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Active sites of the cyclic GMP phosphodiesterase γ-subunit of retinal rod outer segments

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Monoclonal antibodies were prepared to the γ-subunit of the cGMP phosphodiesterase. One of them γp-1, suppresses the activation of phosphodiesterase through the α-subunit of transducin. The γ-subunit fragment 24–45 rich in Arg and Lys residues is involved in γp-1 binding and is essential for the γ-subunit interaction with transducin. Carboxypeptidase Y cleaves off seven amino acid residues from the C-terminus of the γ-subunit resulting in phosphodiesterase activation. Thus, the C-terminal fragment of γ-subunit participates in phosphodiesterase inhibition.

Cyclic-GMP phosphodiesterase; Transducin; Monoclonal antibody; Carboxypeptidase Y

1. INTRODUCTION

The γ-subunit of retinal cyclic GMP phosphodiesterase (PDE) plays a leading role in photoinduced activation of PDE which decreases the cGMP content in the photoreceptor cell and, consequently, changes the Na^+ permeability of the plasma membrane [1,2]. Besides, the γ-subunit was found in no tissues [3] except for retina [4,5]. We showed that the amino acid sequence of the γ-subunit of PDE carried a special site considerably enriched in basic amino acids [6] and focused attention on the functioning of the site in regulation of PDE activity.

Still little is known about the PDE system in the photoreceptor cell though it has been intensively studied. For example, PDE can be modified by cGMP binding to PDE at distance from the catalytic site and by methylation of the enzyme's catalytic subunit [7–9]. The γ-subunit of PDE regulates both the processes. However, their involvement in the mechanism of photoreception has not yet been established. The chemical bases underlying the interaction between the γ-subunit of PDE and the enzyme's catalytic subunits, on the one hand, and the α-subunit of transducin upon the enzyme activation, on the other, are also unknown. This paper presents the data on localization of the regions (active sites) providing a variety of γ-subunit functions. Preliminary results were published in [14].

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2. MATERIALS AND METHODS

The fraction of rod outer segments (ROS) from bovine retinas was isolated according to [15]. PDE and the γ-subunit were isolated and purified as in [6,15]. The γ-subunit was cleaved with *Staphylococcus aureus* protease, trypsin and cyanogen bromide to give peptides S-2, T-11, and CB-1, CB-2, CB-3, respectively. Peptides SP-1 and SP-2 were synthesized.

PDE and the γ-subunit were treated with carboxypeptidase Y in a buffer solution (50 mM Tris-HCl, pH 8.0, 1 mM 2-mercaptoethanol, 2 mM $MgCl_2$) for 15–30 min at 37°C.

Monoclonal antibodies were obtained as in [16] using BALB/c mice for immunization. The thermally treated PDE preparation extracted from ROS was used for injections. The immunization scheme was the following: the first injection, 30 μg of protein per mouse, subcutaneously; 20 days later the

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second subcutaneous injection, 50 μg/mouse; in 10 days the third injection, 50 μg/mouse, intraperitoneally; the same injection was repeated in 2 weeks; 2 weeks later three daily booster injections, 50 μg/mouse, each intraperitoneally. Splenocytes of immunized mice were hybridized with cells of mice myeloma 2/O-Ag 14 on a day after the last immunization. Screening of hybridomas and an investigation of the interaction of peptides with the antibody were carried out by ELISA, using an electrophoretically homogeneous γ-subunit. Monoclonal antibodies were isolated from the ascites liquid and purified on protein A-Sepharose [17].

The PDE activity was determined by the isotopic method and using 5'-nucleotidase [18]. Photoinduced activation of PDE was tested in the presence of GTP and its nonhydrolyzable analogue Gpp(NH)p [19]. High affinity GTPase of rod outer segments was detected at a substrate concentration of 5×10^{-7} M [20].

3. RESULTS AND DISCUSSION

Initially monoclonal antibodies to the γ-subunit of PDE were produced and only one of them, γp-1, was selected. In the presence of GTP and its nonhydrolyzable analogue Gpp(NH)p the photoinduced activation of PDE was virtually suppressed by monoclonal antibody γp-1 (fig.1), but the basal enzyme activity remained the same. Here the GTPase activity intrinsic to transducin, and in particular to its α-subunit, was also retained (not shown). Thus to provide the PDE photoinduced activation, the γ-subunit has a special antibody-blocked site. Immunoglobulins attached to the γ-subunit site prevent the contact of the latter with the α-subunit of transducin, but do not affect other features, in particular, the GTPase activity.

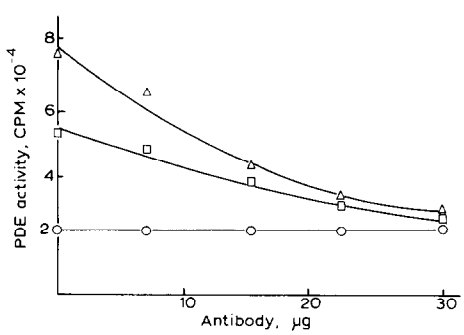

Fig.1. Influence of monoclonal antibody γp-1 on basal activity of PDE (0.25 μg of protein) (○) and PDE photoinduced activation in ROS preparations (10 μg of protein) in the presence of GTP (□) or Gpp(NH)p (△). Concentration of GTP and Gpp(NH)p, 5×10^{-5} M.

Table 1

Interaction of monoclonal antibody γp-1 and PDE γ-subunit peptides

Peptide and its positioning in the molecule	Interaction[a]
BC-1 (2–17)	–
BC-2 (18–57)	+
BC-3 (58–87)	–
T-11 (46–87)	–
S-2 (10–58)	+
SP-1 (24–47)	+
SP-2 (24–35)	±
γCPY[b] (1–80)	+

[a] +, Strong interaction; ±, weak interaction; –, no interaction

[b] γCPY, Carboxypeptidase treated γ-subunit

The protein region responsible for its interaction with the enzyme's catalytic subunits remains intact.

ELISA of some peptides revealed the site of interaction with monoclonal antibody γp-1 on the γ-subunit molecule. Only the peptides composed of the region enriched in basic amino acids (24–45) (table 1) interact with the antibody, i.e. this region is necessary for binding to transducin and, respectively, for photoinduced activation of PDE.

The following experiments provided additional evidence of the important role of this region (24–45) of the γ-subunit of PDE in the enzyme

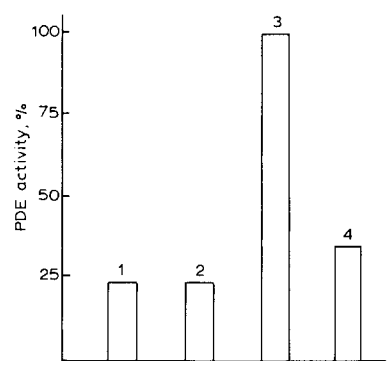

Fig.2. Influence of peptide SP-1 (24–47) (0.08 nmol) on PDE activity in ROS (10 μg of protein). 1, basal activity of PDE in ROS; 2, PDE activity in ROS in the presence of peptide SP-1; 3, PDE activity in ROS in the presence of Gpp(NH)p (10^{-5} M); 4, PDE activity in ROS in the presence of peptide SP-1 and Gpp(NH)p (10^{-5} M).

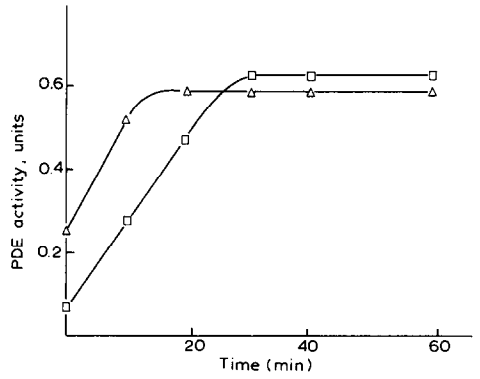

Fig.3. Carboxypeptidase Y activation of purified PDE (0.25 μg of protein) (△) and PDE in ROS preparation (10 μg of protein) (□).

photoactivation. In the presence of peptide SP-1 (amino acid sequence 25–47) and Gpp(NH)p no photoinduced activation of PDE was observed (fig.2), probably due to competition of the peptide and the γ-subunit when interacting with the α-subunit of transducin.

Which site of the γ-subunit of the PDE molecule interacts with the catalytic subunits inhibiting enzyme activity? Treatment of PDE with carboxypeptidase Y was found to cause potent enzyme activation (fig.3). Treatment of ROS with carboxypeptidase Y also results in the activation correlated with the PDE photoinduced activation in the presence of guanyl nucleotides (fig.4). Besides, addition of the carboxypeptidase Y-treated γ-subunit (γCPY) to purified PDE induces no inhibition of the enzyme activity as in the case of the intact γ-subunit (fig.4). Upon treatment of the γ-subunit with carboxypeptidase Y seven amino acid residues (1 Gln, 1 Ala, 1 Gly, 2 Ile, 1 Leu, 1 Tyr) are cleaved off from the C-terminus. The results of the experiments imply participation of the γ-terminal amino acid residues in the PDE inhibition.

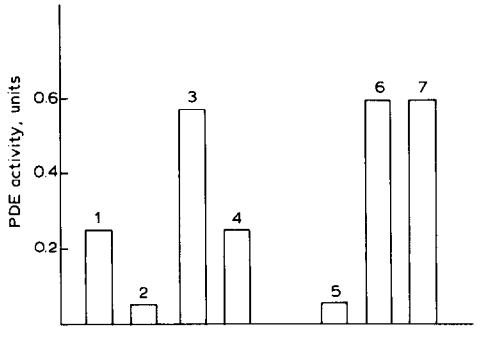

Fig.4. Activation of PDE with carboxypeptidase Y and light. 1, purified PDE (0.25 μg); 2, PDE (0.25 μg) plus γ-subunit (0.05 μg); 3, PDE (0.25 μg) plus CPY (5 μg); 4, PDE (0.25 μg) plus γCPY (0.05 μg); 5, ROS (10 μg of protein); 6, ROS (10 μg of protein) plus Gpp(NH)p (5×10^{-5} M); 7, ROS (10 μg of protein) plus CPY (5 μg).

Thus the C-terminal fragment and the basic region in the middle of the γ-subunit polypeptide chain (24–45) are functionally active sites. The first participates in PDE inhibition and the second in the γ-subunit–transducin interaction. Enzyme activation upon trypsinolysis [10] indicates that this basic region may also be involved in the interaction with the enzyme's catalytic subunits. The functional role of other regions of the γ-subunit of PDE is under investigation.

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