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On the activation of phosphodiesterase by a 26 kDa protein

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The effects of a 26 kDa protein isolated from vertebrate retina rod outer segments (ROS) and its reconstituted analog on the phosphodiesterase (PDE) activity and cGMP-dependent conductance have been studied [Nature 313 (1985) 310–313]. Using the patch-clamp technique it was shown that the 26 kDa protein in concentrations up to 1 μM accelerates hydrolysis of cGMP by near-membrane PDE by 1–2 orders of magnitude. This process is suggested to be mediated by some intracellular agent. At the same concentrations the 26 kDa protein was shown to inhibit cGMP-dependent conductance of the photoreceptor membrane. A possible role of these effects in the processes of phototransduction and adaptation is discussed.

Photoreceptor; cGMP; Phosphodiesterase; Cationic channel; Calcium binding protein

1. INTRODUCTION

Recently the 26 kDa protein (P26 kD) present in ROS preparations was shown to be a calcium-dependent modulator of guanylate cyclase (GC) activity [2,3]. Within the frame of the cyclic nucleotide hypothesis this protein is suggested to participate in the inactivation of the phototransduction cascade. At the same time some indications appeared that calcium-dependent PDE activity can be controlled by a protein of the same molecular mass [4]. In the present work we studied the effects of the P26 kD on PDE activity and cGMP-dependent conductance. The experiments were carried out using the 'patch-clamp' technique on cytoplasm membrane patches incorporating the elements of the phototransduction cascade [5,6].

2. MATERIALS AND METHODS

Rod outer segments (ROS) were isolated from *Xenopus laevis* retina according to [7]. Integral transmembrane conductance of a photoreceptor cytoplasmic membrane patch was recorded by the 'patch-clamp' technique in the 'inside-out' configuration [8]. All experiments were carried out in the light.

In the study we used: 8BrcGMP, a cGMP analog resistant to PDE hydrolysis; HEPES from Boehringer (Germany); IBMX–PDE inhibitor, EDTA (ethylenediaminetetraacetic acid) from Sigma (USA); bovine serum albumin (BSA) and other chemicals from Reakhim (Russia). The physiological solution used was of the following composition (mM): 100 NaCl, 10 KCl, 1 MgCl; 0.1 $CaCl_2$, 10 HEPES; pH 7.4.

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Abbreviations: cGMP, guanosine 3′:5′-cyclic monophosphate; ROS, retina rod outer segment; PDE, phosphodiesterase; IBMX, isobutylmethyl xanthine.

Partially purified P26 kD was isolated as described in [4] using a buffer of low ionic strength. A reconstituted 26 kD protein obtained in the laboratory of Prof. N.G. Abdulaev [9,10] was also used.

3. RESULTS AND DISCUSSION

It has been shown that the P26 kD (i) activates near-membrane PDE and (ii) inhibits cGMP-dependent conductance irrespective of whether PDE activity is changed or not.

3.1. *The P26 kD activates phosphodiesterase*

To study the effect of the P26 kD on near-membrane PDE we selected the patches with pre-existing PDE activity (Fig. 1a). 1 μM P26 kD applied in combination with cGMP (Fig. 1b) considerably accelerates the cGMP hydrolysis by PDE. Restoration of the patch conductance after P26 kD application in the presence of IBMX suggests that the effects observed are just due to extra activation of near-membrane PDE (Fig. 1d). Assuming that the initial rate of the patch conductance decay after application of cGMP or cGMP in combinations with p26 kD is mainly controlled by the rate of cGMP hydrolysis by PDE, the increase in the rate of the hydrolysis due to the application of p26 kD can be estimated by 1–2 orders of the magnitude. Maximal PDE activation is achieved within several minutes after P26 kD application (Fig. 1c,e) and probably related to the slow adsorption of the protein on the membrane. Slow P26 kD adsorption complicated the quantitative description of the phenomenon. According to our estimations (averaged data obtained from 4 series of experiments), a half-maximal effect of the increasing rate of cGMP hydrolysis by PDE is achieved at a P26 kD concentration of about several hundreds nanomoles.

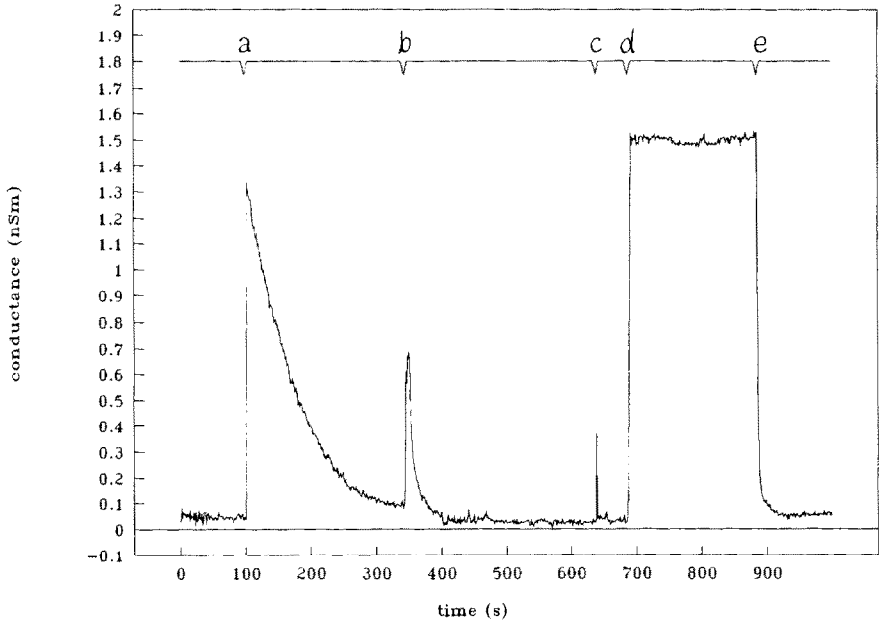

Fig. 1. Effect of p26 kD on PDE activity. Points at which replacement of the perfusion solution was carried out are indicated with marks: a, c, e, application of 100 μM cGMP; b, application of 1 μM p26 kD in combination with 100 μM cGMP; d, application of 1 mM IBMX in combination with 100 μM cGMP.

The effect of PDE activation by P26 kD disappears after numerous cGMP applications and washing with Ringer solution, whereas the PDE activity itself remains at its initial level. We suggest that this phenomenon may point to the fact that PDE activation by P26 kD is mediated via some intracellular agent slowly dissociating from the membrane. The mere absence of such a cofactor may be the reason of weak PDE activation by P26 kD (only 50% at 1 μM free calcium) as described in [4].

Unfortunately, we failed to reliably reveal the effect of calcium ions in PDE activation. In control experiments (the patches prior to P26 kD application) we observed insignificant acceleration of cGMP hydrolysis when the calcium concentration was decreased from 0.1 mM to 10 nM. Approximately similar acceleration of the hydrolysis was observed upon P26 kD application at low calcium as opposed to high calcium concentrations. However, it cannot be excluded that after the patch isolation some P26 kD remains on the membrane and its activity is controlled by calcium.

PDE activity was induced by both partially purified sample of native P26 kD and recombined protein [10]. Efficiency of recombined P26 kD eliminates the probability of any experimental artefacts.

3.2. *The P26 kD inhibits cGMP-dependent conductance*

Application of P26 kD at micromolar concentrations in combination with cGMP leads to quick (less than 1 s) (Fig. 2b) and, as a rule, reversible (Fig. 2d) inhibition of the patch cGMP-dependent conductance (by about 25%). In control experiments with BSA a comparable effect due to non-specific action of the protein was revealed only at BSA concentrations as high as hundreds micromoles, i.e. exceeding the effective concentration of the P26 kD by more than two orders of magnitude. The fact that such an effect cannot be induced by PDE activation is supported by the following: (i) the extent of inhibition did not decrease when instead of cGMP its hydrolysis-resistant analog, 8BrcGMP, was used; (ii) IBMX did not restore the patch conductance (Fig. 2c); and (iii) the inhibition is observed in the patches with both pronounced PDE activity and without it.

The effect of inhibition of cGMP-induced conductance depended on the Ca^{2+} concentration in all experimental series (n = 12). At 10 nM free calcium the extent of inhibition was as a rule two- to fourfold higher than that at 0.1 mM (at the same protein concentration). The dependence of the protein activity on Ca^{2+} concentration points to a probable participation of the effect described in the processes of inactivation and adaptation of photoreceptor.

The question arises whether the process of PDE activation by the P26 kD may play some functional role. There exists a considerable scatter in quantitative description of the mechanism of the photoresponse onset generation. The maximal rate of PDE operation is about 1,250 cGMP molecules per second [11]. The mean Michaelis constant (K_m) is about 100 mM and tends to increase upon illumination [12]. cGMP-free concentration equals units of micromoles [13]. Based on these

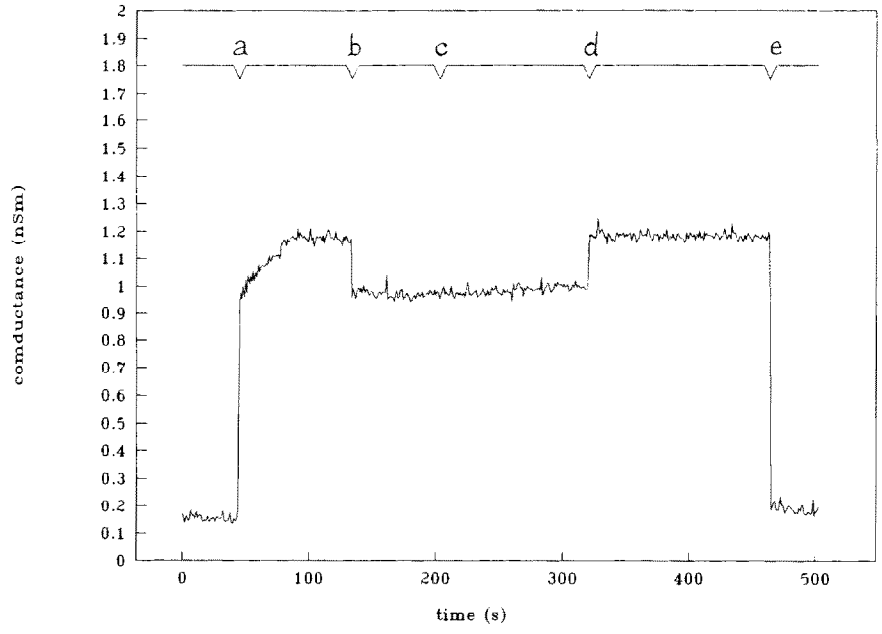

Fig. 2. Effect of p26 kD on cGMP-induced conductance. Points at which replacement of the perfusion solution was carried out are indicated with marks: a, d, application of 100 μM cGMP; b, application of 1 μM p26 kD in combination with 100 μM cGMP; c, application of 1 mM IBMX in combination with 1 μM p26 kD and 100 μM cGMP; e, application of starting physiological solution.

parameters, the mean rate of cGMP hydrolysis by PDE should not exceed a few cGMP molecules per second. This value is at least two orders of magnitude lower than the rate of PDE operation required. From the data obtained (Fig. 1a–c), reasoning from the assumptions made and using the dependence of a given patch conductance on a cGMP concentration at 1 mM IBMX (this dependence appeared to be approximately equal to a standard one [13] and is not shown in this paper), we have estimated that in the absence of P26 kD the rate of cGMP hydrolysis by PDE corresponds to units of molecules per second. However the real rate of hydrolysis in vivo may appear to be considerably higher due to the effect of the P26 kD. At low free cGMP the mechanism of acceleration of cGMP hydrolysis seems to be a decrease of the K_m value of PDE rather than an increase of maximal rate of hydrolysis. This hypothesis correlates well with the data reported in [4] that p26 kD slightly increased the efficiency of PDE activation without any changes in the maximal rate of hydrolysis. Along with the above considerations, the absence of appreciable calcium dependence of this phenomenon suggests the probability of participation of 26 kD protein-dependent PDE activation in the photoresponse rising-phase generation.

It seems surprising that P26 kD can, at the same time, be a calcium-binding protein [9,10], a calcium-dependent modulator of GC activity [2,3] and a strong activator of PDE, the enzyme opposite in action to GC. From this point of view the question arises about identity of recoverin [2] and C-modulin [4].

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