Direct Interaction of the Inhibitory γ-Subunit of Rod cGMP Phosphodiesterase (PDE6) with the PDE6 GAFa Domains†

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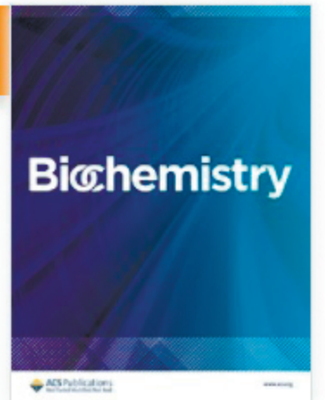

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Abstract

Retinal rod and cone cGMP phosphodiesterases (PDE6 family) function as the effector enzyme in the vertebrate visual transduction cascade. The activity of PDE6 catalytic subunits is controlled by the Pγ-subunits. In addition to the inhibition of cGMP hydrolysis at the catalytic sites, Pγ is known to stimulate a noncatalytic binding of cGMP to the regulatory GAFa–GAFb domains of PDE6. The latter role of Pγ has been attributed to its polycationic region. To elucidate the structural basis for the regulation of cGMP binding to the GAF domains of PDE6, a photoexcitable peptide probe corresponding to the polycationic region of Pγ, Pγ-21–45, was specifically cross-linked to rod PDE6αβ. The site of Pγ-21–45 cross-linking was localized to $Met^{138}Gly^{139}$ within the PDE6α GAFa domain using mass spectrometric analysis. Chimeras between PDE5 and cone PDE6α‘, containing GAFa and/or GAFb domains of PDE6α‘ have been generated to probe a potential role of the GAFb domains in binding to Pγ. Analysis of the inhibition of the PDE5/PDE6α‘ chimeras by Pγ supported the role of PDE6 GAFa but not GAFb domains in the interaction with Pγ. Our results suggest that a direct binding of the polycationic region of Pγ to the GAFa domains of PDE6 may lead to a stabilization of the noncatalytic cGMP-binding sites.