LIGHT ACTIVATION OF BOVINE ROD PHOSPHODIESTERASE BY NON-PHYSIOLOGICAL VISUAL PIGMENTS

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1. Introduction

The direct linkage between the bleaching of the visual pigment rhodopsin and the control of such visual processes as excitation and adaptation is not known. Hence, it has been difficult to design a good biochemical/physiological test for the ability of modified visual pigments to substitute for native pigments. One criterion is to see if non-physiological pigments lose their color after irradiation, i.e., bleach. However, this test is inadequate because the bleaching of the pigment does not insure the ability to initiate a physiological event. Another approach is the creation of non-physiological pigments in the retina by regeneration of bleached opsin in situ with exogeneously applied chromophores [1,2]. With this method, one can ask if the pigments formed can help to restore the threshold of receptor potential, which was raised by the bleaching. Although this technique is a very promising one, it is not clear if all modified chromophores could pass across the rod plasma membrane so as to form the pigment in situ and the experiments are restricted to a single type of the opsin. A new approach to test for the ability of non-physiological pigments to evoke the triggering action of bleached rhodopsin has been opened up by the discovery that light can mediate the activity of enzymes in the retina. These enzymes, a phosphodiesterase and a GTPase operating in close conjuction [3–5], can control cyclic nucleotide concentrations. Exactly what cyclic nucleotides do in vision is still not understood. Nevertheless, there is widespread agreement that they have an important role because of the enormous biochemical machinery that has been devoted to the control of nucleotide concentrations in photoreceptor cells and because it is light that controls these levels [4]. The presence of these light-activated enzymes provides the possibility for a simple test to see if any artificial or non-physiological rhodopsin is physiologically active, i.e., can light absorbed by the pigment lead to the activation of the phosphodiesterase?

2. Materials and methods

We report here tests with three kinds of bovine phosphodiesterase activators:

(i) Pigments consisting of non-physiological isomers of retinal combined with bovine opsin;
(ii) Artificial pigments formed from chemically modified retinals and bovine opsin;
(iii) Non-bovine visual pigments.

The methods used are straightforward. Crude bovine phosphodiesterase prepared as in [5]. This crude preparation has all of the enzyme components needed to give light activation of phosphodiesterase in the presence of rhodopsin-containing membranes. The purified phosphodiesterase cannot be activated by rhodopsin [3,5]; other components in the regulatory system are required and so to test light activation of the phosphodiesterase the crude enzyme system must be used. Phosphodiesterase activity is measured as in [5]. All assays contained 100 μM GTP, a required co-factor [3]. Analogue pigments were prepared by incubating the non-physiological chromophores with bovine opsin. These chromophores include 11-*cis* retinal (as a control), 9-*cis* retinal, 9,13-di*cis* retinal, and the 11-*cis* and 9-*cis* isomers of 13-desmethyl retinal. All of these chromophores have been shown to combine with opsin to form pigments [6,8].

Octopus photoreceptor membranes were prepared as in [9] by sucrose flotation of the microvillar membranes followed by extensive washing. Bacteriorhodopsin (purple membrane) was prepared by the procedure in [10].

3. Results

Table 1 summarizes the results of our experiments. As a control for possible contamination of our crude bovine phosphodiesterase preparation by bovine photoreceptor membranes, we tested to see if there was any light modulation of basal activity of the crude bovine phosphodiesterase alone; none was seen. Completely bleached bovine rhodopsin prepared ⩾24 h before the experiment is unable to activate the PDE. The washed octopus photoreceptor membranes by themselves showed no light-activated phosphodiesterase activity for any of the conditions used here (table 1).

Bovine rhodopsin, as noted, activates the phosphodiesterase well in the presence of light and the co-factor, GTP. So does rhodopsin regenerated from opsin and the isomer found in vivo, 11-*cis* retinal. The amount of this activation in a given experiment depends on such factors as the concentration of the bovine phosphodiesterase [11]. In our experiments the amount of activation was moderate, ~5–7-fold. The pigment prepared by regenerating bovine opsin with the non-physiological isomer 9-*cis* retinal, isorhodopsin I, can activate bovine phosphodiesterase. Isorhodopsin II (9,13-di*cis* rhodopsin) also can activate bovine rod phosphodiesterase (tabel 1).

Next we checked whether artificial visual pigments made with chemically modified retinals would activate bovine rod phosphodiesterase (table 1).
pigments made with the 11-*cis* and 9-*cis* isomers of a retinal missing one of the normal methyl groups-13-desmethyl retinal. Table 1 shows that both of these pigments can activate bovine phosphodiesterase. It is extremely interesting that the 11-*cis* 13-desmethyl pigment shows considerable activation in the dark, while the 9-*cis* 13-desmethyl pigment, like all the other activating pigments, requires light.

Next, we did an entirely different kind of experi-

Table 1
Activation of bovine rod phosphodiesterase (PDE) by non-physiological pigments

Activator[a]	Dark PDE activity[b] (nmol/min)	Light PDE activity[b] (nmol/min)
None	21	20
Opsin[c]	21	21
Octopus rhodopsin without PDE	0	0
Bacteriorhodopsin without PDE	0	0
Rhodopsin	23	120
Regenerated rhodopsin (11-*cis*)	23	121
Isorhodopsin I (9-*cis*)	18	118
Isorhodopsin II (9,13-di*cis*)	14	102
[13-desmethyl] rhodopsin (11-*cis*)	56	108
[13-desmethyl] isorhodopsin (9-*cis*)	27	104
Octopus rhodopsin	17	111
Bacteriorhodopsin	19	19

[a] This result represents a typical experiment. All results have been reproduced at least 3 times. All pigments are based on bovine opsin unless otherwise stated. Free all-*trans* retinal or free 13-desmethyl retinal do not activate the PDE

[b] 100 μM GTP was added to a fixed amount of crude PDE. Pigment, $A \simeq 1.0$, was then added except in the sample labeled 'none'. For light activation, the samples were irradiated for 10 min at 4°C. The assay was started immediately and the samples were incubated for 5 min at 30°C. PDE activity was measured as in [4]

[c] Opsin for the controls and for pigment regeneration was prepared by bleaching rhodopsin in the presence of NH_2OH, freeze-drying and washing with hexane to extract the retinal oxime.

ment using a rhodopsin from another species of animal to activate bovine rhodopsin. We were particularly interested whether invertebrate rhodopsins could activate vertebrate phosphodiesterase because the light control of cyclic nucleotides has not been established in any invertebrate system. Moreover, it is known that the molecular architecture of invertebrate photoreceptor membranes is probably quite different from that of the vertebrate photoreceptor membranes [12]. Table 1 shows quite clearly that an invertebrate rhodopsin, octopus, can activate bovine rod phosphodiesterase.

Although there is no evidence linking rhodopsin to bacteriorhodopsin (the purple membrane protein) we thought it would be interesting to see if light absorbed by this retinal-containing bacterial pigment could activate the phosphodiesterase. No light activation was seen (table 1).

4. Discussion

We have developed a simple way to test non-physiological visual pigments for their ability to substitute for bovine rhodopsin in a physiological visual function, the light-activation of bovine phosphodiesterase.

Three types of non-physiological pigments were studied.

(i) Pigments made with isomers of retinal not found in nature (9-*cis*, 9,13-di*cis*) can activate the rod phosphodiesterase. At least some of the bleaching intermediates of isorhodopsin I are similar to rhodopsin, but the first intermediate of isorhodopsin II is different [13]. Isorhodopsin I [1,2,14] and isorhodopsin II [15] can substitute for rhodopsin in at least some functions, in particular adaptation. It is not known if this is related to their ability to control rod phosphodiesterase.

(ii) The pigments tested having chemically modified chromophores (13-desmethyl retinal) can also express themselves by controlling the activity of the rod phosphodiesterase. An especially interesting finding is that the pigment formed from the 11-*cis* isomer of 13-desmethyl can activate the phosphodiesterase in the dark. This surprising result may be related to the finding in [8] that this 11-*cis* isomer, unlike the 9-*cis* isomer, does not bind tightly to the opsin.

(iii) The ability of octopus rhodopsin to activate bovine phosphodiesterase suggests that: (a) in octopus photoreceptors light absorbed by rhodopsin may be able to control cyclic nucleotide concentrations; and (b) despite their phylogenetic differences, octopus and bovine rhodopsin have key points of similarity.

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