Light-induced conformational change in rhodopsin detected by modification of G-protein binding, GTPγS binding and cGMP phosphodiesterase activation

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CNBr treatment of rod outer segments was performed in dark and in light conditions. With the subsequent modified rhodopsin and opsin the cGMP phosphodiesterase activation system was reconstituted. The recombination systems exhibited greatly reduced G-protein binding, GTPγS binding and cGMP phosphodiesterase activation. The reduction in activity of these three steps of the PDE activation cascade is most significant with modified opsin and is shown to be due to its inability to bind the Gα subunit. The correlation between the localization of CNBr cleavage in dark and light conditions and these results is strongly indicative that a light-induced conformational change occurs in two extradiscal regions of rhodopsin.

Rhodopsin *G-protein* *GTP binding* *cGMP phosphodiesterase* *CNBr digestion* *Conformational change*

1. INTRODUCTION

Three components have been demonstrated to be involved in the cGMP enzymatic cascade of visual transduction in vertebrate photoreceptor cells. These are the light receptor rhodopsin, G-protein (GTP binding protein, transducin) and cGMP phosphodiesterase (PDE). Upon illumination, rhodopsin undergoes a conformational change leading to an active state (R*) shown to be essentially the photoproduct metarhodopsin II [1,2]. R* interacts with the peripheral membrane protein, G-protein, which then exchanges a bound GDP for GTP, inducing dissociation of the R/G-protein complex after a period of milliseconds [3]. The G-protein–GTP complex activates PDE (which hydrolyzes cGMP to 5′-GMP) by displacement of an inhibitory constraint [4]. In the accompanying paper [6] we describe a procedure of CNBr treatments of ROS to investigate the light-induced conformational change in the extradiscal regions of rhodopsin. Here, we have studied the impact of the CNBr modifications of rhodopsin (and opsin) on three different stages of the PDE cascade.

These are its ability to: (i) bind G-protein; (ii) catalyze G-protein GTPγS binding; and (iii) provoke the activation of PDE. At each of these three stages of the PDE-activation cascade we have observed a difference between unbleached ROS treated with CNBr and ROS treated after illumination.

Abbreviations: G-protein, GTP binding protein or transducin; PDE, phosphodiesterase; ROS, rod outer segments; GTPγS, non-hydrolyzable GTP ^{35}S γ-labeled; ROS, rod outer segments

2. MATERIALS AND METHODS

All procedures were performed at 0 or 4°C under dim red light unless otherwise stated.

Bovine ROS were purified according to a standard procedure [5] and were divided into two portions as shown in fig.1. One portion was kept in darkness, the other one illuminated for 1 min at 0°C under white light. ROS samples were treated with CNBr and the resulting modified rhodopsin

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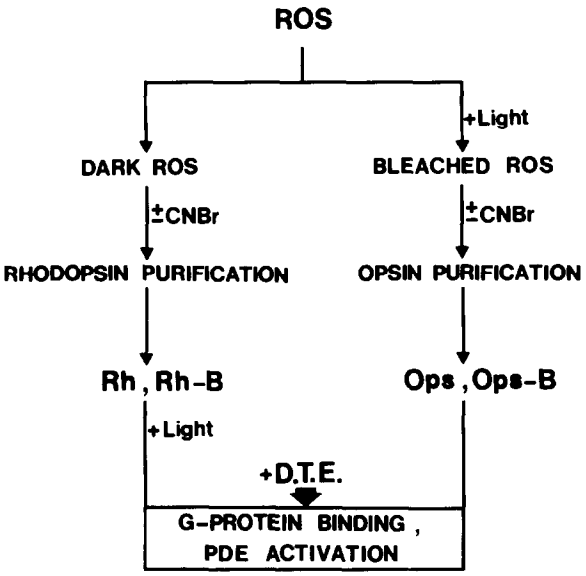

Fig.1. Experimental scheme for monitoring the light-induced conformational change of rhodopsin. The two species of rhodopsin (Rh, Rh-B) and those of opsin (Ops, Ops-B) were prepared as described in the preceding communication. The final step called G-protein binding includes the GTP binding to the G-protein.

(Rh-B) and opsin (Ops-B) were purified as described in the accompanying paper [6].

Soluble ROS proteins were prepared from freshly purified ROS by immediate extraction with 10 mM (pH 7.5) Tris–HCl buffer containing 1 mM EDTA and 1 mM dithioerythritol. This extract termed DTE (Dark Tris Extract) containing the G-protein, PDE and its inhibitor were stored in liquid nitrogen until use.

For G-protein binding experiments modified rhodopsins were recombined with soluble ROS proteins from DTE, each mixture contained 0.4 mg purified rhodopsin and the DTE from ROS corresponding to 2 mg rhodopsin in a (pH 8.0) 100 mM Tris–HCl, 2 mM Mg^{2+} buffer. Each recombined suspension was illuminated according to [7] then immediately centrifuged for 30 min at 100000 × g. The pellet was extracted first with 1 ml of the hypotonic Tris buffer used above for extracting soluble ROS proteins (termed LTE), and then with the same buffer containing 2 mM Mg^{2+} and 100 μM GTP (termed GTP E). The first and the second extracts were submitted to electrophoretic analysis together with the original DTE.

Rhodopsin- or opsin-catalyzed GTPγS binding in DTE was estimated using a nitrocellulose filtration method adapted from that described by Northup et al. [8]. To a preincubated (3 min, 30°C) tube containing 50 μl of 40 mM Tris–HCl (pH 8.0), 2 mM $MgCl_2$, 5 μM GTPγS (250000 cpm) and rhodopsin or rhodopsin derivative (as described in the legend to fig.3) were added 50 μl DTE. After incubation (5 min, 30°C) the sample was applied to a damp nitrocellulose filter under suction then washed and counted for radioactivity according to [8].

The phosphodiesterase activation was estimated by comparing the effect of the different rhodopsins and opsins on the basal PDE activity. Rh and Rh-B suspensions were illuminated for 1 min at 0°C producing 75% bleaching. Bleached rhodopsins and opsins were recombined with DTE in the same conditions as in [9] and the [^{3}H]cGMP hydrolysis was measured according to Thompson and Appleman [10].

3. RESULTS

Fig.2 shows polyacrylamide gels of soluble ROS protein extracts after recombination of modified

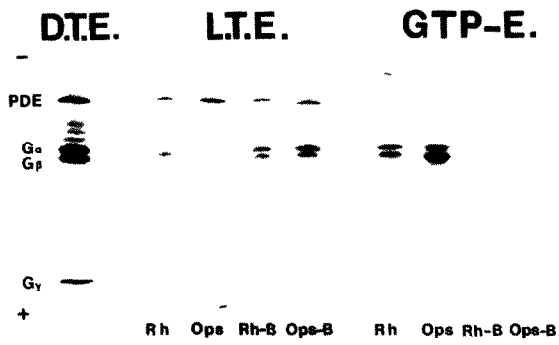

Fig.2. SDS-polyacrylamide gel electrophoresis of soluble extracts from the binding experiment. DTE, hypotonic dark Tris extract obtained directly from dark-kept ROS. DTE was associated as described in fig.1. LTE, 10 mM light Tris extract corresponding to the first extract after the recombination. GTP-E, GTP extract corresponding to the third set of supernatants. Rh, rhodopsin; Ops, opsin; Rh-B, CNBr-treated rhodopsin; Ops-B, CNBr-treated opsin.

or not rhodopsin and opsin with DTE. The DTE profile is shown in the first trace and contains, in addition to PDE, the three subunits of the G-protein [11,12]. After recombination of rhodopsin species with DTE on the bulk of rhodopsin unbound proteins were recovered in the 10 nM LTE. As expected, both illuminated Rh and Ops prevent the extraction of the G-protein and chiefly of the α-subunit in the light Tris extracts (LTE Rh and LTE Ops traces of fig.2). The G-protein complex becomes extractable only in the presence of GTP and magnesium (GTP Rh and GTP Ops traces). On the contrary, in the recombination assays of DTE with CNBr modified rhodopsin and opsin, the G-protein extractability is no longer prevented by light (LTE Rh-B and LTE Ops-B traces). Note that the Gα-subunit is predominant in the extract corresponding to Ops-B. Respective GTP extracts confirm that a weak binding of the G-protein occurs on bleached Rh-B and no binding on Ops-B (GTP-E Rh-B and GTP-E Ops-B traces).

The binding of a non-hydrolyzable GTP analogue (GTPγS) to the G-protein of the DTE extract was studied in the presence of three species of rhodopsin: rhodopsin control (Rh), CNBr-treated rhodopsin (Rh-B) and CNBr-treated opsin (Ops-B) (fig.3).

The ability of DTE to bind GTPγS is greatly enhanced by the addition of 0.5 μg photoactivated rhodopsin. CNBr treatment (Rh-B) greatly diminishes the ability of rhodopsin to catalyze this binding (0.5 μg). Addition of excess of Rh-B (i.e., 5 μg) partially enhances GTPγS-binding suggesting that, in this derivative, this function is partially conserved. In the case of Ops-B, both at 0.5 μg and 5.0 μg, the ability to catalyze GTPγS binding is completely abolished.

The effect of CNBr treatments of ROS is reflected on the light activation of PDE as shown in fig.4. The light activation of PDE is bleached rhodopsin concentration-dependent and the basal PDE activity (in darkness) can be enhanced up to 5–6 times by bleached rhodopsin as well as by opsin. However, the ability of bleached rhodopsin to trigger PDE activation is clearly altered by CNBr modification. Moreover, there is a dramatic difference between the effects obtained with Rh-B and Ops-B: in the case of Rh-B the capacity of bleached rhodopsin to activate PDE is only partially lost (60–70% of the activation of the rhodopsin

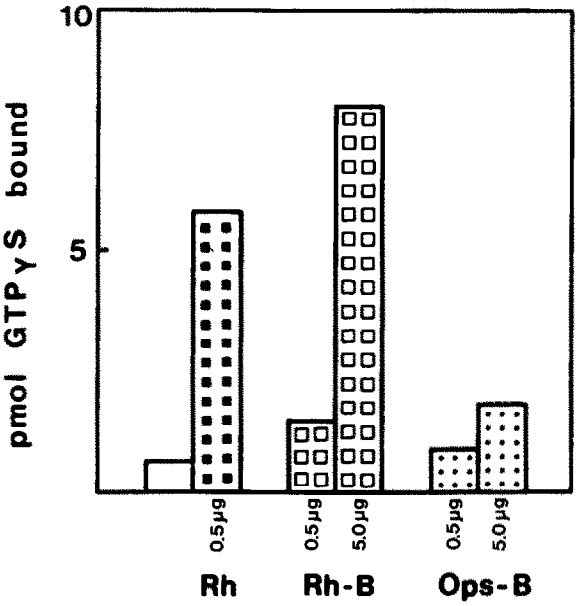

Fig.3. The effect of CNBr treatment on the ability of rhodopsin to catalyze DTE GTPγS-binding. Experiments were performed indifferently under light or dark conditions. Rhodopsin and CNBr-treated rhodopsin and opsin being exposed to white light (5 min, greater than 90% bleaching) before use. Bars: (no symbols) Control without rhodopsin; (■) Rh; (□) Rh-B; (.) Ops-B.

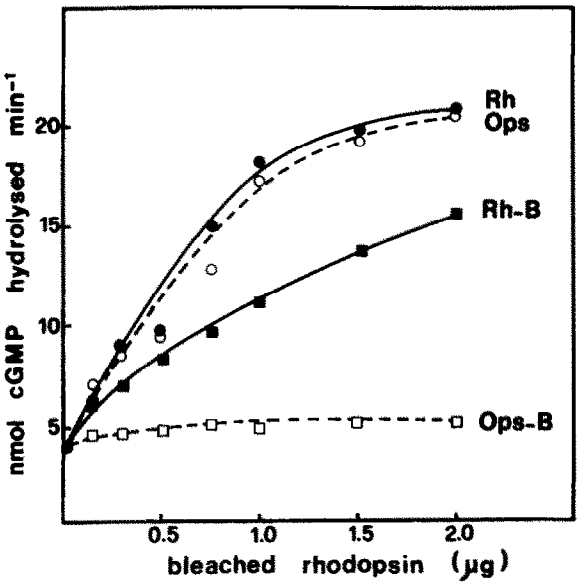

Fig.4. The effect of CNBr treatment on the ability of bleached rhodopsin to activate the cGMP phosphodiesterase. Each DTE was from 0.2 retina, the maximum concentration of bleached rhodopsin species from 0.002 retina (ratio 100).

control) whereas in the case of Ops-B it is completely lost. To avoid the effect of eventual CNBr traces on PDE activity two controls were performed: (i) in some assays containing control rhodopsin or opsin CNBr was incorporated; no change was observed in the rhodopsin-dependent PDE activation; (ii) Rh-B and Ops-B were partially evaporated under vacuum to eliminate CNBr traces and the same values of PDE activity were obtained.

4. DISCUSSION

For monitoring light-induced conformational changes of rhodopsin, we used reconstitution assays with membrane vesicles containing purified visual pigment. Thus we can attest that all the effects observed on the cGMP PDE cascade are due to modified rhodopsin.

In the first investigated stage of the cGMP cascade the light-induced binding of G-protein was affected both with Rh-B and Ops-B indicating that rhodopsin extradiscal regions were modified by CNBr treatment. If we compare the G-protein binding (and chiefly the Gα-subunit) between the two species of rhodopsin and those of opsin, it is obvious that the binding site of G-protein is most affected in Ops-B where it seems to be severely damaged. A reduced binding has been already observed after thermolysin treatment of ROS but the so digested rhodopsin still partially retained its capacity to bind the G-protein [7].

Efficient GTP–GDP exchange occurs on G-protein only if the Gα-subunit in the presence of Gβ and Gγ interacts with photolyzed rhodopsin [13]. This interaction being greatly reduced with Rh-B and prevented with Ops-B, we expected and observed a reduced binding of GTPγS on G-protein with Rh-B and Ops-B. At this second stage of cGMP cascade once again the inhibition effect is more pronounced with Ops-B.

We verified that these differences are still reflected at the final stage of the cascade, i.e., PDE catalytic activity. Recombination of DTE with Ops-B yielded much lower levels of activated PDE than Rh-B. When rhodopsin is modified with 5-times more CNBr the activation of PDE by Rh-B is diminished by 50% but is never suppressed as is the case with Ops-B. Incubation of ROS with CNBr was also performed for time lengths as long as overnight (12 h): not only the yield of cleavage is not significantly increased but the structure of the rhodopsin control is destabilized as attested by its absorption spectrum and the loss of its capacity to activate PDE. On the other hand, the binding site of G-protein is only partially accessible to extradiscal reagents in darkness and becomes exposed upon illumination of rhodopsin.

In the preceding communication we showed that CNBr treatments of disc membranes resulted in modification and cleavage of the C-terminal region, as well as the third and the second extradiscal loops of rhodopsin. We observed an increase in the yield and a qualitative difference in the cleavage when the disc membranes were bleached prior to the CNBr treatment. These cleavage differences are due only to the conformational changes occurring after illumination of rhodopsin. This difference has been monitored here by modifications at three levels of the cGMP cascade. Illumination of rhodopsin causes a higher susceptibility of the C-terminal end to CNBr cleavage [6] or to thermolysin digestion [14] but this region does not participate directly to the binding site of G-protein [7,15]. After a complete cleavage of the third extradiscal loop (without modifying the second one) obtained by limited thermolysin proteolysis [7] the binding of G-protein is not completely suppressed. Although several bonds of rhodopsin are cleaved by CNBr we can deduce from the proceeding observations and the differences between Rh-B and Ops-B that both the third and the second extradiscal loops are involved in the interaction with the G-protein.

The isomerization by light of 11-*cis*-retinal produces a conformational change of the regions involved in the retinal pocket leading presumably to a vertical shift of helices 4 and 6, connected to the second and third extradiscal loops, respectively, towards the extradiscal space. These light-induced conformational changes should be the key of the reversible formation of the G-protein binding site.

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