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Predictive Value of the Analogy Between Hormone-Sensitive Adenylate Cyclase and Light-Sensitive Photoreceptor Cyclic GMP Phosphodiesterase: A Specific Role for a Light-Sensitive GTPase as a Component in the Activation Sequence

Takao Shinozawa, Indira Sen, George Wheeler, and Mark Bitensky

Department of Chemistry, University of New Haven, West Haven, Connecticut (G.W.); and Department of Pathology, Yale University School of Medicine, New Haven, Connecticut (T.S., I.S., M.B.)

We report experiments which involve a light sensitive GTPase in the light dependent activation of retinal rod 3′5′-cyclic guanosine monophosphate (cGMP) phosphodiesterase (PDE). The data suggest that the light activated GTPase is intermediate between rhodopsin and PDE in the light-dependent activation sequence. We list the many striking similarities between hormone sensitive adenylate cyclase and light activated PDE in order to emphasize that the findings presented herein may have predictive value for ongoing studies of the hormone sensitive adenylate cyclase specifically regarding the role of the hormone activated GTPase in the activation sequence.

Key words: **vertebrate photoreceptor, cyclic GMP, cyclic nucleotide regulation, phosphodiesterase, light activated GTPase**

Ever since the classical studies of Rall and Sutherland [1], on the hormone-activated, integral membrane enzyme system, adenylate cyclase, there has been great interest in the molecular details of the transmembrane propagation of the hormonal signal. A significant development in this direction has been the demonstration (by Rodbell's group [2]) of a role for GTP as an obligatory component of the hormonal activation of adenylate cyclase. They have also shown that guanylyl imido-diphosphate (GMP-PNP) can effectively replace GTP in the activation step.

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Since 1970 we have been studying a series of light dependent reactions which appear to govern the levels of cyclic GMP in vertebrate rod photoreceptors. These reactions link photon capture by rhodopsin to a rapid and striking activation of a cGMP PDE. In our attempt to elucidate the mechanism of the light sensitive activation of disk membrane phosphodiesterase, we have observed that GTP is also required as a component of the light-activation of rod outer segment [3]. This observation uncovered a series of striking analogies between the hormone sensitive adenylate cyclase and light sensitive PDE. In our comparison of the two systems, we view light as analogous to the hormone; rhodopsin as analogous to the hormone receptor; and PDE analogous to the cyclase catalytic moiety. In the rod outer segment, it appears that PDE is the dominant force in the regulation of cyclic nucleotide concentrations. We list here 11 additional points of similarity between the hormone activated adenylate cyclase and the vertebrate photoreceptor, light-activated cyclic-GMP PDE:

1. Both systems require two components for the activation of their catalytic moieties. In the case of the hormone stimulated adenylate cyclase, these components are the hormone receptor interaction and GTP. In the case of light activated photo-receptor PDE, these components are the photo-bleaching of rhodopsin and GTP. If GTP is added in the absence of the first signal (hormone or light) significant activation does not occur. If the hormone (or light) is introduced in the absence of GTP, activation also does not occur [4, 5]. Both systems exhibit little activity in the absence of the specific signals and are rapidly activated to a striking extent by the presentation of the specific signal in the presence of GTP [6, 7].

2. The sequence in which the two activating components are presented is critical in both systems. When GTP is added in the absence of light and the membranes washed and then illuminated, no activation of PDE is observed. When washing follows illumination in the presence of GTP, full activation is observed [5]. Similar sequence features are also found for the adenylate cyclase system [8]. These findings suggest the possibility that the binding of GTP to a putative activator site is not possible until either the hormone or light have produced a conformational change which allows this binding.

3. In both systems there is a specific requirement for the guanine nucleotide; other nucleoside triphosphates cannot effectively substitute [5, 9].

4. In both systems GTP functions as an activator at concentrations below 1 micro-molar [5, 10].

5. In both systems the nonhydrolyzable analogue, GMP-PNP, also produces full activation [5, 11].

6. In both systems, the analogue GMP-PNP produces an activated state which does not decay with time [5, 12].

7. There is, in both systems, a signal specific GTPase, ie, the light or the hormone activates GTPase in proportion as it activates PDE or adenylate cyclase respectively [10, 13].

8. Both signal specific GTPases (ie, either light or hormonal activated) can be in-hibited by one micromolar GMP-PNP and the Km's of the GTPase (for GTP) are below one micromolar. This is the same range of concentrations of GTP required for activation of adenylate cyclase or photoreceptor PDE [10, 13].

9. In both systems the signal specific GTPase is only a part of the overall GTPase activity of the cell [5, 10]. We believe that the substrate for the signal specific GTPase must be GTP bound to the specific activator site, since even at Vmax the signal specific GTPase could not significantly diminish the soluble GTP pool size. Hydrolysis of GTP bound

to the activator site by the signal specific GTPase explains why the first signal (light or a hormone) stimulates both the GTPase and the PDE or adenylate cyclase activities, ie, the first signal appears to allow GTP binding to a combined GTP activator and GTP hydrolysis site.

10. Although the hydrolysis of GTP is not necessary for activation of the adenylate cyclase or PDE catalytic moieties (since the nonhydrolyzable analogue GMP-PNP substitutes as an activator) the hydrolysis of GTP (at the activator locus) serves to terminate activation and is a principal element in the turn-off process [3, 12].

11. For both systems there exists a two component turnoff process: The reversal of the first signal (either the binding of hormone or the photo-bleaching of rhodopsin) [4, 14] and the hydrolysis of GTP which is bound to the activator site.

These observed similarities between the light activated PDE and hormone activated adenylate cyclase system have led to a new observation concerning the vertebrate light-activated retinal rod outer segment, PDE system, which we believe may have predictive value for understanding the component interactions of the adenylate cyclase system. We report here that the macromolecular soluble factor (SF), associated with disc membrane preparations which confers GTPase activity on EDTA-washed and illuminated disc membranes [13] is necessary for the light and GTP-dependent activation of rod outer-segment photoreceptor cyclic-GMP PDE.

EXPERIMENTAL PROCEDURE AND RESULTS

We previously found that photoreceptor disc membranes contained a solubilizable, macromolecular heat-labile factor, (SF) which conferred GTPase activity on illuminated but not unilluminated discs [13].

We inquired whether the presence of PDE on the disc membrane is necessary in order for GTPase activity to be expressed as a result of the addition of SF. This question was approached in the following way: Rana Catesbiana disc membranes were prepared as reported previously [7]. Illuminated membranes were extensively washed with 1 mM EDTA and 1 mM dithiothreitol (DTT) (pH 8.1). These disc membranes suspensions were tested both for PDE and GTPase activities as described previously [5]. Such discs show no GTPase activity and negligible PDE activity even in the presence of protamine.

The SF used in this experiment was partially purified as follows: Rod outer-segment disc membranes were incubated in 1 mM EDTA and 1 mM DTT, pH 8.1, for 1 h on ice. The disc membranes were removed by centrifugation and the supernatant, after concentration by lyophilization, was loaded onto a Sephacryl S-200 Pharmacia column, equilibrated with a 10 mM tris buffer (pH 7.5), containing 5 mM $MgSO_4$, 1 mM DTT, 100 mM KCl and 20% sucrose. The column eluate was monitored for GTPase restoring activity by addition to illuminated, EDTA washed-disc membranes. This procedure increases the specific activity of SF two fold as compared to that observed in the unmodified EDTA supernatant.

SF alone has no GTPase or PDE activity. When SF is added to illuminated, EDTA-washed discs, GTPase activity is fully restored (Table I). We cannot yet conclude that the GTPase catalytic moiety is entirely borne by SF since we cannot exclude the possibility that some other membrane protein (or proteins) collaborates with SF to produce this GTPase activity.

Next we inquired whether SF was necessary for the light and GTP-dependent activation of PDE. Partially purified PDE was obtained by sucrose density gradient centrifugation as previously described [7] and was entirely free of SF activity. SF used in this experiment

TABLE I. Restoration of GTPase Activity to Illuminated EDTA-Washed Membranes by the Addition of Partially Purified SF

Components of assay mixture	GTPase activity (pmoles Pi liberated/incubation)
SF alone[a]	0
Disc membranes alone[b]	1
SF + disc membranes	41

The assay mixture containing is a final volume of 100 μl: Tris-HCl, pH 7.5, 100 mM; $MgSO_4$, 10 mM; DTT, 1 mM; and ^{32}P-GTP, 0.5 μM (7×10^5 cpm per incubation) was incubated at 37°C for 2 min. At this substrate concentration all GTPase activity is light-dependent [7]. This reagent blank gave a value of 5 pmoles Pi liberated per incubation, which was subtracted from all values.
[a] 8 μg of sephacryl purified SF were added.
[b] 47.5 μg of EDTA washed disc membranes were added.

TABLE II. Demonstration of the Requirement for SF in the Light and GTP Dependent Activation of PDE

Components of assay mixture	Observed PDE activities[a]	Relative phosphodiesterase activities[b]
SF	0.0	0.00
PDE	17.0	0.19
SF + PDE	17.0	0.19
Disc Membranes	0.0	0.00
Disc Membranes + SF	8.1	0.09
Disc Membranes + PDE	31.9	0.39
Disc Membranes + PDE + SF	65.9	1.08
Disc Membranes + PDE + SF and no GTP	39.3	0.50

PDE activity was assayed as described [7].
[a] Relative PDE activities were calculated as ln(cAMP initial/cAMP final).
[b] Activities are expressed along as n moles of cAMP hydrolyzed/10 min, incubation.
Values given represent the averages of duplicates which agree within 5%. 0.6 μg of SF, 0.05 μg of PDE and 9.5 μg of EDTA-washed disc membranes were added to 40 μl of assay mix as indicated. GTP was added to a concentration of 5 μM except where otherwise indicated.

was further purified by ion exchange chromatography (DEAE-Sephadex, Pharmacia) following the prior purification on gel filtration described above. The SF activity was eluted with a continuous KCl gradient and activity appeared at 0.3M KCl. The capacity of this SF preparation to restore GTPase activity to illuminated, EDTA-washed discs is 13 fold greater than that of the unmodified EDTA supernatent. We found that SF and GTP are both needed to significantly activate sucrose density gradient purified PDE in the presence of Mg^{++} and EDTA-washed, illuminated disc membranes (Table II).

DISCUSSION

These findings, when projected by analogy to the adenylate cyclase system, suggest that the activation of the catalytic moiety depends on the presence of the GTPase moiety and further suggests the possibility that the GTPase moiety and the GTP activator locus

may indeed be the same protein. These data also suggest a simple model, for both the hormone sensitive adenylate cyclase and the photoreceptor PDE, composed of (at least) three principal proteins. These include:

1. The light or hormone receptors (ie, rhodopsin or the outward facing hormone receptor moieties);

2. The component which provides both the activator site for the binding of GTP and the GTPase activity;

3. The modulated catalytic moiety (either PDE or adenylate cyclase).

The data also support a model wherein the photobleaching of rhodopsin confers an active conformation only on the GTPase. It is the latter, in collaboration with GTP, which confers an active conformation on PDE. Such a model is also supported by the previous observation that the light sensitivity and action spectrum for the photoactivation of PDE are identical with those for the photoactivation of the GTPase [5].

In the photoreceptor PDE system, we emphasize that SF confers light activation on PDE and also provides that GTPase activity which is necessary for the reversal of this activation. It would seem that there is a paradox in the finding that SF provides both activation of the photoreceptor PDE and also provides termination of activation. This apparent contradiction can be resolved by the following hypothesis:

While GTP is bound to the activator site, the PDE is enabled to function. However when GTP is hydrolyzed, the system is in the "off" position until another GTP is bound. Thus, if one assays photoreceptor PDE with GTP, the system endures a certain amount of "down time" which is the interval between the hydrolysis of GTP and its replacement by a fresh GTP. On the other hand if one assays the photoreceptor PDE with a nonhydrolyzable analogue like GMP-PNP, the observed activity is greater since there is no down time [3]. This has also been found to be true for the adenylate cyclase system where the use of GMP-PNP produces a more substantial adenylate cyclase activity than is observed with the use of GTP [12]. It should be noted that GDP exhibits only 0.1 percent of the activation provided by GTP in the photoreceptor system.

It would seem that in vivo, an obligatory component of the turnoff system is the removal of the activation signal which prevents further binding of GTP to the activator site, rather than depletion of the GTP pool. The critical role of the GTPase is the hydrolysis of the remaining bound GTP after the activation signal has been removed. Reversal of the activation signal prevents further binding of GTP to the activator/GTPase site. If our proposed analogy has predictive value, one may anticipate that a similar situation will obtain in the participation of GTP in the hormonal activation of adenylate cyclase, where a single protein will also provide both the GTP activator and GTP-hydrolysis site.

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