Control of the Cyclic GMP Phosphodiesterase of Frog Photoreceptor Membranes

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ABSTRACT   The light-activated cyclic GMP phosphodiesterase (PDE) of frog photoreceptor membranes has been assayed in isolated outer segments suspended in a low-calcium Ringer's solution. Activation occurs over a range of light intensity that also causes a decrease in the permeability, cyclic GMP levels, and GTP levels of isolated outer segments. At intermediate intensities, PDE activity assumes constant intermediate values determined by the rate of rhodopsin bleaching. Washing causes an increase in maximal enzyme activity. Increasing light intensity from darkness to a level bleaching 5×10^3 rhodopsin molecules per outer segment per second shifts the apparent Michaelis constant (K_m) from 100 to 900 μM. Maximum enzyme velocity increases at least 10-fold. The component that normally regulates this light-induced increase in the K_m of PDE is removed by the customary sucrose flotation procedures. The presence of 10^{-3} M Ca^{++} increases the light sensitivity of PDE, and maximal activation is caused by illumination bleaching only 5×10^2 rhodopsin molecules per outer segment per second. Calcium acts by increasing enzyme velocity while having little influence on K_m. The effect of calcium appears to require a labile component, sensitive to aging of the outer segment preparation. The decrease in the light sensitivity of PDE that can be observed upon lowering the calcium concentration may be related to the desensitization of the permeability change mechanism that occurs during light adaptation of rod photoreceptors.

INTRODUCTION

Recent studies have indicated that the light-activated cyclic GMP phosphodiesterase (PDE) of vertebrate photoreceptor membranes may be a central element in regulating fast photoreceptor responses to illumination. In isolated frog rod outer segments a light-induced drop in cyclic GMP levels mediated by this enzyme can be extremely rapid, having a $t_{1/2}$ of 125 ms, and the cyclic GMP decrease mimics the light-induced permeability decrease of the plasma membrane in several respects (Woodruff et al., 1977; Woodruff and Bownds, 1979). Yee and Liebman (1978), using a rapid assay technique, have shown that PDE can be activated within milliseconds of light absorption. The cyclic GMP drop apparently is responsible for the dephosphorylation of two minor outer segment proteins. One suggestion has been that these proteins may be

linked to permeability control (Polans et al., 1979). These observations, together with recent electrophysiological studies (Lipton et al., 1977; Miller and Nicol, 1979), have strengthened the hypothesis that cyclic GMP is an intracellular transmitter that mediates between photon absorption by rhodopsin in the disk membrane system and the permeability decrease that occurs in the plasma membrane (Hubbell and Bownds, 1979).

One reason for the present work has been to determine whether the activity of the PDE enzyme can be matched to the behavior of cyclic GMP in intact rod outer segments. We approached this issue by examining PDE in minimally disrupted outer segments in a low-calcium Ringer's solution used in studies on permeability and cyclic GMP decreases in isolated outer segments (Bownds and Brodie, 1975; Woodruff and Bownds, 1979). We hoped that the use of such preparations might reveal the presence of control elements that are removed by the purification and washing procedures used in some of the published studies on this enzyme.

The work reported here demonstrates that gently prepared outer segments can be made permeable enough to permit access of the exogenous radioactive substrate needed to assay PDE while retaining important controlling elements. Under these conditions, several regulatory factors have been found in addition to the GTPase (Wheeler and Bitensky, 1977) and inhibitor components (Dumler and Etingof, 1976; Berman and Usova, 1978; Liu and Wong, 1979; Furaev et al., 1978; Hurley and Ebrey, 1979; Baehr et al., 1979) that have been reported.

MATERIALS AND METHODS

Retinas were removed from dark-adapted bullfrogs (*Rana catesbeiana* or *Rana grylio*, obtained from Lemberger Co., Oshkosh, Wis.) using the procedures described by Woodruff et al. (1977) and Woodruff and Bownds (1979). They were then held by the edge with forceps and gently rinsed in 15 ml of a Ringer's solution: 115 mM NaCl, 2.5 mM KCl, 10 mM HEPES, 2 mM MgCl$_2$, 1 mM dithiothreitol, pH 7.5, calcium concentration buffered to $\sim 10^{-9}$ M by the addition of 10^{-4} M CaCl$_2$ and 2.78 × 10^{-3} M EGTA (Caldwell, 1970). Rod outer segments were detached by shaking the retinas for 2 min in 0.5–1 ml of this Ringer's solution supplemented with 0.8–1 mM GTP (This solution is referred to in the text as GTP-Ringer's). Centrifugation for 1 min at 1,200 g, followed by resuspension of the outer segments in 0.6–0.7 ml of fresh GTP-Ringer's solution, was used both to wash the outer segments and to permeabilize their plasma membranes to ensure access of the [H^3]cyclic GMP used for the PDE assay. All of the outer segments appeared morphologically intact but were permeable as assayed by the fluorescence technique of Yoshikami et al. (1974). After washing and disruption, these preparations were found to retain micromolar levels of ATP as assayed by high-pressure liquid chromatography (Biernbaum and Bownds, 1979). It was also found that 1–5 μM levels of ATP were introduced as a contaminant in the 0.8–1 mM GTP used. In some experiments, outer segments were disrupted by sonication for 5–10 s at 0°C with a Branson Sonifer (Branson Sonic Power Co., Danbury, Conn.). It was found that maximal PDE activity was proportional to the number of permeabilized rods, as assayed using the fluorescent probe technique.

The suspension of washed and permeabilized outer segments then was added to an equal volume of an assay mixture made in the same GTP-Ringer's solution to obtain the following final concentrations: 2–4 mM cyclic GMP (containing trace amounts,

~3 × 10^5 cpm, of 8-[^{3}H]cyclic GMP, 5 Ci/mmol; ICN Chemicals, Irvine, Calif.), 0.8–1 mM GTP, and 4–6 μM rhodopsin. The suspension was then divided into 50 μl portions, which were exposed to various conditions of illumination for 1–5 min, using the calibrated light source previously described (Brodie and Bownds, 1976). The PDE reaction was quenched by adding either 100 μl of 10% trichloroacetic acid (TCA) or by heating for 1 min in a 100°C sand bath. The samples quenched with TCA were neutralized with 10 M KOH. Three portions of the outer segment suspension were reserved for determination of rhodopsin content as previously described (Bownds et al., 1971). Experiments were completed within 30–40 min after the animal was killed to minimize possible time-dependent degradation of the system. All manipulations were performed at room temperature and in the dark under infrared illumination using an infrared image converter (F.J.W. Industries, Mt. Prospect, Ill.).

The amount of cyclic GMP that had been hydrolyzed was determined using procedures adapted from Thompson et al. (1974). Quenched and neutralized samples were added to 200 μl of *Crotalis atrox* venom (2 mg/ml, 50 mM Tris·HCl, pH 7.5), an enzyme mixture that converts [^{3}H]5'-GMP (generated by the PDE from [^{3}H]cyclic GMP) to [^{3}H]guanosine. After 10–15 min of incubation at 37°C, this reaction was quenched by placing the samples in a 100°C sand bath for 1 min. After cooling, 200 or 400 μl of each sample were passed through an anion exchange column (DEAE-Sephadex, A-25, Sigma Chemical Co., St. Louis, Mo., 7.5 × 0.5 cm, equilibrated with 50 mM Tris·HCl, pH 7.5). The radioactivity from the first 6 ml of eluant, which was shown to contain >90% of the [^{3}H]guanosine, was determined by placing a 0.6-ml portion into 5 ml of Aquasol (New England Nuclear, Boston, Mass.) and counting in a Searle Mark II Liquid Scintillation Counter (Searle Analytic, Inc., Des Plaines, Ill.). (As a control, snake venom was omitted from the procedure to demonstrate that the outer segment suspension did not generate guanosine from cyclic GMP by an alternate pathway.) All reagents and enzymes were obtained from Sigma Chemical Co.

RESULTS

Light Sensitivity of Phosphodiesterase

Previous papers in this series (Brodie and Bownds, 1976; Woodruff et al., 1977; Woodruff and Bownds, 1979; Polans et al., 1979; Biernbaum and Bownds, 1979) demonstrate that changes in outer segment permeability and chemistry occur over a range of illumination that bleaches 5 × 10^1 to 5 × 10^4 rhodopsin molecules per outer segment per second. (Each outer segment contains ~3 × 10^9 rhodopsin molecules.) These changes have been measured as responses to continuous illumination, and thus the data in this paper monitor PDE activity as a function of the intensity of continuous illumination.

One goal of these experiments has been to determine whether the activity of the PDE enzyme correlates with the actual changes in cyclic GMP measured in intact outer segments (Woodruff et al., 1977; Woodruff and Bownds, 1979). For this reason, initial measurements were carried out with procedures used in those experiments (no washing or disruption of outer segments, 10% vol/vol calf serum added to the GTP-Ringer's solution). Under these conditions, only 20–40% of the outer segments are disrupted sufficiently to allow access of a fluorescent probe (see Methods), and presumably only this fraction is accessible to the [^{3}H]cyclic GMP substrate used to assay the PDE enzyme.

Breakage of all of the outer segments present by sonication increases the maximum activity threefold to fourfold, but does not influence the form of the data shown in Fig. 1 *a*.

Fig. 1 *a*, then, shows one of five experiments demonstrating that PDE can

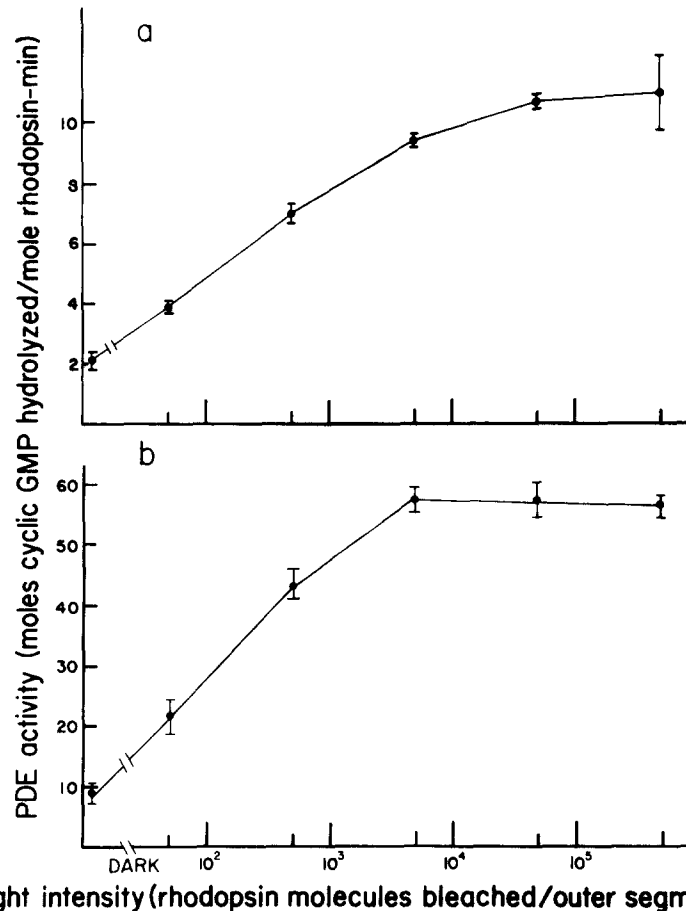Light intensity(rhodopsin molecules bleached/outer segment-s)

FIGURE 1. Cyclic GMP phosphodiesterase activity expressed as a function of light intensity. (*a*) Minimally disrupted outer segments suspended in low-calcium GTP-Ringer's solution (see Methods) that contained 10% calf serum (vol/vol) and [^{3}H]cyclic GMP. Portions of the suspension were illuminated for 5 min at the indicated intensities and then quenched. Each point represents triplicate determinations (±SEM). Data are from one of five similar experiments. (*b*) Outer segments made permeable by sedimentation and resuspension in fresh GTP-Ringer's solution (see Methods). Data are averaged from nine experiments; in each, triplicate points were taken for every intensity. Maximal activities varied from experiment to experiment, possibly because of differential elution of controlling components (cf. Figs. 3 and 4). For this reason, a summary curve was constructed by first averaging the maximal activities from the nine similar experiments and then normalizing the data for each experiment to this maximum. The curve shown was obtained by averaging the separate normalized curves from each experiment. This averaging procedure was also followed in Fig. 5.

be activated over a broad range of illumination. In this experiment, the activity increases over 3–4 log units of continuous light intensity and saturates at an intensity bleaching between 5×10^3 and 5×10^4 rhodopsin molecules per outer segment per second. However, saturation at the higher light level was observed in only two of the five experiments.

The removal of serum and disruption of the outer segments by washing (as described in Methods) does not appear to dramatically change the behavior of the enzyme (Fig. 1 *b*, average of nine separate experiments) except for an increase in activity presumably caused by a more complete permeabilization as well as a stabilization of the saturating level for the light response at 5×10^3 rhodopsin molecules per outer segment per second. Expression of maximal activity requires that both exogenous GTP (0.8–1.0 mM) and 2 mM Mg^{++} be present (cf. Miki et al. [1973] and Chader et al. [1974]). More recent studies demonstrate that the behavior shown in Fig. 1 *a* requires the presence of micromolar levels of ATP. (High-pressure liquid chromatographic analysis revealed, during the course of these experiments, that this ATP was introduced as a contaminant of the 0.8–1 mM GTP added. This ATP requirement will be discussed in more detail in a subsequent paper.)[1]

Intensity-response curves of the sort shown in Fig. 1 *a* and *b* are obtained by incubating separate portions of suspended outer segments at the indicated light intensities. After 5 min, the portions are assayed to determine how much cyclic GMP has been hydrolyzed. It is important to demonstrate that the light-induced hydrolysis is a linear function of time at each intensity tested; otherwise the apparent broad range of response shown in Fig. 1 could be due to exhaustion of substrate or nonlinearity in the light activation process. Fig. 2 demonstrates the required linearity for the range of intensities used and makes clearer the finding that PDE activity reaches a steady-state value for each level of illumination, although illumination and rhodopsin bleaching are continuing. (In this regard, PDE activation is similar to light-induced decreases in permeability, cyclic GMP, and GTP in isolated outer segments. These transitions assume steady-state values at intermediate levels of illumination.) Control experiments that demonstrate linearity were performed for all of the subsequent conditions described in this paper (cf. Fig. 3).

Inhibitor(s) of Phosphodiesterase

Studies from several laboratories have shown the presence in outer segment preparations of an endogenous inhibitor of PDE (Dumler and Etingof, 1976; Berman and Usova, 1978; Liu and Wong, 1979; Furaev et al., 1978; Hurley and Ebrey, 1979; Baehr et al., 1979). Under the conditions of these experiments, it is found that specific activity of phosphodiesterase in the dark (the number of cyclic GMP molecules hydrolyzed per rhodopsin molecule present per min) increases twofold to fivefold as rhodopsin concentration falls below 4 μM. This suggests the dissociation of an inhibitor of the dark activity. However, the specific activity of the enzyme activated by saturating illumination remains constant.

Washing outer segments not only in the GTP-Ringer's solutions used in this work but also in the media used by Yee and Liebman (1978) and Keirns

et al. (1975) enhances both dark and maximal light activity approximately twofold. Enhancement of illuminated activity by washing is illustrated in Fig. 3, which represents one of 23 experiments in which the effect of washing was studied. In this case, the outer segments were sonicated to ensure maximum access of [³H]cyclic GMP and to facilitate elution of inhibitor. The suspension was then divided into two portions, one of which was washed to remove inhibitor and then resuspended in fresh GTP-Ringer's solution. Both were then assayed for PDE activity in response to dim illumination (bleaching 5
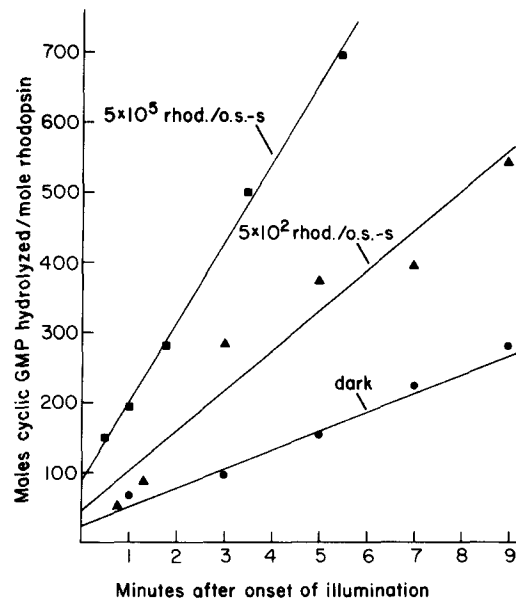

FIGURE 2. Linearity of cyclic GMP hydrolysis at various light intensities. Outer segments suspended in GTP-Ringer's solution were exposed to the indicated levels of illumination, expressed as rhodopsin molecules bleached per outer segment per second (rhod./o.s.-s). Single portions were withdrawn at the indicated times and assayed to determine the amount of cyclic GMP hydrolyzed. The lines were determined by linear regression analysis. Similar data were obtained in two further experiments, and in each the data were linear with r^2 > 0.95.

× 10² rhodopsin molecules per outer segment per second). The washed preparation is twice as active as the unwashed. In numerous preparations this relationship is seen at all intensities tested. (The experiment shown in Fig. 3 was chosen to illustrate the effect of washing because it presents a further demonstration of the linearity of the reaction. The effect of washing on dark activity, however, is obscured by experimental error. The average increase in dark activity caused by washing for the 23 separate experiments was 2.4-fold [±0.6 SEM].)

Outer segment preparations washed one time (as in Fig. 3) yielded the most reproduceable data and were used in all subsequent experiments. Even under

these conditions, variability in the maximum light activity is observed, with some outer segment preparations showing specific activities as high as 250 moles cyclic GMP hydrolyzed per mole rhodopsin per minute.

Kinetic Analysis

The kinetic data shown in Fig. 4 in the form of Lineweaver-Burk graphs reveal a complex picture of the light activation process. Work from several laboratories has suggested that light increases the maximum velocity of the PDE enzyme (V_{max}) without altering the concentration of cyclic GMP substrate
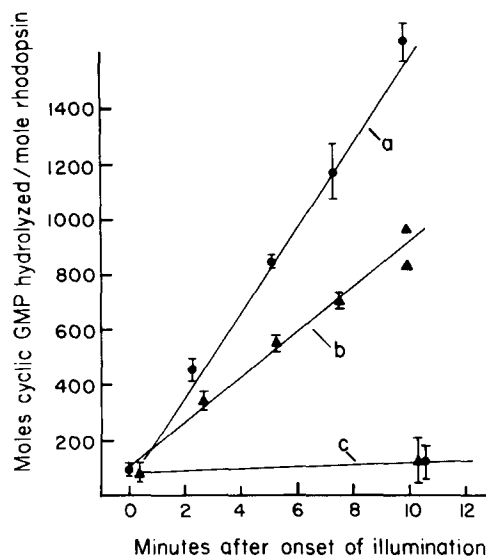

FIGURE 3.   Effect of washing on PDE activity. Curve *a* shows the PDE activity of outer segments after sonication and washing and resuspension in fresh GTP-Ringer's solution. Curve *b* indicates the activity observed in suspended outer segments made permeable by sonication but not washed. Illumination bleaching 5×10^2 rhodopsin molecules per outer segment per second was used. Curve *c* shows PDE activity of washed and unwashed outer segments left in the dark.

required for half-saturation of the enzyme (Michaelis constant, K_m). (Miki et al., 1973; Chader et al., 1974; Yee and Liebman, 1978). In contrast, the present studies demonstrate an increase in the apparent K_m during light activation. The light-activated K_m varies from 0.5 to 1.5 mM in different preparations (for five separate experiments, the average was 0.9 ± 0.2 SEM), perhaps reflecting variation in the concentration of some controlling component. In the experiment shown in Fig. 4 (solid lines), as light intensity increases, a graded increase in V_{max} from 20 to 170 moles cyclic GMP hydrolyzed per mole rhodopsin present per minute and a graded shift in the apparent K_m from 100 to 700 μM is observed. The K_m shift is essentially complete at levels of illumination that bleach 5×10^3 rhodopsin molecules per outer segment per second. Substrate inhibition is observed as cyclic GMP concentration
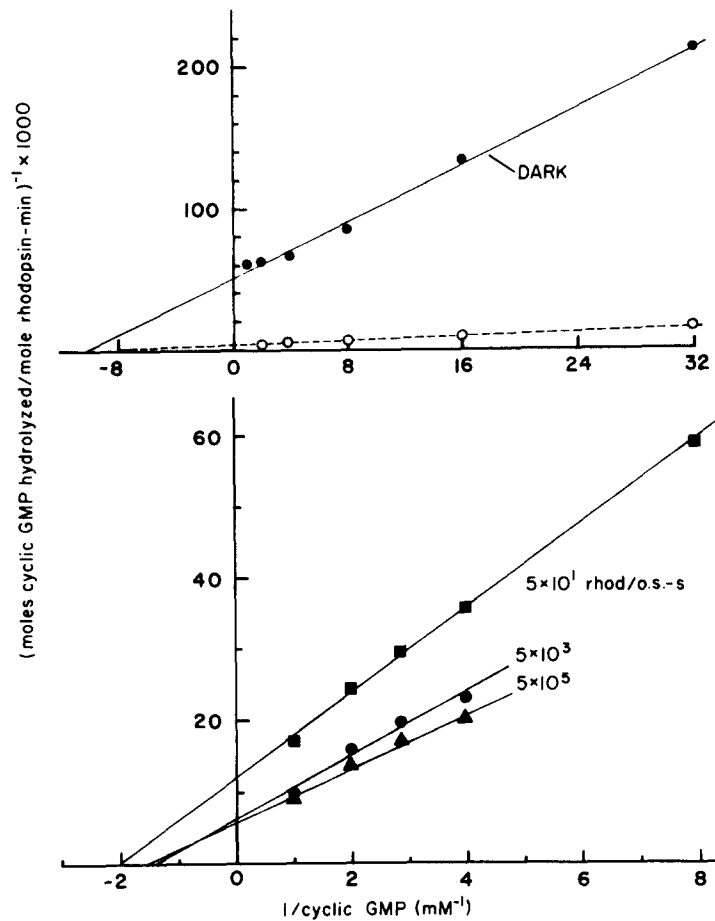
FIGURE 4.   Kinetic analysis of PDE activation. Portions of an outer segment suspension were exposed to illumination at the indicated light intensities (solid lines). After 1 min of illumination [^{3}H]cyclic GMP was added to obtain the indicated concentrations, and the incubations were continued for an additional 30 s to 2 min before the reactions were quenched by the addition of TCA. Each point on the graph represents a single determination. The graph is a Lineweaver-Burk plot showing PDE velocity as a function of the cyclic GMP concentration. The increase in V_{max} is indicated by the decrease in the intercept with the ordinate of the lines obtained for each light intensity (from 20 to 170 moles cyclic GMP per mole rhodopsin per min); and the increase in K_m that occurs upon illumination is indicated by the shift to the right of the intercept with the abscissa (from 100 to 700 μM cyclic GMP). The dashed line shows data for outer segments prepared by sucrose flotation and exposed to saturating illumination. All lines are drawn using linear regression analysis.

approaches 4 mM (data not shown). (Caretta et al. [1979] have also reported a twofold increase in K_m immediately after flash illumination of frog rod outer segments.)

Light-induced shifts in K_m are observed in both washed and unwashed

outer segments. In unwashed outer segment preparations, neither the presence nor absence of serum, nor aging for 1.5 h, significantly influences the light-induced K_m shift. However, the presence of an easily removed component is required. If outer segments are prepared with the customary sucrose flotation and washing procedures (Yee and Liebman, 1978), K_m is found to be 110 μM and little influenced by illumination (Fig. 4, dashed line).

Regulation of Light Sensitivity by Calcium Ions

Fig. 5 demonstrates that the addition of calcium to the low-calcium Ringer's solution used in the experiments described above both increases PDE activity
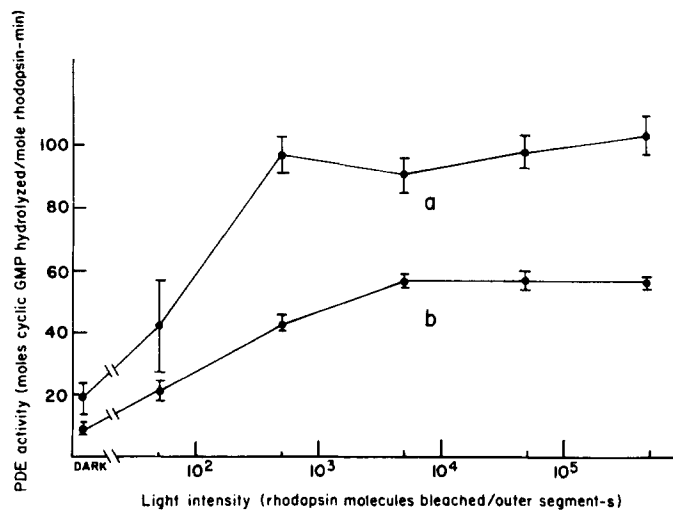

Figure 5. Effect of calcium on light sensitivity of PDE activation. In curve *b*, outer segments were prepared by washing with a low-calcium (10^{-9} M) GTP-Ringer's solution (see Methods). In curve *a*, outer segments were prepared in the same low-calcium solution, but they then were washed by resuspending them in the same solution with the calcium concentration brought to 10^{-3} M. Curves *a* and *b* represent data averaged from four and nine experiments, respectively. Data were normalized as described in Fig. 1.

and causes a sensitization of the light activation process. This is observed in both washed and unwashed outer segments. In 1 mM Ca^{++}, maximum activity is higher, and complete activation is obtained after light intensity has been increased to a level bleaching 5×10^2 rhodopsin molecules per outer segment per second (Fig. 5, curve *a*). In contrast, light activation is not maximal in $\sim 10^{-9}$ M Ca^{++} until the intensity is increased to a level bleaching 5×10^3 rhodopsin molecules per outer segment per second (Fig. 5, curve *b*). In five separate experiments it was determined that the presence of 1 mM Ca^{++} does not have a large influence on the value of K_m or its light-induced shift to higher values (K_m in saturating illumination was 1.0 ± 0.2 mM SEM). Rather, an increase in apparent V_{max} is observed at each intensity tested (between 5×10^1 and 5×10^5 rhodopsin molecules bleached per outer segment per

second). Data for the higher intensity from one of these experiments are shown in Fig. 6. The presence of 1 mM Ca^{++} causes a shift in V_{max} from 200 to 350 moles cyclic GMP hydrolyzed per mole rhodopsin per minute.

The magnitude of the effect of calcium diminishes if outer segments are kept in the GTP-Ringer's solution for more than 1 h, and this decay occurs more rapidly if 10^{-3} M Ca^{++} (rather than 10^{-9} M) is present. This may explain why a calcium effect has not been emphasized previously (cf. Miki et al. [1973] and Yee and Liebman [1978]). (It should also be pointed out that a calcium inhibition of PDE noted by Yee and Liebman [1978] and Chader
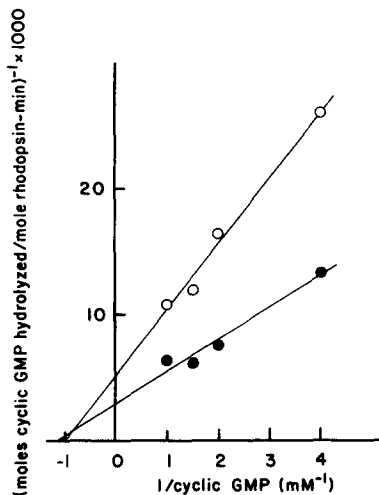

FIGURE 6. Effect of calcium on kinetics of light-activated PDE. Outer segments were prepared as described in Fig. 5 in solutions containing 10^{-3} M Ca^{++} (closed circles) or 10^{-9} M Ca^{++} (open circles) and then exposed to light bleaching 5 × 10^5 rhodopsin molecules per outer segment per second.

et al. [1974] was observed in the absence of Mg^{++}, a required cofactor. In the present experiments Mg^{++} is present at millimolar levels.)

DISCUSSION

The main finding of this work is that a number of different controls appear to regulate the light activation of PDE. In the living rod outer segment, the enzyme might, at different times, have either the desensitized response characteristics shown in Fig. 1 a or the more sensitive response that can be observed in high calcium (Fig. 5, curve a). It is clear that one goal of mechanistic studies must be to isolate and define the factors controlling these variations.

The data in Fig. 1 a and b demonstrate that in outer segment preparations maintained in 10^{-9} M Ca^{++} light activation occurs over at least 3 log units of increasing light intensity (cf. Keirns et al. [1975] Yee and Liebman [1978]). Over this range of illumination, light decreased outer segment permeability (Brodie and Bownds, 1976), cyclic GMP levels (Woodruff and Bownds, 1979),

GTP levels (Biernbaum and Bownds, 1979), and phosphorylation of two small proteins (Polans et al., 1979). At intermediate levels of illumination, PDE activity appears to reach intermediate steady-state values, so that cyclic GMP hydrolysis is a linear function of time rather than of total rhodopsin bleaching (Fig. 2). In current experiments we are attempting to determine what factors (pH, nucleotide concentrations, etc.) control this behavior.

Inhibitor(s) of PDE

This work has provided evidence of an inhibitor component, as has work from other laboratories (Dumler and Etingof, 1976; Liu and Wong, 1979; Berman and Usova, 1978; Yee and Liebman, 1978; Hurley and Ebrey, 1979; Baehr et al., 1979). Detailed comparisons are difficult because those studies did not employ the preparation conditions or the variety of illumination regimens used here. The inhibition of PDE probably involves several factors, and several categories of "inhibition" should be distinguished. Making outer segment suspensions more concentrated inhibits dark PDE activity without altering light activity, suggesting the presence of an inhibitor for the dark activity. Yee and Liebman (1978) have found that increasing rhodopsin concentration to >4 μM does not cause an increase in total PDE activity, also suggesting the presence of an inhibitor of the dark activity. Washing the outer segments, on the other hand, increases both dark and illuminated PDE activity, indicating the removal of an inhibitor that influences both. If work with PDE in other systems is taken as a guide, one expects to find complex controls. In the PDE isolated from rat cerebrum, for example, protein factors that inhibit basal activity are separate from a calcium-binding protein involved in activation (Kanamori et al., 1979).

Kinetics of PDE

The apparent K_m of ~100 M calculated for the PDE enzyme in the dark is in approximate agreement with values obtained in other laboratories (70–160 μM) for both the dark and illuminated enzyme (Miki et al., 1973; Chader et al., 1974; Yee and Liebman, 1978). In this work, however, it has been found that light shifts the K_m to a significantly higher value (~0.9 mM). The K_m shift appears to require an easily removed component, for when outer segments are prepared with the customary sucrose flotation and washing procedures, very little shift in K_m is observed on illumination, in agreement with the work just cited.

One would like to account for the actual behavior of cyclic GMP in intact rods (Woodruff and Bownds, 1979) on the basis of known kinetic parameters for PDE and guanylate cyclase. Unfortunately, calculations that use the kinetic values of Fig. 4 for PDE, and that assume a constant rate of synthesis of cyclic GMP from the cyclase, yield much higher estimates of the light-induced cyclic GMP decline than are actually observed at low light intensities, even though estimates for saturating intensity are correct. This might be explained by the presence in intact outer segments of PDE that is even less sensitive to light than that reported here, or by the presence of a light-sensitive

cyclase. (Little is known about the cyclase in this system, but current evidence suggests that it is not light sensitive [see Pober and Bitensky, 1979, for a review].)

Sensitization of PDE by Calcium Ions

The data presented in Figs. 5 and 6 clearly demonstrate that the light sensitivity of PDE can be shifted by altering the calcium concentration. Dumler (1974) also has reported a slight stimulation of PDE caused by calcium ions in a bovine rod outer segment preparation, and Liu et al. (1979) have suggested that PDE changes from a calcium activator-dependent form to a calcium activator-independent form during normal development of canine retinas. The failure of reports from several laboratories (Miki et al., 1973; Yee and Liebman, 1978; Chader et al., 1974; Goridis and Weller, 1976) to note the effect of calcium reported here may be due to the removal of the calcium binding control element by the purification procedures used.

Fig. 5 indicates an effect of calcium on maximal activity and sensitivity but does not clearly show whether the intensity-response function shifts along the intensity axis. More recent experiments in our laboratory (Kawamura and Bownds)[1] using a pH assay for PDE activity (Yee and Liebman, 1978) have determined that low calcium and ATP cause a net displacement of the curve to the right, with the displacement being intermediate at a calcium concentration of 10^{-6} M. Current experiments are directed at determining whether a distinct calcium binding protein is involved in this process.

Physiological Relevance of the Calcium Effect

It is possible that a calcium-induced shift of PDE sensitivity, such as the one shown in Fig. 5, occurs in the living rod cell. Gold and Korenbrot (1980) and Yoshikami et al. (1980) have demonstrated that light causes an efflux of calcium from photoreceptors. If this efflux causes a lowering of cytoplasmic calcium levels, a desensitization of PDE might be expected. The data of the present paper are of limited usefulness in evaluating this possibility, because PDE sensitivity has been monitored only at calcium concentrations of 10^{-9} and 10^{-3} M. The cytoplasmic levels of calcium in the dark are not known but could be in the 10^{-6} M range commonly assumed (Hagins and Yoshikami, 1974; Wormingthon and Cone, 1978), or even higher. Thus, it would be best to demonstrate that a fall from these levels to 10^{-8} or 10^{-9} M desensitizes the PDE. Such data will be provided in a subsequent paper (Kawamura and Bownds).[1] If cyclic GMP, which is regulated by PDE, controls rod outer segment membrane permeability (Hubbell and Bownds, 1979), this decrease in PDE sensitivity may be part of the molecular mechanism underlying the desensitization that occurs during light adaptation. However, the shifts in sensitivity of PDE seen thus far move its intensity response curve by no more

[1] Kawamura, S., and M. Deric Bownds. Light-adaptation of the cyclic GMP phosphodiesterase of frog photoreceptor membranes mediated by ATP and calcium ions. Submitted for publication.

than 1 log unit of input light intensity and thus fail to offer a complete explanation for the changes over several log units of intensity during light adaptation of living photoreceptors. (A more complete discussion of pathways that might regulate transduction is given elsewhere [Bownds, 1980].)

It should be emphasized that this paper presents only a few of the relevant controls of PDE. A GTPase activity that can be involved in the activation-inactivation sequence has been described by Wheeler and Bitensky (1977). Furthermore, Liebman and Pugh (1979) have observed that bovine PDE activated by a flash of light is inactivated more rapidly if ATP is present. The desensitized behavior of PDE shown in Fig. 5 *b* requires the presence of micromolar levels of ATP, and the light sensitivity of the enzyme is increased by its removal (Kawamura and Bownds).[1] It is possible that rhodopsin phosphorylation by ATP mediates this desensitization. A next step in understanding the control of PDE will be to determine the locus of action of the various components—inhibitor, GTPase, K_m regulator, Ca^{++} regulator, and rhodopsin phosphorylation—now being enumerated.

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