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Modulation of retinal transducin and phosphodiesterase activities by synthetic peptides of the phosphodiesterase γ-subunit

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Synthetic peptides corresponding to various regions of the light-activated guanosine 3′,5′-cyclic monophosphate phosphodiesterase (PDE) γ-subunit (PDEγ) from bovine retinal rod outer segments were synthesized and tested for their ability to inhibit PDE activity, and GTPase activity of transducin. One of these peptides, corresponding to PDEγ residues 31–45, inhibited PDE activity and GTPase activity in a dose-dependent manner. The GTPase activity was inhibited by PDEγ-3 non-competitively. This region of the PDEγ subunit may be involved in the direct interaction of transducin and PDE$\alpha\beta$ with PDEγ.

Phosphodiesterase; Transducin; Synthetic peptide; (Retina)

1. INTRODUCTION

Retinal rod outer segments contain a light-activated guanosine 3′,5′-cyclic monophosphate phosphodiesterase (PDE) which reduces the level of cGMP in the rod outer segment in response to light [1–3]. Accompanied by the decrease in cGMP is a decrease in the Na^+ conductance of the rod outer segment plasma membrane, which hyperpolarizes the rod [4]. There is considerable evidence suggesting that cGMP is responsible for, and directly connected to, the decrease in Na^+ flux through the rod outer segment plasma membrane [4–6].

Bovine rod outer segment PDE is made up of three subunits. The molecular masses are α = 88 kDa, β = 84 kDa and γ = 11 kDa, as determined by SDS-PAGE [7]. The molecular mass derived from the amino acid sequence of the γ-subunit (9.7 kDa) agrees reasonably well with that determined by SDS-PAGE [7,9]. The α-, β- and γ-subunits are present in a 1:1:1 ratio [7]. There is some evidence to indicate that the γ-subunit inhibits PDE activity [8]. Trypsin treatment of PDE leads to a degradation of the γ-subunit, paralleled by an increase in PDE activity [8]. The degree of increase in PDE activity is proportional to the amount of γ-subunit degraded [8]. Addition of purified γ-subunit to trypsin-treated PDE results in a 97% inhibition of PDE activity [8].

When PDE is light activated, the Tα subunit of transducin removes the γ-subunit of PDE from the α- and β-subunits of PDE [10]. The Tα subunit may bind to the α- and β-subunits of PDE, preventing the γ-subunit from reassociating [11].

Recently, the amino acid sequence of the bovine γ-subunit of PDE has been determined by direct

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Abbreviations: PDE (PDEα, PDEβ, PDEγ), light-activated guanosine 3′,5′-cyclic monophosphate phosphodiesterase (α-, β- and γ-subunits); cGMP, guanosine 3′,5′-cyclic monophosphate; Tα, Tβ, Tγ, α-, β- and γ-subunits of transducin; ROS, rod outer segment; SDS-PAGE, SDS-polyacrylamide gel electrophoresis; cDNA, complementary DNA; HPLC, high-performance liquid chromatography; PMSF, phenylmethylsulfonyl fluoride

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protein sequencing and cDNA sequencing [9]. This has enabled us to synthesize peptides corresponding to various regions of the γ-subunit. These peptides were tested for their ability to inhibit PDE and GTPase activities. Specific inhibition studies may help determine which regions of the γ-subunit interact with the α- and β-subunits of PDE and/or with transducin.

2. MATERIALS AND METHODS

Whole bovine eyes were obtained from local slaughterhouses. Tris, $MgCl_2$, and other reagents were from Sigma. [^{3}H]cGMP (19 Ci/mmol) and [γ-^{32}P]GTP (23.8 Ci/mmol) were from ICN Radiochemicals. The *tert*-butoxycarbonyl-amino acids used for peptide synthesis were from Vega Biochemicals and United States Biochemicals while other reagents for peptide synthesis were HPLC grade from Fisher Scientific.

2.1. *Purification of PDE and transducin*

ROS were purified by discontinuous sucrose density-gradient centrifugation under dim red light [12,13]. PDE was extracted by repeated washings under bright light with a buffer containing 10 mM Tris (pH 7.5), 0.1 mM 2-mercaptoethanol, and 0.1 mM PMSF. Transducin was recovered by repeated washings in the above buffer containing 10 μM GTP [12,13].

2.2. *Peptide synthesis and purification*

Peptides were synthesized manually using the solid-state method of Hodges and Merrifield [14], as modified by Gormann [15] except that cleavage of peptides and protecting groups was performed by treatment with HBr and anhydrous trifluoroacetic acid [16]. Peptides were purified and quantitated as in [12,13,17].

2.3. *PDE assay*

PDE activity was determined by the method of Thompson and Appleman [18]. The final concentrations of the reagents in the incubation mixture were 50 mM Tris (pH 7.4), 5 mM $MgCl_2$, 1 mM cGMP, 40 μM GTP, and [^{3}H]cGMP (150000 cpm/tube, spec. act. 19 Ci/mmol) in a final volume of 400 μl. Peptides were added prior to addition of substrate.

2.4. *GTPase assay*

The GTPase assay has been described [12]. Peptides were incubated with GTPase for 5 min before the addition of substrate.

3. RESULTS AND DISCUSSION

Peptides were synthesized which corresponded to various regions of the γ-subunit. Each of these peptides was tested for its ability to inhibit PDE activity. One peptide, PDEγ-3, was found to inhibit PDE activity in a dose-dependent manner (50% inhibition at 0.64 μmol/400 μl, see table 1). The reason why such a large amount of peptide is needed is that very little of the peptide may be in the correct conformation. Since these peptides are very small segments of proteins, the correct 3-dimensional structure cannot be maintained as well as in the PDE γ-subunit, where there are other interactions stabilizing the 3-dimensional structure. This peptide overlapped an α-helical region predicted by the method of Chou and Fasman [19], extending from residues 29–41. A model of this α-helical region predicted one surface containing a large number of positively charged residues (4 lysines and 2 arginines), while the opposite surface contained hydrophobic residues, including 2 phenylalanines. The model was used to determine which amino acids were to be substituted to make peptide analogs of PDEγ-3 (table 1).

Analog 1 was synthesized with a proline replacing a more flexible glutamine, at residue 34, to break any possible α-helical structure (Chou and Fasman prediction). This analog did not inhibit PDE activity, suggesting that the less flexible proline may have altered the secondary structure of the α-helix. The hydrophobicity of the peptide was not changed significantly by the addition of the proline, as determined by Kyte and Doolittle [20], suggesting that the loss of inhibitory activity was not simply due to a change in the hydrophobicity of the peptide. The substitution of a valine for a threonine on one of the hydrophilic surfaces, as in analog 4, also led to a loss in inhibitory activity. This suggested that the hydrophilic nature of this surface needed to be maintained for proper interaction with PDE$\alpha\beta$. The replacement of a charged residue by an uncharged residue, as in analog 3, led to a loss in inhibitory activity, suggesting that ionic interactions with PDE$\alpha\beta$ were

Table 1

Effect of PDEγ peptides on PDE activity[a]

Peptide	Inhibition at 0.64 μmol per 400μl (%)	Residue	Sequence
PDEγ-1	26	1–14	Met-Asn-Leu-Glu-Pro-Pro-Lys-Ala-Glu-Ile-Arg-Ser-Ala-Thr
PDEγ-2	0	16–30	Val-Met-Gly-Gly-Pro-Val-Thr-Pro-Arg-Lys-Gly-Pro-Pro-Lys-Phe
PDEγ-3	50	31–45	Lys-Gln-Arg-Gln-Thr-Arg-Gln-Phe-Lys-Ser-Lys-Pro-Pro-Lys-Lys
PDEγ-4	12	46–60	Gly-Val-Gln-Gly-Phe-Gly-Asp-Asp-Ile-Pro-Gly-Met-Glu-Gly-Leu
PDEγ-5	13	61–74	Gly-Thr-Asp-Ile-Thr-Val-Ile-Cys-Pro-Trp-Glu-Ala-Phe-Asn
PDEγ-6	0	73–87	Phe-Asn-His-Leu-Glu-Leu-His-Glu-Leu-Ala-Gln-Tyr-Gly-Ile-Ile
Analog 1	0		Lys-Gln-Arg-(Pro)-Thr-Arg-Gln-Phe-Lys-Ser-Lys-Pro-Pro-Lys-Lys
Analog 2	0		Lys-Gln-Arg-(Glu)-Thr-Arg-Gln-Phe-Lys-Ser-Lys-Pro-Pro-Lys-Lys
Analog 3	0		Lys-Gln-(Gln)-Gln-Thr-Arg-Gln-Phe-Lys-Ser-Lys-Pro-Pro-Lys-Lys
Analog 4	0		Lys-Gln-Arg-Gln-(Val)-Arg-Gln-Phe-Lys-Ser-Lys-Pro-Pro-Lys-Lys
α-Helix	25	29–41	Lys-Phe-Lys-Gln-Arg-Gln-Thr-Arg-Gln-Phe-Lys-Ser-Lys
Boundary 2	40	35–45	Thr-Arg-Gln-Phe-Lys-Ser-Lys-Pro-Pro-Lys-Lys

[a] Percent inhibition of PDE activity is expressed as percent of a control without added peptide. Results are the average of duplicate experiments and are as described in section 2. In each case, dose-response curves were made

important. The loss of inhibitory activity in analog 2, which had a glutamate replacing a glutamine, suggests that hydrophobic interactions were also important for either the maintenance of the structure of the peptide or for interaction with PDE$\alpha\beta$. Thus it appeared that the intact structure of the α-helix was important for the peptide to recognize PDE$\alpha\beta$, as several kinds of substitutions were all deleterious.

To determine the optimal sequence for interaction, a boundary peptide was synthesized (table 1). PDEγ boundary 2 inhibited PDE activity. PDEγ α-helix, a peptide corresponding to the α-helical region extending from amino acids 29–41, also inhibited PDE activity to some extent (24% inhibition at 0.64 μmol/400 μl).

The α-helical region is flanked by prolines and lysines. It is clear that these lysines and prolines are important for optimal interaction. This is shown by the ability of PDEγ boundary 2 to inhibit PDE activity while PDEγ α-helix, which lacks the prolines and lysines, does not inhibit PDE activity as well. These flanking regions could serve as recognition points, or to maintain a certain secondary structure.

To determine whether the PDEγ peptides had any effect on transducin a GTPase assay was performed. Addition of PDEγ-3 inhibited the GTPase activity of transducin in a dose-dependent manner (fig.1) with 50% inhibition occurring at about 0.12 μmol peptide in a 100 μl assay. Kinetic studies were performed to determine the nature of the interaction of PDEγ-3 with transducin (fig.2). The addition of 0.076 μmol PDEγ-3 decreased the V_{max} from 9.15 to 5.20 μM/min while the K_m was unaffected showing that PDEγ-3 is a non-competitive inhibitor of GTPase activity.

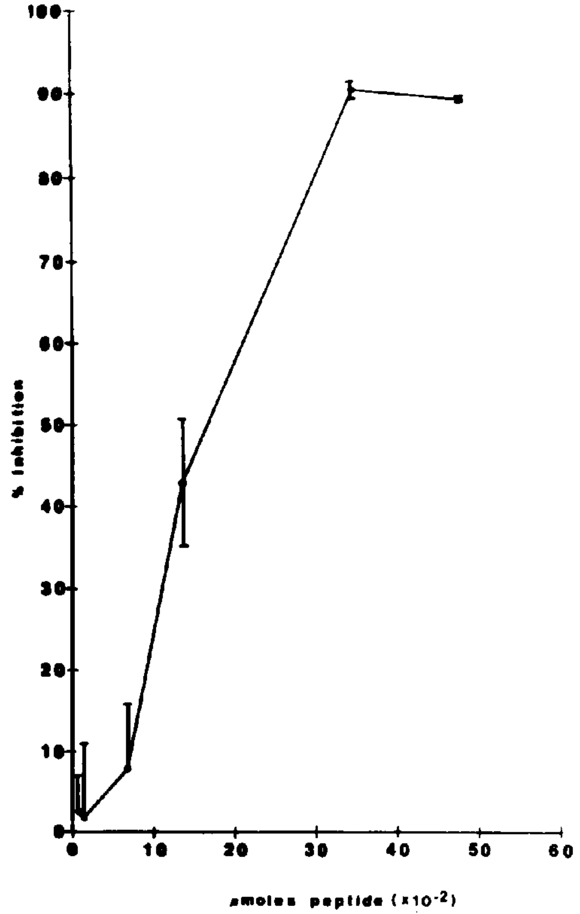

Fig.1. Effect of PDEγ-3 on GTPase activity. Percent inhibition is expressed as percent of a control without peptide. Results are the average of triplicate experiments (± SE) and are as described in section 2.

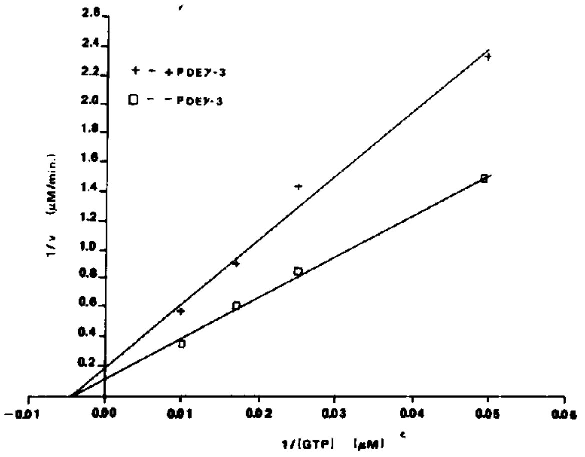

Fig.2. Kinetic studies of PDEγ-3 on GTPase activity. The GTPase assay is described in section 2. Peptide (0.076 μmol per 100 μl assay) was added to the appropriate samples 5 min prior to the addition of substrate. Results are the average of duplicate experiments. Straight lines were plotted by analysing the data with a linear regression program.

The above results suggest that PDEγ may be involved in more than the direct regulation of PDE activity. PDEγ may be involved in the regulation of transducin either directly or indirectly. The decrease in GTPase activity could be due to a decrease in hydrolysis rate, a slower dissociation of GDP or a slower association of GTP to Tα. This could be achieved in several ways. PDEγ could bind to Tα and inhibit its reassociation with the β- and γ-subunits of transducin (Tβγ). Conversely, PDEγ could also bind to Tβγ, preventing reassociation with Tα, or with rhodopsin. In each of these cases, transducin would not be able to bind to rhodopsin. Also, PDEγ could regulate GTPase activity without affecting the binding of Tα with Tβγ. Further investigation is required to determine the role of PDEγ in the visual transduction cascade. We are currently looking at GMPP(NH)P binding and GDP binding to determine the regulatory mechanism by which PDEγ affects transducin.

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