

## Regulation of Rhodopsin Dephosphorylation by Arrestin\*

(Received for publication, June 8, 1989)

Krzysztof Palczewski‡, J. Hugh McDowell‡, Scott Jakes§, Thomas S. Ingebritsen§¶, and Paul A. Hargrave‡||\*\*

From the ‡Department of Ophthalmology and ||Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, Florida 32610-0284 and the §Department of Zoology, Iowa State University, Ames, Iowa 50011-3223

We have characterized the opsin phosphatase activities in extracts of rod outer segments and determined their relationship to known protein phosphatases. The opsin phosphatase activity in the extracts was not due to protein phosphatases 1, 2B, or 2C because it was neither stimulated by  $Mg^{2+}$  or  $Ca^{2+}$ /calmodulin nor inhibited by protein phosphatase inhibitors-1 or -2. Opsin phosphatase activity in rod outer segment extracts was potentially inhibited by okadaic acid ( $IC_{50} \sim 10$  nM), a preferential inhibitor of protein phosphatase 2A. Moreover, during chromatography on DEAE-Sepharose, the opsin phosphatase activity co-eluted with three peaks of protein phosphatase 2A activity, termed protein phosphatases 2A<sub>0</sub>, 2A<sub>1</sub>, and 2A<sub>2</sub>. The opsin phosphatase activity of each peak was stimulated by polylysine, a known activator of protein phosphatase 2A. Finally, treatment of rod outer segment extracts with 80% ethanol at room temperature converted the activity from a high molecular weight form characteristic of the protein phosphatase 2A<sub>0</sub>, 2A<sub>1</sub>, and 2A<sub>2</sub> species to a low molecular weight form characteristic of the protein phosphatase 2A catalytic subunit. We conclude that protein phosphatase 2A is likely to be the physiologically relevant rhodopsin phosphatase.

The 48-kDa rod outer segment protein arrestin (S-antigen) was found to inhibit the dephosphorylation of freshly photolyzed rhodopsin by protein phosphatase 2A but did not inhibit the dephosphorylation of unbleached rhodopsin. Arrestin has no effect on the dephosphorylation of phosphorylase  $\alpha$ , indicating that the effect was substrate-directed. It appears that dephosphorylation of the photoreceptor protein phosphorhodopsin occurs only after decay of the photoactivated protein and that this may be regulated *in vivo* by arrestin. The binding of arrestin to photolyzed phosphorylated rhodopsin, *i.e.* the binding of a regulatory protein to a protein phosphatase substrate to form a

complex resistant to dephosphorylation represents a novel mechanism for the regulation of protein phosphatase 2A.

Rhodopsin is the photoreceptor protein of rod cells in the retina. Absorption of light energy by rhodopsin causes it to become activated and leads to a series of events culminating in neural signaling. Photolyzed rhodopsin activates the G-protein transducin which in turn activates a cGMP-phosphodiesterase. Hydrolysis of cGMP leads to closing of cation channels in the plasma membrane, completing the sequence of events in the signal transduction process (reviewed by Stryer, 1986).

Rhodopsin deactivation occurs as a result of its phosphorylation on specific serine and threonine residues by rhodopsin kinase (reviewed by Kühn, 1984). Phosphorylated rhodopsin can activate transducin only poorly, and in the presence of a 48-kDa protein, arrestin, rhodopsin is completely deactivated (Kühn *et al.*, 1984; Miller *et al.*, 1986; Wilden *et al.*, 1986a). Rhodopsin is regenerated from photolyzed rhodopsin by its combination with 11-*cis*-retinal and by the hydrolysis of phosphate groups from the protein, thus allowing it to again participate in photoreception.

Recently, we showed that protein phosphatase 2A (PrP-2A),<sup>1</sup> one of four known protein serine/threonine phosphatases, efficiently dephosphorylates phosphoropsin. In this manuscript we demonstrate that PrP-2A accounts for most, if not all, of the opsin phosphatase activity in rod outer segment extracts and show the novel way in which the action of PrP-2A on phosphoropsin is regulated. Since rhodopsin is the best studied member of the family of seven transmembrane helix receptors that function via G-proteins (Applebury and Hargrave, 1986), these findings may have general significance for understanding the functions of other members of this important receptor family.

### MATERIALS AND METHODS

ATP, poly-L-Lys:HBr (*M*, 17,300), Tris, calmodulin, and other chemicals were purchased from Sigma. Sangivamycin was obtained from National Institutes of Health, National Cancer Institute (Natural Products Branch), Division of Cancer Treatment. [ $\gamma$ -<sup>32</sup>P]ATP was purchased from Du Pont-New England Nuclear. 11-*cis*-Retinal was prepared by photoisomerization of all-*trans*-retinal and separation of 11-*cis* isomer using a Maxisil 5 Si-60 (Phenomenex) column as described earlier (Wang *et al.*, 1980). Okadaic acid was the generous gift of Dr. Toshio Kitazawa.

**Isolation of Rod Outer Segments and Extraction of Protein Phosphatases**—Rod outer segments (ROS) were prepared from fresh bovine retinas by the method of Schnetkamp and Daemen (1982).

The extract containing protein phosphatase activity was obtained from rod outer segments using buffer A (50 mM Tris/HCl, pH 7.0, at 25 °C, containing 10% glycerol, 50 mM KCl, and 10  $\mu$ g/ml each of the following inhibitors of proteolysis: pepstatin, benzamide, leupeptin, and aprotinin). ROS (10 mg of rhodopsin) were sonicated with 3 ml of buffer A. The supernatant containing soluble phosphatases was collected after centrifugation. For localization of the phosphatase to the soluble or membrane fractions, buffer A contained up to 1 M KCl. The suspension was centrifuged (average 40,000  $\times$  g; 20 min) and the supernatant was assayed for activity using phosphoropsin and histone

\* This work was supported in part by Grants EY 6625 and EY 6626 from the National Eye Institute (to P. A. H.), an unrestricted grant to the Department of Ophthalmology, University of Florida, from Research to Prevent Blindness, Inc., Grant NP-608 from the American Cancer Society (to T. S. I.), and grants from the Office of Biotechnology at Iowa State University (to T. S. I.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¶ An Established Investigator of the American Heart Association.

\*\* Supported in part by a Jules and Doris Stein Professorship from Research to Prevent Blindness, Inc.

<sup>1</sup> The abbreviations used are: PrP, protein phosphatase; ROS, rod cell outer segments; SDS, sodium dodecyl sulfate; EGTA, [ethylenedibis(oxyethylenenitrilo)]tetraacetic acid.

H1 as substrates. The pellet was resuspended in the same volume of buffer (3 ml) and also assayed for phosphatase activity. KCl had no effect on the distribution of activity between soluble and membrane fractions.

**Protein Preparations**—Arrestin was extracted from bovine retinas according to the method of Wilden *et al.* (1986b). The extract was further purified on a Mono Q column (fast protein liquid chromatography, Pharmacia LKB Biotechnology Inc.) as described by Zigler *et al.* (1984). Purity of the arrestin was greater than 95% as determined by SDS-polyacrylamide gel electrophoresis and gel filtration.

Phosphorylated opsin was prepared from urea-washed ROS as described by Palczewski *et al.* (1989). Regeneration of phosphorylated opsin was performed as described elsewhere (Palczewski *et al.*, 1988a).

Phosphorylase *b* (Fischer and Krebs, 1958), phosphorylase kinase (Cohen, 1973), inhibitor-2 (Foulkes and Cohen, 1980), inhibitor-1 (Nimmo and Cohen, 1978), and catalytic subunits of PrP-1 and PrP-2A (Resink *et al.*, 1983; Tung *et al.*, 1984) were purified to homogeneity from rabbit skeletal muscle. PrP-2A was free of PrP-1 as judged by lack of inhibition by protein inhibitor-1 and -2. Histone H1 was purified to homogeneity from calf thymus (Sanders, 1977). Protein kinase C was partially purified from fresh rat brain as described in Jakes and Schlender (1988). The enzyme was further purified by a modification of the method of Wolf *et al.* (1985). Protein kinase C (DEAE-Sepharose eluate) was incubated with inside-out human erythrocyte vesicles for 30 min at 2°C in solution A (20 mM Tris/HCl (pH 7.5 at 25°C), 14 mM 2-mercaptoethanol, 5 mM benzamide, 0.5 mM phenylmethylsulfonyl fluoride, 1 µg/ml pepstatin A, 5 µg/ml leupeptin) containing 90 mM NaCl, 3 mM MgCl<sub>2</sub>, 1 mM CaCl<sub>2</sub>. The mixture was centrifuged at 40,000 × *g* for 15 min, and the pellet was resuspended in solution A containing 50 mM NaCl, 1 mg/ml polyethylene glycol 20,000, 2 mM EGTA, 2 mM EDTA (solution B). The mixture was incubated for 30 min at 2°C and centrifuged at 40,000 × *g* for 15 min. The supernatant containing protein kinase C was dialyzed overnight against 1 liter of solution B containing 0.1 mM EDTA and stored at 2°C.

[<sup>32</sup>P]Phosphorylase *a* (1.0 mol/mol) and [<sup>32</sup>P]histone H1 (0.5 mol/mol) were prepared using phosphorylase kinase (Shenolikar and Ingebritsen, 1984) and protein kinase C (Jakes and Schlender, 1988).

**Gel Filtration of Phosphatases**—Gel filtration of the catalytic subunit of PrP-2A was performed as described by Palczewski *et al.* (1989). Gel filtration on Sepharose CL-6B 200 was used to determine the molecular weight of the native forms of ROS protein phosphatases. A 1.6 × 100-cm column was prepared using gel equilibrated with buffer A. The column was developed with this buffer at a flow rate of 15 ml/h, and 1-ml fractions were collected. The column was standardized using pyruvate kinase (*M<sub>r</sub>* = 237,000), aldolase (*M<sub>r</sub>* = 160,000), bovine serum albumin (*M<sub>r</sub>* = 67,000), and carbonic anhydrase (*M<sub>r</sub>* = 30,000) with blue dextran to indicate void volume and sodium azide to mark the included volume. The column was monitored for phosphatase activity by withdrawing aliquots of the fractions and measuring the activity using phosphorylated (by protein kinase C) histone H1, phosphorylase *a*, and phosphorylated rhodopsin as substrates.

**Enzyme Assays**—Opsin phosphatase assays were performed as described previously (Palczewski *et al.*, 1989). PrP-2A was assayed using either [<sup>32</sup>P]phosphorylase *a* as substrate in the presence of an excess of inhibitor-2 (to block PrP-1 activity) (Shenolikar and Ingebritsen, 1984), or using [<sup>32</sup>P]histone H1 phosphorylated by protein kinase C as substrate. PrP-2A is the only protein phosphatase which is active toward the latter substrate (Jakes and Schlender, 1988). PrP-1 was assayed using [<sup>32</sup>P]phosphorylase *a* as substrate (Shenolikar and Ingebritsen, 1984). Inhibitor-1 and inhibitor-2 were assayed by their ability to inhibit PrP-1 (Shenolikar and Ingebritsen, 1984). For all protein phosphatase assays, 1 unit of activity is that amount which catalyzes the release of 1.0 nmol of inorganic phosphate/min from the appropriate substrate.

**Miscellaneous Methods and Procedures**—Other methods were described in recent publications (Palczewski *et al.*, 1988a, 1988b; Palczewski *et al.*, 1989).

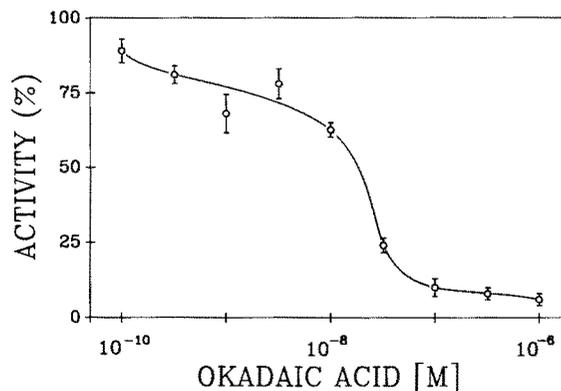
## RESULTS AND DISCUSSION

**Protein Phosphatase 2A Is the Major Rhodopsin Phosphatase of Rod Outer Segments**—There are four known serine/threonine protein phosphatases, termed protein phosphatases 1, 2A, 2B, and 2C. We have previously shown that the catalytic subunit of PrP-2A from rod outer segments or rabbit skeletal

muscle efficiently dephosphorylates phosphoopsin, whereas the catalytic subunit of PrP-1 is not active toward this substrate (Palczewski *et al.*, 1989). In the present study we have carried out experiments to identify the opsin phosphatase activities in extracts of rod outer segments and to determine the relationship of these activities to known protein phosphatases. The opsin phosphatase activity in the extracts is not stimulated by Mg<sup>2+</sup> (5 mM), Ca<sup>2+</sup> (0.1 mM), or Ca<sup>2+</sup>/calmodulin (0.1 mM/0.2 µM) and is not inhibited by inhibitor-1 (10 nM) or inhibitor-2 (100 nM) as compared to regular assay (with 100 µM EDTA; data not shown). This indicates that the activity is not due to PrP-2B or PrP-2C which are dependent on Ca<sup>2+</sup> or Mg<sup>2+</sup>, respectively, for activity, nor is it due to PrP-1 which is selectively inhibited by the two inhibitor proteins. Consequently the opsin phosphatase activity in rod outer segments must be due to PrP-2A and/or to a previously unidentified protein phosphatase.

Several other lines of evidence also indicate that opsin phosphatase activity is due to PrP-2A. First, when opsin phosphatase activity was measured in the soluble and membrane fractions from rod outer segments, 80% of the activity was in the soluble fraction. Phosphatase activity toward histone H1, a substrate dephosphorylated only by PrP-2A, showed the same distribution between soluble and membrane fractions. This distribution was not affected by adding up to 1.0 M KCl to the extraction buffer.

Second, opsin phosphatase activity in rod outer segment extracts is potently inhibited by okadaic acid (Fig. 1). The value of IC<sub>50</sub> of ~10 nM is intermediate between reported IC<sub>50</sub> values for inhibition of the holoenzyme form and catalytic



**FIG. 1. Effect of okadaic acid on dephosphorylation of phosphorylated rhodopsin.** ROS (1.2 mg of rhodopsin) was homogenized in buffer A (1.1 ml) containing 50 mM 2-mercaptoethanol, 2 mM Mg(OAc)<sub>2</sub>, and 50 µM ouabain. [<sup>32</sup>P]ATP was added to a final concentration of 100 µM (30 µCi) and the sample was illuminated for 10 min at 30°C. Then, sangivamycin, a potent inhibitor of rhodopsin kinase, was added to block further phosphorylation. The 50 µM sangivamycin used completely inhibits rhodopsin kinase under these conditions (*K<sub>i</sub>* for sangivamycin was 180 nM; K. Palczewski, N. Kahn, J. W. Shriver, and P. A. Hargrave, manuscript in preparation). Aliquots (100 µl) of the ROS suspension were added to 5 µl of okadaic acid in dimethyl sulfoxide yielding from 0 to 1 µM okadaic acid (final concentration). The extent of rhodopsin phosphorylation was 0.8 Pi/Rho as determined by trichloroacetic acid precipitation of 2 aliquots taken immediately after adding sangivamycin. The other samples were incubated at 30°C for 60 min and dephosphorylation was measured using SDS-polyacrylamide gel electrophoresis. 50 µl of 5% SDS was added to each sample and SDS-polyacrylamide gel electrophoresis was performed using a 12% polyacrylamide gel with the Laemmli buffer system (Laemmli, 1970). The rhodopsin band was visualized by Coomassie Brilliant Blue, cut out, and counted to determine the radioactivity remaining bound. 100% activity was taken as the loss of radioactivity from the rhodopsin band in the presence of dimethyl sulfoxide without okadaic acid. The data points are the averages of four experiments.

form of PrP-2A but much lower than the values reported for PrP-1, -2B, and -2C (Bialojan and Takai, 1988). Thus, we conclude that PrP-2A is likely to be the only enzyme that dephosphorylates phosphorylated rhodopsin *in vivo*.

Third, as previously found in other tissues, three forms of PrP-2A are resolved after chromatography of the soluble fraction from rod outer segments on DEAE-Sephacrose. The elution positions of the three peaks, termed PrP-2A<sub>0</sub>, -2A<sub>1</sub>, and -2A<sub>2</sub>, were monitored either by assaying the phosphorylase phosphatase activity of the fractions in the presence of inhibitor-2 (Fig. 2A) or by assaying the fractions using histone H1 (phosphorylated by protein kinase C) as substrate (results not shown). Similar elution profiles are obtained by either method. Other studies have established that each of the three PrP-2A peaks consists of the PrP-2A catalytic subunit in a complex with one or two other subunits (Tung *et al.*, 1985). The PrP-2A<sub>0</sub> and PrP-2A<sub>1</sub> species are believed to exist *in vivo* whereas PrP-2A<sub>2</sub> is thought to arise during the DEAE-Sephacrose chromatography as a consequence of proteolysis and/or dissociation of one of the other subunits.

Three peaks of opsin phosphatase activity were also detected after DEAE-Sephacrose chromatography (Fig. 2B). The elution positions of the three peaks of opsin phosphatase activity correspond to those of PrP-2A<sub>0</sub>, -2A<sub>1</sub>, and -2A<sub>2</sub>. Polylysine, a potent stimulator of PrP-2A, markedly increased the activity of each peak using either phosphorylase *a* or phosphoopsin as substrate (Fig. 2). The activity of each of the peaks towards phosphoopsin was also stimulated by freezing and thawing the fractions in the presence of 2-mercaptoethanol (not shown). This latter treatment is known to dissociate the catalytic subunit of PrP-2A from other subunits in the PrP-2A<sub>0</sub>, -2A<sub>1</sub>, and -2A<sub>2</sub> species which suppress activity toward some substrates (Tamura and Tsuiki, 1980; Ingebritsen *et al.*, 1983).

When the ROS extract was loaded on Sepharose CL-6B 200, the phosphatase activity eluted near the aldolase standard at 160 kDa with some heterogeneity noted on the descending limb of the peak. This is consistent with the gel filtration behavior expected from a mixture of PrP-2A<sub>0</sub>, -2A<sub>1</sub>, and -2A<sub>2</sub>. When the total pool containing the phosphoopsin (or phos-

phorylase *a*) phosphatase activity was subjected to ethanol treatment, one single peak of rhodopsin phosphatase activity was observed. That activity coelutes with the *catalytic subunit* of rabbit muscle PrP-2A between carbonic anhydrase (30 kDa) and ovalbumin (43 kDa; data not shown).

In a recent paper (Palczewski *et al.*, 1989), we tested different forms of phosphorylated photoreceptor as substrates for the catalytic subunit of PrP-2A. No difference was found in the rate of dephosphorylation of phosphoopsin, phosphorhodopsin, or freshly photolyzed phosphorylated rhodopsin (Palczewski *et al.*, 1989). In the present work using the PrP-2A *holoenzyme*, the rate of dephosphorylation of the different forms of rhodopsin are indistinguishable (data not shown).

The opsin phosphatase specific activity in ROS extracts (0.3 units/amount of ROS containing 1 mg rhodopsin) (Table I) is about 1/10th the amount of rhodopsin kinase activity under optimal conditions. This may explain why the dephosphorylation is much slower than the phosphorylation either *in vivo* or *in vitro* (Kühn, 1974; Weller *et al.*, 1975; Miller *et al.*, 1977) and why many investigators found very little or no opsin phosphatase activity in rod outer segment extracts. The opsin phosphatase activity in extracts is increased about 3-fold by treatment with 80% ethanol at room temperature. This is presumably due to the dissociation of other subunits present in the PrP-2A<sub>0</sub>, -2A<sub>1</sub>, and -2A<sub>2</sub> species that appear to suppress opsin phosphatase activity. Based on the phosphorylase phosphatase specific activity, the levels of PrP-1 plus PrP-2A in ROS extracts are comparable to those observed in other tissues.

**Regulation of the Opsin Phosphatase Activity of Protein Phosphatase 2A by Arrestin**—Arrestin is a 48-kDa protein that binds to freshly bleached phosphorylated rhodopsin. Binding of arrestin blocks the activation of the G-protein transducin by rhodopsin and prevents further phosphorylation of rhodopsin by rhodopsin kinase. The results in Fig. 3 show that arrestin inhibits the dephosphorylation of freshly bleached phosphorylated rhodopsin by PrP-2A. Arrestin inhibits the dephosphorylation of freshly bleached rhodopsin by all three forms of PrP-2A (Fig. 2B). Significantly, arrestin does not inhibit the dephosphorylation of phosphorylated rhodopsin when the reaction is carried out in the dark (Fig. 3). Neither does arrestin inhibit the dephosphorylation of phosphorylase *a* (Fig. 3, *inset*), suggesting that the effect is due to the binding of arrestin to photolyzed rhodopsin rather than to the interaction of arrestin directly with PrP-2A. This idea is further supported by the observation that a stoichiometric amount of arrestin with respect to rhodopsin is enough for the complete inhibition (Fig. 3).

**Conclusions**—The results presented above show that PrP-2A is the major, if not the only, phosphatase that acts on

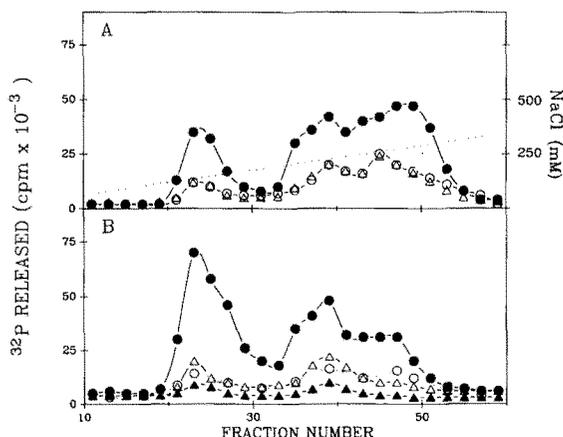


FIG. 2. Separation of different forms of protein phosphatase 2A from ROS on DEAE-Sephacrose. ROS (16 mg of rhodopsin) were extracted with the buffer A (20 ml). The extract was loaded at a flow rate of 1 ml/10 min on a column (1.6 × 16 cm) of DEAE-Sephacrose 6B pre-equilibrated with buffer A. Protein phosphatase forms were eluted by a continuous linear gradient of 50–400 mM NaCl (50 ml each). Fractions (1.8 ml) were collected and assayed for protein phosphatases with phosphorylase *a* (2 μM) (A) or freshly bleached phosphorylated rhodopsin (10 μM) (B) as substrate. The fractions were assayed with no additions (○), in the presence of 20 μM arrestin (▲), 10 μM poly-L-Lys (●), and 50 nM inhibitor-2 (△).

TABLE I

*Activity of protein phosphatase in rod outer segments*

The rates of dephosphorylation of phosphorylase *a* and phosphorylated opsin were determined from the time course of the dephosphorylation of these substrates where a linear relationship was observed between dephosphorylation and time. The concentrations of substrates were: phosphorylase *a*, 10 μM; phosphorylated opsin, 50 μM. The assays were performed at 30 °C. The phosphatase(s) was extracted from intact ROS or from ethanol-treated ROS (Palczewski *et al.*, 1989). The amount of phosphate released is expressed per amount of rod outer segments that contain 1 mg of rhodopsin.

Substrate	Protein phosphatase activity	
	Intact ROS	EtOH-treated ROS
Phosphorylase <i>a</i>	0.297	0.933
P-opsin	0.368	1.10

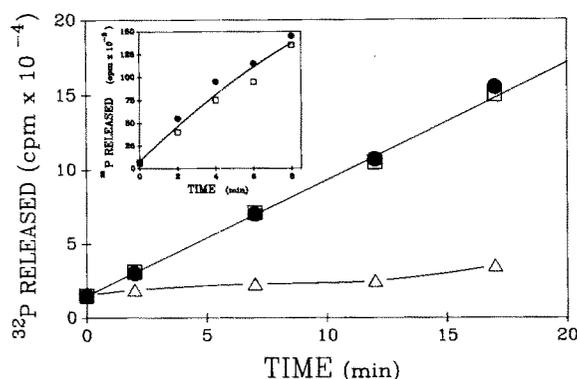


FIG. 3. Inhibition of dephosphorylation of freshly photolyzed phosphorhodopsin by arrestin. Phosphorylated and regenerated rhodopsin was used as a substrate for ROS protein phosphatase in the presence of arrestin and/or light. Phosphorylated rhodopsin ( $20 \mu\text{M}$ ) with an average of 2.1 Pi/Rho was incubated with  $20 \mu\text{M}$  arrestin for 20 min in the dark. The sample was bleached and phosphatase extract from rod outer segments was immediately added. The reaction was terminated by addition of an equal volume of 20% trichloroacetic acid and the  $^{32}\text{P}$  released as a function of time was measured ( $\Delta$ ). In the control samples, dephosphorylation was carried out with arrestin but without bleaching ( $\bullet$ ) or without arrestin ( $\square$ ). Using phosphorylase  $\alpha$  as substrate (inset), dephosphorylation proceeds as well in the presence of arrestin ( $\bullet$ ) as in its absence ( $\square$ ).

rhodopsin in rod outer segments. In addition, the results suggest an elegant mechanism for arrestin to control rhodopsin phosphorylation in response to light. Liebman *et al.* (1987) predicted that arrestin is involved in the regulation of rhodopsin phosphatase action. In order to understand how arrestin can be the regulatory protein, we must first examine the role of rhodopsin phosphorylation. Upon light activation, rhodopsin is able to activate the G protein transducin. This activated form of rhodopsin is relatively long-lived, surviving for minutes. Phosphorylation of rhodopsin is the process in the visual transduction cascade that serves to partially turn off the activation of transducin (Wilden *et al.*, 1986a; Miller *et al.*, 1986). The activation of transducin is completely quenched by the binding of arrestin to the phosphorylated, but still activated rhodopsin. Now, if the phosphates were removed from the complex of activated rhodopsin and arrestin, the still activated rhodopsin would activate transducin and the whole deactivation process would be ineffective. What we observed, however, was that arrestin prevents dephosphorylation of only the freshly photolyzed phosphorylated rhodopsin, suggesting that binding occurs only to activated rhodopsin. As the photolyzed-rhodopsin becomes inactive, arrestin is released, allowing the phosphatase to remove phosphates from the now inactive opsin. This is consistent with the fact that arrestin is released from the membrane more slowly than any other protein that is bound in a light-dependent manner (Liebman and Zimmerman, 1989). Thus, arrestin is a very important clock-protein that regulates the dephosphorylation of rhodopsin, allowing the dephosphorylation to occur only after the photoactive state of rhodopsin has decayed.

Of the four protein serine/threonine phosphatases, PrP-1 and PrP-2B are known to be regulated by physiologically significant mechanisms. The present experiments have uncovered a novel mechanism for regulating PrP-2A activity. The regulation is based on removing the substrate by forming an inactive complex with the regulatory protein. Regulation of dephosphorylation reactions at the substrate level is ap-

pealing because it explains how dephosphorylation reactions catalyzed by protein phosphatases having broad substrate specificity (e.g. protein serine/threonine phosphatase 2A) can be specifically regulated. While this regulatory mechanism has not been observed elsewhere for PrP-2A, it may have broader application, especially with the membrane bound protein receptors that are similar to rhodopsin such as the  $\beta$ -adrenergic receptor, muscarinic receptor, etc. (Sibley *et al.*, 1987).

*Acknowledgments*—We wish to thank Hanke van der Wel for technical assistance in preparing rod cell outer segments and Mabel Wilson for manuscript preparation.

## REFERENCES

- Applebury, M. L., and Hargrave, P. A. (1986) *Vision Res.* **26**, 1881–1895
- Bialojan, C., and Takai, A. (1988) *Biochem. J.* **256**, 283–290
- Cohen, P. (1973) *Eur. J. Biochem.* **44**, 1–14
- Fischer, E. H., and Krebs, E. G. (1958) *J. Biol. Chem.* **231**, 65–71
- Foulkes, J. G., and Cohen, P. (1980) *Eur. J. Biochem.* **105**, 195–203
- Ingebritsen, T. S., Foulkes, J. G., and Cohen, P. (1983) *Eur. J. Biochem.* **132**, 263–274
- Jakes, S., and Schlender, K. K. (1988) *Biochim. Biophys. Acta* **967**, 11–16
- Kühn, H. (1974) *Nature* **250**, 588–590
- Kühn, H. (1984) *Prog. Retinal Res.* **3**, 1123–1156
- Kühn, H., Hall, S. W., and Wilden, U. (1984) *FEBS Lett.* **176**, 473–478
- Laemmli, U. K. (1970) *Nature* **227**, 680–685
- Liebman, P. A., Parker, K. R., and Dratz, E. A. (1987) *Annu. Rev. Physiol.* **49**, 765–791
- Liebman, P. A., and Zimmerman, R. W., Jr. (1989) *Invest. Ophthalmol. Vis. Sci. Suppl.* **30**, 170
- Miller, J. A., Paulsen, R., and Bownds, M. D. (1977) *Biochemistry* **16**, 2633–2639
- Miller, J. L., Fox, D. A., and Litman, B. J. (1986) *Biochemistry* **25**, 4983–4988
- Nimmo, G. A., and Cohen, P. (1978) *Eur. J. Biochem.* **87**, 341–351
- Palczewski, K., McDowell, J. H., and Hargrave, P. A. (1988a) *Biochemistry* **27**, 2306–2313
- Palczewski, K., McDowell, J. H., and Hargrave, P. A. (1988b) *J. Biol. Chem.* **263**, 14067–14073
- Palczewski, K., Hargrave, P. A., McDowell, J. H., and Ingebritsen, T. S. (1989) *Biochemistry* **28**, 415–419
- Resink, T. J., Hemmings, B. A., Tung, H. Y. L., and Cohen, P. (1983) *Eur. J. Biochem.* **133**, 455–461
- Sanders, C. (1977) *Biochem. Biophys. Res. Commun.* **78**, 1034–1042
- Schnetkamp, P. P. M., and Daemen, F. J. M. (1982) *Methods Enzymol.* **81**, 110–116
- Shenolikar, S., and Ingebritsen, T. S. (1984) *Methods Enzymol.* **107**, 102–129
- Sibley, D. R., Benovic, J. L., Caron, M. G., and Lefkowitz, R. J. (1987) *Cell* **48**, 913–922
- Stryer, L. (1986) *Annu. Rev. Neurosci.* **9**, 87–119
- Tamura, S., and Tsuiki, S. (1980) *Eur. J. Biochem.* **111**, 217–224
- Tung, H. Y. L., Resnik, T. J., Hemmings, B. A., Shenolikar, S., and Cohen, P. (1984) *Eur. J. Biochem.* **138**, 635–641
- Tung, H. Y. L., Alemany, S., and Cohen, P. (1985) *Eur. J. Biochem.* **148**, 253–263
- Wang, J. K., McDowell, J. H., and Hargrave, P. A. (1980) *Biochemistry* **19**, 5111–5117
- Weller, M., Virmaux, N., and Mandel, P. (1975) *Proc. Natl. Acad. Sci. U. S. A.* **72**, 381–385
- Wilden, U., Hall, S. W., and Kühn, H. (1986a) *Proc. Natl. Acad. Sci. U. S. A.* **83**, 1174–1178
- Wilden, U., Wüst, E., Weyand, I., and Kühn, H. (1986b) *FEBS Lett.* **207**, 292–295
- Wolf, M., Cuatrecasas, P., and Sahyoun, N. (1985) *J. Biol. Chem.* **260**, 15718–15722
- Zigler, J. S., Jr., Mochizuki, M., Kuwabara, T., and Gery, I. (1984) *Invest. Ophthalmol. Vis. Sci.* **25**, 977–980