Direct Allosteric Regulation between the GAF Domain and Catalytic Domain of Photoreceptor Phosphodiesterase PDE6*  

Photoreceptor cGMP phosphodiesterase (PDE6) is the central enzyme in the visual transduction cascade. The PDE6 catalytic subunit contains a catalytic domain and regulatory GAF domains. Unlike most GAF domain-containing cyclic nucleotide phosphodiesterases, little is known about direct allosteric communication of PDE6. In this study, we demonstrate for the first time direct, inter-domain allosteric communication between the GAF and catalytic domains in PDE6. The binding affinity of PDE6 for pharmacological inhibitors or for the C-terminal region of the inhibitory γ subunit (Pγ), known to directly inhibit PDE6 catalysis, was increased ~2-fold by ligands binding to the GAF domain. Binding of the N-terminal half of Pγ to the GAF domains suffices to induce this allosteric effect. Allosteric communication between GAF and catalytic domains is reciprocal, in that drug binding to the catalytic domain slowed cGMP dissociation from the GAF domain. Although cGMP hydrolysis was not affected by binding of Pγ1–60, Pγ lacking its last seven amino acids decreased the Michaelis constant of PDE6 by 2.5-fold. Pγ1–60 binding to the GAF domain increased vardenafil but not cGMP affinity, indicating that substrate- and inhibitor-binding sites do not totally overlap. In addition, prolonged incubation of PDE6 with vardenafil or sildenafil (but not 3-isobutyl-1-methylxanthine and zaprinast) induced a distinct conformational change in the catalytic domain without affecting the binding properties of the GAF domains. We conclude that although Pγ-mediated regulation plays the dominant role in visual excitation, the direct, inter-domain allosteric regulation described in this study may play a feedback role in light adaptational processes during phototransduction.

The photoreceptor cyclic nucleotide phosphodiesterase (PDE6) is the central enzyme in the vertebrate visual signaling pathway in rods and cones. Phototransduction is initiated when light induces the isomerization of the 11-cis-retinal chromophore of rhodopsin, which leads to activation of the photoreceptor-specific G-protein, transducin. Activated transducin then causes activation of PDE6, which results in rapid lowering of cGMP levels, closure of cGMP-gated ion channels, and hyperpolarization of the cell membrane (1–3). Hydrolysis of cGMP by PDE6 must be precisely regulated to control the amplitude and kinetics of the photoreponse. Furthermore, each of these parameters undergoes additional modulation in response to ever-changing conditions of ambient illumination.

The PDE6 holoenzyme consists of a catalytic dimer of α and β subunits (Pαβ) and two inhibitory γ subunits (Pγ) that are tightly bound to Pαβ. Transducin activation of PDE6 results from displacement of the inhibitory constraint of Pγ upon activated transducin binding to PDE6. The affinity of Pγ for the Pαβ catalytic dimer is also modulated in a reciprocal manner by noncatalytic cGMP binding to PDE6 at sites distinct from the catalytic site (Ref. 4 and reviewed in Ref. 5).

Photoreceptor PDE6 is one of five members of the class I phosphodiesterase superfamily that contain tandem regulatory GAF domains (i.e. GAFa and GAFb; (6)). The GAF domains were originally named for their presence in cGMP-regulated PDEs, certain adenylyl cyclases, and the transcription factor Hh1A of bacteria (7). The GAF domains of the vertebrate PDE members contain a functional cyclic nucleotide binding pocket. cGMP is the ligand for PDE2, PDE5, PDE6, and PDE11 (8–13), whereas cAMP is the ligand for PDE10 (13). For PDE6, the noncatalytic cGMP-binding site has been localized to the N-terminal GAFa domain (14, 15) (Fig. 1A).

Direct allosteric regulation of catalytic activity induced by binding of cyclic nucleotides to the GAF domains has been well documented for PDE2 and PDE5. For both PDE families, cGMP binding to the GAF domains induces a conformational change that relieves inhibition of catalysis in the active sites, causing stimulation of the enzyme (10, 16, 17). Furthermore, the binding affinity of inhibitors to the catalytic domains of PDE5 is increased by cGMP addition (18, 19). As predicted, this allosteric regulation between the GAF and catalytic domains is reciprocal. For example, in PDE5, some inhibitors enhanced cGMP binding to the GAF domains (11, 20). In addition to this direct allosteric communication between GAF and catalytic domains, it has been reported that PDE5 inhibitors can induce a conformational change in the catalytic domain that enhances inhibitor binding affinity in a time-dependent manner (21).

Based on the many similarities between PDE5 and PDE6 (22), direct, inter-domain allosteric communication between the GAF and catalytic domains is predicted for PDE6. However, previous work evaluating whether cGMP binding could influ-
ence the catalytic properties of PDE6 has not revealed a direct parallel to the allosteric control exerted on PDE5. For example, cGMP binding to the GAF domains fails to alter either the $K_{on}$ or $K_{cat}$ of the enzyme (4, 23, 24). Instead, attention has focused on the allosteric control mediated by Py on both GAF and catalytic domains. The N-terminal region of Py (Fig. 1B) is known to interact with the GAF domains of the Paβ catalytic dimer with a 50-fold higher affinity than the affinity of the C-terminal region of Py for the catalytic domain of Paβ (24–28). The C-terminal region of Py (Fig. 1B) is responsible for blocking the catalytic activity by binding to the catalytic domains of Paβ (27–31). The ability of Py to interact with both GAF and catalytic domains of Paβ serves to allosterically link the regulatory and catalytic domains of PDE6 in two ways as follows: 1) Py binding to the catalytic dimer enhances the binding affinity of cGMP to the GAF domain (32, 33); 2) cGMP occupancy of the GAF domain enhances Py affinity to Paβ (4, 23, 24).

In this study, we first document that direct allosteric communication between the GAF domains and catalytic domains of PDE6 does indeed occur. Ligand binding to the GAF domains enhances the affinity of inhibitors and Py63–87 (i.e. amino acids 63–87 of the Py sequence) binding to the catalytic dimer. This inter-domain allosteric mechanism is reciprocal, in that inhibitor binding to catalytic domains increases the binding affinity of cGMP to the GAF domains. The magnitude of this direct allosteric regulation in PDE6 is comparable with that seen in PDE5, and it may play a role in modulating PDE6 activity during persistent activation of rod photoreceptors that occurs during normal daytime illumination conditions.

**EXPERIMENTAL PROCEDURES**

**Materials**—Bovine retinas were purchased from W. L. Lawson, Inc. Synthetic peptide Py63–87 was purchased from New England Peptide. Vardenafil and sildenafil were provided by Bayer Healthcare AG. Ultima Gold scintillation fluid was from PerkinElmer Life Sciences. Filtration membranes were from Millipore, bicinechoninic acid protein assay reagents were from Pierce, and all other chemicals were from Sigma. Stock solutions of PDE inhibitors were prepared in DMSO and diluted to less than 1% final concentration before use in assays.

**PDE6 and Paβ Purification and Functional Assays**—Bovine rod PDE6 was purified from bovine retinas as described (34). Paβ catalytic dimers lacking Py were prepared by limited trypsin proteolysis and re-purified by gel filtration chromatography prior to use (34). PDE6 catalytic activity was measured in 20 mM Tris, 10 mM MgCl$_2$, 0.5 mg/ml bovine serum albumin either with a phosphate release microplate assay or with a radiotracer assay (35). The PDE6 concentration was estimated based on the rate of cGMP hydrolysis of trypsin-activated PDE6 and a knowledge of the $k_{cat}$ of the enzyme (5600 mol of cGMP hydrolyzed per Paβ per s (36)); this estimate was validated by determining stoichiometric binding of $[^3H]cGMP$ to PDE6 with a filter binding assay (37). The inhibition potency (IC$_{50}$) of PDE5/6 inhibitors or Py63–87 was determined in the presence or absence of Py mutants using either 2 mM cGMP or 0.1 mM cAMP as substrates.

**Purification of Py and Py Mutants**—Several Py truncation mutants (Fig. 1C) were generated from the full-length coding sequence using standard methods, introduced into the pET11a (Novagen) expression vector, and nucleotide sequences were verified. Recombinant Py and mutants (Py1–45, Py1–60, and Py1–80) were expressed in *Escherichia coli* BL21(DE3). The bacterial extract was partially purified by cation exchange chromatography using SP-Sepharose, followed by C4 reverse-phase high pressure liquid chromatography (38). The purity (≥95%) and size of these proteins were evaluated by SDS-PAGE. The inhibitory activity of Py was assessed by its ability to stoichiometrically inhibit Paβ catalytic dimers (2 Py per Paβ) (24). Protein concentrations were determined by the bicinchoninic acid protein assay (39) using bovine γ-globulin as a standard. Loading cGMP on the GAF Domains of Activated PDE6—Purified Paβ was preincubated with 10 mM EDTA in binding buffer (100 mM Tris, 2 mM MgCl$_2$, and 0.5 mg/ml bovine serum albumin) for 2 h at 22 °C to inhibit cGMP breakdown before addition of 1 μM $[^3H]cGMP$ and N-terminal region Py peptides (24). The amount of cGMP bound to PDE6 under these conditions was verified to be 1.7–2.0 cGMP per Paβ. The hydrolytic activity of PDE6 was restored by adding 10 mM MgCl$_2$ immediately prior to assaying cyclic nucleotide hydrolysis. For experiments in which $[^3H]cGMP$ dissociation kinetics from Paβ were measured in the absence of Py, purified Paβ was preincubated for 2 h with 10 mM EDTA plus 20 mM dipicolinic acid to abolish residual catalytic activity (40). EDTA alone was unable to protect $[^3H]cGMP$ from breakdown by Paβ (lacking Py peptides) during the binding assay.) Release of $[^3H]cGMP$ from Paβ was monitored following addition of 1 mM unlabeled cGMP (containing 10 mM MgCl$_2$ and 5 mM ZnSO$_4$), and the time course of $[^3H]cGMP$ dissociation was monitored in the presence or absence of 100 μM vardenafil.

**Data Analysis**—Dose-response experiments were analyzed using nonlinear regression analysis (Sigmplot) to fit experimental data to a three-parameter logistic dose-response function: $y = a/(1 + (x/x_b)^b)$, where $a$ is the amplitude, $b$ is the slope factor, and $x_b$ is the IC$_{50}$ (41). For other experiments, curve-fitting models are described in the figure legends. Except where noted, all experiments were repeated at least three times. Tests of statistical significance for the curve fitting results used the Student’s $t$ test to calculate probability values, as indicated in the figure legends.

**RESULTS**

**Binding of Ligands to the Regulatory GAF Domain Enhances the Ability of Vardenafil to Bind to the Catalytic Domain**—Motivated by the analogy of PDE6 with other GAF-containing PDEs, we first explored whether ligand binding to the GAF domains could allosterically alter the properties of the active site of PDE6.

Because both cGMP and the N-terminal region of Py are known to bind to the GAF domains of the PDE6 catalytic dimer (Paβ; see Introduction and Fig. 1), we first examined whether the catalytic properties of PDE6 were altered upon binding of these ligands to the GAF domains. The first 60 amino acids of Py (Py1–60) lack the ability to inhibit catalysis (data not shown), and the primary sites of interaction are confined to the GAF domains (28, 42). We therefore used the truncated mutant Py1–60 to determine whether the binding affinity of vardenafil
Immediate occupancy of the GAF domains by vardenafil 

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FIGURE 1. Interaction sites between catalytic and inhibitory subunits of PDE6 and domain organization of Pγ mutants. A, Catalytic α or β subunit of PDE6 consists of the regulatory domain and the catalytic domain. The regulatory domain includes two tandem GAF domains. The inter-domain allosteric communication between regulatory and catalytic domain is the subject of this study. The asterisk represents the prenylated, membrane-anchored C terminus of PDE6. B, 10-kDa Pγ inhibitory subunit has two major functional domains as follows: the proline-rich and polycationic region (amino acids 18–45, light gray box) serves as primary interaction sites with the GAF domains of the PDE6 catalytic dimer; the C-terminal domain of Pγ (amino acids 70–87, dark gray box) directly interacts with the catalytic domain to block cGMP hydrolysis. C, schematic diagram of the Pγ truncation mutants and the Pγ63–87 synthetic peptide used in this study.

FIGURE 2. Pγ1–60 binding to the PDE6 GAF domains enhanced vardenafil binding affinity to the catalytic sites. Purified Pαβ (2 nmol) was preincubated with 10 mM EDTA for 2 h to inhibit PDE activity, followed by incubation with 1 μM cGMP and 2 μM Pγ1–60 (●), 2 μM Pγ1–60 only (▲), or no addition (▲). 10 mM MgCl2 was then added to restore catalytic activity, and the inhibitory potency of vardenafil was measured using 0.1 mM cAMP as substrate with the radiotracer assay (see “Experimental Procedures”). cGMP binding assays confirmed retention of bound cGMP during the experiment. The data are the mean (±S.E.) of five experiments. The solid lines represent the fit to a three-parameter logistic dose-response equation with IC50 values of 4.3 ± 0.3 nM (no addition), 2.2 ± 0.2 nM (Pγ1–60), and 2.3 ± 0.1 nM (Pγ1–60 plus cGMP). The asterisks indicate that the IC50 value was statistically significant (p < 0.05) from the control value.

to the catalytic domain of Pαβ was altered when Pγ1–60 or Pγ1–60 plus cGMP were bound. To evaluate catalytic activity while simultaneously testing the effect of occupancy of the GAF domains, cAMP was used as a substrate for this experiment because it binds very poorly to the GAF domain even at high concentrations (12). Fig. 2 demonstrates that the inhibitory potency of vardenafil was increased ~2-fold (IC50 shifted from 4.3 ± 0.3 to 2.2 ± 0.2 nM) by Pγ1–60 binding to the GAF domain. Experiments using a shorter N-terminal fragment of Pγ (Pγ1–45) showed the same 2-fold enhancement of vardenafil binding as were seen with Pγ1–60 (data not shown), confirming that the GAF-interacting region of Pγ (localized to amino acid residues 18–45 (24)) was responsible for this inter-domain allosterism observed in Fig. 2.

Inclusion of cGMP with Pγ1–60 so as to occupy the GAF cGMP binding pocket failed to further enhance the allosteric effect on vardenafil binding to the Pαβ active site (Fig. 2). (Unfortunately, we were unable to directly measure the allosteric effects of cGMP binding in the absence of the Pγ N-terminal region, because the high catalytic rate of Pαβ led to hydrolysis of cGMP unless GAF-interacting Pγ peptides were present to stabilize cGMP binding to the GAF domain.) It is possible that either Pγ binding to the GAF domain or cGMP occupancy of the GAF binding pocket induce the same conformational change in the GAF domains that is transmitted to the catalytic domain.

Binding of the N-terminal Region of Pγ to the GAF Domains Enhances the Binding Affinity of Pγ63–87 to the Catalytic Domain—The C-terminal region of Pγ inhibits PDE6 catalysis by interacting with amino acid residues lining the entrance to the catalytic pocket (43); these residues are predicted to be distant from those stabilizing vardenafil binding to the active site (44). Therefore, we questioned whether binding of the N-terminal region of Pγ to the GAF domains could alter the binding affinity of the C-terminal region of Pγ to the catalytic domains of Pαβ. Fig. 3 shows that the dose-response curve for Pγ63–87 inhibition of cGMP hydrolysis was shifted about 2-fold when Pγ1–45 or Pγ1–60 was bound to the GAF domains. The finding that both vardenafil and Pγ63–87 affinity were increased ~2-fold when the N-terminal half of Pγ bound to the PDE6 GAF domains indicates that this inter-domain allosteric change is likely to affect the global conformation of the PDE6 catalytic domain.

Conformational Changes in Substrate Affinity to the Active Site Require Pγ Interactions with the Catalytic Domain, Not the GAF Domains—These novel allosteric effects of Pγ on vardenafil and Pγ63–87 binding to PDE6 differ from earlier work in which cGMP and/or Pγ1–45 binding to the GAF domains failed to allosterically alter the kinetic parameters for substrate hydrolysis of the Pαβ active site (4, 24). This is unexpected, because cGMP and vardenafil are likely to share some interaction sites within the catalytic pocket, as judged by comparison of crystal structures of PDE5 complexed with 5′-GMP and vardenafil (44, 45). To examine this more closely, we measured the Michaelis constant (Km) for cGMP in the presence of two Pγ truncation mutants, Pγ1–60 and Pγ1–80. Pγ1–60 was chosen because it cannot inhibit catalysis, and its primary sites of
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Interaction with Paβ are within the GAF domain, whereas Py1–80 partially inhibits catalysis (∼60% reduction in $V_{\text{max}}$; see Ref. 30) and has been shown to interact with sites within the catalytic domain (28, 42) (see Fig. 1). Whereas Py1–60 failed to affect the $K_m$ (or $V_{\text{max}}$) value for cGMP hydrolysis (Fig. 4) (consistent with previous work with Py1–45 (24)), binding of Py1–80 to Paβ increased the $K_m$ value for cGMP from 9 ± 0.9 to 23 ± 1.5 μM (Fig. 4). The differences in how cGMP (Fig. 4) and vardenafil (Fig. 2) are affected by Py binding to Paβ may reflect a local conformational change within the catalytic domain upon binding amino acids 61–80 of Py that is distinct from the inter-domain communication between the GAF and catalytic domains that is induced by the GAF-interacting region of Py.

Binding of Vardenafil to the Catalytic Domains Enhanced cGMP Binding to the GAF Domains—Having established in the previous sections the direct allosteric regulation by the GAF domains on the catalytic domain of PDE6, the principle of allosteric linkage (46) requires that this allosteric communication be reciprocal; occupancy of the active site should induce conformational changes in the GAF domains. In the earliest study of cGMP binding to the GAF domains of PDE6, the nonspecific PDE inhibitor 3-isobutyl-1-methylxanthine (IBMX) was reported to allosterically enhance cGMP binding to partially purified, nonactivated frog PDE6 (9). To examine this behavior with our highly purified, activated bovine Paβ catalytic dimer, we took special precautions to prevent [3H]cGMP breakdown with our highly purified, activated bovine Paβ catalytic dimer, and we took special precautions to prevent [3H]cGMP breakdown with our highly purified, activated bovine Paβ catalytic dimer, and we took special precautions to prevent [3H]cGMP breakdown with our highly purified, activated bovine Paβ catalytic dimer.
The idea that binding of PDE inhibitors to the catalytic domain of the PDE6 catalytic dimer can induce an allosteric change in the cGMP binding pocket located within the GAFα domain that enhances cGMP binding affinity.

A Second Conformational Change Occurs upon Vardenafil Binding—Because PDE5 undergoes a slow, time-dependent conformational change in its catalytic domain upon drug binding to the active site (21), we sought evidence for a similar allosteric effect on PDE6. Fig. 6 shows that both vardenafil and sildenafil, but not zaprinast or IBMX, increase their own affinities for the active site of PDE6 by 2-fold when incubated for 16 h compared with a 20-min drug incubation. The ability of sildenafil (Fig. 6A), but not zaprinast (Fig. 6B), to induce this shift in inhibitory potency cannot be ascribed to the overall affinity of drug for the active site, because both drugs have similar inhibition constants for rod PDE6 (48), but rather must reflect differences in how the inhibitors contact residues within the active site.

FIGURE 6. Certain PDE inhibitors induced a slow conformational change in the catalytic domain. 0.2 nM PDE6 was incubated with the indicated PDE inhibitors for 20 min (closed symbol) or overnight (open symbol) before assaying catalytic activity with 2 μM cGMP. The data (mean ± S.E., n = 3) were fit to the logistic equation with the following IC50 values: A, vardenafil, 0.3 ± 0.02 μM (20 min incubation) and 0.2 ± 0.02 μM (overnight incubation); sildenafil, 3.0 ± 0.2 μM (20 min) and 2.0 ± 0.1 μM (overnight); B, zaprinast, 5.8 ± 0.2 μM (20 min) and 5.6 ± 0.2 μM (overnight); IBMX, 1.2 ± 0.08 μM (20 min) and 1.2 ± 0.01 μM (overnight). The asterisk indicates that the IC50 value for overnight incubation of drug was statistically significant (p < 0.05) from the corresponding 20-min incubation.

To test whether this slow conformational change in the catalytic domain is transmitted allosterically to the GAF domain, we examined cGMP dissociation after preincubating PDE6 with vardenafil overnight. We found that cGMP dissociation rates were the same regardless of the length of time PDE6 was incubated with vardenafil (data not shown). We conclude that this slowly achieved conformational change in the catalytic domain is not communicated to the cGMP-binding site on the GAFα domain of PDE6.

DISCUSSION

This study represents the first demonstration that photoreceptor PDE6 has the ability to undergo allosteric regulation within its catalytic subunits. For over 2 decades it was thought that unlike other GAF domain-containing PDEs, the catalytic properties of PDE6 are not affected by direct allosteric regulation by the cGMP-binding GAF domains. However, our experiments reveal that there is a mutual allosteric reciprocity between the GAF and catalytic domains. The binding of the N-terminal portion of PDE6 to the GAF domains alters the catalytic domain conformation, resulting in enhanced affinity of drugs that occupy the active site as well as enhancing the affinity of the C-terminal portion of PDE6 that binds to amino acid residues in the vicinity of the active site. Conversely, binding of drugs to the active site induces a conformational change in the GAF domains that enhances their binding affinity for cGMP. This direct allosteric regulation occurs in addition to PDE6 activation, and may represent a secondary mechanism for its catalytic control.

Analogies between PDE5 and PDE6 Allostery—Direct allosteric control of catalytic activity is well documented for PDE5 (see the Introduction). Binding of cGMP to the GAF domain of PDE5 increases cGMP hydrolysis or inhibitor binding affinity by 2–5-fold (17–19). Conversely, cGMP binding affinity to the GAF domains of PDE5 is enhanced 2-fold by inhibitors binding to the catalytic domain (11, 49). The magnitude of inter-domain allosteric regulation reported for PDE5 is thus comparable with what we describe in this study for PDE6.

However, there are two major differences between the allosteric relations within PDE5 and PDE6. First, whereas the cross-regulation of the catalytic and noncatalytic domains in PDE5 extends to both its natural substrates (cyclic nucleotides) and inhibitors, this regulation in PDE6 is restricted only to inhibitors (both its regulatory Py subunit and chemical compounds such as sildenafil and vardenafil). It appears that in the case of PDE6, direct allosteric regulation by the GAF domain induces a conformational change in the catalytic domain that is sensed by inhibitors but not by substrates. The ability of Py1–80, but not Py1–60, to shift the Michaelis constant for cGMP hydrolysis (Fig. 4) suggests that local conformational changes can be induced by the C-terminal domains of Py that do alter the substrate binding pocket. We will discuss the potential structural basis for this below.

A second major difference between PDE5 and PDE6 is that PDE5 activity is also regulated by phosphorylation of its N-terminal region. Phosphorylation of serine 102 in human PDE5 induces a conformational change in the neighboring GAFα domain, resulting in a 4–10-fold increase in binding affinity of
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cGMP (50, 51). This conformational change is further communicated to the catalytic domain, as judged by 2-fold elevated cGMP hydrolytic rate (51–53). This mode of regulation has not been documented for PDE6. Instead, PDE6 has evolved a quite distinct mechanism for regulating its catalytic activity. PDE6 relies on a separate regulatory protein, Py, whose binding is regulated by activated transducin as well as by occupancy of the PDE6 GAFa domains by cGMP (reviewed in Refs. 5, 54).

Structural Basis for the Conformational Changes in the Catalytic Domain of PDE6—Three distinct effects on the catalytic domain of PDE6 have been observed in this study as follows: 1) a reciprocal, ligand-mediated allosteric communication between the cGMP-binding GAF domains and the catalytic domain; 2) an elevation of the $K_m$ value for cGMP hydrolysis induced only by the Py1–80 truncation mutant; and 3) a slowly occurring intra-domain increase in vardenafil or sildenafil affinity that is not observed for zaprinast or IBMX. Lacking structural data for the PDE6 catalytic domain, we wondered whether the known PDE5 catalytic domain structure (44, 45, 55) might provide insights on the structural basis for these conformational changes. To examine this, we performed structural homology modeling by threading the PDE6 catalytic domain sequence onto the PDE5 catalytic domain crystal structures (56) containing either no ligand (57), bound vardenafil (58), bound IBMX (55), or bound 5'-GMP (45).

When comparing the unliganded model of the catalytic domain to that of the vardenafil-containing structure, these two structures are observed to have a very similar $\alpha$-helical domain structure overall. Variability in the conformation of the so-called M-loop and H-loop (defined in Ref. 55) suggests that these less structured loop regions are conformationally sensitive (57, 58). Py-interacting sites are also located in the vicinity of the M-loop (amino acids 750–760) (43, 59). Although both the M- and H-loops are believed to provide stabilizing contacts for drug binding, most of the residues interacting with the cGMP substrate are found in the H-loop. This interpretation is further supported by site-directed mutagenesis studies of PDE5, which suggest that residues in the H-loop are more important for stabilizing substrates than inhibitors (57). This might explain why cGMP affinity to the catalytic domains is not affected by Py1–60 (Fig. 4), whereas vardenafil or Py63–87 inhibition potency is increased by Py1–60 (Figs. 2 and 3). We speculate that interactions of Py with the PDE6 GAF domains may induce conformational changes that are transmitted to the H- and/or M-loops in the catalytic domain. In a reciprocal manner, inhibitor binding to the active site of PDE6 may induce conformational changes in the H- or M-loops that are communicated to the GAF domains to alter the conformation of the cGMP binding pocket in the GAFa domain (Fig. 5). Further work is clearly needed to determine the structural basis for the inter-domain allosterism we report for PDE6 in this study.

We also showed that certain inhibitors (i.e. vardenafil and sildenafil but not zaprinast or IBMX) induce a slowly developing increase in drug binding affinity (Fig. 6) that is not communicated to the GAF domains of PDE6. This slow shift in drug affinity was observed for PDE5 as well (21). The structure of IBMX-bound PDE5 shows that IBMX interacts primarily with residues in the vicinity of the M-loop but lacks significant contacts with the H-loop (55). In contrast, vardenafil binding to the catalytic domain of PDE5 causes movement of a portion of the H-loop toward the center of the drug-binding site, with no major change in M-loop structure (58). We speculate that the conformation of the PDE6 H-loop is affected by vardenafil but not IBMX binding, causing an intra-domain enhancement of drug binding affinity for vardenafil.

Physiological Implications of Direct Allosteric Regulation of PDE6—It is well established that transducin activation of PDE6 is the primary regulatory mechanism for the pathway of visual excitation that leads to hyperpolarization of the photoreceptor cell. Specifically, the PDE6 holoenzyme (aβγγ) becomes activated when activated transducin α subunit displaces the C-terminal region of Py. A likely scenario is that the terminal region of Py remains associated with PDE6 catalytic dimer upon transient activation of PDE6 (reviewed in Ref. 5). However, upon persistent illumination, PDE6 may remain activated by transducin for a sufficient time for the allosteric communication between the catalytic and GAF domains to trigger the dissociation of the N-terminal half of Py (and bound cGMP) from the GAF domains of an appreciable fraction of PDE6 molecules. Release of Py and dissociation of bound cGMP from the PDE6 catalytic subunits have been demonstrated previously to speed up the rate at which activated transducin hydrolizes its bound GTP, which results in a faster rate of transducin inactivation (and hence PDE6 re-inhibition (54)). In this way, allosteric communication between domains of the PDE6 catalytic dimer could influence the lifetime of activated PDE6 and provide a distinct mechanism of photoreceptor light adaptation characterized by shortened, less sensitive responses to light.

Mutations of PDE6 catalytic subunits are known to cause congenital stationary night blindness or retinal degeneration (reviewed in Ref. 5). Of particular interest to this study are those instances where mutations occurring outside the catalytic domain disrupt PDE6 function and lead to visual disturbances or retinal degeneration. One example is the mutation in GAFa (H258N) found in the Rambusch form of autosomal dominant congenital stationary night blindness (60) that alters the affinity of Py for the catalytic subunits of rod PDE6 (61, 62). We speculate that mutations within the GAF domains of PDE6 could also impair inter-domain allosteric communication between the GAF and catalytic domains of PDE6, possibly leading to disruptions in the visual signaling pathway and, ultimately, photoreceptor cell death.

Conclusion—This work is the first demonstration of direct allosteric communication within the PDE6 catalytic dimer, and it is consistent with allosteric regulation occurring with other PDE families that contain regulatory GAF domains. This inter-domain allosteric communication between the GAF domains and the catalytic domains is reciprocal, in that ligand binding to one domain alters the properties of the other domain. Unlike other PDE families, direct allosteric control of PDE6 is much less important than the G-protein-mediated regulation of PDE6 resulting from binding of the Py subunit to the PDE6 catalytic dimer. Although dis-inhibition of Py by transducin dominates PDE6 regulation during visual excitation, this newly discovered inter-domain communication between the GAF and catalytic domains may be relevant for fine-tuning the
extent and lifetime of PDE6 activation, particularly during conditions of prolonged light adaptation.

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