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The γ Subunit of Rod cGMP-Phosphodiesterase Blocks the Enzyme Catalytic Site*

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Cyclic GMP phosphodiesterase (PDE) is the effector enzyme in the visual transduction cascade of vertebrate photoreceptor cells. In the dark, the activity of the enzyme catalytic α and β subunits (Pαβ) is inhibited by two γ subunits (Pγ). Previous results have established that approximately 5–7 C-terminal residues of Pγ comprise the inhibitory domain. To study the interaction between the Pγ C-terminal region and Pαβ, the Pγ mutant (Cys^{68} → Ser, and the last 4 C-terminal residues replaced with cysteine, Pγ-1–83Cys) was labeled with the fluorescent probe 3-(bromoacetyl)-7-diethylaminocoumarin (BC) at the cysteine residue (Pγ-1–83BC). Pγ-1–83BC was a more potent inhibitor of PDE activity than the unlabeled mutant, suggesting that the fluorescent probe in part substitutes for the Pγ C terminus in PDE inhibition. HoloPDE ($P\alpha\beta\gamma_2$) had no effect on the Pγ-1–83BC fluorescence, but the addition of Pαβ to Pγ-1–83BC resulted in an approximately 8-fold maximal fluorescence increase. A K_d for the Pγ-1–83BC-Pαβ interaction was 4.0 ± 0.5 nM. Zaprinast, a specific competitive inhibitor of PDE, effectively displaced the Pγ-1–83BC C terminus from its binding site on Pαβ (IC_{50} = 0.9 μM). cGMP and its analogs, 8-Br-cGMP and 2′-butyryl-cGMP, also competed with the Pγ-1–83BC C terminus for binding to Pαβ. Our results provide new insight into the mechanism of PDE inhibition by showing that Pγ blocks the binding of cGMP to the PDE catalytic site.

In the visual transduction cascade of rod photoreceptor cells, the photoexcited visual receptor, rhodopsin, interacts with the rod G-protein, transducin, and stimulates the exchange of GTP for bound GDP. The GTP-bound α subunit of transducin dissociates from rhodopsin and the transducin βγ subunits and activates the effector enzyme, cGMP phosphodiesterase (PDE),[1] by relieving the inhibitory constraint imposed by two identical inhibitory subunits of PDE (Pγ) on the enzyme αβ catalytic subunits (Pαβ) (for review see Refs. 1–4).

Insights into the Pγ-Pαβ interaction are critical for understanding the mechanisms of PDE inhibition by Pγ and PDE activation by transducin. Approximately 5–7 C-terminal amino acid residues of Pγ are involved in the inhibitory interaction with Pαβ (5–9). Recently, using a cross-linking approach we have identified a site on Pα for binding of the Pγ C terminus as a region Pα-751–763 within the PDE catalytic domain (10). The finding suggests that the Pγ C terminus either occupies the site for binding and catalysis of cGMP or induces local conformational changes of the PDE catalytic site that block cGMP hydrolysis. Here, we study the interaction between the C terminus of Pγ and Pαβ using a novel fluorescence assay to elucidate the mechanism of PDE inhibition by Pγ.

EXPERIMENTAL PROCEDURES

Materials—cGMP was obtained from Boehringer Mannheim. 3-(bromoacetyl)-7-diethylaminocoumarin (BC) was purchased from Molecular Probes, Inc. Trypsin and soybean trypsin inhibitor were from Worthington. Zaprinast and all other reagents were purchased from Sigma.

Preparation of Trypsin-activated PDE, Pγ, and Pγ Mutants—Bovine rod outer segment (ROS) membranes were prepared by the method of Papermaster and Dreyer (11). PDE was extracted from ROS membranes as described in Ref. 12. PDE and trypsin-activated PDE (tPDE) were prepared and purified as described previously (6). The purified proteins were kept in 40% glycerol at −20 °C. The Pγ subunit was expressed in *Escherichia coli* and purified as described in Ref. 9. The Pγ mutants PγCys^{68} → Ser and Pγ-1–83Cys (Cys^{68} → Ser, and the last 4 C-terminal residues, Tyr-Gly-Ile-Ile, replaced with a single cysteine) were obtained as described in Ref. 10.

Preparation of Pγ-1–83BC, PγBC, and Pγ-24–45BC—To obtain Pγ-1–83BC and PγBC, a 5 mM stock solution of BC (final concentration, 200 μM) was added to either 100 μM Pγ-1–83Cys or 100 μM Pγ, each in 20 mM HEPES buffer (pH 7.6), and the mixture was incubated for 30 min at room temperature. Pγ-1–83BC and PγBC were then passed through a PD-10 column (Pharmacia Biotech Inc.) equilibrated with 20 mM HEPES buffer (pH 7.6) containing 100 mM NaCl and purified by reversed-phase HPLC on a C-4 column Microsorb-MW (Rainin) using a 0–100% gradient of acetonitrile, 0.1% trifluoroacetic acid. The preparations of Pγ-1–83BC and PγBC contained no free BC. Using ϵ_{445} = 53,000 for BC, the molar ratio of BC incorporation into Pγ and Pγ-1–83BC was greater than 0.8 mol/mol. Pγ-24–45BC was prepared by labeling of peptide Pγ-24–45Cys and purified as described in Ref. 13. A Pγ mutant, PγCys^{68} → Ser, and a peptide, Pγ-24–45, that contain no cysteine were not derivatized with BC under similar conditions, suggesting the selectivity of cysteine labeling.

Fluorescent Assays—Fluorescent assays were performed on a F-2000 Fluorescence Spectrophotometer (Hitachi) in 1 ml of 80 mM Tris-HCl buffer (pH 7.6) containing 2 mM $MgCl_2$. Fluorescence of Pγ-1–83BC, PγBC, or Pγ-24–45BC was monitored with excitation at 445 nm and emission at 495 nm. The assays were carried out at equilibrium, which was typically reached less than 3 s after mixing of the components. The concentration of labeled polypeptides was determined using ϵ_{445} = 53,000. Where indicated, zaprinast was added from 1 mM stock solution to an assay buffer. The K_d values in Figs. 2 and 4 were calculated by fitting the data to Equation 1,

$$\frac{F}{F_0} = 1 + \frac{\left[\frac{F}{F_0}^{max} - 1\right] \times X}{K_d + X} \qquad \text{(Eq. 1)}$$

where F_0 is a basal fluorescence of Pγ-1–83BC or Pγ-24–45BC, F is the fluorescence after additions of tPDE, F/F_{0max} is the maximal relative increase of fluorescence, and X is a concentration of free tPDE.

The IC_{50} values in Figs. 3 and 5 were calculated by fitting the data to the one site competition equation with variable slope:

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[1] The abbreviations used are: PDE, rod outer segment cGMP phosphodiesterase; tPDE, phosphodiesterase activated with limited trypsin digestion to remove γ subunits; ROS, rod outer segment; BC, 3-(bromoacetyl)-7-diethylaminocoumarin; HPLC, high performance liquid chromatography.

$$\frac{F}{F_0} = 1 + \frac{\frac{F}{F_0}_{max} - 1}{1 + 10^{[X\text{-}\log IC_{50}]\times H}} \quad \text{(Eq. 2)}$$

where X is a concentration of a competing ligand (zaprinast, cGMP, or cGMP analogs) and H is a Hill slope.

Analytical Methods—The PDE activity was measured using the proton evolution assay of Liebman and Evanczuk (14). The assay was performed at room temperature in 200 μl of 10 mM HEPES (pH 7.8) containing 100 mM NaCl and 1 mM $MgCl_2$. The reaction was initiated by the addition of cGMP. The pH was monitored with a pH microelectrode (Microelectrode, Inc.). Protein concentrations were determined by the method of Bradford (15) using IgG as a standard or using calculated extinction coefficients at 280 nm. SDS-polyacrylamide gel electrophoresis was performed by the method of Laemmli (16) in 12% acrylamide gels. Fitting of the experimental data was performed with nonlinear least squares criteria using GraphPad Prizm Software. The K_i, K_d, and IC_{50} values are expressed as the means ± S.E. of three independent measurements.

RESULTS

Inhibition of tPDE Activity by Pγ-1–83Cys, Pγ-1–83BC, and PγBC—To study the inhibitory interaction between the Pγ C terminus and Pαβ, we have replaced C-terminal amino acid residues of Pγ with a fluorescent probe by labeling the Pγ-1–83Cys mutant with BC. To determine if the fluorescent probe can substitute for the Pγ C terminus, the ability of the labeled and unlabeled mutant to inhibit tPDE was tested. Limited proteolysis of holoPDE with trypsin removes intrinsic Pγ subunits and small farnesylated and geranyl-geranylated C-terminal fragments of Pα and Pβ, respectively, leaving mainly intact active catalytic Pαβ subunits (17, 18). The Pγ-1–83Cys mutant was, as expected, a weak inhibitor of tPDE activity. It inhibited maximally only ~50% of tPDE activity with an apparent K_i value of 13 nM (not shown). The labeled mutant, Pγ-1–83BC, was a significantly more potent inhibitor of tPDE than Pγ-1–83Cys. Pγ-1–83BC almost completely inhibited tPDE activity with an apparent K_i of 5.4 ± 0.5 nM (Fig. 1*A*). Free BC had no effect on tPDE activity at tested concentrations up to 20 μM (not shown). This suggests that when the Pγ C-terminal residues, Gly-Ile-Ile, are replaced with the fluorescent probe BC, the probe binds into a complimentary hydrophobic pocket on Pαβ and substantially restores the inhibitory potential of the Pγ-1–83Cys mutant. Furthermore, the apparent K_i for tPDE inhibition by Pγ-1–83BC was influenced by the cGMP concentration in the assay. Decrease in cGMP concentration from 3 to 0.3 mM resulted in a reduction of the apparent K_i value from 5.4 to 2.1 nM (Fig. 1*A*). In control experiments, labeling of wild-type Pγ with BC at Cys^{68} did not notably affect the ability of Pγ to inhibit tPDE (not shown). Both Pγ and PγBC fully inhibited tPDE with K_i values less than 0.25 nM (not shown), and the K_i values were not affected by the concentration of cGMP in the assay (not shown). Next we examined effects of Pγ-1–83BC and Pγ on K_m values of cGMP hydrolysis by tPDE. A K_m of 90 μM was calculated from the Michaelis-Menten plot for tPDE (Fig. 1*B*). This K_m is consistent with earlier estimates (17). In the presence of 3.5 and 8 nM of Pγ-1–83BC, the apparent K_m values were 215 and 480 μM, and V_{max} values were 95 and 80%, respectively (Fig. 1*B*). The inhibitory interaction between Pγ-1–83BC and Pαβ was not purely competitive with cGMP, because the V_{max} values were also affected by Pγ-1–83BC (Fig. 1*B*). In agreement with previous data (17), the addition of Pγ did not significantly change the K_m for cGMP hydrolysis by tPDE (not shown).

Binding of Pγ-1–83BC to Pαβ—Addition of tPDE to Pγ-1–83BC increased the fluorescence of the probe many fold in a dose-dependent manner (Fig. 2). The binding curve shows a single class of binding sites with K_d = 4.0 ± 0.5 nM and a maximal fluorescence enhancement F/F_0 = 8.4 ± 0.2. No

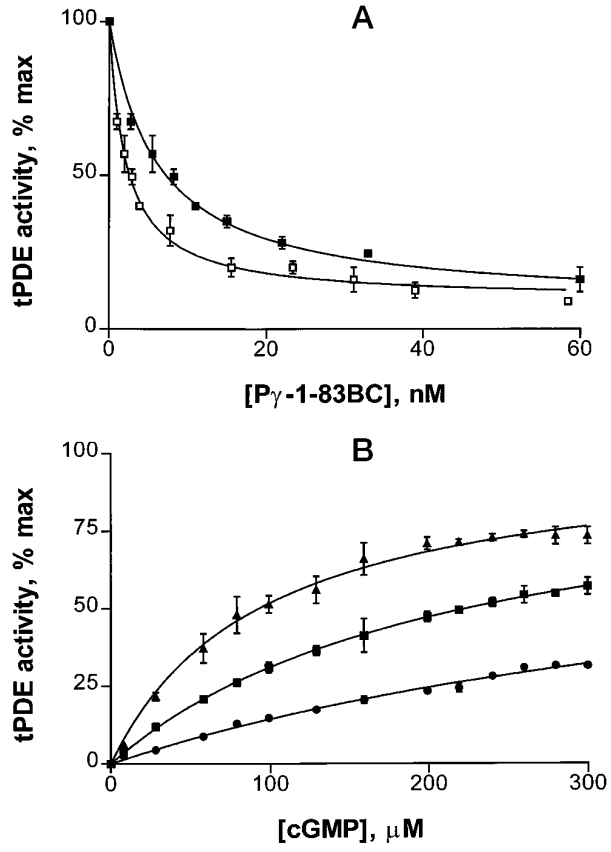

FIG. 1. *A*, inhibition of tPDE activity by Pγ-1–83BC. The activity of tPDE (5 nM) was determined upon addition of increasing concentrations of Pγ-1–83BC. The reaction was initiated by addition of 3 (■) or 0.3 mM cGMP (□). The percentage of maximal tPDE activity (3500 mol $cGMP \cdot s^{-1} \cdot mol\ PDE^{-1}$ at 3 mM cGMP and 2700 mol $cGMP \cdot s^{-1} \cdot mol\ PDE^{-1}$ at 0.3 mM cGMP) is plotted as a function of the free Pγ concentration. The inhibition curves (■, K_i = 5.4 ± 0.5 nM; □, K_i = 2.1 ± 0.2 nM) each fit the data with r value of 0.98. *B*, effect of Pγ-1–83BC on the apparent K_m values for cGMP hydrolysis by tPDE. The activity of tPDE (2.5 nM) alone (▲) or in the presence of 3.5 (■) and 8 nM of total Pγ-1–83BC (●) was determined at varying concentrations of cGMP. The curves (▲, K_m = 90 ± 10 μM; ■, K_m = 215 ± 23 μM, V_{max} = 95%; and ●, K_m = 480 ± 76 μM, V_{max} = 80%) fit the Michaelis-Menten equation with r values of 0.99, 0.99, and 0.98, respectively.

change in the fluorescence of Pγ-1–83BC was detected upon the addition of holoPDE or tPDE reconstituted with Pγ. Furthermore, addition of Pγ to the Pγ-1–83BC-Pαβ complex readily reduced fluorescence to a basal F_0 level, suggesting that the fluorescent increase reflects a specific interaction between Pαβ and Pγ-1–83BC (not shown). Fluorescence of PγBC was not notably affected in the presence of tPDE. It appears that in the PγBC-Pαβ complex the probe is oriented away from the Pαβ subunits, whereas in the Pγ-1–83BC-Pαβ complex the probe occupies the pocket for binding of the Pγ C terminus.

Effects of Zaprinast on the Interaction between Pγ-1–83BC and Pαβ—Zaprinast is a well characterized competitive inhibitor of photoreceptor PDEs and cGMP-binding, cGMP-specific PDE (19, 20). We have investigated effects of zaprinast on the interaction between Pγ-1–83BC and Pαβ. Zaprinast had no effect on the basal fluorescence of Pγ-1–83BC. Addition of increasing concentrations of zaprinast resulted in a complete reversal of the fluorescent enhancement of Pγ-1–83BC bound to Pαβ (IC_{50} of 0.9 μM) (Fig. 3). Zaprinast was effective in blocking the Pγ-1–83BC-Pαβ interaction within a pharmacologically relevant range of concentrations. A K_d value of 140 nM for zaprinast binding to rod PDE catalytic sites was calculated based on inhibition of PDE activity by zaprinast (19). Our assay does not allow us to calculate the true K_d value for the zapri-

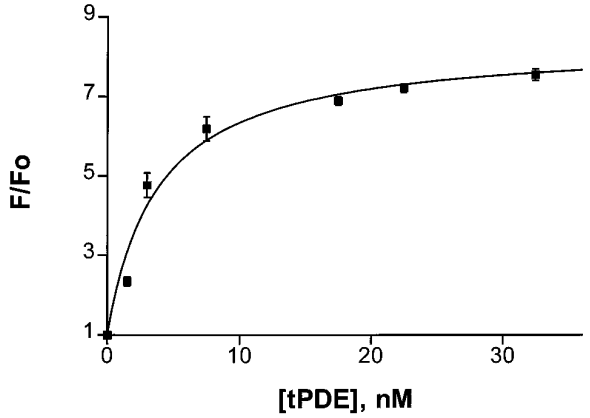

FIG. 2. **Binding of Pγ-1–83BC to tPDE.** The relative increase in fluorescence (F/F_0) of Pγ-1–83BC (10 nM) was determined after the addition of increasing concentrations of tPDE and is plotted as a function of the free tPDE concentration. The binding curve (K_d = 4.0 ± 0.5 nM, maximum F/F_0 = 8.4 ± 0.2) fits the data with r = 0.98.

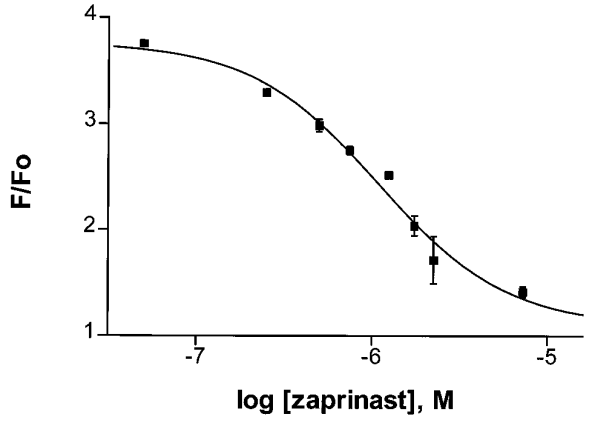

FIG. 3. **Competition between Pγ-1–83BC and zaprinast for binding to Pαβ.** tPDE (total concentration, 6 nM) was added to Pγ-1–83BC (10 nM), and then the fluorescence was measured before and after the addition of increasing concentrations of zaprinast. The relative fluorescent change (F/F_0) is plotted as a function of zaprinast concentration. The curve (IC_{50} = 0.90 ± 0.02 μM and Hill slope = 1.1) fits the data with r = 0.98.

nast binding from the curve in Fig. 3 because zaprinast does not compete for the Pγ-24–45BC-Pαβ interaction (see below) and cannot completely displace Pγ-1–83BC from Pαβ. A K_d value for the zaprinast-PDE interaction lower than an IC_{50} value of 0.9 μM (Fig. 3) can be predicted.

To examine the effects of zaprinast on the apparent K_d of Pγ-1–83BC binding to Pαβ, the binding curves were obtained in the presence of different concentrations of zaprinast (Fig. 4). Increasing concentrations of zaprinast reduced the fluorescent enhancement of Pγ-1–83BC by Pαβ and increased the apparent K_d of the Pγ-1–83BC binding to Pαβ. The apparent K_d calculated from the binding curves in the presence of 2 and 4 μM of zaprinast were 14.7 ± 0.9 and 22.6 ± 1.3 nM, respectively. The decrease in fluorescence of the Pγ-1–83BC-Pαβ complex and the increase in apparent K_d values suggest that zaprinast competitively displaces the fluorescently labeled Pγ-1–83BC C terminus from the binding pocket on Pαβ. Dipyridamole, another potent competitive inhibitor of photoreceptor PDEs (19), was unsuitable for studies using our assay. Dipyridamole is highly fluorescent with maximal emission at 480 nm.

Effects of Zaprinast on the Interaction between Pγ-24–45BC and Pαβ—To investigate if zaprinast can compete for binding between the polycationic region of Pγ, Pγ-24–45, and Pαβ, we utilized an assay of interaction between a synthetic peptide,

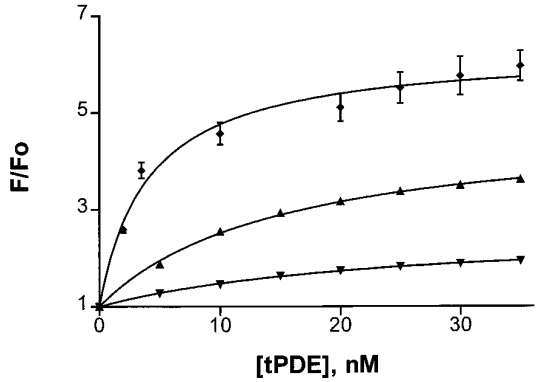

FIG. 4. **Effect of zaprinast on apparent K_d for Pγ-1–83BC binding to Pαβ.** Zaprinast was added to Pγ-1–83BC (10 nM) at indicated concentrations, and then the relative increase in fluorescence (F/F_0) of Pγ-1–83BC was determined after the addition of increasing concentrations of tPDE. The calculated apparent K_d values for Pγ-1–83BC binding to tPDE in the presence of 0.5, 2 and 4 μM of zaprinast were 5.1 ± 0.7, 14.7 ± 0.9, and 22.6 ± 1.3 nM, respectively. ♦, 5×10^{-7} zaprinast; ▲, 2×10^{-6} zaprinast; ▼, 4×10^{-6} zaprinast.

Pγ-24–45Cys, labeled with BC and tPDE (13). Zaprinast at concentrations that completely reversed the fluorescent enhancement of the Pγ-1–83BC-Pαβ complex had no effect on the fluorescent increase of Pγ-24–45BC caused by its binding to tPDE (not shown). At a higher concentration (10 μM), zaprinast reduced the maximal increase in fluorescence of the Pγ-24–45BC-Pαβ complex by only ~15% without affecting the K_d . The K_d values for Pγ-24–45BC binding to Pαβ in the presence of 10 μM of zaprinast or with no zaprinast added were indistinguishable (~26 nM) (not shown). Interestingly, the apparent K_d values for Pγ-1–83BC binding to Pαβ at higher concentrations of zaprinast (Fig. 4) approached the K_d for the Pγ-24–45BC-Pαβ complex. This supports the notion that zaprinast competes with the Pγ-1–83BC C terminus for binding to Pαβ and does not affect the interaction of the polycationic region, Pγ-24–45, with Pαβ.

Effects of cGMP and Its Analogs on the Interaction between Pγ-1–83BC and Pαβ—The relative potency of zaprinast in competition with the Pγ-1–83BC C terminus for binding to Pαβ indicated that cGMP and its analogs might be effective as well. Effects of cGMP on the Pγ-1–83BC-Pαβ interaction were tested in the presence or the absence of Mg^{2+}. Mg^{2+} participates in binding of cGMP to the PDE catalytic site and is critical for cGMP hydrolysis. In the presence of Mg^{2+}, cGMP reversed the fluorescent enhancement of Pγ-1–83BC bound to Pαβ with an apparent IC_{50} of 0.77 mM (Fig. 5*A*). Presumably, cGMP would be significantly more effective in the absence of cGMP hydrolysis. In the presence of EDTA, cGMP was ~2.5-fold less potent (IC_{50} of 1.9 mM, Fig. 5*A*). Because cGMP is not hydrolyzed in the presence of EDTA, the data suggest that Mg^{2+} enhances affinity of cGMP for the catalytic site by more than 2.5-fold. We also tested two cGMP analogs, 8-Br-cGMP and 2′-butyryl-cGMP, because they block ROS-PDE activity with a relatively high affinity (21). Both cGMP analogs competed with the Pγ-1–83BC C terminus for binding to Pαβ (Fig. 5*B*). The IC_{50} values from the competition curves for 8-Br-cGMP and 2′-butyryl-cGMP were 0.42 mM and 0.58 mM, respectively. Perhaps, an IC_{50} for 2′-butyryl-cGMP was higher than an IC_{50} for 8-Br-cGMP because tPDE hydrolyzed 2′-butyryl-cGMP with a rate of ~15% of cGMP hydrolysis, whereas 8-Br-cGMP was resistant to the hydrolysis (not shown).

DISCUSSION

Interaction between the inhibitory γ subunits of PDE and the catalytic Pαβ subunits is essential for blocking PDE activity in

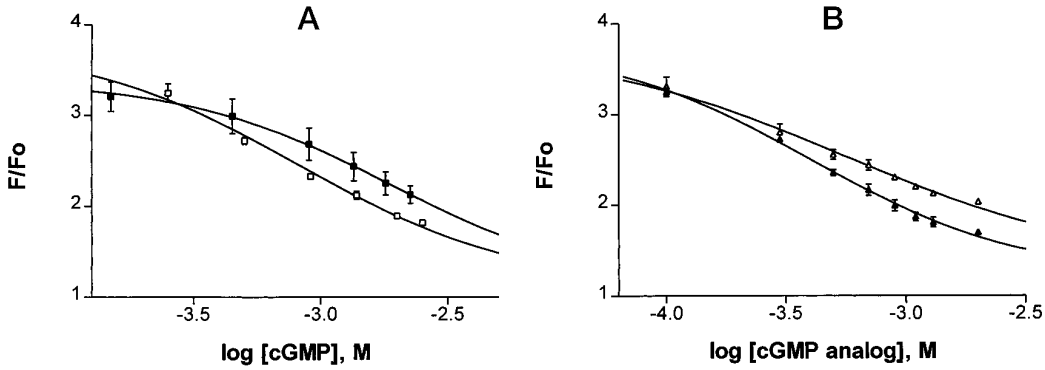

FIG. 5. **Effects of cGMP and its analogs on Pγ-1–83BC binding to Pαβ.** *A*, fluorescence of Pγ-1–83BC (10 nM) in the presence of tPDE (total concentration, 6 nM) was measured before and after addition of increasing concentrations of cGMP. An assay buffer contained 2 mM Mg^{2+} (□) or 1 mM EDTA instead of Mg^{2+} (■). The curves in the presence of Mg^{2+} (IC_{50} = 0.77 ± 0.01 mM; Hill slope = 0.9) and in the presence of EDTA (IC_{50} = 1.9 ± 0.3 mM; Hill slope = 1.1) fit the data with *r* values of 1.0 and 0.96, respectively. *B*, fluorescence of Pγ-1–83BC (10 nM) in the presence of tPDE (total concentration, 6 nM) was measured before and after addition of increasing concentrations of 8-Br-cGMP (▲) or 2′-butyryl-cGMP (△). The curves for 8-Br-cGMP (IC_{50} = 0.42 ± 0.03 mM; Hill slope = 1) and for 2′-butyryl-cGMP (IC_{50} = 0.58 ± 0.06 mM; Hill slope = 0.85) each fit the data with *r* value of 0.99.

the dark and for inactivation of the enzyme upon recovery of the photoreceptor cell from light stimulation. The Pγ subunits bind to Pαβ with very high affinity (K_d< 100 pM) (22). The high affinity of the Pγ-Pαβ interaction is provided by two major binding sites on Pγ, the central polycationic region, Pγ-24–45, and the C-terminal 5–7 amino acid residues (5–9). The main role of the Pγ-24–45 region is to enhance the affinity of Pγ interaction with Pαβ. The C terminus of Pγ is critical for PDE inhibition. Truncations of the Pγ C-terminal residues lead to a loss of the Pγ inhibitory function (5, 8, 9). Peptides corresponding to the C-terminal region of Pγ can fully inhibit PDE activity (6, 7, 9). Recently, we have shown that the C-terminal region of Pγ binds within the catalytic domain of PDE (10). This finding raised the possibility that Pγ may inhibit PDE activity by physically blocking the binding site for cGMP. An alternative mechanism of PDE inhibition by Pγ would be a local conformational change of the PDE catalytic site that prevents cGMP hydrolysis. Standard analysis for competitive (noncompetitive) inhibition of cGMP hydrolysis may not discriminate between the two mechanisms because Pγ binds to Pαβ very tightly (K_d <100 pM) compared with cGMP binding (K_m for cGMP is within 17–80 μM range) (17, 19, 22). The large differences in affinity and very slow off-rates of Pγ from Pαβ (>10 min) (8, 22) may not allow cGMP to compete with Pγ bound to Pαβ. It has been shown previously that the addition of Pγ to tPDE caused very little change in the apparent K_m value (17). Indeed, in our experiments the K_m value of 90 μM was unaffected by the addition of Pγ.

To study the mechanism of PDE inhibition by Pγ, we developed an assay that reports binding of the Pγ C terminus to Pαβ. The assay utilizes a Pγ mutant with the C-terminal amino acid residues replaced with a fluorescent probe, BC. The fluorescently labeled mutant, Pγ-1–83BC, was a more potent inhibitor of PDE activity than the unlabeled mutant, Pγ-1–83Cys, suggesting that the probe interacts with the inhibitory pocket on Pαβ. Addition of Pγ-1–83BC to Pαβ led to a dose-dependent increase of the apparent K_m values for cGMP hydrolysis. Binding of Pγ-1–83BC to Pαβ produced a large ~8-fold increase in the probe fluorescence. Zaprinast, a specific competitive inhibitor of photoreceptor PDEs, effectively competed for the interaction between Pγ-1–83BC and Pαβ, but had no effect on binding of the polycationic region, Pγ-24–45, to Pαβ. Perhaps the fact that Pγ-1–83BC binds to Pαβ (K_d of 4 nM) less tightly than Pγ has helped zaprinast to compete for the Pγ-1–83BC-Pαβ interaction. cGMP and its analogs, 8-Br-cGMP and 2′-butyryl-cGMP, were also effective in blocking the interaction between the Pγ-1–83BC C terminus and Pαβ using the fluorescent assay. Effects of cGMP and its analogs on the Pγ-1–83BC binding to Pαβ cannot be attributed to the noncatalytic cGMP-binding sites of PDE, because bovine rod PDE contains two molecules of tightly bound cGMP with an extremely slow off-rate ($t_{1/2}$ = ~4 h) (23).

Overall, our data strongly suggest that Pγ inhibits PDE activity by physically blocking access of the substrate, cGMP, to the PDE catalytic site. The region of Pα, Pα-751–763, that interacts with the C terminus of Pγ (10) is adjacent to the NKXD motif. In G-proteins, the NKXD motif specifies binding of the GTP guanine ring (24, 25). Based on our results, it is likely that the NKXD motif is involved in the binding of cGMP by photoreceptor PDEs.

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The γ Subunit of Rod cGMP-Phosphodiesterase Blocks the Enzyme Catalytic Site

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