Stimulation of Rhodopsin Phosphorylation by Guanine Nucleotides in Rod Outer Segments†

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ABSTRACT: Porcine rod outer segment (ROS) proteins were phosphorylated in the presence of [γ-^{32}P]ATP and Mg^{2+}, separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, and detected by autoradiography. The phosphorylation of rhodopsin, the major protein-staining band (M_r ~34000–38000), was markedly and specifically increased by exposure of rod outer segments to light; various guanine nucleotides (10 μM) including GMP, GDP, and GTP also specifically increased rhodopsin phosphorylation (up to 5-fold). Adenine nucleotides (cyclic AMP, AMP, and ADP at 10 μM) and 8-bromo-GMP (10 μM) or cyclic 8-bromo-GMP (10 μM) had no detectable stimulatory effect on rhodopsin phosphorylation. GTP increased the phosphorylation of rhodopsin at concentrations as low as 100 nM, and guanosine 5′-(β,γ-imidotriphosphate), a relatively stable analogue of GTP, was nearly as effective as GTP. Maximal stimulation of rhodopsin phosphorylation by GTP was observed at 2 μM. GMP and GDP were less potent than GTP. Both cyclic GMP and GMP were converted to GTP during the time period of the protein phosphorylation reaction, suggestive of a GTP-specific effect. Transphosphorylation of guanine nucleotides by [^{32}P]ATP and subsequent utilization of [^{32}P]GTP as a more effective substrate were ruled out as an explanation for the guanine nucleotide stimulation. With increasing concentrations of ROS proteins, the phosphorylation of rhodopsin was nonlinear, whereas in the presence of GTP (2 μM) linear increases in rhodopsin phosphorylation as a function of added ROS protein were observed. These results suggest that GTP stimulates the phosphorylation of rhodopsin by ATP and that a GTP-sensitive inhibitor (or regulator) of rhodopsin phosphorylation may be present in ROS.

Light-induced phosphorylation of rhodopsin in retinal rod outer segments (ROS)[1] has been studied extensively since its discovery by Kuhn & Dreyer (1972) and Bownds et al. (1972). However, both the mechanism of light activation of rhodopsin phosphorylation and the function of the phosphorylation remain to be determined. A mechanism proposed by several laboratories (Frank et al., 1973; Kuhn et al., 1973; Weller et al., 1975; McDowell & Kuhn, 1977) suggests that light activation of phosphorylation is due to exposure of phosphorylation sites on the rhodopsin molecule during bleaching; this is supported by the observation that purified rhodopsin kinase is not activated by light (Shichi & Somers, 1978). However, the simple mechanism of substrate activation does not explain several observations; these include (a) that regeneration of rhodopsin from opsin and 11-*cis*-retinal does not influence the rate of phosphorylation (Kuhn et al., 1973; Miller et al., 1977) and (b) that the quantum efficiency of rhodopsin phosphorylation is higher at lower levels (<10%) of bleaching [up to 40 mol of phosphate incorporated per mol of bleached rhodopsin (Miller et al., 1977); there are only nine phosphorylation sites on rhodopsin (Wilden & Kuhn, 1982)]. In order to explain these latter observations, Miller et al. (1977) have suggested that bleached rhodopsin interacts cooperatively with unbleached rhodopsin to result in an unbleached rhodopsin which is a more effective substrate.

In 1976, Chader et al. reported that GTP-dependent phosphorylation of rhodopsin was enhanced to a greater extent by light than ATP-dependent phosphorylation. This suggested either that GTP acted as a more effective substrate than ATP or that GTP served as an activator in addition to serving as a substrate. As compared to ATP, GTP is reported to be a less effective substrate (higher K_m, lower V_{max}) for purified rhodopsin kinase (Shichi & Somers, 1978). A nucleotide binding site which can bind GTP as well as ATP has been implicated in the regulation of rhodopsin phosphorylation (Hermolin et al., 1982). Whether this proposed nucleotide binding site is on rhodopsin, rhodopsin kinase, or some other protein is not known. Here, we report on the effects of guanine nucleotides on the phosphorylation of rhodopsin in isolated porcine rod outer segments. GTP was an effective activator of rhodopsin phosphorylation at micromolar concentrations. A part of this work has been presented at a recent meeting (Swarup & Garbers, 1982).

Experimental Procedures

Materials. Nonradioactive nucleotides were purchased from Sigma. Cyclic [8-^{3}H]GMP, [8-^{3}H]GMP, [8-^{3}H]GMPPNP, and [γ-^{32}P]GTP were from Amersham. GMPPNP was from P-L Biochemicals. [γ-^{32}P]ATP was prepared by the procedure of Walseth & Johnson (1979).

Isolation of Rod Outer Segments. ROS from pig retinas were isolated in the dark room in the presence of dim red light. The animals were not dark adapted, but the eyes were immediately kept in the dark after removal from the animals. Retinas were removed in the presence of dim red light from 40–50 pig eyes and suspended in ice-cold, 38.4% sucrose (w/w) prepared in 25 mM Tris-HCl, pH 7.5, containing 5 mM $MgCl_2$. After being stirred gently for 10 min, the suspension was filtered through four layers of cheesecloth. The filtrate was centrifuged at 10000 rpm in a Beckman SW-27 rotor for 3 h, and the floating layer of (red-colored) ROS was collected, suspended in 20 mM Tris, pH 7.0, containing 1 mM dithiothreitol, and pelleted by centrifugation at 100000*g* for 30 min. The pellet was suspended in 20 mM Tris-HCl, pH 7.0, containing 1 mM dithiothreitol and stored frozen at −35 °C in 200-μL aliquots. This preparation of ROS showed rodlike

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[1] Abbreviations: ROS, rod outer segments; GMPPNP, guanosine 5′-(β,γ-imidotriphosphate); Tris, tris(hydroxymethyl)aminomethane; $NaDodSO_4$, sodium dodecyl sulfate; EDTA, ethylenediaminetetraacetic acid.

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structures (before hypotonic shock) with little contamination when viewed by scanning electron or light microscopy. After hypotonic shock this preparation showed disklike structures. Polyacrylamide gel electrophoresis of this preparation of ROS showed (Figure 1) one major band of ~34000–38000 daltons (rhodopsin).

Phosphorylation Procedures. ROS were incubated at 30 °C in 40 mM Tris-HCl, pH 7.0, containing 5 mM $MgCl_2$, 1 mM dithiothreitol, and 40 μM [γ-^{32}P]ATP (1000–2000 cpm/pmol) in a total volume of 50 μL. In some cases the ATP concentration was increased to 400 μM, and in other cases 1–40 μM [γ-^{32}P]GTP was substituted for the radiolabeled ATP. The other nucleotides were added as indicated in the text. The phosphorylation reactions were started by the addition of an aliquot of ROS (20–40 μg of protein) after the desired amount of rhodopsin was bleached. The reaction was terminated at the indicated times by the addition of 50 μL of a solution containing 40 mM Tris-HCl, pH 7.5, 200 mM dithiothreitol, 6% $NaDodSO_4$, and 25% glycerol.

Polyacrylamide Gel Electrophoresis and Autoradiography. Polyacrylamide gel electrophoresis of ROS samples prepared as described above was carried out in the presence of 0.1% $NaDodSO_4$ in a slab gel apparatus with the buffer system of Laemmli (1970). The acrylamide concentration was 10% in the separating gel and 5% in the stacking gel. After electrophoresis for 4–5 h at 20 mA/gel the gels were stained in 0.1% Coomassie blue and destained in methanol (30%), acetic acid (10%), and H_2O (60%). Autoradiography of the dried gel was carried out overnight at −70 °C in a Du Pont Cronex cassette with intensifying screens by using Du Pont X-ray film.

Conversion of Cyclic GMP and GMP to GTP. Cyclic [8-^{3}H]GMP (10 μM) or [8-^{3}H]GMP (10 μM) was incubated with ROS and 40 μM ATP (nonradioactive) under the same conditions as described above for phosphorylation. At the end of the incubation period (3 min) the reaction was stopped with 0.5 mL of 0.5 N perchloric acid. After freezing and thawing, the samples were centrifuged, and the clear supernatant fluid (0.4 mL) was applied to a Dowex 50 (H^+ form) column (0.7 × 25 cm) equilibrated with 0.1 N HCl. Nucleotides were eluted with 0.1 N HCl at a flow rate of 20 mL/h. Two-milliliter fractions were collected and counted for radioactivity. Control experiments where no ROS were added were run in an identical manner.

Stability of GMPPNP. [8-^{3}H]GMPPNP (1 μM) was incubated with ROS as described above for 3 min. At the end of the incubation (3 min) the reaction was stopped with 0.5 mL of 0.5 N perchloric acid. The chromatography of these samples on Dowex 50 (H^+ form) columns was carried out as described above for cyclic GMP and GMP.

Protein Estimation. Protein concentration of ROS samples was measured by the method of Bradford (1976) with γ-globulin as standard.

Results

Isolated rod outer segments were incubated with [γ-^{32}P]-ATP and $MgCl_2$, and the resulting phosphorylated proteins were separated by polyacrylamide gel electrophoresis in the presence of $NaDodSO_4$. An autoradiograph of the gel showed several phosphoprotein bands (Figure 1). However, only the phosphorylation of a major protein staining band of about M_r 34000–38000 (Figure 1) was increased by light; up to 20-fold increases in phosphorylation of this band were observed in the presence of continuous white light (i.e., complete bleaching of rhodopsin). Rhodopsin is known to be an integral membrane protein which constitutes more than 50% of the total ROS proteins. When phosphorylated ^{32}P-labeled ROS were

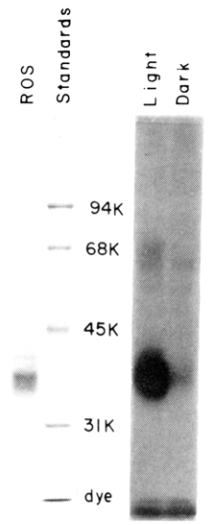

FIGURE 1: (Left panel) Polyacrylamide gel electrophoresis of porcine ROS proteins (8.4 μg) in the presence of $NaDodSO_4$. After electrophoresis the gel was stained with Coomassie blue and destained as described under Experimental Procedures. Standard proteins and their molecular weights are the following: phosphorylase *b*, 94K; bovine serum albumin, 68K; ovalbumin, 45K; carbonic anhydrase, 31K; soyabean trypsin inhibitor, 21K. (Right panel) An autoradiograph of $NaDodSO_4$–polyacrylamide gel showing the effect of light on the phosphorylation of proteins in ROS. The phosphorylation of ROS proteins (28 μg) was carried out with 40 μM [γ-^{32}P]ATP for 2 min in the dark or in the presence of continuous white light as described under Experimental Procedures.

washed with hypotonic buffer (5 mM Tris, pH 7.5, 1 mM EDTA, 1 mM dithiothreitol, 10 mM NaF) at 0 °C followed by centrifugation, the major phosphoprotein band was recovered in the pellet. The EDTA wash, however, solubilized several other proteins as shown by gel electrophoresis (not shown). These results suggest that the major phosphoprotein of M_r 34000–38000 is rhodopsin. The nature of the phosphorylated amino acids was determined by electrophoresis (pH 3.5) of the acid hydrolysate of phosphorylated rhodopsin (removed from polyacrylamide gels). Phosphoserine and phosphothreonine but no phosphotyrosine were detected (not shown).

The effect of various guanine nucleotides on the phosphorylation of proteins in ROS by [γ-^{32}P]ATP is shown in Figure 2. Phosphorylation of rhodopsin was markedly increased by GTP, GDP, and GMP at micromolar concentrations; this response, similar to that observed with light, was specific for the rhodopsin band (Figure 2). Adenine nucleotides (AMP, ADP, and cyclic AMP) at 10 μM showed no effect on rhodopsin phosphorylation (not shown). Table I shows the effect of guanine nucleotides and their analogues on the phosphorylation of rhodopsin. GTP was the most effective nucleotide in increasing the phosphorylation of rhodopsin. GMPPNP, a relatively stable analogue of GTP, was very effective at micromolar concentrations. The order of potency was GTP > GDP = GMPPNP > GMP (Table I). The time course of phosphorylation of rhodopsin in the presence and absence of GTP is shown in Figure 3, and the concentration–response curves for GTP and GMPPNP are shown in Figure 4. GTP was effective even at 0.1 μM concentrations and resulted in half-maximal responses at 0.3 μM. GTP caused 3–5-fold stimulations of rhodopsin phosphorylation at 2 μM concentrations. GMPPNP showed half-maximal responses at 1.0 μM concentrations and at 10 μM concentrations gave 2–5-fold stimulations. Rhodopsin phosphorylation increased with increasing GTP concentrations up to 2 μM. At higher concentrations of GTP (10–100 μM) a decrease in rhodopsin

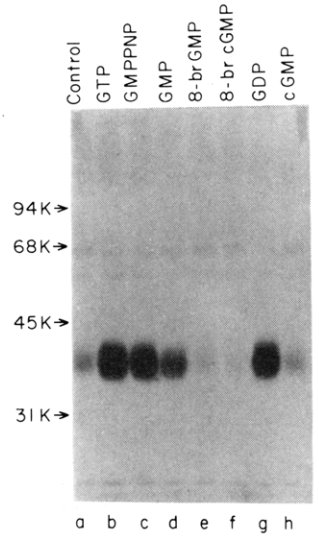

FIGURE 2: An autoradiograph of a $NaDodSO_4$–polyacrylamide gel showing the effect of various guanine nucleotides on the phosphorylation of proteins in porcine ROS. Phosphorylation of ROS proteins (28 μg) with 40 μM [γ-^{32}]ATP was carried out for 1 min after 1% of the rhodopsin was bleached. Phosphorylated samples were subjected to polyacrylamide gel electrophoresis and autogradiography as described under Experimental Procedures: (a) control; (b) GTP, 2 μM; (c) GMPPNP, 10 μM; (d) GMP, 10 μM; (e) 8-bromo-GMP, 10 μM; (f) cyclic 8-bromo-GMP, 10 μM; (g) GDP, 10 μM; (h) cyclic GMP, 10 μM.

Table I: Effect of Various Guanine Nucleotides on the Phosphorylation of Rhodopsin[a]

guanine nucleotide added	pmol of $^{32}P_i$ incorporated in rhodopsin min^{-1} (mg of ROS protein)$^{-1}$	%
none	42.6	100
GTP	194.5	456
GMPPNP, 10 μM	156.1	366
GMP, 10 μM	79.3	186
8-bromo-GMP, 10 μM	25.8	60
cyclic 8-bromo-GMP, 10 μM	24.0	56
GDP, 10 μM	157.7	370
cyclic GMP, 10 μM	33.3	78

[a] Phosphorylation of ROS was carried out in the presence of 40 μM [γ-^{32}P]ATP for 1 min after 1% of the rhodopsin was bleached as described under Experimental Procedures. Phosphorylated proteins were separated by polyacrylamide gel electrophoresis. Following autoradiography, the rhodopsin band was removed from the dried gel and counted for radioactivity.

phosphorylation was observed (Figure 4); this decrease in the effectiveness of GTP at 10–100 μM concentrations is perhaps due to competition of nonradioactive GTP with [^{32}P]ATP as a substrate since the phosphorylation of other ROS proteins was also decreased (not shown).

Cyclic GMP at 10 μM concentrations showed slight inhibition of rhodopsin phosphorylation in ROS at low levels (1%) of bleaching (Figure 2 and Table I); however, at higher levels of bleaching (>5%) and after longer time periods of incubation cyclic GMP caused 2–3-fold stimulation of rhodopsin phosphorylation (not shown). Maximum stimulation of rhodopsin phosphorylation by cyclic GMP was obtained at 10 μM concentrations; higher or lower concentrations gave less or no stimulation. Cyclic GMP had no effect on the phosphorylation of any other protein in ROS (Figure 2).

The ability of various guanine nucleotides to serve as apparent activators suggested the possible conversion of the nucleotides to one common metabolite. It seemed likely either

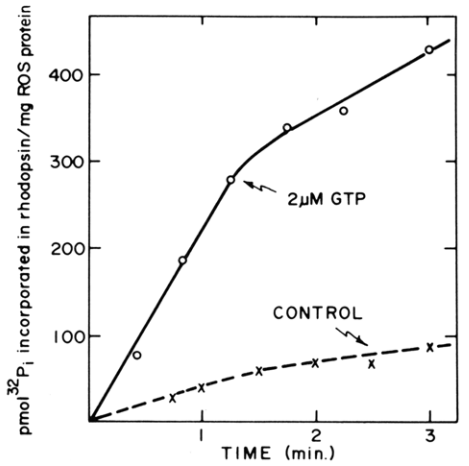

FIGURE 3: Time course of phosphorylation of rhodopsin in the presence and absence of 2 μM GTP. Phosphorylation of ROS (28 μg of protein) by [γ-^{32}P]ATP was carried out for the indicated times with or without GTP after 5% of the rhodopsin was bleached. After the phosphorylation reactions were stopped, the samples were subjected to polyacrylamide gel electrophoresis and autoradiography as described under Experimental Procedures. The rhodopsin band (the major Coomassie blue staining band) was removed from the dried gel after autoradiography and counted for radioactivity.

that a guanine nucleotide was an activator or that [^{32}P]GTP formed by transphosphorylation of GMP or GDP by [γ-^{32}P]ATP was a much more effective substrate than ATP. Since GTP was the most potent nucleotide (and GMPPNP was very effective), the phosphorylation of various guanine nucleotides to GTP (or possibly GDP) was suspected. To determine the metabolic fate of cyclic GMP during the normal phosphorylation reaction, we measured the metabolic products of cyclic [8-^{3}H]GMP (10 μM) after incubation with ROS for 3 min (substituting nonradioactive ATP for [^{32}P]ATP). The ^{3}H-labeled products were separated by chromatography on Dowex 50 (H^+) columns; 50–60% of cyclic [^{3}H]GMP was hydrolyzed within 3 min. GMP was the major radiolabeled product, but some radioactivity (~2%) also appeared in the early fractions corresponding to the position of GTP. This observation suggested the possibility that the apparent effect of added cyclic GMP on rhodopsin phosphorylation was due to one of its metabolites. When [8-^{3}H]GMP was incubated with ROS in a similar manner, radioactivity (~2%) at the position of GTP again was observed. Very little if any radioactivity was observed at the position of cyclic GMP. In similar experiments, [8-^{3}H]GMPPNP (1 μM) was incubated with ROS; its degradation products also were separated on a Dowex 50 (H^+) column. Approximately 90% of the GMPPNP was recovered undegraded, and less than 5% of the nucleotide was recovered as cyclic GMP or GMP. On the basis of these observations, it seemed likely that GTP was the effector for increased rhodopsin phosphorylation and that GDP, GMP, and cyclic GMP had their effects due to their conversion to GTP. This is not surprising since ROS are known to have high activities of cyclic-GMP phosphodiesterase, guanylate kinase, and nucleosidediphosphate kinase (Sosamma et al., 1980).

To this point the experimental evidence for GTP serving as an activator rather than as a much more effective substrate than ATP was (1) extremely low concentrations of GTP were effective, (2) GMPPNP was effective, and (3) the time course of apparent activation with GTP was linear to concave downward rather than concave upward (Figure 3). For further evidence for the proposed activator role of GTP, ATP concentrations were increased to 400 μM. GTP was just as potent

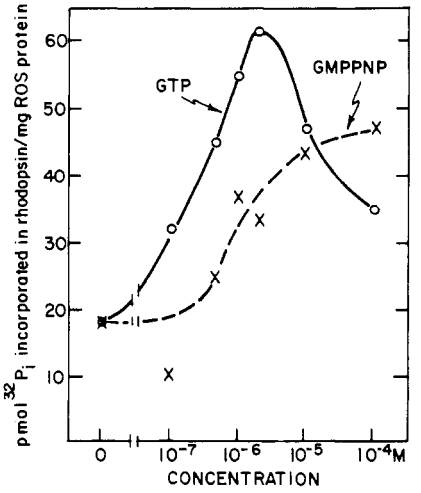

FIGURE 4: Effect of guanine nucleotide concentration on the phosphorylation of rhodopsin in ROS. Phosphorylation of ROS (39 μg of protein) was carried out with 40 μM [γ-^{32}P]ATP for 1 min in the presence of the indicated concentrations of GTP or GMPPNP. After electrophoresis and autoradiography, the rhodopsin band was removed from the dried gel and counted for radioactivity.

at high ATP concentrations (not shown). Furthermore, when [γ-^{32}P]GTP (1 μM) was used as a substrate (in the presence or absence of 40 μM unlabeled ATP) less than 2% phosphorylation of rhodopsin was observed compared with [γ-^{32}P]ATP (40 μM) as substrate. [γ-^{32}P]GTP at 40 μM concentrations was not a better substrate (1900 cpm incorporated in rhodopsin) than [γ-^{32}P]ATP at the same concentration (2600 cpm in rhodopsin). Thus, transphorylation of GMP or GDP by [γ-^{32}P]ATP to form [γ-^{32}P]GTP does not explain the apparent activating effect of the guanine nucleotides.

It also remained possible that the guanine nucleotides served as inhibitors of phosphorhodopsin phosphatase activity; therefore, ROS were phosphorylated, and nonradioactive ATP (800 μM) was then added with or without GMPPNP (10 μM) during the time of dephosphorylation. During a 15-min incubation period, approximately 40% of the rhodopsin was dephosphorylated, but GMPPNP had no detectable effect on the rate of dephosphorylation (not shown).

The effect of variable amounts of rhodopsin bleaching on its phosphorylation in the presence and absence of GTP is shown in Figure 5. The degree of stimulation of rhodopsin phosphorylation by GTP was higher at low levels of bleaching; at low levels of bleaching 2 μM GTP caused up to 9-fold stimulation (Figure 5). Stimulation of rhodopsin phosphorylation by GTP was seen even in the dark (not shown), but it is relevant to point out that even dark-adapted mammalian ROS have significant amounts of bleached rhodopsin (Pober & Bitensky, 1979).

During the course of this work, it was observed that rhodopsin phosphorylation was linear over only a restricted range of ROS protein concentrations; addition of 2 μM GTP, however, resulted in an extension of the linear portion of rhodopsin phosphorylation as a function of protein concentrations (Figure 6). The concave downward pattern observed in the absence of GTP was not due to depletion of ATP since the time course of rhodopsin phosphorylation was linear at the highest protein concentrations in either the presence or absence of GTP. This observation suggests the presence of an inhibitor of rhodopsin phosphorylation in ROS.

Bleached ROS membranes are known to lose their phosphorylation ability on incubation in the dark. ROS membranes were bleached in the presence or absence of GMPPNP (or GTP) and incubated in the dark at 22 °C in order to find out

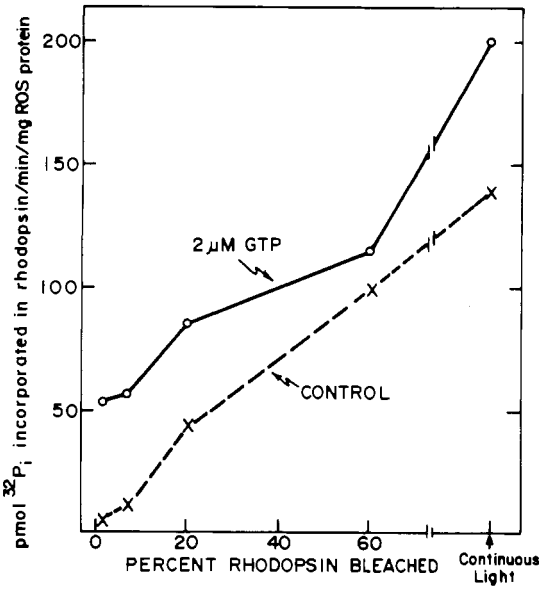

FIGURE 5: Effect of bleaching on the phosphorylation of rhodopsin in the presence or absence of 2 μM GTP. Phosphorylation of ROS proteins (28 μg) was carried out with 40 μM ATP for 2 min after the indicated amount of rhodopsin was bleached. Following electrophoresis and autoradiography, the rhodopsin band was removed from the dried gel and counted for radioactivity.

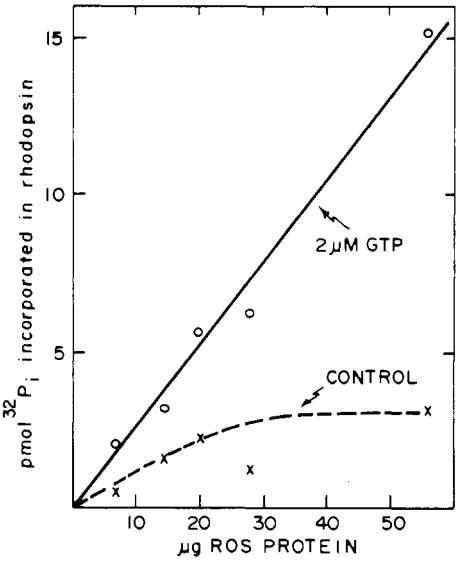

FIGURE 6: Effect of increasing ROS protein concentration on the phosphorylation of rhodopsin in the presence (O) or absence (×) of 2 μM GTP. The phosphorylation of different amounts of ROS was carried out for 2 min with 40 μM [γ-^{32}P]ATP as described under Experimental Procedures. The time course of phosphorylation was nearly linear at the various amounts of ROS protein used.

whether GMPPNP or GTP has any effect on the decay of phosphorylation ability. As shown in Figure 7, GMPPNP (or GTP) did not inhibit the time-dependent decay of phosphorylation ability of bleached ROS membranes.

Discussion

From the data presented in this paper it is clear that added guanine nucleotides stimulate the phosphorylation of rhodopsin. Concentration dependence and other experiments suggest that GTP may be the actual activator. That GTP is the activator nucleotide is strongly supported by the finding that GMPPNP at micromolar concentrations is nearly as good an activator as GTP. Other guanine nucleotides are required in higher concentrations and appear to be converted, at least

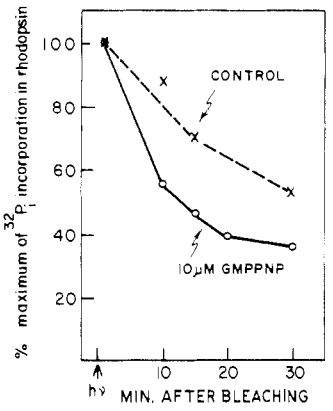

FIGURE 7: Decay of phosphorylation ability of rhodopsin following bleaching. After 5% of the rhodopsin was bleached, ROS were incubated in the dark in the presence or absence of GMPPNP (10 μM). Phosphorylation was initiated at indicated times by the addition of 40 μM [γ-^{32}P]ATP and continued for 2 min. After electrophoresis and autoradiography, the rhodopsin band was removed from the dried gel and counted for radioactivity.

in part, to GTP. The activation of rhodopsin phosphorylation by guanine nucleotides resembles, at least in some respects, the guanine nucleotide dependent light activation of cyclic-GMP phosphodiesterase (Wheeler & Bitensky, 1977) in ROS: (a) in both cases the maximal effective concentration of GTP is about 1–2 μM, (b) GMPPNP can replace GTP, suggesting that hydrolysis of GTP is not required for the activation process, (c) GDP at micromolar concentrations is nearly as effective as GTP, and (d) rhodopsin phosphorylation like cyclic GMP hydrolysis by phosphodiesterase appears to be under some inhibitory constraint which is removed by GTP. On the basis of these similarities it seems possible that the light activation of rhodopsin phosphorylation and light activation of cyclic-GMP phosphodiesterase may involve a common step. However, there is one important difference with respect to the effect of GMPPNP. In the presence of GMPPNP (but not GTP) the activation of cyclic-GMP phosphodiesterase is persistent and does not decay with time (Wheeler & Bitensky, 1977). In contrast, activation of rhodopsin phosphorylation by GMPPNP (or GTP) decays with time (Figure 7). This may suggest that the complex of GTP with GTP binding protein (which is the activator for cyclic-GMP phosphodiesterase) is perhaps not the activator for rhodopsin phosphorylation. An alternative interpretation is that hydrolysis of GTP is not required for the decay of phosphorylation ability of bleached rhodopsin.

Nonlinear increases in rhodopsin phosphorylation with increasing ROS protein concentration (in the absence of GTP) suggests the presence of a GTP-sensitive inhibitor (or regulator) of rhodopsin phosphorylation. The nature of this inhibitor is not known, but one possible candidate for this GTP-sensitive inhibitor is the GTP binding protein (Kuhn, 1980; Godchaux & Zimmerman, 1979) which is also called transducin (Fung et al., 1981). In the absence of light the GTP binding protein (with bound GDP) remains largely bound to rhodopsin (Fung et al., 1981). In the presence of bleached rhodopsin the complex of GTP with the GTP binding protein is formed which dissociates from rhodopsin (Godchaux & Zimmerman, 1979; Fung et al., 1981; Kuhn et al., 1981). The release of bound GTP binding protein may result in the transient exposure of phosphorylation sites on rhodopsin. The phosphorylation sites would be then available for interaction with rhodopsin kinase. This hypothesis is supported by the finding of Kuhn (1978) that rhodopsin kinase is readily extracted from dark-adapted ROS but not from bleached ROS.

Recently Hermolin et al. (1982) have reported that in frog ROS both GTP (0.5 mM) and ATP (0.5 mM) stimulate rhodoposin phosphorylation (at 10 μM [γ-^{32}P]ATP as substrate). On the basis of this observation it was suggested that a nucleotide binding site on rhodopsin or rhodopsin kinase is involved in the regulation of rhodopsin phosphorylation. The maximum stimulation of rhodopsin phosphorylation by GTP was observed at 0.5 mM concentration (Hermolin et al., 1982); this concentration is much higher than that used by us in the present study. We have not observed any stimulation of rhodopsin phosphorylation by ATP, and thus our results suggest that the nucleotide binding site involved in the regulation of rhodopsin phosphorylation is specific for guanine nucleotides.

Acknowledgments

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Registry No. GTP, 86-01-1; GDP, 146-91-8; GMP, 85-32-5; guanosine 5′-(β,γ-imidotriphosphate), 34273-04-6; cyclic GMP, 7665-99-8; 8-bromo-GMP, 21870-09-7; cyclic 8-bromo-GMP, 31356-94-2.

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