

Characteristics of Photoreceptor PDE (PDE6): similarities and differences to PDE5

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Phosphodiesterase 6 (PDE6) is highly concentrated in the retina. It is most abundant in the internal membranes of retinal photoreceptors, where it reduces cytoplasmic levels of cyclic guanosine monophosphate (cGMP) in rod and cone outer segments in response to light. The rod PDE6 holoenzyme comprises α and β catalytic subunits and two identical inhibitory γ subunits. Each catalytic subunit contains three distinct globular domains corresponding to the catalytic domain and two GAF domains (responsible for allosteric cGMP binding). The PDE6 catalytic subunits resemble PDE5 in amino-acid sequence as well as in three-dimensional structure of the catalytic dimer; preference for cGMP over cyclic adenosine monophosphate (cAMP) as a substrate; and the ability to bind cGMP at the regulatory GAF domains. Most PDE5 inhibitors inhibit PDE6 with similar potency, and electroretinogram studies show modest effects of PDE5 inhibitors on visual function—an observation potentially important in designing PDE5-specific therapeutic agents.
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PDE6, the central effector of visual transduction in rods and cones

The retina contains cones, which operate under daylight conditions and perform color discrimination, and rods, which operate in dim light. Three types of cones exist in humans and in many primates, each one with a peak absorption at a wavelength corresponding to blue, green, or red light. In mammals, rods comprise approximately 90% of the photoreceptors, and cones the remaining 10%.

Both rods and cones contain light-sensitive pigments (opsins) that photoactivate on exposure to light. The photoexcited visual pigments stimulate the cell membranes of the rods and cones, triggering signals transmitted to the inner retina and via the optic nerve into the brain. In the dark, a circulating current flows from the inner segment of the photoreceptor cell to the outer segment. When illumination occurs, processes in the outer segment of the photoreceptor cell interrupt this current. The plasma membrane of the outer segment encloses

a stack of several thousand physically separate disc membranes, on which the initial events of the visual transduction pathway occur. Changes in cGMP levels in the outer segment transmit this signal from the disc membrane to the plasma membrane.¹

PDE6 is the primary regulator of cytoplasmic cGMP concentration in rod and cone photoreceptors (Figure 1). In the dark, PDE6 exists in an inactive form, and cGMP levels in the rod outer segment are relatively high (several micromolar). This permits a fraction of the cGMP-gated ion channels in the plasma membrane to remain open, allowing a current to circulate through the photoreceptor cell. Photoexcitation of the visual pigment, rhodopsin, activates the photoreceptor G-protein, transducin. The activated α subunit of transducin binds to PDE6, and displaces the PDE6 inhibitory γ subunit from the active site of PDE6. The resulting subsecond drop in cGMP concentration causes closure of cGMP-gated ion channels, resulting in membrane hyperpolarization.^{2,3}

Precise regulation of cGMP levels is essential for normal operation of the visual transduction cascade. Indeed, a persistent imbalance in cGMP metabolism (either in its synthesis or degradation) will disrupt the visual signaling pathway and eventually lead to photoreceptor cell death and retinal degeneration (eg, retinitis pigmentosa).

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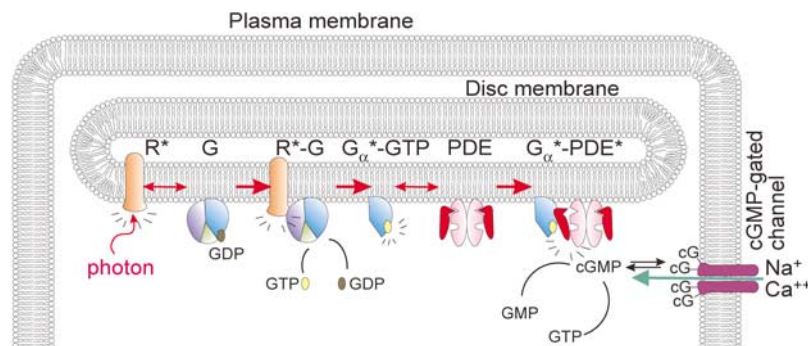


Figure 1 Visual excitation pathway in rod photoreceptors. The initial events of phototransduction occur on the physically separate disc membranes in the outer segment portion of the cell. Photoactivation of rhodopsin (R^*) catalyzes the activation of hundreds of heterotrimeric G-proteins (G). Nucleotide exchange on the G α -subunit is accelerated, and the dissociated G_{α}^* -GTP subunit then interacts with the PDE6 holoenzyme (PDE). Displacement of the PDE6 γ subunit from the catalytic site by G_{α}^* -GTP relieves the inhibition of catalysis at one catalytic subunit, resulting in rapid hydrolysis of cytoplasmic cGMP. The drop in cGMP levels in the outer segment causes dissociation of cGMP from the cGMP-gated ion channels in the plasma membrane, causing their closure. The reduced entry of cations into the outer segment causes membrane hyperpolarization, and ultimately, generation of the receptor potential at the photoreceptor synapse. Reactions involved in the recovery of the photoresponse and desensitization of the light response are not shown.

Subunit composition and structure of the PDE6 holoenzyme

The rod PDE6 holoenzyme is a tetramer consisting of α and β catalytic subunits to which two identical inhibitory γ subunits bind ($\alpha\beta\gamma_2$).^{4,5} Cone PDE6 differs from rod PDE6 in that its catalytic dimer is composed of two identical α' subunits. Also, the low molecular weight cone inhibitory γ' subunits differ slightly in size (9.4 vs 9.7 kDa) and amino-acid composition from the rod γ subunits.⁶

Each γ subunit interacts with at least two distinct sites on the catalytic subunit, and the affinity of this interaction is regulated by cGMP binding to the regulatory GAF domains of PDE6.^{7,8} When cGMP is bound to the GAF domain, the two γ molecules bind with different affinities to the catalytic dimer. When these regulatory sites are empty, both molecules of γ bind to $\alpha\beta$ with the same, albeit reduced, affinity. Two major domains on γ interact independently with the PDE6 catalytic dimer. The extreme C-terminal residues of γ bind directly to the active sites of PDE6, blocking access of substrate to the catalytic core.⁹ The N-terminal half of γ binds to $\alpha\beta$ with an affinity 50 times greater than its C-terminal half, and is responsible for the cGMP-dependent modulation of γ affinity by the GAF domain.⁸

While not yet fully elucidated, structural differences between the α and β catalytic subunits, differences in cGMP binding to the GAF domains, and cGMP-dependent modulation of γ affinity for $\alpha\beta$, all contribute to the precise regulation of the extent and lifetime of PDE6 activation during rod visual transduction.

Electron microscopic analysis of purified rod PDE6 catalytic dimers at 2.8 nm resolution reveals a three-dimensional (3-D) structure comprised of three distinct globular domains.¹⁰ The largest

domain represents the catalytic domain, while the two smaller domains correspond to the two tandem GAF domains. While the resolution in the analysis was insufficient to detect low molecular weight proteins bound to the PDE6 catalytic dimer, the results unequivocally show that the primary dimerization site of the α and β catalytic subunits resides in the N-terminal GAF α domain.

The molecular organization of PDE5 closely approximates that of PDE6, both in terms of its primary amino-acid sequence¹¹ and its 3-D structure¹⁰ (Figure 2). For both PDE families, the two GAF domains and the catalytic domain are each likely to fold into independent globular structures, as predicted by the crystal structures of the catalytic domain of PDE4,¹² and the tandem GAF domains of PDE2.¹³

Similarities and differences between PDE5 and PDE6

Of the 11 mammalian PDE families, not only is PDE6 most closely related to PDE5 structurally, but it also shares several similarities in its biochemical properties. Both PDE5 and PDE6 strongly prefer cGMP over cyclic adenosine monophosphate (cAMP) as a substrate at the catalytic site.¹⁴ The PDE5 and PDE6 catalytic mechanisms share a requirement for divalent cations, including high-affinity binding sites for zinc ions that likely serve a structural role as well.^{15,16} Both PDE families bind cGMP with high affinity at the regulatory GAF domains, and most PDE5-selective pharmacological inhibitors also potentially inhibit PDE6 catalysis.

However, there are several aspects of the enzymology and regulation of PDE6 that are not found

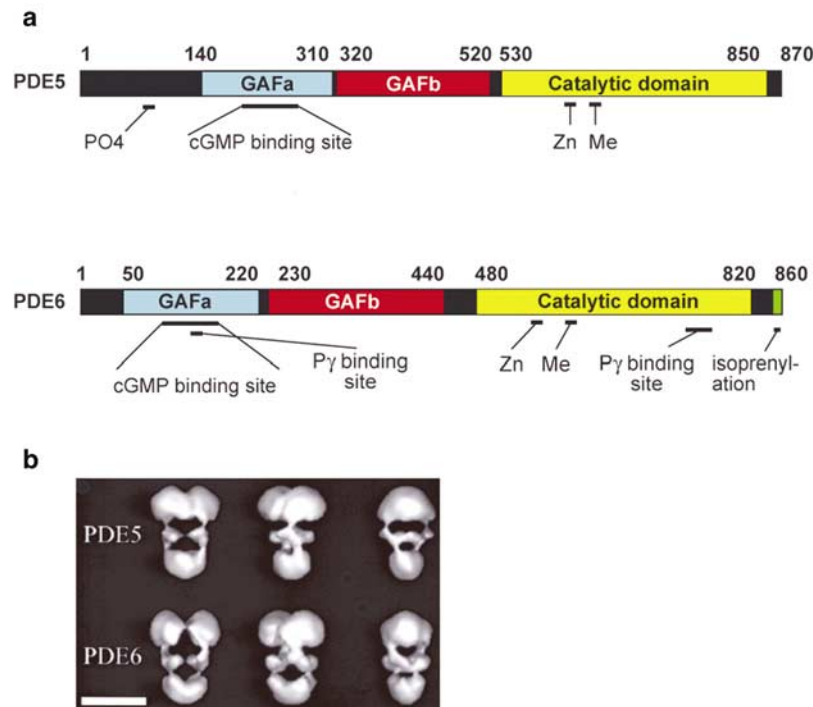


Figure 2 PDE5 and PDE6 share a similar domain organization and three-dimensional holoenzyme structure. (a) The domain organization of the catalytic subunits of PDE5 and PDE6 are compared. Both enzymes contain regulatory domains with tandem GAF domains that share significant sequence homology. For PDE5, the GAFa domain has been shown to be responsible for cGMP binding. The homologous domain for PDE6 is depicted but experimental proof is still lacking. In addition, PDE6 GAFa contains a region that interacts with the inhibitory γ subunit. The catalytic domains of PDE5 and PDE6 are also highly homologous, with a similar catalytic core containing metal ion binding sites. PDE6 contains a unique region at the entrance to the catalytic site responsible for inhibition of catalysis by γ binding. PDE5 is regulated by phosphorylation in the N-terminal region; no similar regulatory site has been identified for PDE6 to date. PDE6 contains a consensus sequence for farnesylation (α subunit) or geranylgeranylation (β subunit) at its C-terminus. (b) The 3-D surface representation of the PDE5 and PDE6 holoenzymes shows a high degree of conservation. The largest lobe at the top represents the catalytic domain. The primary dimerization site appears to be the GAFa domain, with the smaller GAFb domains linking GAFa and the catalytic domain. The low molecular weight γ subunit or the prenyl binding protein of PDE6 cannot be visualized at this resolution. The three images of each enzyme are 45° rotations in the vertical axis. The bar represents 10 nm. (Taken from Kameni *et al*, 2001; Figure 4.)

in PDE5. First, while the maximum rate of cGMP hydrolysis by PDE6 achieves catalytic perfection^{17,18,7} (6000–8000 cGMP hydrolyzed per second) and operates at the diffusion-controlled limit, the catalytic constant for PDE5 is lower by almost three orders of magnitude.¹⁹ This extraordinary catalytic power of PDE6 may have evolved from the need of photoreceptor cells to generate a receptor potential on the millisecond time scale. Second, displacement of the inhibitory γ subunit from the active site by activated transducin mediates the primary mechanism of PDE6 activation.³ For PDE5, enzyme activation most likely proceeds via phosphorylation of the catalytic subunits and allosteric changes in cGMP binding to the GAF domains.^{20,21} (While PDE5 has been reported to copurify with the PDE6 γ subunit,²² the physiological significance is uncertain because the γ subunit lacks binding or inhibitory activity toward PDE5 *in vitro*.²³)

Functional chimeric phosphodiesterases have been constructed using the GAF domains from cone PDE6 and the catalytic domain of PDE5 in order to

identify structural and functional differences of the two PDE families. The original PDE6/PDE5 chimera retained the catalytic properties of PDE5 and the cGMP binding properties of PDE6.²³ Site-directed mutagenesis using the PDE6/PDE5 chimera has identified two sites on PDE5 (Ala(608) and Ala(612)) that accelerate catalysis 10-fold when substituted for glycine residues (found at positions 562 and 566 of cone PDE6).²⁴ These residues are near the metal binding motif¹⁵ that represents the catalytic site of the enzyme. The unique sites of interaction of the γ subunit with the PDE6 catalytic sites have also been probed by substituting a stretch of cone PDE6 sequence (amino acids 737–784) into the catalytic domain of PDE5 and demonstrating that inhibition by the γ subunit occurred.²⁶ Three hydrophobic residues at the entry to the catalytic core (Met(758), Phe(777), and Phe(781)) have been found to stabilize γ subunit binding and promote enzyme inhibition.²⁴ Another γ -binding site has been identified in the GAFa domain of PDE6 that further stabilizes binding

of this inhibitory subunit.²⁵ These studies have provided insights into the unique features of transducin-activated PDE6 that distinguish PDE6 catalytic and regulatory properties from those of PDE5.

Regulation of PDE5 and PDE6 by post-translational modifications

PDE6 is unique among the 11 PDE families in that it undergoes a post-translational modification resulting in carboxymethylation and isoprenylation of the C-terminus of the catalytic subunits. The incorporation of a farnesyl group (rod PDE6 α subunit) or a geranylgeranyl group (rod PDE6 β subunit) accounts for the high-affinity interaction of rod PDE6 with photoreceptor membranes.^{27,28} A 17-kDa prenyl binding protein (PrBP), originally referred to as the 'delta' (δ) subunit of PDE6,²⁹ can bind to PDE6 and release the holoenzyme from its membrane-associated state.³⁰ PrBP principally interacts with PDE6 at its prenylated C-terminus.³¹ In contrast to the catalytic and inhibitory subunits of PDE6, PrBP is widely expressed in a variety of tissues.^{30,32} It is also highly conserved through evolution.³³ While PrBP has been shown to interact with many other binding partners,³⁴ neither PDE5 nor other phosphodiesterases have been reported to interact with this prenyl binding protein.

In PDE5, phosphorylation at serine 92 of the bovine enzyme correlates with enhanced catalytic activity of the enzyme as well as conformational changes in the regulatory GAF domains.^{20,35,36} The γ subunit of PDE6 also acts as a substrate for phosphorylation at several distinct sites within the central region of this 10 kDa protein. Phosphorylation of γ at Thr(22) or Thr(35) has little effect on the PDE6 holoenzyme itself, but greatly diminishes the ability of activated transducin to bind the γ subunit and relieve inhibition of catalysis.³⁷ Phosphorylation of γ at Thr(62) in nonretinal tissue has been reported to regulate mitogenic signaling via interactions with proteins other than PDE6 catalytic subunits (whose expression is confined to the retina and pineal gland).³⁸ Little information is available on potential regulation of PDE6 catalytic subunits by phosphorylation.

Drug selectivity for PDE5 and PDE6

One of the challenges in developing PDE5-specific inhibitors for therapeutic purposes is the similarity in the catalytic sites of PDE5 and PDE6 with respect to drug binding. For example, sildenafil is a highly selective inhibitor of PDE5 ($K_i = 4$ nM) with one exception, namely its potent inhibition of rod PDE6 ($K_i = 30$ nM).³⁹ This is physiologically relevant,

since one well-documented side effect of sildenafil treatment is a transient disturbance in visual function.⁴⁰ PDE5 inhibitors are not known to cross the blood-brain barrier but they do cross the blood-retina barrier. Electroretinogram studies have shown that PDE5 inhibitors exert a modest effect on visual function.⁴¹ While it is likely that visual disturbances induced by sildenafil are a direct consequence of PDE6 inhibition, other modes of action must also be considered.

Important concepts

- (1) *What are the differences in the drug interaction sites within the catalytic domains of PDE5 and of the various PDE6 isoforms?* Rational drug design of compounds, which better discriminate PDE5 from PDE6, requires a deeper understanding of the molecular architecture of the active sites of PDE5, rod PDE6, and cone PDE6. Improved specificity of PDE5 inhibitors will likely reduce or eliminate the adverse effects on visual function reported with current PDE5 inhibitors.
- (2) *Can the reported changes in visual function following PDE5 inhibitor administration be ascribed to action of the drug on PDE6 within cone photoreceptors?* While cones are 10-fold less abundant than rod photoreceptors in the human retina, alterations in color perception reported following sildenafil usage may reflect preferential targeting of cone PDE6. More specifically, further research is required to distinguish the susceptibility of each of the three classes of cone photoreceptor in the human retina to the action of PDE5 inhibition.
- (3) *Could the presence of PDE5 in ocular tissues account for some of the adverse side effects of PDE5 inhibitors?* We currently lack knowledge of the prevalence and functions of PDE5 in retinal cells, the retinal vasculature, and in other tissues of the eye. For example, it is as yet unknown to what extent the vasodilatory effects of PDE5 induce altered visual performance by increasing blood flow to the retina.
- (4) *Does the retina itself possess unique features that might alter the pharmacokinetics and bioavailability of PDE5 inhibitor delivery to the rod and cone photoreceptors, compared to the effects of these agents on other tissues and organs?* A paucity of data exist on how metabolism of PDE5 inhibitors might differ in the retina vs other tissues. We also require more information about the efficacy with which PDE5 inhibitors penetrate the retinal vasculature, are delivered to the photoreceptor layer of the retina, and subsequently enter the rod and cone photoreceptors to act on PDE6.

(5) *What are the short- and long-term consequences of PDE5 inhibitor therapy for individuals with retinal diseases, particularly retinitis pigmentosa and macular degeneration?* Obviously, people with genetic defects in rod or cone PDE6 do not qualify as candidates for PDE5 inhibitor therapy, since inhibition of PDE6 is likely to exacerbate abnormal cGMP metabolism within the photoreceptor cells of these individuals. Before PDE5 inhibitors can be utilized as long-term therapy in this subset of patients, the effects on retinal function must first be carefully evaluated.

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