Photoreceptor cGMP Phosphodiesterase δ Subunit (PDEδ) Functions as a Prenyl-binding Protein*

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Bovine PDEδ was originally copurified with rod cGMP phosphodiesterase (PDE) and shown to interact with prenylated, carboxymethylated C-terminal Cys residues. Other studies showed that PDEδ can interact with several small GTPases including Rab13, Ras, Rap, and Rh6, all of which are prenylated, as well as the N-terminal portion of retinitis pigmentosa GTPase regulator and Arl2/Arl3, which are not prenylated. We show by immunocytochemistry with a PDEδ-specific antibody that PDEδ is present in rods and cones. We find by yeast two-hybrid screening with a PDEδ bait that it can interact with farnesylated rhodopsin kinase (GRK1) and that prenylation is essential for this interaction. In vitro binding assays indicate that both recombinant farnesylated GRK1 and geranylgeranylated GRK7 co-purify with a glutathione S-transferase-PDEδ fusion protein. Using fluorescence resonance energy transfer techniques exploiting the intrinsic tryptophan fluorescence of PDEδ and dansylated prenyl cysteines as fluorescent ligands, we show that PDEδ specifically binds geranylgeranyl and farnesyl moieties with a 4\(K_D\) of 19.06 and 0.70 \(\mu\)M, respectively. Our experiments establish that PDEδ functions as a prenyl-binding protein interacting with multiple prenylated proteins.

Photoreceptor cGMP phosphodiesterase (PDEδ, termed PDEδ hereafter) is a member of the large and diverse PDE family (1) and a key enzyme in vertebrate phototransduction (2). The holoenzyme of PDE in rod photoreceptors consists of two catalytic subunits, PDEα and PDEβ (3), and two inhibitory subunits PDEγ (4, 5). Rod PDE is a peripherally membrane-associated protein anchored in the plasma membrane via the farnesyl and geranylgeranyl chains at the C termini of PDEα and PDEβ, respectively (6–8). A 17-kDa protein was copurified with rod PDE (9) and considered the fourth subunit of PDE (PDEδ). Native and recombinant PDEδ solubilize PDE from the rod outer segment disc membrane, thereby reducing the efficacy of phototransduction and light-activated cGMP hydrolysis but without affecting PDE catalytic activity (10, 11). The binding of PDEδ to PDE was shown to be blocked by short prenylated and carboxymethylated peptides derived from the C terminus of PDEα and PDEβ, and this effect required that the peptides be prenylated and methylated, indicating that PDEδ interacts directly with the prenylated C-terminal cysteine methyl ester residue (33). In contrast to PDE, which is expressed exclusively in photoreceptors, PDEδ is expressed in other tissues in addition to the retina (10, 12). A PDEδ orthologue is present in eyeless invertebrates such as Caenorhabditis elegans (13). These expression patterns of PDEδ suggested that it may not be a PDE subunit and may have other functions.

Yeast two-hybrid (y2h) screening yielded the first clues that PDEδ may participate in vesicular transport. PDEδ was identified as a Rab13 binding partner when PDEδ was used as bait to screen a HeLa cDNA library (12). Rab proteins belong to the Ras-related small GTPase superfamily (14) and have been shown to be necessary for the regulation of vesicle fusion and interaction with molecular motors (15). The recombinant PDEδ has the capacity to dissociate Rab13 from membranes, possibly by interacting with the C-terminal tail of Rab13, which is prenylated (12). Another y2h screening demonstrated that PDEδ was able to interact with ARF-like proteins, Arl2 and Arl3, which belong to the ARF small GTPase family (16, 17). Components of the ARF protein family are believed to participate in vesicle formation and transport (14). Y2h also showed that PDEδ can interact with retinitis pigmentosa GTPase regulator (RPGR) (18). Mutations in the RPGR gene located on the X chromosome are linked to retinitis pigmentosa 3 and cone dystrophy (19, 20). In COS cells, transiently expressed RPGR protein is localized to the Golgi complex, one hub for vesicle formation, targeting, and fusion (21), raising the possibility that PDEδ could be colocalized with RPGR in the Golgi complex. The bulk of PDE in photoreceptors is found in the connecting cilium, suggesting a role in protein transport from the inner to outer segment (22). More recently, additional small

GST, glutathione S-transferase; FRET, fluorescence resonance energy transfer; ARF, ADP-ribosylation factor; LiAc, lithium acetate.
GTPases of the Ras superfamily, including Ras, Rap (23), Rho6, and Rheb (24), were added to the growing family of PDEδ-interacting proteins. Because PDEδ is able to regulate the association of Rab13 to the membrane, which is similar to the function of GDI (GDP dissociation inhibitor), similarities among PDEδ, RhoGDI, and RabGDI were investigated (23). By analysis of the predicted secondary structure of PDEδ and the crystal structure of RhoGDI, it was found that PDEδ and RhoGDI shared striking structural similarity, whereas the sequence identity is low. However, a number of amino acid residues within the geranylgeranyl binding pocket of RhoGDI are conserved particularly well in PDEδ. The crystal structure of PDEδ verified the presence of a hydrophobic domain packed by two opposite β-sheets, and the overall β-sandwich fold is identical to that of RhoGDI (24). The binding of Arl2 to PDEδ was found to be independent of lipids, suggesting that PDEδ can interact with proteins in two distinct ways: 1) through a lipid binding pocket and 2) β-sheet/β-sheet interactions (24).

In terms of prenylated ligand specificity, PDEδ is more promiscuous than GDI. Although only GTPases of the Ras family have been shown to interact with RhoGDI, PDEδ interacts with various prenylated proteins including protein kinases, PDE subunits, and GTPases. To examine interactions of PDEδ with components present in the retina, we screened a 2.7 amino acid retina expression library with a PDEδ bait and specifically explored PDEδ interactions with two photoreceptor-specific protein kinases: rhodopsin kinase GRK1 (farnesylated) and its homologue GRK7 (geranylgeranylated). We found that the prenyl groups covalently linked to their C-terminal cysteines mediate GRK/PDEδ interactions. We then investigated interactions among PDEδ, dannelsylated farnesyl, and geranylated cysteines in the absence of polyolpeptides and found that PDEδ interacts with farnesyl but to a lesser extent with geranylgeranyl side chains. We conclude that PDEδ is a promiscuous prenyl-binding protein targeting hydrophobic prenylated C termini of a variety of polyolpeptides.

**EXPERIMENTAL PROCEDURES**

**Polyclonal Antibody Preparation and Confoval Immunolocalization—**Anti-PDEδ antibody (directed against the N-terminal peptide MSAKDGERARL)GFLC supplied by Brent Rollman, Affinity BioReagents, Inc., Golden, CO) was purified by affinity chromatography. To verify specificity by immunoblotting, a small piece of fresh bovine retina was sonicated briefly in PBS and centrifuged at 20,000 × g for 10 min at 4 °C to remove insoluble debris. An aliquot of the supernatant was subjected to 4% SDS-PAGE and transferred to nitrocellulose, and the membrane was blotted using anti-PDEδ polyclonal antibody as the primary antibody and HRP-anti-rabbit IgG as second antibody and visualized by chemiluminescence (ECL kit, Amersham). 

**Polyclonal Antibody Preparation and Confoval Immunolocalization—**Polyclonal Antibody Preparation and Confoval Immunolocalization—The full-length PDEδ cDNA was amplified by RT-PCR and cloned in-frame into pGEX-2T expression vector (Amersham Biosciences). The expression construct was verified by DNA sequencing and transformed into E. coli ER2566 (New England Biolabs). A transconjugant was selected and resuspended in double-distilled H2O and spread on synthetic dropout medium plates lacking tryptophan, histidine, adenine, and leucine (THAL medium). The plates were incubated at 30 °C overnight. The overnight culture was transferred to 50 ml of warm YPDA (YPD supplemented with adenine hemisulfate and grown at 30 °C overnight. The overnight culture was transferred to 50 ml of warm YPDA (YPD supplemented with adenine hemisulfate) medium with a final volume of 0.5 ml. The tubes were vortexed vigorously to completely disperse the cells. The tubes were incubated at 30 °C for 30 min and shook-at 40 °C for 30 min. The culture was resuspended in double-distilled H2O and spread on synthetic dropout medium plates lacking tryptophan, histidine, adenine, and leucine (THAL medium). The plates were incubated at 30 °C. After 4–5 days, the colonies growing up from the selective plates were transferred to the same selective plates but containing 0.2 mM α-X-Gal. Blue colonies were inoculated into media, and the DNA from each positive clone was sequenced using T7/T7 primers.

**Expression of PDEδ and Purification GST-PDEδ Fusion Protein—**Full-length mouse PDEδ cDNA was amplified by RT-PCR and cloned in-frame into pGEX-2T expression vector (Amersham Biosciences). The expression construct was verified by DNA sequencing and transformed into E. coli ER2566 (New England Biolabs). A transconjugant was selected and resuspended in double-distilled H2O and spread on synthetic dropout medium plates lacking tryptophan, histidine, adenine, and leucine (THAL medium). The plates were incubated at 30 °C. After 4–5 days, the colonies growing up from the selective plates were transferred to the same selective plates but containing 0.2 mM α-X-Gal. Blue colonies were inoculated into media, and the DNA from each positive clone was sequenced using T7/T7 primers.

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Synthesis of N-Acetyl S-Farnesyl Cysteine Methyl Ester (AFCME) and N-Acetyl S-Geranylgeranyl Cysteine Methyl Ester (AGGCME)—AFCME and AGGCME were synthesized as described previously and stored as a stock solution in MeSO (Fig. 1) (26). Synthesis of N-Dansyl S-Geranylgeranyl Cysteine Methyl Ester (DGGCME) and N-Dansyl S-Farnesyl Cysteine Methyl Ester (DFCME)—S-Geranylgeranyl cysteine methyl ester and S-farnesyl cysteine methyl ester were prepared according to a method reported previously (27). To synthesize DGGCME, a solution containing 1.32 mg (4.91 μmol) of dansyl chloride, S-geranylgeranyl cysteine methyl ester (2 mg, 4.91 μmol), and N,N-diisopropylpropylamine (1 μl, 7.5 μmol) in 0.3 ml of N,N-dimethylformamide was stirred at room temperature overnight. The reaction mixture was purified on SiO\textsubscript{2} (ethyl acetate-hexane 1:4). Finally, 1.92 mg (61%) of oil was obtained. Similarly, 0.34 mg of DFCME was prepared. The molecular structures of both compounds were confirmed by 1H NMR. The fluorescence properties were determined by a SPECTRAmax Gemini XS multiplate spectrofluorimeter.

**Competition Assay**—For competition experiments, an excess amount of compounds in stock solution were diluted into 100 μM PBS separately and incubated with 7 μg of GST-PDE\textsubscript{6} overnight at room temperature. The preincubated GST-PDE\textsubscript{6} was mixed with H5 cell lysate containing expressed GRK1 or GRK7 in the presence of excess AFCME or AGGCME. The mixtures were incubated for 1 h at room temperature. The pull-down assays were carried out as described above.

**Fluorescence Resonance Energy Transfer (FRET) to Determine Dissociation Constants of PDE\textsubscript{6} Binding to Prenyl Groups**—For FRET assay of PDE\textsubscript{6} in a total volume of 100 μl of PBS, 2.8 μM PDE\textsubscript{6} was mixed with 10 μM AFCME or DGGCME and incubated overnight at room temperature. AFCME or DGGCME without protein was used as control. The fluorescence emission spectrum was recorded with a fixed λ\textsubscript{ex} = 282 nm from 315 to 550 nm.

To determine the dissociation constant of PDE\textsubscript{6} binding to DFCME, a series of increasing concentrations of DFCME in 100 μl of PBS was excited at 282 nm and the fluorescence spectrum for each concentration was measured. The values of fluorescence intensity at λ = 505 nm were retrieved and plotted against the concentrations of the fluorescence probe. Their relations can be described in Equation 1, where $F$ is the measured relative fluorescence intensity, $C$ the concentration of probe, and $k_1$ stands for a coefficient that was determined by curve fitting using Origin software. DFCME at different concentrations then was incubated with or without 2.8 μM PDE\textsubscript{6} in 100 μl of PBS at room temperature for 4 h. The fluorescence spectrum between 315 and 540 nm for each mixture was measured. The dissociation constant of PDE\textsubscript{6} binding to DFCME was fit to Equation 2 using Origin software.

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\Delta F = F_i - F = F_i - k_1 \times C_i
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where $F_i$ is the emission fluorescence of the protein and probe mixture at 505 nm, $F_i$ is the fluorescence of DFCME without adding protein, $C_i$ is the concentration of added DFCME, $k_2$ is the coefficient, $k_1$ is the dissociation constant, and $P_i$ is the initial concentration of added protein. The dissociation constant of PDE\textsubscript{6}-binding DGGCME was determined in a similar way with the exception that the protein and fluorescence probe mixture were incubated overnight.

**RESULTS**

**PDE\textsubscript{6} Is Expressed in Rods and Cones**—Multiple tissue Northern blots showed that many tissues express the PDE\textsubscript{6} gene at low levels, but among the tissues tested, the retina expressed the highest levels (10, 12). At the protein level, the PDE\textsubscript{6} polypeptide immunolocalized to rod but not cone outer segments (10). In view of our hypothesis that PDE\textsubscript{6} may interact with multiple prenylated proteins, we re-investigated the distribution of PDE\textsubscript{6} in the bovine retina with a polyclonal antibody raised against an N-terminal polypeptide of the bovine sequence (Fig. 2, A–F). In a Western blot of bovine retina extract, anti-PDE\textsubscript{6} recognized the 17-kDa PDE\textsubscript{6} polypeptide nearly exclusively (Fig. 2G). Although immunolabel attributable to PDE\textsubscript{6} is present at low levels throughout all of the layers of transversely sectioned retina (results not shown), the most intense label is associated with photoreceptors. Specifically, strong signals originate in the rod inner segments and rod outer segments colocalizing with rhodopsin (Fig. 2, D–F), consistent with previous results (10). However, PDE\textsubscript{6} was also observed in cone photoreceptors (Fig. 2, A–C), including outer segments and synaptic pedicles. Cone photoreceptors were identified specifically with an anti-cone arrestin antibody (mAb 7G6) (28), a widely used marker for primate and bovine cones. The presence of PDE\textsubscript{6} in the cytoplasm of cones is demonstrated clearly when cones are double-labeled with mAb 7G6 and anti-PDE\textsubscript{6} antibody (Fig. 2F). A similar result was obtained with a second monospecific antibody raised against recombinant PDE\textsubscript{6} (FL, results not shown). Like PDE\textsubscript{6}, cone arrestin is a soluble protein that associates with membranes under certain conditions. PDE\textsubscript{6} is distributed throughout the cone cells, consistent with cytoplasmic distribution. Results of our immunolocalization studies show that PDE\textsubscript{6} is present in bovine rod and cone photoreceptors and in much lower concentrations in other retinal cell types as well (data not shown), supporting the hypothesis that PDE\textsubscript{6} function is not limited to its association with rod PDE.

**Identification of Retina Proteins Interacting with PDE\textsubscript{6}**—We next used full-length bovine PDE\textsubscript{6} (mouse and bovine PDE\textsubscript{5} polypeptides are 98.5% identical) as a bait to identify PDE\textsubscript{6}-interacting proteins by screening a y2h bovine retina cDNA library. In total, we screened approximately 4 million clones and found that >1,000 colonies were able to grow on selective medium and turned blue on selective THAL medium containing α-X-gal. We randomly picked 95 colonies and isolated the library plasmids (Table I) from these yeast clones that contained both PDE\textsubscript{6} bait plasmid and library plasmid. Sequencing and in-frame translation revealed several clones encoding Arl2 and PDE\textsubscript{6} that were known PDE\textsubscript{6}-interacting partners, and seven clones represented partial sequences of GRK1 (rhodopsin kinase). These seven clones probably originated from two independent clones that corresponded to 165 (GRK1L) and 69 (GRK1S) amino acid residues at the C terminus of GRK1, respectively. Full-length bovine GRK1 consists of 561 amino acid residues with a predicted mass of 63 kDa. To verify spec-
ificity of the interaction between the C termini of GRK1 and PDE\textsubscript{H9254}, the two independent GRK1 clones (encoding the 165 and 69 amino acid residues of the GRK1 C terminus, respectively) were co-transformed into yeast with bovine RG4/Unc-119, a retina-dominant protein that shares 23\% identity with PDE\textsubscript{H9254} in its C-terminal 153 amino acids (29, 30). We found that partial GRK1 polypeptides encoded by these two clones only interacted with PDE\textsubscript{H9254} but not with RG4/Unc-119, suggesting that PDE\textsubscript{H9254} specifically interacts with GRK1.

In Vitro Interaction of PDE\textsubscript{H9254} with GRK1 and GRK7—To test whether the farnesylation of GRK1 is required for its interaction with PDE\textsubscript{H9254}, the cysteine residue of the CAAX box motif of GRK1L was mutated to serine, a mutation that disables farnesylation of GRK1L at the C terminus. The library plasmid encoding mutant GRK1L was cotransformed into yeast with PDE\textsubscript{H9254} bait. The yeast cells did not grow on the selective THAL medium. This result suggests that farnesylation is essential and required for the interaction between PDE\textsubscript{H9254} and GRK1.

Similarly, Rab13 did not bind to PDE\textsubscript{H9254} when the CAAX box was deleted (12). To further confirm the interaction between PDE\textsubscript{H9254} and full-length GRK1 protein, we performed in vitro pull-down assays with PDE\textsubscript{H9254} expressed in E. coli as a GST fusion protein (Fig. 3A). Because the post-translational farnesylation is required for the interaction between PDE\textsubscript{H9254} and GRK1L in yeast, we expressed GRK1 in H5 cells (these cells prenylate cysteines of the CAAX box motif, whereas bacteria are unable to perform this C-terminal modification). In addition, we also expressed GRK7, another photoreceptor-specific G protein-coupled receptor kinase that was recently identified in several species (25, 31, 32). Upon the incubation of GST-PDE\textsubscript{H9254} protein with the crude extract of H5 cells infected by GRK-expressing viruses, GRK1 (Fig. 3A) and GRK7 (Fig. 3B) bound to GST- PDE\textsubscript{H9254} immobilized by glutathione-agarose, whereas the recombinant kinases could not be coprecipitated with GST alone. These results demonstrate that PDE\textsubscript{H9254} can bind specifically both GRK1 and GRK7 in vitro.
PDEδ Functions as a Prenyl-binding Protein

Prep. Text

Prenyl Chains Compete with the Binding of PDEδ to GRK1 or GRK7—We suspected that farnesyl or geranylgeranyl side chains alone can be capable of binding to PDEδ. Therefore, we designed experiments in which recombinant GRK1 and GRK7 were incubated with PDEδ in the presence of excess of isoprenoid compounds. AFCME and AGGCME, representing the modified C termini of prenylated proteins, were synthesized (see “Experimental Procedures”). GST-PDEδ was preincubated with these two synthetic compounds, and the pull-down binding assay was performed as described above. The presence of AFCME could completely abolish the binding of GST-PDEδ to (farnesylated) GRK1 and strongly compete with the binding of GST-PDEδ to (geranylgeranylated) GRK7. AGGCME also strongly competed with the binding of GST-PDEδ to GRK1 but only weakly prevented the binding of GST-PDEδ to GRK7 (Fig. 3). In a control experiment, ACME (the backbone of AFCME and AGGCME) did not compete (Fig. 3). These results demonstrate that PDEδ interacts with GRK1 and GRK7 predominantly through prenyl moieties at their C termini. The shorter farnesyl side chain (C15) has higher binding affinity for PDEδ than the longer geranylgeranyl chain (C20). The inability of AGGCME to chase GRK7 binding to GST-PDEδ suggests that additional binding sites exist on the GRK7 polypeptide that stabilizes the interaction.

Binding Constants of PDEδ Bound to Prenyl Side Chains—Our experiments indicate PDEδ can function as a prenyl-binding protein. Therefore, we decided to use FRET to measure the PDEδ/prenyl-chain dissociation constants, taking advantage of intrinsic tryptophan fluorescence of the PDEδ polypeptide.

Fig. 3. GST-PDEδ pull-down assays of recombinant GRK1 and GRK7. A, pull-down assay of GRK1. Polypeptides bound to the beads and remaining in the supernatant were analyzed by immunoblot using anti-GRK1 antibody as described in under “Experimental Procedures.” Lane 1, supernatant following the incubation of GST with GRK1 crude extract; lane 2, supernatant following the incubation of GST-PDEδ with GRK1 crude extract (in lanes 1 and 2, GRK1 is in large excess); lane 3, pellet of GST beads; lane 4, pellet of GST-PDEδ beads. B, pull-down assay of GRK7 by GST-PDEδ. Lanes 1–4 are as in Fig. 1A but GRK1 replaced GRK1. C, competition of GRK1 binding to PDEδ by prenyl groups. GST-PDEδ was incubated with excess amount of AFCME or AGGCME. The preincubated GST-PDEδ then was mixed with crude extract of insect cell expressing GRK1 for pull-down assay in the presence of excess amount of AFCME and AGGCME, respectively. The coprecipitated proteins along with GST-PDEδ were analyzed by Western blot using anti-GRK1 antibody. MeSO4, the solvent, and ACME, the backbone of the isoprenoid compounds, were used as controls. The four lanes represent the solvent or the different compounds used for competition. Lane 1, DMSO; lane 2, ACME; lane 3, AFCME; lane 4, AGGCME. D, identical sequence of experiments in which GRK1 was replaced by GRK7. Note that competition of geranylgeranylated GRK7 is weaker, consistent with the higher Kd values.

Fig. 4. Binding of dansyl farnesyl and dansyl geranylgeranyl to PDEδ. A, FRET assay of PDEδ binding to DFCME. 9 μM DFCME in the presence or absence of 2.8 μM PDEδ was excited at λ = 282 nm simultaneously, and the emission fluorescence spectrum from 315 to 530 nm was recorded. The intrinsic fluorescence of PDEδ was also measured. ■, the fluorescence from DFCME probe only; ○, the fluorescence from the protein and DFCME mixture; ▲, the intrinsic fluorescence from protein without DFCME. R, non-linear curve fitting of the binding of PDEδ to DFCME or DGGCME. Various concentrations of fluorescence probe were mixed with 2.8 μM PDEδ or a control buffer without protein. For each concentration, the fluorescence probe or the probe-protein mixture was excited at λ = 282 nm and the fluorescence spectra were recorded. Each point in the figure represents the difference between the relative fluorescence intensity from probe-protein mixture and from the probe alone at λ = 505 nm for each corresponding concentration of fluorescence probes. The dissociation constants were fit by computer software as described under “Experimental Procedures.” When PDEδ is excited at 282 nm, a strong tryptophan fluorescence can be observed with a peak emission at λ = 335 nm. The four tryptophan residues present in PDEδ are distributed uniformly in its binding pocket and serve as reporters for ligand binding. For the PDEδ FRET assay, we synthesized two isoprenoid compounds in which dansyl was conjugated to S-farnesyl cysteine methyl ester and S-geranylgeranyl cysteine methyl ester, representing the C termini of prenylated proteins. Fluorescence spectra of synthetic DFCME and DGGCME indicated that the maximal excitation is around 340 nm, close to the maximal emission of PDEδ. The maximal fluorescence emission of both DFCME and DGGCME is at λ = 505 nm.

The FRET assay was carried out by mixing proper amounts of PDEδ with DFCME or DGGCME and exciting the mixture at λ = 282 nm. Significant energy transfer was seen for DFCME almost immediately after the mixing (Fig. 4A), whereas FRET was observed for DGGCME only after extended incubation. As expected, concomitant with FRET, quenching of the intrinsic
tryptophan fluorescence of PDEδ by DFCMε or DGGMε was observed (Fig. 4A) because of the energy transferred from PDEδ to the fluorescent probes. No FRET was observed for the mixture of PDEδ with a control dansyl probe (dansyl alanine cycloheximide, no prenyl chain was present). Thus, PDEδ can specifically bind farnesyl or geranylgeranyl. We next set out to determine the binding affinity of PDEδ to DFCMε or DGGMε. When a series of concentrations of fluorescent probes were incubated with PDEδ, different intensities of FRET were seen (Fig. 4B). Calculations (see “Experimental Procedures”) of the dissociation constants (Kd) revealed that half-maximal binding of PDEδ to farnesyl is at 0.70 ± 0.27 μM, whereas that of PDEδ to geranylgeranyl is at 19.06 ± 2.41 μM. The binding constant of farnesyl to PDEδ is higher than that of another prenyl-binding protein, RhoGDI (Kd = 4.8 μM), whereas gera

nylgeranyl has approximately 10 times less affinity for PDEδ compared with RhoGDI (Kd = 1.6 μM) (26).

**DISCUSSION**

We show that PDEδ can interact with both farnesyl (C15) and geranylgeranyl (C20) side chains in the absence of polyphemides. The following independent results support this hypothesis. 1) Almost all of the PDEδ-binding proteins identified by yeast screening are prenylated (as exceptions, the N-terminal region of RPGR and Arl2/Arl3 have no lipid modification). 2) Mutations in the CAXX box motif-disabling prenylation eliminate the binding of GRK1 to PDEδ, suggesting that for some interacting partners the lipid attachment is essential. 3) An excess of prenyl side chains prevents stable interactions between GST-PDEδ and prenylated GRKs in GST pull-downs assays. 4) Dansylated farnesyl and geranylgeranyl chains stably bind to PDEδ with binding constants in the millimolar range (dansyl alone is unable to bind). The binding affinity between PDEδ and farnesyl is comparable to RhoGDI-binding farnesyl, whereas the binding affinity between PDEδ and geranylgeranyl is much weaker, consistent with our competition assay data (Fig. 3). An analysis of the prenyl-binding data revealed that PDEδ binds farnesyl and geranylgeranyl groups with a stoichiometry of 1:1. Thus, it is very likely that GRK1 and GRK7 bind to PDEδ with a ratio of 1:1, whereas for multiple subunit proteins with more than one prenyl tail such as rod PDEδ, it is predicted that each subunit can interact with one PDEδ.

In addition to these experiments, other groups showed that the deletion of 13 amino acid residues at the C terminus of Rab13 eliminated binding to PDEδ (12) and prenylated/carboxymethylated peptides corresponding to the C termini of PDE catalytic subunits blocked PDE/PDEδ interaction while non-prenylated polypeptides had no effect. Recent crystallographic data of PDEδ show that PDEδ forms a hydrophobic pocket between two β-sheet propellers, providing a structural basis for the binding between PDEδ and prenyl chains (24). The hydrophobic pocket within PDEδ is shallower than the similar pocket in RhoGDI, which is designed to bind geranylgeranylated proteins of the Rho family, consistent with weaker binding of geranylgeranyl of PDEδ. Despite weaker PDEδ/geranylgeranyl association, the interaction between PDEδ and geranylgeranylated RK7 appears stronger than the interaction between PDEδ-RGR and geranylgeranyl RK7 because neither the excess of farnesyl nor that of geranylgeranyl could completely compete off PDEδ binding to RK7, whereas excess of farnesyl could completely compete off PDEδ binding to RK1. It is conceivable that within GRK7, there exists a second PDEδ binding site apart from its geranylgeranylated C terminus, which may have higher affinity for PDEδ than its C terminus. A second binding site in GRK7 is undefined at the molecular level but may be akin Arl2/Arl3 interaction sites or similar to interaction with RGR, which has a high affinity PDEδ binding site (Kd = 90 nm) within its N-terminal half (13, 18). It has been shown that PDEδ has C-terminal SRV and FVY motifs that may be involved in interacting with PDZ (PSD95, Dig, ZO-1) domain-containing proteins (12). When PDEδ C-terminal sequences were deleted, interaction with prenylated targets was disrupted (13) and colocalization with vesicular structures was disrupted (12). Therefore, it is conceivable that PDEδ has several major interacting domains in addition to the hydrophobic pocket binding to prenylated proteins.

Although both PDEδ and RhoGDI can bind prenyl groups and they share structural similarity in their hydrophobic pocket, their distinct sequences suggest that they assume different functions. RhoGDI was originally identified as a Rho GTPase-binding protein. RhoGDI prefers the GDP-bound form of Rho family proteins, serving as Rho GDP dissociation inhibitor while PDEδ prefers Arl2 and Arl3 in the active form with GTP bound. As small GTPases, ARF proteins participate in a variety of intracellular transport processes. Arl2 and Arl3 may interact with PDEδ to target a set of prenylated proteins to their destination membrane. The localization of PDEδ in the cytoplasm of cells (Fig. 2) supports this hypothesis. Thus, one function of PDEδ may be that of a soluble transport factor interacting with a large number of prenylated proteins steered by a number of factors like Arl proteins. Protein trafficking is particularly important for photoreceptors where high turnover of outer segments housing the phototransduction machinery requires unusually active protein transport. In ~100 million photoreceptors present in the human retina (see webvision.med.utah.edu/facts.html), an entire outer segment must be replaced once per week, an enormous task for protein transport and correct membrane targeting. Since many of the membrane-associated proteins participating in phototransduction are prenylated (rod and cone PDE, GRK1/7, Ty subunit), it is conceivable that PDEδ, being more abundant in photoreceptors than in other cell types, may exert a prominent role in membrane targeting of prenylated phototransduction components in photoreceptors. Consistent with this model, preliminary experiments with a PDEδ knock-out mouse² suggest that in the absence of PDEδ GRK1 remains mostly in the inner segment where biosynthesis occurs and is not transported to its outer segment destination.

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