Activation of cGMP phosphodiesterase by purified green rod pigment from frog retina

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Activation of cGMP phosphodiesterase(PDE) of frog rod outer segments (ROS) by purified green rod pigment (GRP) was analyzed. GRP activated PDE in a similar manner to purified rhodopsin. This activation required illumination of the pigment and presence of GTP.

Frog retina *Green rod pigment* *Rhodopsin* *cGMP* *Phosphodiesterase* *Visual excitation*

1. INTRODUCTION

The blue-sensitive rods, so called 'green rods', are found in amphibian retina and constitute about 10% of total rod cells [1]. Electrophysiological [2] and behavioural studies [3] demonstrate that green rods participate in colour coding in frog vision. Green rods respond to light with hyperpolarization in the same way as red rod and cone cells [4]. However, no biochemical study has been conducted on the transduction mechanism of green rods. The blue-sensitive visual pigment of green rods has been purified by affinity chromatography, and it was demonstrated that the pigment has properties intermediate between rhodopsin and cone pigment, iodopsin [5,6]. We investigate here the activation of cGMP PDE by purified GRP.

2. MATERIALS AND METHODS

2.1. *Chemicals*

[^{3}H]cGMP was purchased from New England Nuclear. cGMP and GTP were obtained from Sigma. Concanavalin A–Sepharose 4B and Sephadex G-100 were obtained from Pharmacia Fine Chemicals. Tris base, Na_2SO_4 and $BaCl_2$ were obtained from Wako Pure Chemicals. All other reagents were of the highest analytical grade.

Abbreviations: PDE, phosphodiesterase; ROS, rod outer segments; GRP, green rod pigment; SDS–PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis

2.2. *GRP and rhodopsin*

GRP and rhodopsin from bullfrog (*Rana catesbeiana*) retina were prepared under dim red light ($\lambda > 600$ nm) as in [5,6]. GRP was separated from rhodopsin by Concanavalin A–Sepharose 4B column chromatography in digitonin solution. The GRP fraction was applied to a Sephadex G-100 column and the pigments were eluted with 0.2% digitonin in 20 mM K-phosphate buffer at pH 6.8. The GRP fraction contained 94% GRP and 6% rhodopsin as determined by the NH_2OH destruction of GRP as described in [6]. The rhodopsin fraction contained no GRP. GRP and rhodopsin fractions were also analyzed by staining with Coomassie blue R-250 after SDS–PAGE and the purity of both fractions was more than 95%. Illumination of GRP or rhodopsin was carried out on ice with use of a fluorescent lamp until complete bleach.

2.3. *Preparation of frog ROS suspension and assay of PDE activity*

These procedures were carried out under in-

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frared light with an infrared light image converter (Type 5156, N.V. Optische Industrie, Delft, The Netherlands). ROS suspension for PDE activity assay was prepared by a modified sucrose (43%, w/w) floatation method [7]. ROS paste, formed at the air–sucrose interface by centrifugation at 112000 × *g* for 1 h, was suspended in a solution containing 20 mM Tris–HCl (pH 7.5), 4 mM $MgCl_2$ and 2 mM DTT. The ROS were disrupted by being drawn 4 times into a syringe with a No.25 needle (TERUMO, N N-2525R). ROS suspension was incubated with illuminated or unilluminated GRP (or rhodopsin) at 30°C for 3 min in the dark in 100 μl of a solution containing 15 mM Tris–HCl (pH 7.5), 3 mM $MgCl_2$, 1.5 mM DTT, 2 mM cGMP, 25 mM K-phosphate and 0.05% digitonin with or without 25 μM GTP. At the end of incubation, the reaction mixture was heated in boiling water for 2 min and supplemented with 750 μl of 80 mM $BaCl_2$ and 500 μl of 110 mM Na_2SO_4, resulting in the formation of a white precipitate of $BaSO_4$. As described in [8], the precipitate of $BaSO_4$ adsorbed [^{3}H]GMP which was produced by the hydrolysis of [^{3}H]cGMP by PDE, but did not adsorb [^{3}H]cGMP. After separation of the precipitate by centrifugation at 1300 × *g* for 30 min, 200 μl of the supernatant fraction was isolated. The amount of [^{3}H]cGMP in that fraction was measured in 10 ml of scintillator (ACS II, Amersham) by a Packard Tri-Carb liquid scintillation spectrometer (Model 2405). The amount of non-specific adsorption of [^{3}H]cGMP to the precipitate was also assayed without ROS. All of the assays were done in duplicate and the results agreed within 5% error.

2.4. *Determination of the amounts of GRP, rhodopsin and protein*

The amount of GRP or rhodopsin was determined by the absorption spectrum as already described [6]. Concentration of protein was determined by the method of [9].

3. RESULTS AND DISCUSSION

Fig.1 shows the effect of addition of purified GRP or purified rhodopsin to frog ROS suspension on the activity of PDE. The amount of GRP required for the activation of PDE was the same as that of rhodopsin. The necessity of the illumination and the presence of GTP for the activation of PDE by GRP was similar to that by rhodopsin.

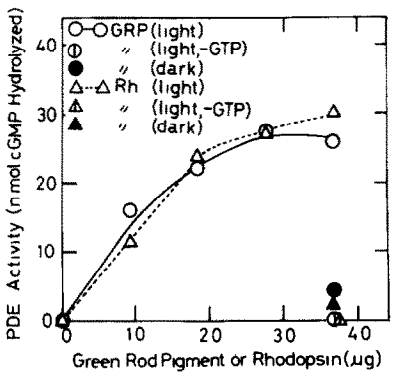

Fig.1. Activation of cGMP PDE by purified GRP and rhodopsin. Frog ROS suspensions (containing 32 μg rhodopsin), supplemented with different amounts of (○—○) illuminated GRP or (△—△) illuminated rhodopsin, were incubated for PDE assay with 25 μM GTP at 30°C for 3 min in the dark. PDE activity was measured with supplementation of 3.8 μg of (●) unilluminated GRP or (▲) unilluminated rhodopsin. PDE activity in the absence of GTP was also measured with (Ⓘ) 3.8 μg of illuminated GRP or (◭) illuminated rhodopsin.

These results show that the activation of PDE by GRP is similar to that by rhodopsin [10] or iodopsin [11]. The results also suggest that the cGMP PDE is present in green rod cells and that several phenomena concerning cGMP metabolism caused by light in green rod cells are similar to those in red rod cells.

The activation mechanism of PDE by illuminated rhodopsin and the metabolism of cGMP in red rod cells have already been analyzed [12,13]. However, such information regarding green rod cells and cone cells is very scarce. Further clarification of the activation mechanism of PDE by GRP and the characterization of PDE in green rod cells seems to be important from the viewpoint of the excitation mechanism of photoreceptor cells.

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