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Calcium-sensitive control of rhodopsin phosphorylation in the reconstituted system consisting of photoreceptor membranes, rhodopsin kinase and recoverin

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Abstract Rhodopsin phosphorylation in the reconstituted system consisting of urea-washed photoreceptor membranes, rhodopsin kinase and recoverin is regulated by Ca^{2+}: the process takes place at low [Ca^{2+}] but is suppressed at high [Ca^{2+}]. In the absence of recoverin, rhodopsin kinase is active irrespective of the cation concentration used. Hence, recoverin is an inhibitor (at high [Ca^{2+}]) but not an activator of rhodopsin kinase. Based jointly on these data obtained on the reconstituted system and on our preceding experiments on rod outer segments suspension, one may conclude that (i) the function of recoverin in retina rod cells is the Ca^{2+}-sensitive control of rhodopsin phosphorylation and (ii) the presence of recoverin is essential and sufficient to provide rhodopsin kinase with the Ca^{2+} sensitivity.

Key words: Photoreception; Phosphorylation; Rhodopsin; Rhodopsin kinase; Recoverin; Calcium; Bovine retina rod cell

1. Introduction

Photon absorption by a retina rod cell initiates signal transduction in cascade rhodopsin–transducin–cGMP-phosphodiesterase and as a result a decrease in the cycloplasmic concentration of a photoreceptor second messenger, cGMP. Since cGMP keeps open cationic channels in the ROS plasma membrane, the fall of its concentration causes a closure of the channels and hyperpolarization of the membrane ([1] for review). In addition the channels' closure is accompanied by a decrease in the cytoplasmic free [Ca^{2+}] [2], which in turn controls the cell recovery by modulating the activity of guanylate cyclase [3–6]. Another point of Ca^{2+} action upon the process recovery is believed to be rhodopsin phosphorylation [7–9]. Previously [9], using a bovine ROS suspension, we showed that antibodies to a calcium-binding protein from bovine ROS, recoverin [4,10], stimulate rhodopsin phosphorylation at high [Ca^{2+}] but have no effect upon the reaction at low [Ca^{2+}]. Based on this result, we suggested that recoverin is an inhibitor (at high [Ca^{2+}]) but not an activator (at low [Ca^{2+}]) of RK. Now we have found that rhodopsin phosphorylation in the reconstituted system consisting of urea-washed ROS membranes, RK and recoverin responds to Ca^{2+} in the same manner as it does in the ROS suspension. The elimination of recoverin from the system results in a loss of the Ca^{2+} sensitivity of RK which is permanently active irrespective of the [Ca^{2+}] used. Hence, recoverin does act as an inhibitor of RK and its presence is essential and sufficient to provide RK with the Ca^{2+} sensitivity.

2. Experimental

ROS prepared from fresh or frozen bovine retinas [11] under dim red light were frozen in liquid nitrogen and stored at −70°C. Before use in the RK assay, ROS membranes were washed with 5 M urea. Rhodopsin concentration was determined by the difference in the optical densities of the samples at 500 nm before and after illumination in the presence of 1% Triton X-100, taking $\varepsilon = 42{,}000$ [12]. The Coomassie blue binding method [13] was used to determine protein concentrations. Homogeneous recoverin preparations and monospecific antibodies to recoverin were obtained as described in [6] and [4,10], correspondingly. RK was purified according to [14] except that $Mg(OAc)_2$ and dithiotreitol in the buffers were substituted with $MgCl_2$ and dithioerythritol, respectively.

RK was assayed at 25°C in the reaction mixture (200 μl) containing 50 mM Tris-HCl, pH 7.4, 100 mM NaCl, 3 mM $MgCl_2$, 1 mM dithiotreitol, 100 μM [γ-^{32}P]ATP (4.7×10^4 cpm/nmol), 1 mM EGTA, about 1 μg RK, urea-washed ROS membranes (70 μg rhodopsin), recoverin (concentrations are shown in the figures); none, 0.7, 0.8, 0.9, or 1.0 mM $CaCl_2$ was added to obtain < 1, 104, 178, 252, 399 nM or 5.6 μM free [Ca^{2+}], respectively [15]. The reaction was carried out under illumination (100-W bulb, at a distance of 2 meters) for 10 min and terminated by the addition of 10% trichloroacetic acid containing 10 mM H_3PO_4. The samples were centrifuged ($10{,}000 \times g$, 15 min), the pellet obtained was washed with the latter mixture and ^{32}P incorporation in the pellet was estimated by Cherenkov counting in plastic tubes. The main portion of the radioactivity of the pellet is present in rhodopsin [9].

3. Results and discussion

Like the bovine ROS suspension [9], the reconstituted system consisting of urea-washed ROS membranes, RK and recoverin demonstrates a dependence of rhodopsin phosphorylation on [Ca^{2+}]: the level of ^{32}P incorporation into the membranes decreases as free [Ca^{2+}] is changed from <1 nM to 5,6 μM (Fig. 1). K_{50} of the effect is equal to 250 nM that is about twice as much as the value of 140–150 nM obtained in the case of the ROS suspension.

It can be seen from Fig. 2 that (i) rhodopsin phosphorylation in the system is strictly light-dependent; (ii) at low [Ca^{2+}] the level of rhodopsin phosphorylation is maximal and independent of the recoverin concentration; (iii) at high [Ca^{2+}] rhodopsin phosphorylation is inhibited by recoverin in a concentration dependent manner; the K_{50} value for recoverin is equal to about 6 μM. Since in the absence of recoverin the maximal level of rhodopsin phosphorylation was observed irrespective of the free [Ca^{2+}] used, we may conclude that recoverin is an inhibitor of RK effective at high [Ca^{2+}]. The same conclusion follows from the experiment with recoverin-specific antibodies which,

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Abbreviations: ROS, rod outer segments; cGMP, 3′,5′-cyclic guanosine monophosphate; RK, rhodopsin kinase.

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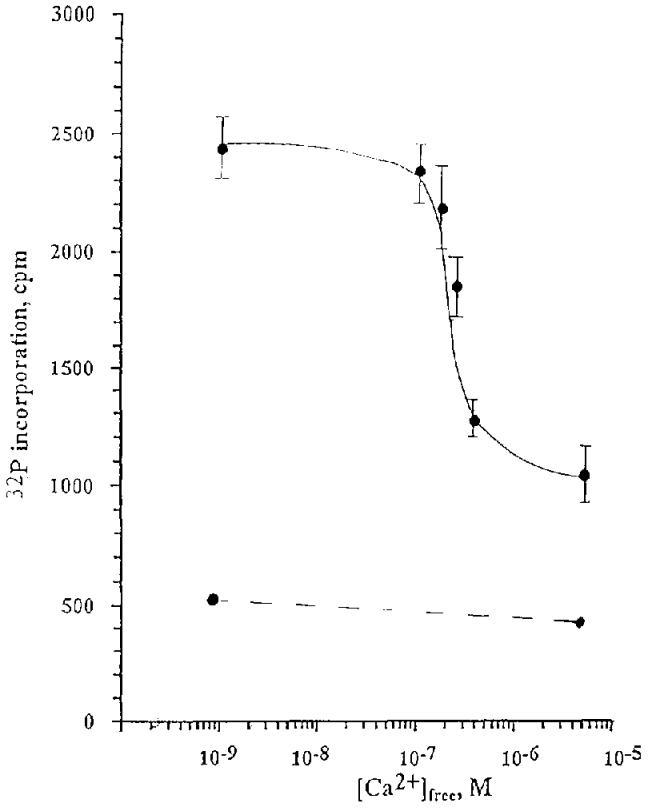

Fig. 1. Dependence of rhodopsin phosphorylation on free [Ca^{2+}] in the reconstituted system RK, urea-washed ROS membranes and recoverin. 10 μM recoverin was present in the reaction mixture. The reaction was carried out under light (solid line) or in the dark (dashed line). Points represent the average value ($n = 2$) with S.D. bars.

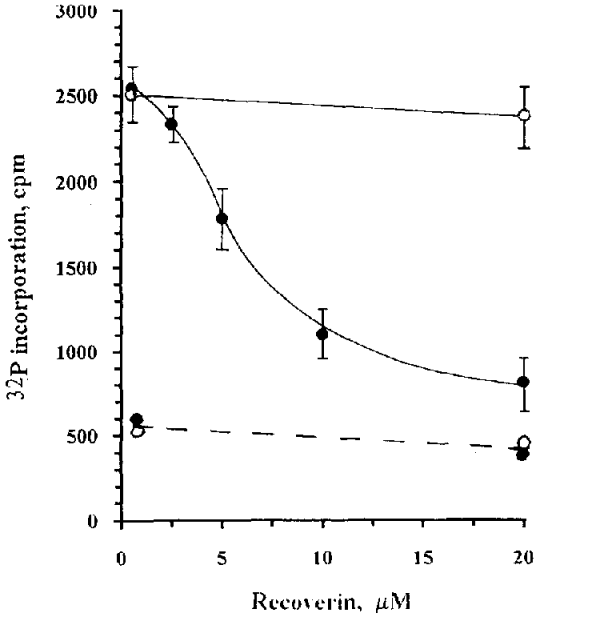

Fig. 2. Dependence of rhodopsin phosphorylation on recoverin concentration in the reconstituted system RK, urea-washed ROS membranes and recoverin. < 1 nM and 5.6 μM free [Ca^{2+}] are referred to as 'low' (○) and 'high' (●) [Ca^{2+}], respectively. The reaction was carried out under light (solid line) or in the dark (dashed line). Points represent the average value ($n = 2$) with S.D. bars.

in the case of the reconstituted system, as in the case of the ROS suspension [9], activate rhodopsin phosphorylation at high [Ca^{2+}] but have no effect at low [Ca^{2+}] (data not shown). Notice that according to a recent preliminary communication of another group [16] recombinant recoverin also acts as an inhibitor of rhodopsin phosphorylation at high [Ca^{2+}].

Our previous experiments performed on the ROS suspension [9] did not allow us to exclude the possibility that the mechanism of the Ca^{2+}-sensitive control of RK is more complicated and includes, besides recoverin, an additional factor needed to provide RK with the sensitivity to Ca^{2+}. The present data obtained on the reconstituted system containing only urea-washed membranes and two purified proteins, RK and recoverin, exclude such a possibility. Therefore, it may be inferred that recoverin is an essential and sufficient factor for providing RK with the Ca^{2+} sensitivity.

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