Cyclic guanosine 5'-monophosphate (cGMP) phosphodiesterase (PDE) regulates the level of cGMP in vertebrate photoreceptor cells. Two identical inhibitory PDE γ subunits (Pγ) block catalytic activity of PDE-α and -β subunits (Pαβ) in the dark. The primary regions of Pγ involved in the interaction with Pαβ are a central polycationic region, Pγ24–45, and a C-terminal region of Pγ. Recently, we have shown that the C-terminal region of Pγ, which is the major Pγ inhibitory domain, blocks PDE activity by binding to the catalytic site of PDE (Artemyev, N. O., Natochin, M., Busman, M., Schey, K. L., and Hamm, H. E. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 5407-5412). Here, we localize the site on the rod cGMP PDE α subunit that binds to the central polycationic domain of Pγ. This site is located within a region that links a second noncatalytic cGMP binding site with the catalytic domain of PDE. A polypeptide corresponding to this region, Pα-461-553, expressed as a glutathione S-transferase fusion protein in Escherichia coli and isolated after cleavage of the fusion protein with thrombin, blocks inhibition of PDE activity by Pγ. In addition, Pα-461-553 binds to the Pγ24–45 region (Kd, 7 μM), as measured by a fluorescent increase in a Pγ24–45Cys peptide labeled with 3-(bromoaacetyl)-7-diethylaminocoumarin. The Pα-461-553 region was further characterized by using a set of synthetic peptides. A peptide corresponding to residues 517-541 of Pα (Pα517-541) effectively suppressed inhibition of PDE activity by Pγ and bound to Pγ24–45Cys labeled with 3-(bromoaacetyl)-7-diethylaminocoumarin (Kd, 22 μM). Pα-517-541 also competes with the activated rod G-protein γ-subunit for binding to Pγ labeled with lucifer yellow vinyl sulfone. This suggests that light activation of rod PDE by the G-protein transducin involves competition between transducin α-guanosine 5'-triphosphate and Pα-517-541 for binding to Pγ24–45 region. Based on the results, we propose a linear model of interactions between catalytic and inhibitory PDE subunits.

In vertebrate photoreceptor cells, light-activated rhodopsin catalyzes GDP-GTP exchange on the rod G-protein α-subunit (Gαon), which in turn activates cGMP phosphodiesterase (PDE).

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The abbreviations used are: Gαon, α-subunit of the photoreceptor G-protein transducin; PDE, rod outer segment cGMP phosphodiesterase; Pα, Pβ, and Pγ, α, β, and γ subunits of PDE; taPDE, trypsin-activated PDE; BC, 3-(bromoaacetyl)-7-diethylaminocoumarin; LY, lucifer yellow vinyl sulfone; HPLC, high performance liquid chromatography; GST, glutathione S-transferase; GTP-γS, guanosine 5′-O-(thiotriphosphate); PBS, phosphate-buffered saline; cGMP-PDE, cGMP-binding, cGMP-specific PDE.
EXPERIMENTAL PROCEDURES

Materials—cGMP, GTP·S, and T4 DNA ligase were obtained from Boehringer Mannheim. Restriction enzymes were from New England Biolabs. 3-(Bromocarboxyl)·7-diethylaminoacoumarin was purchased from Molecular Probes, Inc. Trypsin and soybean trypsin inhibitor were from Worthington. All other reagents were purchased from Sigma.

Preparation of Gₛ·GTP·S, Trypsin-activated PDE, and Pᵧ—Bovine rod outer segment membranes were prepared by the method of Papae and Dreyer (1974). The Gₛ·GTP·S was extracted from membranes with GTP·S and purified using chromatography on a Blue-Sepharose CL-6B column as described by Kleuss et al. (1987). PDE was extracted from rod outer segment membranes as described by Baehr et al. (1982). PDE was purified, and trypsin-activated PDE (taPDE) was prepared as described earlier (Artemyev and Hamm, 1992). The purified proteins were kept in 40% glycerol at −20°C. The Pᵧ subunit was expressed in E. coli and purified on a SP-Sepharose fast flow column and on a C-4 HPLC column (Microsorb-MW, Rainin) as described by Skiba et al. (1995).

Construction and Expression of Pₛ GST Fusion Proteins—A vector, containing cDNA coding for the full-length Pₛ subunit (Ovchinnikov et al., 1987), was kindly provided by Dr. N. Skiba (University of Illinois, Chicago, IL). Fragments of Pₛ cDNA corresponding to sequences 2–553, 2–854, 92–553, 92–854, 461–854, and 461–553 were amplified by polymerase chain reaction with appropriate upstream primers containing the XbaI site. These fragments were digested with XbaI and SalI and ligated into the pGEX-KG expression vector (Guan and Dixon, 1991), which was cut with the same enzymes. The GST-Pₛ-461–553 cDNA sequence was verified by automated DNA sequencing at the University of Iowa DNA Core Facility using the 5' pGEX sequencing primer (Pharmacia Biotech Inc.). A single silent nucleotide C → T substitution was found in AGC codon for Ser-549. All DNA manipulations were performed using standard techniques (Maniatis et al., 1989). Expression of Pₛ GST fusion proteins even at a low temperature (25°C) and a low isopropl-1-thio-β-galactopyranoside concentration (20 μM) resulted in the accumulation of recombinant protein in inclusion bodies. We attempted to refold the GST fusion proteins after dissolving the inclusion bodies in 6 M urea. The GST-Pₛ-461–553 polyepitide was relatively stable in solution after removing urea. Typically, E. coli cells, after a 5-h induction with 0.5 mM isopropyl-1-thio-β-galactopyranoside, were spun down and resuspended in PBS at a ratio of 1:20. The cells were disrupted by ultrasonication, and pellets were collected after the cell lysate had been centrifuged for 30 min at 50,000 × g. Insoluble inclusion bodies were separated from cells and membranes by five consecutive washes in PBS buffer containing 0.5% Triton X-100 and five washes in PBS with 1% Nonidet P-40. After the final wash with PBS, inclusion bodies were dissolved in 6 M urea, and β-mercaptoethanol was added to a final concentration of 10 mM. The urea was removed on a Sephadex G-25 column equilibrated with PBS. GST-Pₛ-461–553 fusion protein was then digested with thrombin (1 NIH unit/2 mg of protein) overnight at room temperature. Proteins were then precipitated with 10% trichloroacetic acid (TCA). The pellet was washed three times with 20 mM Tris-HCl (pH 7.4) and dissolved in 20 mM Tris-HCl buffer (pH 7.4) containing 6 M urea and 100 mM NaCl. The Pₛ-461–553 polyepitide was purified on a Superose 12 (Pharmacia) column equilibrated with the same buffer. Fractions containing Pₛ-461–553 were desalted on a PD-10 column (Pharmacia), equilibrated with double distilled H₂O, lyophilized on a SpeedVac concentrator (Savant), and dissolved in buffer A (10 mM HEPES, pH 7.8, 100 mM NaCl, and 1 mM MgSO₄).

Peptide Synthesis—Peptides corresponding to Pₓ residues 21–31, 467–491, 492–516, and 517–541 (Ovchinnikov et al., 1987) and Pᵧ residues 24–45Cys (Ovchinnikov et al., 1986) were synthesized by the solid-phase Merrifield method on an Applied Biosystems automated peptide synthesizer. The extra cysteine was added to the C terminus of the Pᵧ-24–45 sequence as a site for the introduction of the environmentally sensitive fluorescent probe 3-(bromocarboxyl)-7-diethylaminoacoumarin (BC). Each peptide was purified by reverse-phase HPLC on a preparative Aqueapor Octyl column (25 × 1 cm; Applied Biosystems). The purity and chemical formula of each peptide were confirmed by fast atom bombardment mass spectrometry and analytical reverse-phase HPLC.

Preparation of Pᵧ-24–45BC and Pᵧ-LY—The Pᵧ-24–45Cys peptide (0.5 mg) was dissolved in 0.3 ml of 10 mM HEPES (pH 7.8). A fold molar excess of 3-(bromocarboxyl)-7-diethylaminocoumarin in acetonitrile was added to the peptide solution, and the mixture was incubated for 15 min at room temperature. The Pᵧ-24–45BC was purified by reverse-phase HPLC on a Microsorb-MW C-4 column (Rainin). Pᵧ was labeled at its single cysteine (Cys-68) with lucifer yellow vinyl sulfone (Pᵧ-LY) and purified as described by Artemyev et al. (1992).

Fluorescent Assays—Fluorescent assays were performed on a F-2000 fluorescence spectrophotometer (Hitachi) in 1 ml of buffer A. The fluorescence of Pᵧ-24–45BC was monitored with excitation at 445 nm and emission at 500 nm. The concentration of Pᵧ-24–45BC was determined using ε290 = 53,000. The assay of interaction between Pᵧ-LY and Gₛ·GTP·S was carried out essentially as described by Artemyev et al. (1992).

Analytical Methods—The PDE activity was measured using the protein-evolution assay of Lieberman and Evanutz (1982). The assay was performed at room temperature in 200 μl of buffer A. The reaction was initiated by addition of cAMP (3 mM final concentration). The pH was monitored with a pH microelectrode (Microelectrode, Inc.). Protein concentrations were determined by the method of Bradford (1976) using IgG as a standard or using calculated extinction coefficients at 280 nm. Sodium dodexyl sulfate-polyacrylamide gel electrophoresis was performed by the method of Laemmli (1970) in 14% acrylamide gels.

RESULTS

Expression and Purification of Pₛ-461–553—Pₓ sequences corresponding to residues 2–553, 2–854, 92–553, 92–854, 461–854, and 461–553 were cloned into the pGEX-KG vector and overexpressed as GST fusion proteins in E. coli. The recombinant proteins were found primarily in the inclusion bodies. Solubilization of inclusion bodies in 6 M urea, followed by removal of urea and cleavage of GST fusion proteins with thrombin, resulted in the formation of recombinant Pₓ polypeptides with their expected molecular masses. However, all the Pₓ polypeptides, except Pₛ-461–553, were unstable and aggregated into complexes that migrated with high molecular mass on gel-filtration columns. Formation of inclusion bodies with GST-Pₛ-461–553 led to a very high yield of this protein. After washing, a fraction of the inclusion bodies (~1 g/liter of culture) contained ~90% GST-Pₛ-461–553 (Fig. 1, lane 1). Complete cleavage by thrombin resulted in the formation of three major protein bands at ~27, 25, and 13 kDa (Fig. 1, lane 2). Formation of the 25-kDa band may reflect additional nonspecific cleavage of the proteolytically sensitive poly-Gly loop connecting GST and Pₛ-461–553. The Pₛ-461–553 (13 kDa) protein was purified on a Superose 12 gel-filtration column (Fig. 1, lane 3).

Pₛ-461–553 Blocks Inhibition of taPDE by Pᵧ—To elucidate whether the Pₛ-461–553 region contains a site for Pᵧ interaction, we assayed whether it could compete with Pᵧ by measuring the effects of purified Pₛ-461–553 on inhibition of taPDE
by Pγ. Limited proteolysis of PDE with trypsin removes intrinsic Pγ subunits and small farnesylated and geranyl-geranylated C-terminal fragments of Pα and Pβ, respectively (Hurley and Stryer, 1982; Catty and Deterre, 1991), leading to significantly increased activity compared with holoenzyme. Subsequent addition of Pγ rehinders PDE activity. The activity of taPDE was determined following addition of increasing concentrations of Pγ in the absence or presence of the Pα-461–553 peptide. At 10 μM Pα-461–553, the EC₅₀ of taPDE inhibition by Pγ shifts from 0.22 to 6 nM (Fig. 2).

Pα-461–553 Binds to Pγ-24–45—As was recently demonstrated, the C terminus of Pγ interacts with the catalytic domains of Pα and, likely, Pβ (Artemyev et al., 1996). To determine whether Pα-461–553 binds to another known region of the Pαβ-Pγ interface, namely that of Pγ-24–45, we developed a fluorescent assay to monitor interaction between Pγ-24–45 and Pαβ. A peptide, Pγ-24–45Cys, was synthesized and labeled with the environmentally sensitive fluorescent probe 3-(bromoacetyl)-7-diethylaminocoumarin. Addition of taPDE to Pγ-24–45BC significantly increased the fluorescence of the probe in a dose-dependent manner (Fig. 3A). The binding curve shows a single class of binding sites with a Kₐ of 37 nM. Addition of excess Pγ reversed the fluorescent increase, indicating that the assay is specific (data not shown). Similarly, when Pα-461–553 was added to Pγ-24–45BC, there was a dramatic increase in probe fluorescence (maximum f/f₀, 7.2) (Fig. 3B). The affinity of Pγ-24–45BC binding to Pα-461–553 (Kₐ, 7.3 μM) is lower than that of Pα-24–45BC/Pαβ interaction (Kₐ, 37 nM). This suggests that the polycationic region of Pγ may have an additional binding site on Pαβ. Alternatively, Pα-461–553 could have a tendency to self-association, which would explain some cooperativity seen in its binding to Pγ-24–45BC (Fig. 3B).

Effects of Synthetic Peptides from the Pα-461–553 Region on Inhibition of taPDE Activity by Pγ—Peptides corresponding to sequences Pα-467–491, Pα-492–516, and Pα-517–541 were synthesized and tested for their ability to suppress the taPDE inhibition by Pγ. In the presence of 1, 3, or 10 μM Pα-517–541, the EC₅₀ of taPDE inhibition by Pγ shifted in a linear fashion from −0.22 to 4, 15, or 41 nM, respectively (Fig. 4A). Complete folding of the larger Pα-461–553 polypeptide may explain in part why the shorter Pα-461–541 peptide is more effective in blocking inhibition of taPDE activity by Pγ.

The Pα-467–491 peptide was effective only at relatively high concentrations. When present at 100 μM, it shifts the EC₅₀ for taPDE inhibition by Pγ to 5 nM (not shown). This result indicates that Pα-467–491 might be a part of the Pγ binding site, however, the Pα-467–491 sequence contains a patch of acidic residues, ECREEE (Fig. 6B), which most likely interacts with the positively charged polycationic region of Pγ. Therefore, it is not clear whether the effects of Pα-467–491 at high concentrations are functionally significant. Peptides Pα-492–516 and Pα-21–31 (control) had no effect at concentrations up to 100 μM. Peptide Pα-21–31 was used in control experiments, as it is a part of the proposed N-terminal Pγ binding domain on Pα (Oppert et al., 1991).

Peptide Pα-517–541 Binds to Pγ-24–45—Next we examined binding of peptides Pα-467–491, Pα-492–516, Pα-517–541, and Pα-21–31 to the polycationic region of Pγ using the fluorescent assay. Addition of Pα-517–541 to Pγ-24–45BC caused a significant increase (maximum f/f₀, 7.4) in the probe fluorescence, similar to the effects of taPDE and Pα-461–553. Pα-517–541 bound to Pγ-24–45BC with a Kₐ of 22 μM (Fig. 4B). Unlabeled peptide Pγ-24–45Cys reversed the fluorescent increase of Pγ-24–45BC caused by binding of Pα-517–541 (not shown). Other peptides at concentrations up to 100 μM had no effect on Pγ-24–45BC fluorescence. The affinity of the Pα-517–541/Pγ-24–45BC interaction is somewhat lower than what might have been expected based on the potency of this peptide to block taPDE inhibition by Pγ. Pα-517–541 may have additional interactions with Pγ outside the Pγ-24–45 region, or the fluorescent probe may interfere to some extent with the interaction.

Pα-517–541 Competes with GαGTP-Y5 for the Interaction with Pγ—Evidence suggests that the polycationic region of Pγ contains binding sites for both Pαβ and GαGTP (Artemyev and Hamm, 1992; Artemyev et al., 1992; Brown, 1992; Takemoto et al.)
The interfaces was able to fully reverse the fluorescent increase of \(P\) in the presence of 1, 3, or 10 \(\mu M\) \(P\)-517-541. The relative increase of fluorescence \((f/f_0)\) of \(P\)-24-45BC (50 \(nM\)) was determined after addition of increasing concentrations of \(P\)-517-541. The curve of the relative fluorescent increase \((K_d, 22 \mu M; \text{maximum } f/f_0, 7.4)\) fits the data, with \(r = 0.99\). 

To determine whether \(P\)-517-541 can compete with \(G_\alpha\)-GTP\(\gamma\)S for binding to \(P\)-24-45, we used a fluorescent assay of the interaction between \(G_\alpha\)-GTP\(\gamma\)S and \(P\) labeled with lucifer yellow vinyl sulfone (Artemyev et al., 1995). \(P\)-517-541 had no effect on the basal fluorescence of \(P\)-LY but was able to fully reverse the fluorescent increase of \(P\)-LY that resulted from addition of \(G_\alpha\)-GTP\(\gamma\)S (Fig. 5). A \(K_d\) of 3 \(\mu M\) for the \(P\alpha\)-517-541-P-\(\gamma\)LY complex was calculated from the competition curve based on the \(K_d\) of 36 \(nM\) for the \(G_\alpha\)-GTP\(\gamma\)S-P-\(\gamma\)LY complex (Artemyev et al., 1995). Peptides \(P\alpha\)-467-491, \(P\alpha\)-492-516, and \(P\alpha\)-21-31 at concentrations up to 100 \(\mu M\) did not compete with \(G_\alpha\)-GTP\(\gamma\)S for the interaction with \(P\)-LY.

DISCUSSION

In rod photoreceptor cells, GTP-bound transducin \(\alpha\) subunits interact with a PDE complex of two catalytic \(\beta\) subunits and two inhibitory \(\gamma\) subunits, leading to displacement of the inhibitory subunits and subsequent PDE activation. The interfaces of critical interactions between \(P\\gamma\) and \(P\alpha\beta\) or \(P\gamma\) and \(G_\alpha\)-GTP have been analyzed in a number of studies (Lipkin et al., 1988; Artemyev and Hamn, 1992; Artemyev et al., 1992, 1993; Oppert et al., 1991, 1992; Brown, 1992; Takenoto et al., 1992; Skiba et al., 1995, 1996). Although functional sites of \(P\gamma\) have been elucidated, the interactive surfaces on \(G_\alpha\)-GTP and \(P\alpha\beta\) are not well defined.

The central polycationic region of \(P\gamma\), residues 24-45, together with the C terminus, is involved in the interaction between \(P\gamma\) and the PDE catalytic subunits. The primary role of the \(P\)-24-45 region is to enhance the affinity of the \(P\gamma\)-\(P\alpha\beta\) interaction. To map the \(P\)-24-45 binding site on the \(P\alpha\) subunit, we have expressed a region linking a second noncatalytic \(G_\gamma\)GTP\(\gamma\)S-binding site with the catalytic domain of \(P\alpha\) as a GST fusion protein in E. coli. This selection was made for the following reasons: this region: (a) appears to be unique for photoreceptor PDEs; (b) contains several patches of acidic amino acid residues (Fig. 6B), and (c) has unknown function. The choice is consistent with the unique ability of photoreceptor PDE to be inhibited by \(P\gamma\) and with the polycationic nature of the \(P\)-24-45 region. The \(P\alpha\)-461-553 polypeptide, purified after cleavage of the GST fusion protein, blocks inhibition of PDE activity by \(P\gamma\). In addition, \(P\alpha\)-461-553 binds to fluorescently labeled \(P\)-24-45BC and induces a maximal increase in fluorescence, similar to that of taPDE binding with \(P\)-24-45BC.

Synthetic peptides were used to localize the \(P\)-24-45 binding to residues 517-541 of \(P\alpha\). Peptide \(P\)-517-541 effectively suppressed inhibition of PDE activity by \(P\gamma\) and bound to \(P\)-24-45BC in the fluorescent assay. Despite the fact that \(P\alpha\)-517-541 contains 4 acidic and 3 basic residues and \(P\alpha\)-467-491 contains 8 acidic and 3 basic residues, the latter was able to interfere with the \(P\gamma\) inhibition only at high concentrations and did not show binding to \(P\gamma\)-24-45BC. This implies that interactions other than electrostatic ones are very important for the \(P\gamma\)-24-45 binding to \(P\alpha\). It also eliminates a concern one might have regarding the specificity of the initially observed interaction between the overall negatively charged region \(P\alpha\)-461-553 and the polycationic region \(P\gamma\)-24-45. Nevertheless, \(P\alpha\)-467-491 may represent an additional, weaker interaction site between \(P\alpha\) and \(P\)-24-45. Results of the study by Oppert et al. (1991) indicated that a weak interaction between region \(P\alpha\)-453-563 and \(P\gamma\) may complement major interactions that involve the N-terminal regions of \(P\alpha\), \(P\alpha\)-16-30, and \(P\alpha\)-78-90. Our data suggest that the \(P\alpha\)-517-541 domain is the major site of \(P\alpha\)-\(P\gamma\) interaction that binds to \(P\gamma\)-24-45. Different methods used in this study and the study by Oppert et al. (1991) could in part account for the different conclusions. Also, peptides \(P\alpha\)-16-30 and \(P\alpha\)-78-90 are basic and may at high concentrations bind to \(P\alpha\) and compete with \(P\gamma\) by a mechanism similar to known nonspecific effects of histones and protamines on PDE (Miki et al., 1975). The conclusion that highly dissimilar regions of \(P\alpha\) and \(P\beta\) interact with \(P\gamma\) (Oppert et al., 1991, Oppert and Takenoto, 1991) appear to be inconsistent with the evidence that both \(P\alpha\) and \(P\beta\) interact with...
identical sites on Pγ with high affinity (Wensel and Stryer, 1990; Artemyev and Hamm, 1992; Brown, 1992).

Analysis of the sequences from the regions of Pb and cone Pα′ that correspond to Pα-517–541 indicates that Pα and Pb are more than 80% identical in this region, whereas Pα′ has only ~40% homology to Pα or Pb (Fig. 6A). Most likely, Pb-515–539 binds Pγ-24–45, as is seen with Pα-517–541. The lower homology between rod PDE and cone PDE in this region may explain why rod Pγ inhibits rod PDE more effectively (Kd, 80 pM) than cone PDE (Kd, 600 pM) (Hamilton et al., 1993). The high level of homology between the Pα and Pb sites that bind the Pγ C terminus (Artemyev et al., 1996) and Pγ-24–45 favors a model with similar, although not necessarily identical, affinities for the Pγ-Pα and Pγ-Pb binding sites (Wensel and Stryer, 1990). Fig. 6C shows a linear model for the Pγ interaction with Pα and Pb based on our results.

Previous studies have demonstrated that the Pγ-24–45 site is involved in the interaction with Gα-GTP (Lipkin et al., 1988; Artemyev et al., 1992; Takemoto et al., 1992). Here we show that Pα-517–541 effectively competes with Gα-GTPγS for the binding to PγLY. The question still to be answered is whether a very tight binding of Pγ to Pb with a Kd < 50 pm (Wensel and Stryer, 1990) does not allow for Pγ dissociation from Pb during the time of photoresponse (0.2 s). However, noncompetitive binding of Gα-GTP to the Pγ-63–76 region near the major Pγ inhibitory domain Pγ-77–87 (Skiba et al., 1995) may induce a conformational change of Pγ, resulting in the availability of the Pγ-24–45 region for the interaction with Gα-GTP. This would increase the affinity of Gα-GTP-Pγ interaction in the active complex Gα-GTP-Pγ-Pβ-Pγ-Gα-GTP.

Current classification of cyclic nucleotide phosphodiesterases separates all known mammalian PDEs into seven families (Beavo et al., 1994). All PDEs contain a highly conserved catalytic domain within the C-terminal part of the enzyme. Photoreceptor PDEs have similar general domain organization with two PDE families: cGMP-stimulated PDE and cGMP-binding, cGMP-specific PDE (cGB-PDE). These PDEs have two internally homologous repeats for noncatalytic cGMP binding (Li et al., 1990; Lipkin et al., 1990; Trong et al., 1990; McAllister-Lucas et al., 1993, 1995). Direct binding studies have shown that only two molecules of cGMP are bound to the dimeric PDE molecule, which has four cGMP binding segments (Gillespie and Beavo, 1989; Thomas et al., 1990; Stoop and Beavo, 1991). Perhaps, each cGMP binding site is formed by two analogous motifs from both catalytic PDE subunits (McAllister-Lucas et al., 1995). A segment of approximately 60–70 amino acid residues connects the second noncatalytic cGMP binding site with the PDE catalytic domain. The functional role of this domain for different cGMP-binding PDEs is not known. We performed a local alignment search (BLAST) to compare sequences from bovine photoreceptor PDEs corresponding to this “linker” region against protein sequence data bases. This search revealed 65–70% homology between the linker regions of Pα and Pb from different species and 45–50% homology between Pα and Pb and cone Pα′. Interestingly, the cGB-PDE also has a significant level of homology to photoreceptor PDEs within this region. The sequence corresponding to residues 524–583 of bovine cGB-PDE is 35–37% identical (51–55% similar) to the Pα-481–540 and Pγ-479–538 regions (Fig. 6B). Thus far, no protein modulators of activity of cGB- and cGMP-stimulated PDEs have been identified. The universal Ca2+-binding protein modulator calmodulin stimulates activity of calmodulin-dependent PDEs through binding to two sites located N-terminally to the catalytic domain (Sonnenburg et al., 1995). It is tempting to speculate that the linker regions in PDEs with noncatalytic cGMP binding sites could represent potential sites for PDE regulation by other proteins.

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Fig. 6. A, comparison of the putative domains for binding of Pγ-24–45 in photoreceptor PDEs. B, sequence homology within the regions of Pα (Ovchinnikov et al., 1987) and cGB-PDE (McAllister-Lucas et al., 1993) that link a second noncatalytic cGMP binding site and the catalytic domain. C, linear model of Pα–Pγ–Pβ interactions.
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