The effect of rhodopsin phosphorylation on the light-dependent activation of phosphodiesterase from bovine rod outer segments

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Received 14 December 1984

ATP quenches light-dependent phosphodiesterase (PDE) activation in rod outer segments presumably due to rhodopsin phosphorylation. Here we compared the efficiency of phosphorylated and non-phosphorylated rhodopsins as PDE activators in a reconstituted cell-free system. It is shown that the ability of phosphorylated membranes to activate this enzyme is supressed compared with non-phosphorylated ones.

Rhodopsin phosphorylation *Light-dependent phosphodiesterase*

1. INTRODUCTION

Light absorption by rhodopsin is accompanied by an increase in PDE activity and a decrease in the cGMP concentration in ROS. It leads to a lowering of Na^+ permeability and to hyperpolarization of the ROS plasma membrane; a rod photoreceptor response is the final result of these events [1–4]. It has been shown that the rhodopsin effect on PDE activity is mediated by a GTP-dependent protein TD: at first the bleached rhodopsin catalyzes the exchange of TD-bound GDP for GTP, and then TD-GTP activates PDE. Since TD possesses GTPase activity, the bound GTP is hydrolyzed, and the TD-GDP can be reactivated by rhodopsin again [5].

Liebman et al. [6,7] have demonstrated that ATP inhibits the light-dependent activation of PDE both in ROS and in a system containing disk membranes, TD, PDE and partly purified rhodopsin kinase, and postulated that the ATP-dependent inhibition of PDE is due to rhodopsin phosphorylation. However, the ATP-dependent quenching of PDE stimulation in ROS has been demonstrated in conditions when rhodopsin phosphorylation is not revealed [8], and so the authors suppose that the inhibitory effect of ATP is not due to the rhodopsin phosphorylation.

To answer the question whether rhodopsin phosphorylation is involved in the inhibition of a light-dependent PDE reaction, we have investigated the efficiency of phosphorylated rhodopsin in PDE activation in a reconstituted cell-free system containing photoreceptor membranes, TD and PDE, but without rhodopsin kinase and ATP.

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Abbreviations: ROS, rod outer segments; PDE, cyclic nucleotide phosphodiesterase; TD, transducin

2. MATERIALS AND METHODS

2.1. *Isolation of photoreceptor membranes and enzymes from ROS*

ROS and photoreceptor disks were obtained by the method of [9] in the cold, under dim red illumination. The rhodopsin concentration was assayed by the difference in the absorbance of a membrane solution in 1% cetyltrimethylammonium bromide before and after bleaching. The

Published by Elsevier Science Publishers B.V. (Biomedical Division)
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A_{280}/A_{500} ratio for the photoreceptor disks was 1.95–2.10.

PDE and TD were extracted according to [10] and purified by the method of [11]. PDE activity was estimated as in [12] by using cAMP as substrate.

To obtain crude rhodopsin kinase, ROS were extracted in darkness according to [13], and extract was concentrated by precipitation with ammonium sulphate [14]. Protein concentration was determined with Coomassie blue G as in [15] using bovine serum albumin as the standard.

2.2. *Rhodopsin phosphorylation*

The phosphorylation of rhodopsin was performed in conditions described in [16]. Disks were incubated in light for 1 h at 30°C in 70 mM potassium phosphate buffer (pH 7.4), containing 3 mM $MgCl_2$, 3 mM ATP, 0.5 mg/ml rhodopsin, 1.5 mg/ml rhodopsin kinase. The phosphorylation efficiency was assayed by the method of [13] with the aid of [γ-^{32}P]ATP; 2.8–3.2 mol phosphate per mol protein were incorporated in light; in the dark the proportion was no more than 0.1 mol phosphate per mol protein. Control non-phosphorylated membranes were obtained by incubation of disks under similar conditions, but without ATP. After the incubation the membranes were regenerated with 11-*cis*-retinal and washed free from peripheral proteins.

2.3. *Isoelectric focusing*

Isoelectric focusing of rhodopsin preparations was carried out as in [17] in darkness. The gels were densitometred at 510 nm using the UA-5 monitor with a gel scanner ('ISCO', USA). After isoelectric focusing non-phosphorylated membranes indicate a single rhodopsin band with an isoelectric point of 6.0, the phosphorylated membranes show the band of non-phosphorylated

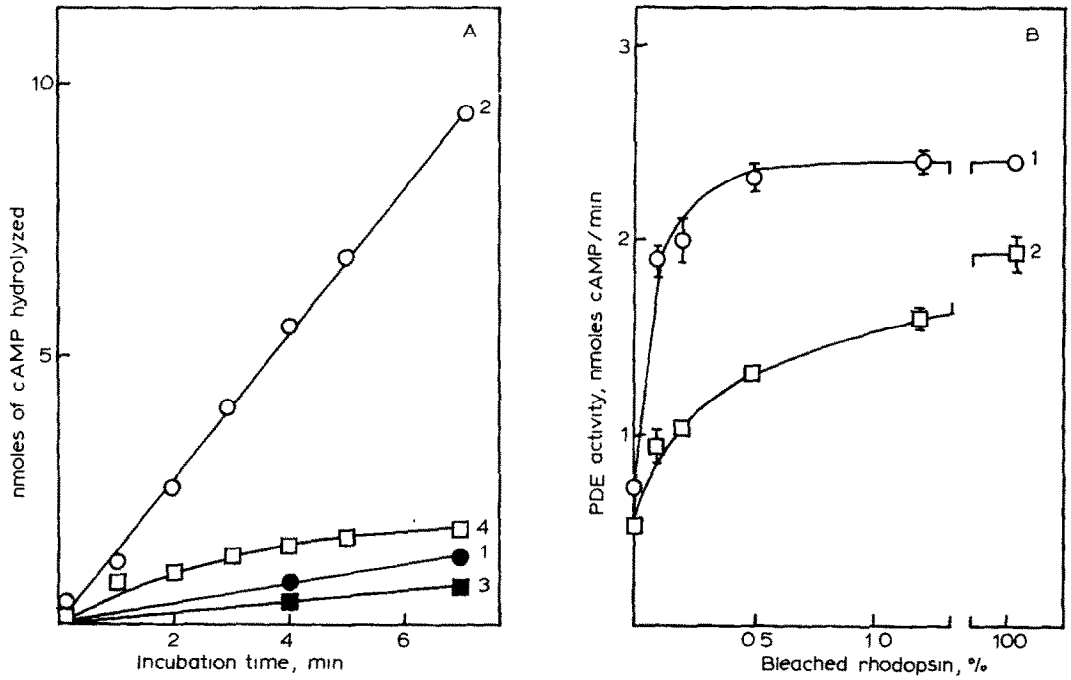


Fig.1. Light-dependent PDE activation by phosphorylated and non-phosphorylated photoreceptor membranes. The reaction mixture contained 10 mM Tris–HCl (pH 8.0), 140 mM NaCl, 5 mM $MgCl_2$, 1 mM dithiothreitol, 10 μM GTP, 0.9 mM [^{3}H]cAMP (2 Ci/mmol), 10 μg rhodopsin, 1 μg PDE, 16 μg TD; final volume, 120 μl; temperature, 20°C. Ten-μl samples were taken from the reaction mixture to determine the amount of hydrolyzed cAMP. (A) PDE activation at the bleaching of 0.1% rhodopsin; (1,2) dark and illuminated control membranes; (3,4) dark and illuminated phosphorylated membranes. (B) PDE activity dependence on the bleached rhodopsin proportion. Incubation time, 6 min. (1) Control membranes; (2) phosphorylated membranes.

rhodopsin (30% of total protein) and a set of its phosphorylated forms (isoelectric points 4.5–5.0) with a phosphate content of 2–5 mol per mol protein.

3. RESULTS

Rod disk membranes of two types were used to study the efficiency of phosphorylated rhodopsin as PDE activator: (i) preparations containing phosphorylated rhodopsin – obtained by incubating the membranes in light in the presence of rhodopsin kinase and ATP; (ii) control preparations containing only non-phosphorylated rhodopsin – obtained by incubating the disk membranes in the same conditions, but without ATP. After incubation, both preparations were washed and regenerated with 11-*cis*-retinal.

A direct comparison of the efficiency of the phosphorylated and non-phosphorylated rhodopsins as PDE activators was made in a reconstituted cell-free system which contained membranes, TD and PDE. As seen in fig.1, the bleaching of 0.1% rhodopsin in non-phosphorylated membranes causes a significant PDE activation. In the case of the phosphorylated membranes containing 70% of phosphorylated rhodopsin, the activation is about 70% lower; i.e., the inhibiting effect correlates with the proportion of phosphorylated rhodopsin in these membranes. Therefore the PDE stimulation observed in this experiment is mainly (or exceptionally) due to non-phosphorylated rhodopsin. The inhibiting effect is revealed at low bleaching, when the PDE reaction rate is limited by the amount of bleached rhodopsin.

ACKNOWLEDGEMENTS

This study was carried out within the framework of the Rhodopsin research program sponsored by Professor Yu.A. Ovchinnikov under the auspices of the USSR Academy of Sciences and the Moscow State University. We wish to express our thanks to Professor V.P. Skulachev and Dr V.A. Tkachuk for their helpful discussions and to Dr B.I. Mitsner for his generous gift of 11-*cis*-retinal.

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