis strategy (24). All constructs were confirmed by DNA sequencing.

Expression plasmids for human PLCβ_2 and bovine Gβ_1 and Gγ_1 have been described (5).

Transfection. COS-7 cells were seeded at a density of 1×10^5 cells per well in 12-well dishes 1 day before transfection. One microgram of DNA was used in each well. The molar ratio of DNA used for transfection of PLCβ_2, Gβ_1, Gγ_1, and Phd was 1. Wherever necessary a plasmid expressing the β-galactosidase gene under the control of the cytomegalovirus promoter was cotransfected to keep the total amount of DNA constant; 0.5 ml of Opti-MEM (GIBCO/BRL) containing 1 μg of DNA along with 5 μl of LipofectAmine (GIBCO/BRL) was added to each well. Five hours later, the medium was replaced with 1 ml of Dulbecco's modified Eagle's medium containing 10% fetal calf serum. Transfected cells were labeled the next day with 10 μCi of *myo*-[2-^{3}H]inositol per ml (NEN) (1 Ci = 37

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Abbreviations: G protein, guanine nucleotide-binding protein; Gβγ, β and γ subunits of G protein; PLCβ_2, phospholipase C β_2 isoform; βARK, β-adrenergic receptor kinase; GST, glutathione *S*-transferase; Phd, phosducin; SPR, surface plasmon resonance; HA, hemagglutinin.

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GBq) in 0.4 ml of inositol-free medium containing 10% dialyzed fetal calf serum. After 24 hr, the levels of [^{3}H]inositol phosphate were determined as described (5).

SDS/PAGE, Western Blot Analysis, and Antiserum. Gel electrophoresis was carried out in SDS/12% polyacrylamide slab gels according to Laemmli (25). The proteins were then transferred to nitrocellulose membranes at 100 V for 1 hr in 25 mM Tris/192 mM glycine/20% (vol/vol) methanol and subsequently detected by specific antibodies with the ECL Western blotting system (Amersham). The $G\beta_1$ antibody was raised against the N-terminal 14 amino acid residues of bovine $G\beta_1$. The anti-HA tag antiserum HA11 was purchased from Berkeley Antibody (Richmond, CA).

***In Vitro* Binding of Gβγ to Glutathione *S*-Transferase (GST)–Phd Fusion Proteins.** Phd wild-type and mutant cDNAs were placed in-frame downstream of the GST cDNA in the pGEX-3X vector (Pharmacia) using the *Bam*HI and *Eco*RI sites. The fusion proteins GST–Phd1–245 and GST–Phd1–63 contain full-length Phd and amino acid residues 1–63 of Phd, respectively, with the first 2 amino acid residues converted to proline and glycine. The fusion protein GST–Phd112–245 contains amino acid residues 112–245. Plasmids were transformed into *Escherichia coli* stain DH10B or BL21. Expression of the GST fusion proteins was induced with isopropyl, β-D-thiogalactoside at a final concentration of 1 mM for 2 hr after the A_{600} reading of the culture reached 0.8. The cells were pelleted and resuspended in phosphate-buffered saline (PBS) containing 1% Triton *X*-100 followed by mild sonication. The soluble fractions were incubated with the glutathione Sepharose 4B beads (Pharmacia) at 4°C for 2 hr followed by washing three times with PBS.

For affinity chromatography, purified Gβγ, which was prepared as described by Bigay and Chabre (26) from the bovine retina and diluted in PBS containing 0.01% lubrol, was then added. After the GST fusion protein and Gβγ were incubated for another 2 hr at 4°C, the beads were washed four times with PBS containing 0.01% lubrol. Finally, the beads were eluted with the SDS/PAGE sample buffer and the proteins were separated on a SDS/12% polyacrylamide gel and analyzed by Western blotting.

For analysis of Phd–Gβγ interactions using a surface plasmon resonance (SPR) biosensor, purified Gβγ was biotinylated with NHS-LC-biotin (Pierce) and coupled to the sensor surface of Pharmacia biosensor chip SA5 with covalently immobilized streptavidin. In a standard experiment, 2000 ± 200 resonance units (RU) of Gβγ was attached to the surface. Binding of the biotinylated Gβγ heterodimer was very stable: only 1–5 RU of the bound protein dissociated per minute. This allowed kinetic analysis of the interactions of Gβγ with the recombinant Phd as well as with Gα and βARK (V.Z.S., unpublished data). GST–Phd fusion proteins were eluted from the glutathione Sepharose beads in 50 mM Tris·HCl (pH 8.0) with 10 mM reduced glutathione. Different concentrations of fusion proteins were flown through the surface with coupled Gβγ. The running buffer contains 10 mM NaHepes, 120 mM NaCl, 2.5 mM $MgCl_2$, 0.5 mM EDTA, and 0.01% Tween 20 (pH 7.4). The injections lasted for 8 min at a flow rate of 5 μl/min. The kinetic analysis of the resulting binding curves (sensograms) was performed with BIAevaluation software, Version 2.0 (Pharmacia Biosensor).

RESULTS

The N Terminus of Phd Is Required for Inhibition of Gβγ-Mediated PLCβ$_2$ Activation. The $G\beta_1\gamma_1$ complex with a fully modified γ_1 subunit has been shown to activate PLCβ$_2$ (4, 5); thus, the presence of Phd may inhibit Gβγ-mediated PLCβ$_2$ activation by scavenging Gβγ. To test this notion, we cotransfected COS-7 cells with bovine $G\beta_1$ and $G\gamma_1$ subunits, with human PLCβ$_2$, and with or without bovine Phd. Cells transfected with Gβγ and PLCβ$_2$ accumulated a higher level of inositol phosphate than those transfected with Phd along with Gβγ and PLCβ$_2$ (see Fig. 2*A*). This indicates that the wild-type Phd inhibits Gβγ-mediated PLCβ$_2$ activation. This phenomenon was used as an assay system in the following study to detect interaction between Gβγ and Phd mutants. Several deletion mutants of bovine Phd (Fig. 1) were prepared by PCR. The mutants were cotransfected with Gβγ and PLCβ$_2$ into COS-7 cells and PLCβ$_2$ activity was measured. As shown in Fig. 2*A*, the mutant that consists of the N-terminal 120 residues (Phd1–120) was able to inhibit 68% of Gβγ-mediated PLCβ$_2$ activity, while the mutant that contains the C-terminal half of Phd extending from residues 112 to 245 (Phd112–245) showed no inhibition. This suggests that the Gβγ binding regions of Phd are located in its N-terminal half. Additional deletions revealed that the mutant containing the first 63 residues (Phd1–63) was able to inhibit 39% of Gβγ-mediated PLCβ$_2$ activity. The larger residual portion of the protein (Phd55–245), however, showed little (<8%) inhibition. This further suggests that the N terminus of Phd plays an important role in Gβγ binding. It is worth noting that the phosphorylation site on Phd, Ser-73 (18), is not included in mutant Phd1–63. Two more deletion mutants were prepared and their activities confirmed our initial results. The inhibitory activity of the C-terminal deletion mutant (Phd1–90) fell between the activities of the previous two mutants (Phd1–63 and Phd1–120). The N-terminal deletion mutant (Phd88–245) failed to inhibit PLCβ$_2$ activity as predicted by our earlier results (data not shown). Phd did not show specificity toward different γ subunits since it inhibited PLCβ$_2$ activities stimulated by $G\beta_1$

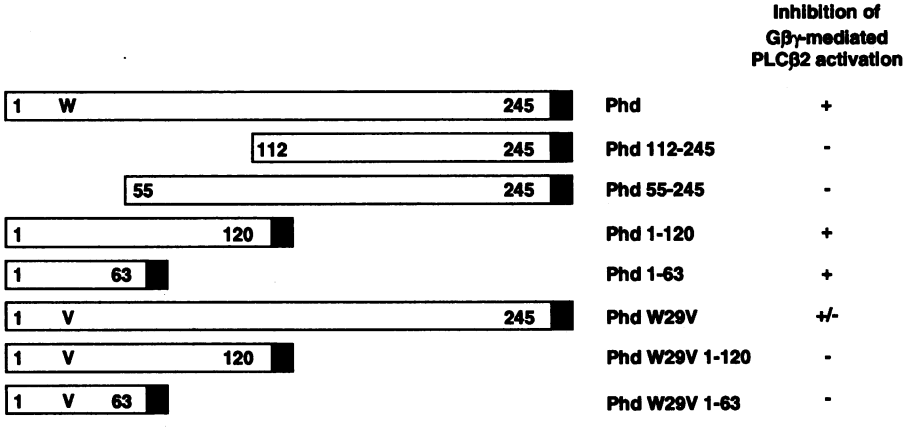

FIG. 1. Summary of Phd mutant constructs and their ability to inhibit Gβγ-mediated PLCβ$_2$ activation. Solid box, HA tag; numbers, first and last amino acid residues in each construct; W, tryptophan at position 29; V, valine substitution; +, ability to inhibit; −, inability to inhibit.

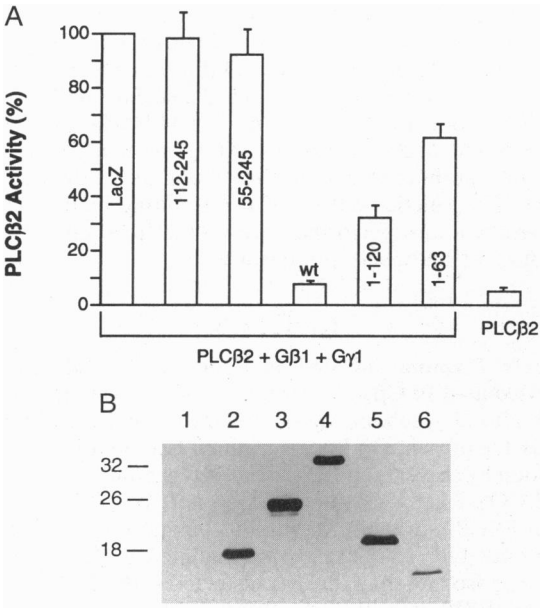

FIG. 2. Deletion mutants. (*A*) Activities of deletion mutants in the cotransfection assay. Transfections were done and PLCβ_2 activities were measured as described. G$\beta\gamma$-activated PLCβ_2 activity was set at 100%. Means + SD from at least three experiments are presented. Numbers indicate first and last amino acid residues in each construct. wt, Wild type; LacZ, plasmid expressing β-galactosidase gene. (*B*) Western blot analysis using HA11 antiserum. COS-7 cells were transfected as described and lysed in SDS/PAGE sample buffer. One-twentieth of cell lysate from one well of a 12-well dish was loaded for the wild-type Phd as the control amount. SDS/polyacrylamide gel was electroblotted onto nitrocellulose membrane (pore size, 0.45 μm) (Costar). Lanes: 1, lacZ (20-fold the control amount of cell lysate was loaded); 2, Phd112–245 (twice the control amount of cell lysate was loaded); 3, Phd55–245 (twice the control amount of cell lysate was loaded); 4, Phd wild type, control; 5, Phd1–120 (20-fold the control amount of cell lysate was loaded); 6, Phd1–63 (20-fold the control amount of cell lysate was loaded). Molecular mass standards (kDa) are marked on the left.

in combinations of various Gγ subunits, including Gγ$_1$, Gγ$_2$, Gγ$_3$, Gγ$_5$, and the Gγ subunit from the cone photoreceptor cells (data not shown).

To verify that the differences in the inhibitory effects of these Phd mutants did not result from differences in their expression levels in COS-7 cells, Western blotting analysis was performed. The wild-type and mutant Phd proteins were labeled at their C terminus with a 9-amino acid epitope tag sequence, YPYDVPDYA, derived from human influenza virus HA (HA tag) (22). Insertion of the tag into Phd did not affect the inhibitory function of Phd on G$\beta\gamma$-mediated PLCβ_2 activation (data not shown). The anti-HA polyclonal antiserum, HA11, which recognizes the 9-amino acid sequence, was used in the Western blot analysis. As shown in Fig. 2*B*, the N-terminal deletion mutants were expressed at higher levels than the C-terminal deletion mutants. Despite an ≈20-fold higher level of expression, the C-terminal portion of Phd was unable to inhibit PLCβ_2 activity. We therefore conclude that the N-terminal part of Phd plays a crucial role in inhibiting the G$\beta\gamma$-activated function of PLCβ_2. Phd and its various mutant forms have no effects on the basal PLCβ_2 activity (data not shown). The Western blot experiments using antisera against Gβ_1 and PLCβ_2 demonstrated that cotransfection of Phd and its mutants did not affect the expression levels of recombinant G$\beta\gamma$ and PLCβ_2 (data not shown).

The Tryptophan Residue Is Important. The first 63 residues are apparently required for inhibiting G$\beta\gamma$-mediated PLCβ_2 activation. In this region, there is an 11-amino acid stretch that is conserved in all Phd molecules found in different vertebrate species as well as in the Phd-like protein (27). Within this 11-amino acid sequence resides the only tryptophan residue found in Phd. Conserved tryptophan residues have been found in other protein homology motifs, including the pleckstrin homology (PH) domains (28) and the WD repeats (29). And the highly conserved tryptophan residue in subdomain 6 of the PH domain of Bruton tyrosine kinase has been shown to play a critical role in binding of G$\beta\gamma$ to this kinase (30). Thus, the tryptophan residue in Phd was replaced with a valine residue in the wild type (PhdW29V) and in the two C-terminal deletion mutants (PhdW29V1–120 and PhdW29V1–63) (Fig. 1). As shown in Fig. 3*A*, the point mutation severely impaired the inhibitory ability of Phd. The full-length Phd carrying this point mutation could inhibit only 17% of G$\beta\gamma$-activated PLCβ_2 activity compared to 93% by the wild type. The point mutation completely abolished the inhibitory effects when introduced into the C-terminal deletions (PhdW29V1–120 and PhdW29V1–63) (Fig. 3*A*). Western analysis (Fig. 3*B*) showed that all of the mutant proteins were expressed at levels that are equal to or higher than their counterparts without the point mutation. Thus, the losses of inhibitory effects were not due to lower levels of protein, suggesting that the tryptophan residue is critical for the inhibition of G$\beta\gamma$-mediated PLCβ_2 activation.

The First 63-Amino Acid Residue Fragment of Phd Binds to G$\beta\gamma$ *in Vitro*. To obtain more direct evidence for interaction between Phd and G$\beta\gamma$, Phd and its mutants were expressed in *E. coli* as GST fusion proteins (Fig. 4*A*). The cDNAs were cloned into the vector pGEX-3X (Pharmacia). Protein expression was induced by isopropyl β-D-thiogalactoside. The soluble fractions were immobilized on glutathione Sepharose 4B beads (Pharmacia). Purified G$\beta\gamma$ from the bovine retina was then incubated with the GST fusion proteins. G$\beta\gamma$ retained by the beads was detected by Western analysis. As shown in Fig.

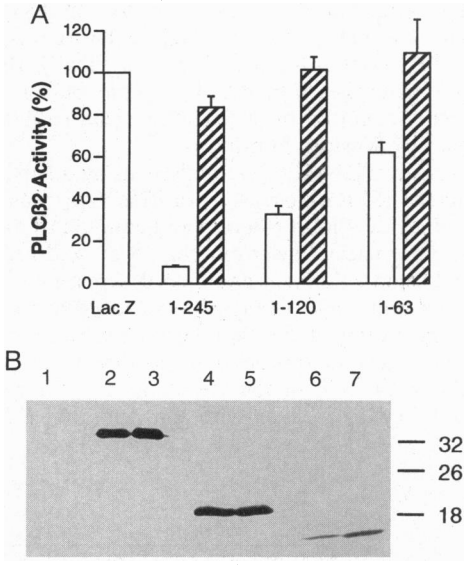

FIG. 3. Tryptophan-to-valine substitution at position 29. (*A*) Activities of mutants in the cotransfection assay. Transfections were done and PLCβ_2 activities were measured as described. G$\beta\gamma$-activated PLCβ_2 activity was set at 100%. Means + SD from at least three experiments are presented. Open and hatched bars, wild type and mutant counterparts, respectively; numbers, first and last amino acid residues in each construct. (*B*) Western blot analysis using HA11 antiserum. COS-7 cells were transfected as described and lysed in SDS/PAGE sample buffer. One-twentieth of cell lysate from one well of a 12-well dish was loaded for full-length constructs (20 times the amount of cell lysate was loaded for the remaining samples). SDS/polyacrylamide gel was electroblotted onto nitrocellulose membrane (pore size, 0.45 μm). Lanes: 1, lacZ; 2, Phd; 3, PhdW29V; 4, Phd1–120; 5, PhdW29V1–120; 6, Phd1–63; 7, PhdW29V1–63. Molecular mass standards (kDa) are marked on the right.

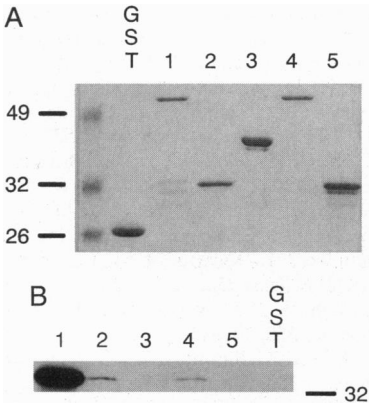

FIG. 4. *In vitro* binding of GST–Phd fusion proteins and Gβγ in affinity chromatography. Experiments were done as described. (*A*) Coomassie blue staining of expressed GST fusion proteins. (*B*) Western blot analysis of GST fusion protein and Gβγ binding with anti-β_1 antiserum. About 5 μg of fusion protein and 400 ng of purified Gβγ were used for each sample. Lanes: 1, GST–Phd; 2, GST–Phd1–63; 3, GST–Phd112–245; 4, GST–PhdW29V; 5, GST–PhdW29V1–63. Molecular mass standards (kDa) are marked on the left (*A*) and right (*B*).

4*B*, wild-type Phd bound to $G\beta_1$ very well. The GST fusion protein containing the first 63 residues of Phd was able to bind to $G\beta_1$, as predicted by the results obtained in cotransfection assays. Its binding affinity for Gβγ is apparently lower than the wild type because lower levels of $G\beta_1$ were retained. In contrast, the C-terminal half of Phd was not able to bind to $G\beta_1$ under the same conditions. In addition, tryptophan-to-valine substitution in the full length drastically decreased, and this substitution in the 63-residue fragment completely abolished, $G\beta_1$ association. These observations are in agreement with the transfection data. The above results strongly suggest that the binding site of Phd for Gβγ is in the N terminus. As a negative control, in the absence of Phd sequences GST alone did not bring down any Gβγ; thus, the binding of Gβγ is specific for Phd and not for GST or for the Sepharose beads.

The interaction of Phd with Gβγ was further studied by a series of SPR measurements on the Pharmacia Biosensor BIAcore instrument (31, 32). In these experiments, the Gβγ complex from bovine retina was immobilized on the sensor chip and solutions of purified GST–Phd fusion proteins were introduced to the activated surface in a flow cell. Fig. 5 demonstrates the increase of the SPR signal during the injections of wild-type and mutant forms of Phd fusion proteins into the flow cell with immobilized Gβγ. Wild-type Phd, the N-terminal 63-amino acid residue fragment (Phd1–63), and the tryptophan-to-valine (PhdW29V) mutant bound Gβγ under these conditions, while no binding was detected with the C-terminal fragment of Phd (Phd112–245) or with the 63-residue fragment bearing the substitution (PhdW29V1–63) (data not shown). In the control experiment, no binding was detected with GST. Both association and dissociation of Phd were slow—the apparent rate constants for wild-type Phd were measured as $k_a = 3.8 \pm 0.5 \times 10^3\ M^{-1}s^{-1}$ ($n = 2$) and $k_d = 3.2 \pm 0.1 \times 10^{-4}$ ($n = 2$). The apparent equilibrium constant K_D is 86 ± 4 nM, which was determined as the ratio between k_d and k_a. Phd1–63 fragment bound to Gβγ with ≈3-fold lower affinity than the full-sized protein ($K_D = 318 \pm 21$ nM) with both reduced association ($k_a = 1.8 \pm 0.5 \times 10^3\ M^{-1}s^{-1}$; $n = 3$) and increased dissociation rate ($k_d = 5.6 \pm 0.2 \times 10^{-4}$; $n = 3$) apparently caused by the truncation. The PhdW29V mutant showed very low affinity to Gβγ primarily because of a more rapid dissociation. At the highest concentration of this mutant (30 μM), the binding reached steady state at relatively low levels (150 RU); therefore, it was difficult to achieve reliable quantitative determination of the binding constants. The K_D was estimated as 1–5 μM, with $k_a \approx 100$–$1000\ M^{-1}s^{-1}$ and $k_d > 3 \times 10^{-3}$ ($n = 3$). The data obtained with the SPR biosensor are in good agreement with estimates made by both affinity chromatography on the glutathione Sepharose beads and COS cell cotransfection assay.

DISCUSSION

In summary, a series of specific deletion and substitution mutations were made in bovine Phd. Three types of assays were used to address its interaction with Gβγ. In the cotransfection assay, it was shown that the N-terminal 63 amino acid residues were required for inhibition of Gβγ-mediated $PLC\beta_2$ activation (Fig. 1). Without the N terminus, Phd lost its ability to inhibit. Substitution of the sole tryptophan residue (Trp-29) in the N-terminal region for a valine residue severely impaired Phd inhibitory function. When the fragment of Phd consisting of the first 63 residues was expressed as a GST fusion protein, it was able to bind to Gβγ *in vitro* when tested by both affinity chromatography and SPR biosensor technology. The tryptophan-to-valine substitution drastically decreased Phd binding to Gβγ. Data gathered in all three assays agreed very well and

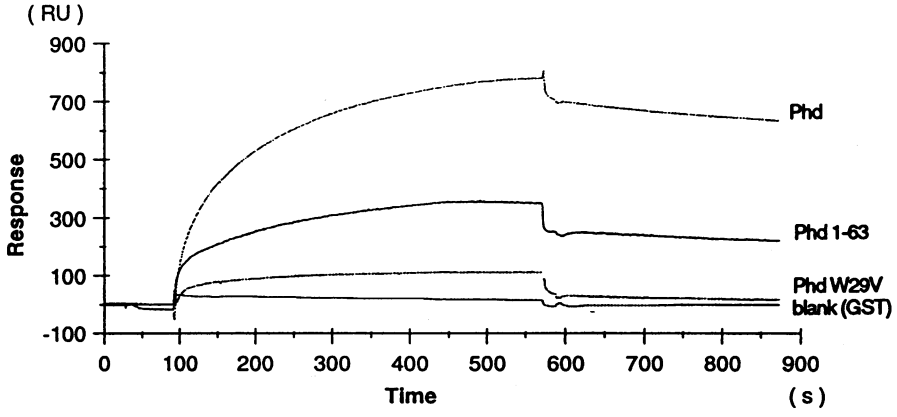

FIG. 5. Analysis of binding of GST–Phd fusion proteins and Gβγ using a BIAcore biosensor. Solutions of purified recombinant Phd were introduced by flow across the surface of the sensor chip SA5 (Pharmacia Biosensor) with immobilized Gβγ complex. Increase of the SPR signal (*y* axis) during the time of injection (*x* axis) indicates the binding of Phd. Concentration of the Phd preparations was 3 μM for GST–Phd and GST–Phd1–63, 5 μM for GST–PhdW29V, and 9 μM for GST. Data were collected from independent sensor chip channels on which 2000 ± 200 RU of Gβγ was immobilized. A representative of *n* independent experiments is shown (see text for values of *n*). Binding curves were analyzed after subtracting the background (injection of GST). The resulting curve for GST–Phd1–63 was multiplied by a factor of 2 in order to normalize with the full-size GST–Phd fusion protein, which is twice the size of the truncated form.

the results indicate that the primary Gβγ binding site of Phd is located in the first 63 residues and the 11-amino acid conserved stretch, which includes the critical tryptophan residue, plays an important role.

Our data do not exclude the possibility that other regions of Phd also contribute to Gβγ binding. Phd1–63 inhibited PLCβ$_2$ activation moderately while Phd1–120 and Phd1–245 were more efficient. The GST–Phd1–63 fusion protein retained much less of the Gβγ proteins and showed lower affinity for Gβγ than the intact molecule. Although the C terminus could neither inhibit nor bind Gβγ, it may still play a role in formation and stabilization of the tertiary structure of Phd that enables Phd to interact more efficiently with Gβγ. Furthermore, the phosphorylation site that regulates Phd binding to Gβγ has been shown to be at Ser-73 (18). Modifications at this site could lead to changes in the configuration of the protein and thus act to prevent Gβγ interaction. Alternatively, the phosphorylation may have a local steric or electrosteric effect on Gβγ binding. In any event, measurements of Phd–Gβγ binding suggest that it represents a high-affinity interaction. The N-terminal region of Phd is important for binding activity. However, it is not clear how effective Phd would be as a competitive inhibitor of the GDP-bound form of the α subunit of transducin since *in vivo* this would depend a great deal on relative local concentrations in the photoreceptor cells. The *in vitro* binding system may allow us to design molecules that could be introduced into transgenic animals to test this hypothesis.

While this manuscript was in preparation, Hawes *et al.* (33) reported that the first 105 amino acid residues in human retinal Phd contain Gβγ binding activity. Our data agree with and confirm their results. We further identify the first 63-amino acid residue fragment as required for binding and show that the sole tryptophan residue makes an important contribution. Recently, the rat Phd-like protein, an ethanol-responsive potential modulator of G-protein function (27), was coexpressed with Gβγ and PLCβ$_2$ in COS-7 cells and it was found to inhibit Gβγ-mediated PLCβ$_2$ activation. The nature of the regions involved in interaction with Gβγ and whether they share homology with those in Phd remain to be investigated.

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