Omenn, G. S., Fontana, A., & Anfinsen, C. B. (1970) *J. Biol. Chem. 245*, 1895.
Rosenthal, C. J., & Franklin, E. C. (1975) *J. Clin. Invest. 55*, 746.
Segrest, J. P., Pownall, H. J., Jackson, R. L., Glenner, G. G., & Pollock, P. S. (1976) *Biochemistry 15*, 3187.
Selinger, M. J., McAdam, K. P. W. J., Kaplan, M. M., Sipes, J. D., Vogel, S. N., & Rosenstreich, D. L. (1980) *Nature (London) 285*, 498.
Sletten, K., & Husby, G. (1974) *Eur. J. Biochem. 41*, 117.
Tarr, G. E., Beecher, J. F., Bell, M., & McKean, D. J. (1978) *Anal. Biochem. 84*, 622.
Yaoi, J., Titani, K., & Narita, K. (1964) *J. Biochem. (Tokyo) 56*, 222.

Autophosphorylation of Rhodopsin Kinase from Retinal Rod Outer Segments†

Rehwa H. Lee,* Bruce M. Brown, and Richard N. Lolley

ABSTRACT: Rhodopsin kinase has been identified as a 68K protein that is more readily extracted from dark-adapted rod outer segments (dark-extract) than from illuminated rod outer segments (light-extract). We observed that a 68K protein is phosphorylated by endogenous protein kinase of dark- or light-extract of bovine rod outer segments and that the amount of incorporated radioactivity (^{32}P) was greater in the dark- than in the light-extract. Phosphorylation of the 68K protein is neither stimulated by cyclic nucleotides nor affected by the light or dark conditions of the phosphorylation reaction. Light- and dark-extracts were centrifuged simultaneously on individual sucrose density gradients revealing that the 68K phosphoprotein cosediments with endogenous rhodopsin kinase activity and that both greater ^{32}P incorporation and higher rhodopsin kinase activity are found in dark-extract as compared to light-extract. These findings suggest strongly that the 68K phosphoprotein and rhodopsin kinase are identical and that rhodopsin kinase undergoes autophosphorylation.

Protein phosphorylation is an important mechanism by which cellular metabolism or function is regulated (Greengard, 1978). It participates in the control of carbohydrate and lipid metabolism as well as synaptic transmission and muscle contraction (Kebabian, 1977; Nimmo & Cohen, 1977). On the molecular level, the phosphorylation of enzymes is known to alter the conformation of enzymes and, thereby, modulate their activity or kinetic characteristics (Krebs & Beavo, 1979).

In rod photoreceptors of bovine retina, phosphorylation of two endogenous proteins has been described. The phosphorylation of a 33K soluble protein is catalyzed by a cyclic nucleotide dependent protein kinase (CNPK)[1] which is present in the rod outer segment (ROS) (Lolley et al., 1977). The activity of CNPK is modulated by light-induced changes in cGMP concentrations which are high in the dark and low in the light (Woodruff & Bownds, 1979; Farber et al., 1978; Yee & Liebman, 1978; Fletcher & Chader, 1976).

Phosphorylation of the ROS membrane protein, rhodopsin, is catalyzed by a rhodopsin kinase (RK), the activity of which is affected by neither cyclic nucleotides nor light (Frank & Buzney, 1975; Weller et al., 1975). The RK phosphorylates only freshly bleached rhodopsin, but not dark-adapted rhodopsin or commonly used protein kinase substrates such as histone, protamine, or casein (Lee et al., 1981; Shichi & Somers, 1978). This enzyme is a 68K protein which exists in the cytoplasmic compartment of ROS in the dark and binds to the ROS membranes in the light (Kühn, 1978). It is suggested that light stimulates phosphorylation of rhodopsin by inducing conformational changes of the visual pigment, thereby providing RK with an appropriate binding site on the ROS membranes as well as a suitable substrate for phosphorylation.

In this paper, we report the phosphorylation of a 68K ROS protein by an endogenous ROS protein kinase. On the basis of (a) the molecular weight (68K) of this endogenous substrate, (b) its light-dependent affinity for ROS membranes, (c) its cosedimentation with RK activity during centrifugation, and (d) the absence of effects by light or cyclic nucleotides on its phosphorylation, we propose that this 68K phosphoprotein is phosphorylated rhodopsin kinase and that the enzyme undergoes autophosphorylation.

Experimental Procedures

Materials

Dark-adapted bovine retinal ROS, protein kinase substrate (PKS) from rat intestine, and alum-treated ROS membranes were prepared as described previously (Lee et al., 1981). [γ-^{32}P]ATP was purchased from New England Nuclear (2.5–4 Ci/μmol). Cronex X-ray film was from Du Pont.

Methods

Preparation of Light- or Dark-Extract from Bovine ROS. Peripheral proteins were extracted from light- or dark-adapted ROS membranes by the procedure of Kühn (1978). Briefly, a ROS pellet, which was prepared from six dark-adapted bovine retinas and kept in darkness at −70 °C until use, was homogenized in the dark in 1 mL of 20 mM Tris-HCl, pH

† From the Department of Anatomy, University of California at Los Angeles School of Medicine, Los Angeles, California 90024, and the Developmental Neurology Laboratory, Sepulveda Veterans Administration Medical Center, Sepulveda, California 91343. *Received September 17, 1981.* This work was supported by National Science Foundation Grant BNS 79-26806, National Institutes of Health Grant EY00395, and the Medical Research Service of the Veterans Administration.

* Address correspondence to this author at the Developmental Neurology Laboratory (151B9), Sepulveda Veterans Administration Medical Center.

[1] Abbreviations: ROS, rod outer segment(s); cAMP, adenosine cyclic 3′,5′-phosphate; cGMP, guanosine cyclic 3′,5′-phosphate; CNPK, cyclic nucleotide dependent protein kinase; PKS, protein kinase substrate; RK, rhodopsin kinase; $NaDodSO_4$, sodium dodecyl sulfate; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid.

0006-2960/82/0421-3303$01.25/0

7.6, containing 50 mM β-mercaptoethanol and 2 mM EDTA. Two aliquots of 0.45 mL each were removed and put into separate centrifuge tubes which were incubated for 5 min in a 22–24 °C water bath. One tube was then exposed to laboratory illumination for 3 min, while the other was kept in the dark. The tube containing bleached ROS was returned to darkness, and both tubes were cooled in an ice bucket for 1 min, before centrifugation at 100000*g* for 5 min. The supernatants in each tube were collected individually and centrifuged again for 1 h. The resultant supernatants obtained from the bleached or dark-adapted ROS were called light- or dark-extract, respectively. The extracts were kept in the dark at 4 °C until they were used in the phosphorylation reaction.

Assay of RK. The activity of RK was determined by using alum-treated ROS membranes as the substrate. The alum-treated ROS membranes were devoid of protein kinase activities, but they retained the ability of the membrane protein, rhodopsin, to serve as the substrate of RK. Immediately before the start of the assay, the reaction mixture of 90 μL containing 50 mM potassium phosphate, pH 7.0, and 10 mM magnesium acetate, 20 μL of alum-treated ROS membranes (approximately 400 μg of protein), and an appropriate amount of RK was preincubated at 30 °C for 5 min in the dark (dark control) or under laboratory illumination (bleached rhodopsin samples). The protein kinase reaction was initiated by adding 10 μL of 0.1 mM [γ-^{32}P]ATP (specific activity about 2000 cpm/pmol). After 10–30 min of incubation, the phosphorylation reaction was terminated by transferring a 50-μL aliquot of the reaction mixture to a premarked Whatman filter paper disk which was then dropped into ice-cold 20% trichloroacetic acid (Cl_3CCOOH). The disks were washed with four changes of fresh 5% Cl_3CCOOH (Lee et al., 1981), before they were counted in a PCS–xylene (Amersham; 2:1 mixture) scintillation cocktail. Blanks were prepared by using buffer or boiled enzyme to substitute for the RK enzyme. The activity of RK was determined by light-stimulated phosphate incorporation into protein. The $NaDodSO_4$–polyacrylamide gel electrophoresis and autoradiography confirmed that this incorporation was into rhodopsin. The amount of phosphorylation was calculated from the specific activity of [γ-^{32}P]ATP. The activity of RK was also evaluated by a filtration technique using either glass-filter (GF/C) or Millipore (HAWP)-filter membranes. It was found that the recovery of the phosphorylated ROS membranes by the batch-washing technique used in this study was 15–20% higher than by the filtration technique.

Assay of Cyclic Nucleotide Dependent Protein Kinase (CNPK). The basal and cAMP-dependent protein kinase activities were assayed in a reaction mixture of 100 μL, containing 50 mM potassium phosphate, pH 7.0, 10 μM [γ-^{32}P]ATP, 10 μM magnesium acetate, 0.1 mM isobutylmethylxanthine, 400 μg of protein kinase substrate (PKS), and an appropriate amount of CNPK, in the absence or presence of 1 μM cAMP, respectively. The reaction was started by the addition of either ATP or CNPK. Incubations were carried out at 30 °C for 3–30 min. The amount of phosphate incorporation into protein was determined as described above.

Endogenous Phosphorylation of Soluble Proteins in the Light- or Dark-Extract of ROS. The phosphorylation of soluble ROS proteins by endogenous kinases was carried out, using 40 μL of light- or dark-extract (containing 70–90 μg of protein), in a 100-μL reaction mixture containing 50 mM potassium phosphate, pH 7.0, 10 μM [γ-^{32}P]ATP, 10 μM magnesium acetate, and 0.1 mM isobutylmethylxanthine. The incubation was carried out at 30 °C for 10 min in the presence or absence of cAMP (1 μM) and in light or darkness as specified under Results. The phosphorylation reaction was stopped by adding 19 μL of stopping solution (25% $NaDodSO_4$, 25% β-mercaptoethanol, 0.2% bromophenol blue, and 30% glycerol), followed by boiling at 100 °C for 2 min. The phosphorylated proteins were separated by electrophoresis on an $NaDodSO_4$–polyacrylamide gel (10%, slab gel) according to Laemmli (1970). After Coomassie blue staining, the gel was dried and exposed to Cronex X-ray film for 16–48 h. The ^{32}P-labeled proteins were identified by their molecular weights, and the isotope incorporation was quantitated by densitometric scanning of the autoradiographs. Measurements were made under conditions in which the optical density is proportional to the radioactivity as determined by direct scintillation counting of the sliced polyacrylamide gels.

Sucrose Density Gradient Centrifugation of Light- or Dark-Extract of ROS. Light- or dark-extract of ROS was centrifuged in parallel on individual 5–20% linear sucrose density gradients. The sedimentation was carried out according to Martin & Ames (1961) in a Beckman SW40Ti rotor at 40 000 rpm at 3 °C for 40 h. The gradients were prepared in 5 mM 2-(*N*-morpholino)ethanesulfonate (Mes), pH 6.9, 100 mM NaCl, 0.2 mM EDTA, and 15 mM β-mercaptoethanol. Two hundred microliters of ROS extracts was layered on top of the gradients, and following centrifugation, the gradients were fractionated into 500-μL portions and assayed for RK activity.

Endogenous phosphorylation was examined in each of the fractions from sucrose density gradients by incubating 200 μL of eluate with 1 μM [γ-^{32}P]ATP (4.7×10^6 cpm), 18.2 mM potassium phosphate, pH 7.0, and 9.1 mM magnesium acetate for 10 min at 4 °C. The reaction was stopped with the addition of 23 μL of 25% $NaDodSO_4$ and 25% mercaptoethanol (220-μL reaction mixture, final concentration 2.3%), and the tubes were boiled immediately for 5 min. The samples were then dialyzed overnight in 2 L of 0.1% $NaDodSO_4$ and 0.1% mercaptoethanol, lyophilized, reconstituted in 45 μL of sample buffer, and boiled for 1 min (sample buffer: 0.125 M Tris-HCl, pH 6.8, containing 5% β-mercaptoethanol, 1% $NaDodSO_4$, and 5% glycerol with bromophenol blue). Forty microliters of each sample was then loaded on a 10% $NaDodSO_4$ gel, with the light and dark samples run on adjacent wells. The gels were stained and then destained before autoradiography, as described above for identification of the phosphorylated proteins.

Results

Extraction of RK and CNPK from Light- or Dark-Adapted ROS. The ROS contain several soluble proteins which interact with ROS membranes in a light-dependent manner. We have extracted ROS with 20 mM Tris-HCl, pH 7.6, containing 2 mM EDTA and 50 mM mercaptoethanol, in either the light or dark as described by Kühn (1978). Table I shows the RK activity in light- or dark-extract as measured by light-dependent phosphorylation of alum-treated ROS membranes. The RK activity was 2 times higher in dark-extract than in light-extract when measured in the presence of bleached rhodopsin. On the other hand, activities of CNPK in light- and dark-extracts are the same.

Endogenous Phosphorylation of Soluble Proteins in Light- or Dark-Extract of ROS. Soluble proteins of ROS which are phosphorylated by endogenous protein kinases from either the light- or dark-extract were separated by $NaDodSO_4$ gel electrophoresis and detected by autoradiography. The ^{32}P-labeled proteins were identified by their molecular weights, and the amount of phosphorylation was quantitated by densitometric scanning of the autoradiographs. Figure 1 shows

Table I: CNPK and RK Activities in Dark- and Light-Extracts of ROS[a]

enzyme	assay condition	dark extract	light extract
RK	dark	31.4 ± 12.2	20.8 ± 13.2
	light	156.0 ± 25.4	78.6 ± 15.6
CNPK	basal	99.6 ± 14.7	88.6 ± 10.0
	cAMP	327.4 ± 47.6	303.6 ± 46.9

[a] The light- or dark-extracts of ROS were prepared as described under Methods. Rhodopsin kinase was assayed in the light or in the dark, whereas CNPK was assayed in the presence or absence of 1 μM cAMP, as described under Methods. The activities were expressed as picomoles of phosphate incorporated per minute per milligram of protein. The protein concentration was determined according to Ross & Schatz (1973). Values in this table represent mean ± SEM from three experiments run in duplicate.

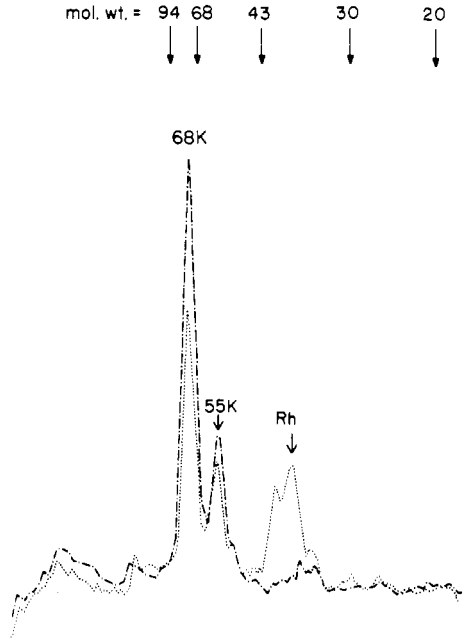

FIGURE 1: Endogenous phosphorylation of soluble proteins in the light- or dark-extracts of ROS. The phosphorylation of soluble ROS proteins by endogenous kinases was carried out in the dark and in the absence of exogenous cyclic nucleotides. The phosphorylated proteins were analyzed by $NaDodSO_4$ gel electrophoresis and autoradiography, as described under Methods. Densitometric scannings of the ^{32}P-labeled proteins in the dark- (---) and light-extracts (···) were superimposed for comparison. The phosphoproteins were identified by their molecular weights in reference to standard proteins of known molecular weights, as indicated at the top of the figure.

the densitometric scans of the ^{32}P-labeled proteins when light- or dark-extract was incubated in the dark and in the absence of exogenous cyclic nucleotides. Two proteins, with molecular weights of 68K and 55K, respectively, were phosphorylated in the light- and dark-extracts. The amount of phosphorylation of the 68K protein in the dark-extract was about 2 times that of the light-extract, whereas the amount of phosphorylation of the 55K protein was essentially the same in both extracts. Another peak of phosphoprotein, with molecular weights ranging between 35K and 40K, was observed only in the light-extract of ROS. As will be described in later sections, this peak appeared to be phosphorylated rhodopsin.

Effect of Light on the Endogenous Phosphorylation of Soluble ROS Proteins. Figure 2 shows the densitometric scans of ^{32}P-labeled proteins when the dark-extract of ROS was incubated either under laboratory illumination or in darkness. The amount of phosphorylation of both the 68K and the 55K proteins was essentially the same under either reaction condition. Another peak of phosphoprotein, with molecular weights ranging between 35K and 40K, was observed when the phosphorylation reaction was carried out in the light. On the basis of its molecular weight and its light-dependent phosphorylation, it was concluded that the 35K phosphoprotein was a trace amount of contaminating rhodopsin which was phosphorylated by the rhodopsin kinase present in the ROS extract.

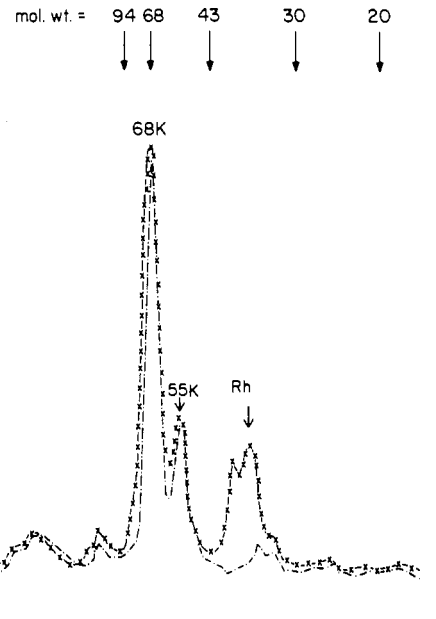

FIGURE 2: Effect of light on the endogenous phosphorylation of proteins in the dark-extract. The dark-extract of ROS was incubated, in the absence of exogenous cyclic nucleotide, either under laboratory illumination (-×-) or in darkness (---). The densitometric scans of the ^{32}P-labeled proteins were superimposed.

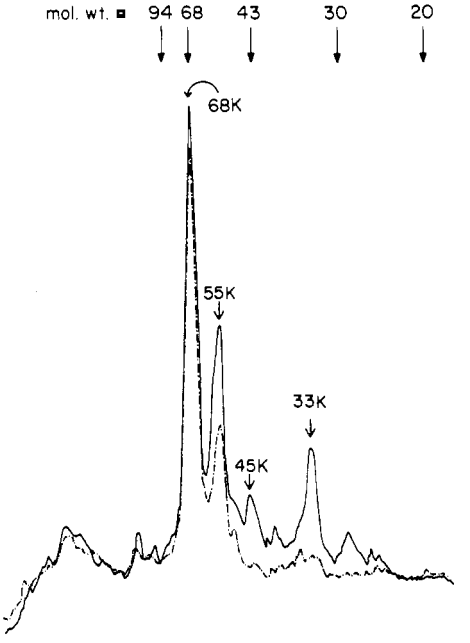

FIGURE 3: Effect of cAMP on the endogenous phosphorylation of proteins in the dark-extract. The dark-extract of ROS was incubated, in the dark, in the presence (—) or absence (---) of 10^{-6} M cAMP. The densitometric scans of the ^{32}P-labeled proteins were superimposed.

When the light-extract of ROS was used, phosphorylation of the 68K and 55K proteins was also not affected by the light/dark conditions of incubation (results not shown). However, the ^{32}P-labeled rhodopsin peak was observed whether the incubation was carried out in the light or in darkness,

apparently because rhodopsin in the light-extract, after its exposure to light during the extraction procedure, remains a good substrate for rhodopsin kinase.

Effect of cAMP on the Endogenous Phosphorylation of Soluble ROS Proteins. Figure 3 depicts the densitometric profiles of ^{32}P-labeled proteins when the dark-extract of ROS was incubated, in the dark, in either the presence or absence of 1 μM cAMP. The amount of phosphorylation of the 68K protein was not affected by the presence of exogenous cAMP, suggesting that the 68K protein was not a substrate of cyclic nucleotide dependent protein kinases. The amount of phosphorylation of the protein with 55K molecular weight was increased by the presence of cAMP. The phosphorylation of two additional proteins, with molecular weights of 45K and 33K, respectively, was also stimulated by cAMP.

The cyclic nucleotide dependent phosphorylation of the ROS proteins was further evaluated by carrying out the incubation of the dark-extract in the presence or absence of skeletal muscle inhibitor, which was a specific inhibitor of the cAMP-dependent protein kinase. It was found that the phosphorylation of the 45K and 33K proteins was completely inhibited, while the extent of phosphorylation of the 55K protein was reduced to that obtained in the absence of added cAMP (results not shown). The presence in bovine ROS of a photoreceptor-specific 33K protein, as an endogenous substrate of the cyclic nucleotide dependent protein kinase, has been previously reported by our laboratory. The properties of the 45K protein have yet to be studied. There appeared to be more than one protein comigrating on the $NaDodSO_4$ gel in the region of 55K molecular weight, which can be phosphorylated by endogenous protein kinases. One 55K phosphoprotein (as shown in Figures 1 and 2) was phosphorylated in a cyclic nucleotide independent manner, while another 55K phosphoprotein (Figure 3, cAMP-stimulated phosphorylation) was a substrate of a cAMP-dependent protein kinase.

The effect of cAMP on the phosphorylation of proteins in the light-extract was studied in the same manner (results not shown). The extent of phosphorylation of the 68K protein, which is about half of that in the dark-extract, was not affected by cAMP. The phosphorylation of the 55K, 45K, and 33K proteins is stimulated by the exogenous cyclic nucleotide, the extent of which is the same as that in the dark-extract, indicating that extraction of the 55K, 45K, and 33K proteins was not affected by light.

Sedimentation of Rhodopsin Kinase and the 68K Protein on Sucrose Density Gradient. The 68K phosphoprotein appears to have characteristics that are similar to those of RK, e.g., comparable molecular weights and light-affected extraction from ROS. Simultaneous centrifugation experiments were performed for the light- and dark-extracts by using individual 5–20% linear sucrose density gradients in order to discern RK from any other 68K phosphoprotein which might have comigrated on the $NaDodSO_4$ gel. The gradients were fractionated, and each fraction was assayed for RK activity. As shown in Figure 4A, the RK activity in the dark-extract was twice as high as that in the light-extract.

Aliquots from each fraction of the sucrose density gradient were also incubated with [γ-^{32}P]ATP in the absence of exogenous protein kinases or substrate. Proteins in each fraction, phosphorylated by the endogenous protein kinases, were then identified by $NaDodSO_4$ gel electrophoresis and autoradiography. Densitometric scanning of the autoradiographs (Figure 5) showed that in the peak fraction of RK activity (fraction 16), a single 68K protein was most extensively phosphorylated.

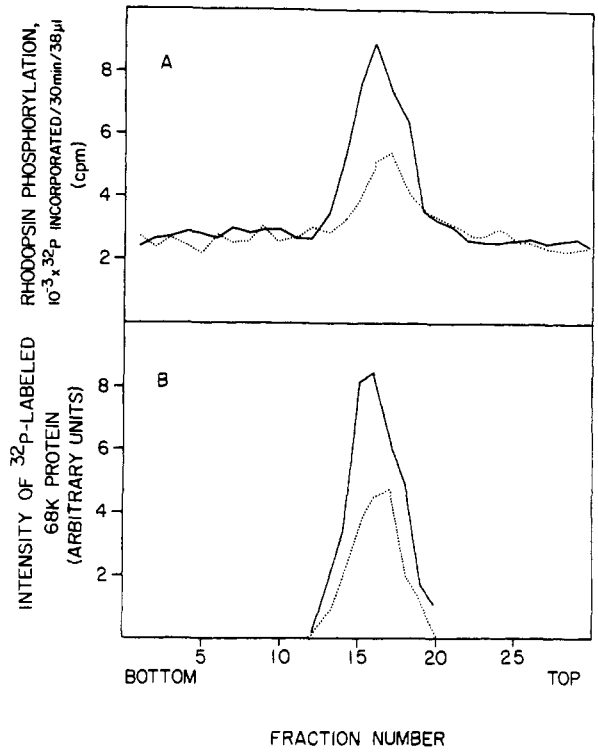

FIGURE 4: Sedimentation of rhodopsin kinase and the 68K protein on sucrose density gradients. (A) The light- or dark-extracts of ROS were centrifuged in parallel on individual 5–20% sucrose density gradients, and the resultant fractions were assayed for rhodopsin kinase activities as described under Methods. Rhodopsin kinase in the dark- (—) or light-extracts (···) was measured by the phosphorylation of bleached rhodopsin. (B) The soluble proteins in the fractions of the sucrose density gradients were allowed to undergo endogenous phosphorylation, and phosphorylated 68K protein was identified on the $NaDodSO_4$ gel by its molecular weight and then quantitated by the height of its densitometric scan as described under Methods. The quantities of 68K phosphoproteins in the light- (—) or dark-extracts (···) were plotted for each fraction of the sucrose gradients.

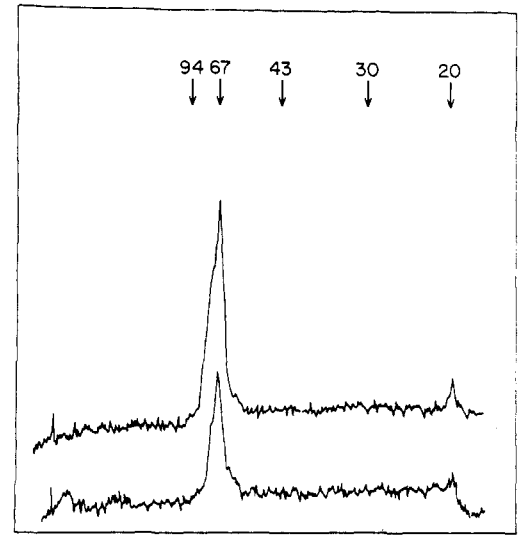

FIGURE 5: Densitometric scanning of the 68K phosphoprotein in fraction 16 of the sucrose density gradient (Figure 4). Soluble proteins in the fractions of the sucrose density gradients were allowed to undergo endogenous phosphorylation, and the phosphorylated proteins were then analyzed as described under Methods. Densitometric scannings of the autoradiographs for fraction 16 of the light (bottom scan) or dark (top scan) extracts were lined up so that the quantity of the 68K phosphoprotein could be compared.

The amount of the 68K phosphoprotein was twice as high in the dark- as in the light-extract. Furthermore, the distribution of the 68K phosphoprotein on the sucrose gradient (quantitated

by the height of the scanning peak) coincided with that of the RK activity (Figure 4B).

Discussion

While the endogenous substrates for protein kinases that exist in the soluble fraction of ROS were being investigated, it was observed that a 68K protein was extensively phosphorylated. The phosphorylation was neither activated by cyclic nucleotides nor inhibited by the skeletal muscle inhibitor, indicating that the 68K protein was not a substrate of the cyclic nucleotide dependent protein kinases. Also, the level of the 68K protein phosphorylation was not affected by the light or dark conditions of the reaction. It was observed further that the amount of the 68K phosphoprotein was lower in the light-extract than in the dark-extract of ROS. Sedimentation experiments showed not only that the distribution of the 68K phosphoprotein on the sucrose density gradient correlated closely with that of the RK but also that the relative quantities of 68K phosphoprotein in the light- and dark-extracts correlated with the relative catalytic activities of RK in the two extracts. We therefore concluded that the 68K phosphoprotein was phosphorylated RK. These latter observations further indicated that the endogenous protein kinase which phosphorylated the 68K protein (RK) also comigrated with RK during the sedimentation. Considering the similarity between the catalytic characteristics of the phosphorylation reaction and those of the RK, we concluded that RK is apparently phosphorylated by itself; in other words, RK is capable of autophosphorylation. Still, it is possible that a distinct cyclic nucleotide independent protein kinase, which has sedimentation and extraction properties similar to those of RK, may be responsible for the phosphorylation of RK.

It is known that type II cAMP-dependent protein kinase and cGMP-dependent protein kinase both undergo autophosphorylation (Lincoln et al., 1978). Autophosphorylation of the regulatory subunit of CNPK II was postulated to regulate the reassociation and inactivation of the catalytic and regulatory subunits and, thereby, modulate the enzyme response to cyclic nucleotides (Rangel-Aldao & Rosen, 1976). By analogy, phosphorylation of RK could induce a conformational state that differs from that of the unphosphorylated protein. Kühn (1978) suggested that light-dependent interaction between RK and ROS membranes may serve as a regulatory mechanism for the activity of RK. The regulation of RK may be more sophisticated than a simple binding/dissociation reaction between RK and the bleached ROS membranes. It seems likely that RK will exhibit different enzyme or binding characteristics than phosphorylated RK, and phosphorylation may represent one of the mechanisms for the control of rhodopsin phosphorylation.

Our experiments on light/dark extraction of ROS confirm the observations of Kühn (1978) that RK interacts with the ROS membrane in a light-dependent manner. The cAMP-dependent phosphorylation of 33K protein (previously estimated as 30K) by cyclic nucleotide dependent protein kinase was reported earlier by our laboratory (Lolley et al., 1977). Phosphorylation of two additional proteins with molecular weights of 55K and 45K was also stimulated by cAMP. It appears that none of these substrates for CNPK interacted with ROS membranes in a light-dependent manner, since neither the phosphorylated protein nor the CNPK was extracted in a light-affected manner. Further characterization of these three phosphoproteins is in progress.

Acknowledgments

We thank Louise V. Eaton for her assistance in manuscript preparation.

References

Farber, D. B., Brown, B. M., & Lolley, R. N. (1978) *Vision Res. 18*, 497–499.

Fletcher, R. T., & Chader, G. J. (1976) *Biochem. Biophys. Res. Commun. 70*, 1297–1302.

Frank, R. N., & Buzney, S. M. (1975) *Biochemistry 14*, 5110–5117.

Greengard, P. (1978) *Science (Washington, D.C.) 199*, 146–152.

Kebabian, J. W. (1977) *Adv. Cyclic Nucleotide Res. 8*, 421–508.

Krebs, E. G., & Beavo, J. A. (1979) *Annu. Rev. Biochem. 48*, 923–959.

Kühn, H. (1978) *Biochemistry 17*, 4389–4395.

Laemmli, U. K. (1970) *Nature (London) 227*, 680–682.

Lee, R. H., Brown, B. M., & Lolley, R. N. (1981) *Biochemistry 20*, 7532–7538.

Lincoln, T. M., Flockhart, D. A., & Corbin, J. D. (1978) *J. Biol. Chem. 253*, 6002–6009.

Lolley, R. N., Brown, B. M., & Farber, D. B. (1977) *Biochem. Biophys. Res. Commun. 78*, 572–578.

Martin, R. G., & Ames, B. N. (1961) *J. Biol. Chem. 236*, 1372–1379.

Nimmo, H. G., & Cohen, P. (1977) *Adv. Cyclic Nucleotide Res. 8*, 145–266.

Rangel-Aldao, R., & Rosen, O. M. (1976) *J. Biol. Chem. 251*, 3375–3380.

Ross, E., & Schatz, G. (1973) *Anal. Biochem. 54*, 304–306.

Shichi, H., & Somers, R. L. (1978) *J. Biol. Chem. 253*, 7040–7046.

Weller, M., Virmaux, N., & Mandel, P. (1975) *Proc. Natl. Acad. Sci. U.S.A. 73*, 381–385.

Woodruff, M. L., & Bownds, M. D. (1979) *J. Gen. Physiol. 73*, 629–653.

Yee, R., & Liebman, P. A. (1978) *J. Biol. Chem. 253*, 8902–8909.