Light-Induced Phosphorylation of Rod Outer Segments by Guanosine Triphosphate

Gerald J. Chader, R.T. Fletcher and G. Krishna
Laboratory of Vision Research, National Eye Institute and
Laboratory of Chemical Pharmacology, National Heart and Lung
Institute, National Institutes of Health, Bethesda, Maryland 20014

Received March 24,1975

Summary: Significant GTP-dependent protein kinase activity is present in isolated photoreceptors of the retina. No transfer of the γ-^{32}P group from GTP-γ-^{32}P to ATP was detected. Although no stimulation of GTP-kinase activity by cyclic GMP or cyclic AMP was observed, exposure of dark-adapted photoreceptors to light resulted in a 46-fold increase in protein phosphorylation

Isolated rod outer segments (ROS), the photoreceptor units of the neural retina, exhibit high activities of guanylate cyclase (1-3) and cyclic 3',5'-GMP phosphodiesterase (4,5). Extraordinarily high concentrations of cyclic 3',5'-GMP are also present in rod outer segments (6,7). In view of this apparent specificity for guanine nucleotide metabolism and since an ATP-dependent protein kinase activity has been reported in outer segments (8-10), we decided to investigate the possibility that GTP-dependent protein kinase activity was present in isolated bovine rod outer segments.

Materials and Methods

Dark-adapted bovine rod outer segments were prepared by discontinuous sucrose gradient centrifugation as previously described (5). When appropriate, 100 µl samples of dark-adapted ROS containing about 100 µg protein were exposed to light (100-foot-candles) for specific periods of time just before the assay. Assays for protein kinase were in a volume of 110µl containing 50-100 µg ROS protein in 40mM tris buffer, pH 7.4, containing 5mM $MgCl_2$ and 1 X 10^{-4}M ATP-γ-^{32}P or GTP-γ-^{32}P (New England Nuclear Corp., Boston, Mass.) at 75-100 cpm/p mole. When appropriate, cyclic AMP or cyclic GMP was added at a final concentration of 1 X 10^{-8}M to 1 X 10^{-6}M.

Incubation was conducted under dim red light for 30 seconds at 37°C; the addition of 0.5 ml of 10% CCl_3COOH stopped the reaction. Precipitated protein was collected on a Millipore filter paper (0.45 micron pore size), washed with 5 ml of 10% CCl_3COOH, dried, 1 ml cellosolve added and assayed for radioactivity using 10 ml Aquasol (New England Nuclear Corp.). Incubations containing denatured rod outer segment protein were conducted; blank values were 10-12 pmoles and were subtracted from the experimental values.

High pressure liquid chromatography was conducted using DuPont AAX anion exchange resin. Elution was with 0.25 M KH_2-PO_4 buffer, pH 2.8

Results and Discussion

Figure 1 shows the influence of light on phosphate transfer activity

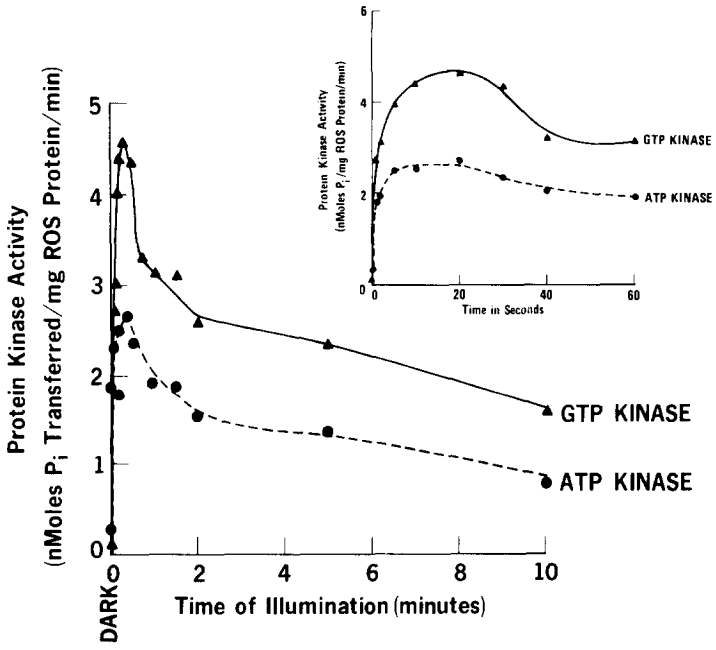

Figure 1 Effect of light on phosphorylation in isolated rod outer segments. Dark-adapted rod outer segments were illuminated for appropriate times immediately prior to assay. The incubation mixture contained 1 X 10^{-4}M GTP-γ-^{32}P or ATP-γ-^{32}P, 40 mM tris buffer, pH 7.4, 5 mM $MgCl_2$ and 50-100 ug protein in 110 ul. Incubation was for 30 seconds at 37° [2] in the dark and was halted by the addition of 0.5 ml 10% CCl_3COOH. Each time point is the average of three separate determinations which [3]agreed within 15%. The insert is an expanded view of the events taking place within the first 60 seconds after light exposure.

of outer segments using ATP-γ-^{32}P or GTP-γ-^{32}P. In dark-adapted outer segments, phosphorylation was low, 0.3 nmoles/min/mg protein with ATP and 0.1 nmoles/min/mg protein with GTP. Exposure of outer segments to light dramatically increased phosphorylation. A one second exposure increased Pi transfer from ATP and GTP to 1.8 and 2.7 nmoles/min/mg respectively (see insert of Figure 1). Maximal activity was observed after a 20 second exposure to light with 2.7 nmoles/min/mg and 4.6 nmoles/min/mg Pi transfered from ATP and GTP respectively. A 9-fold increase in ATP phosphorylation and a 46-fold increase in GTP phosphorylation was thus observed. After 10 minutes of illumination, the initial rate of phosphorylation was decreased by approximately 65% of the maximum for both ATP and GTP. No stimulatory effect on phosphorylation was observed with 1 X 10^{-8}M to 1 X 10^{-6}M cyclic AMP or cyclic GMP. At higher concentrations (10^{-6}-10^{-4}M), cyclic AMP inhibited phosphorylation by GTP.

Using the technique of high pressure liquid chromatography, no transfer of the γ-^{32}P group from GTP-γ-^{32}P to ATP was observed. After incubation of GTP-γ-^{32}P with light-adapted rod outer segments, approximately 96% of the ^{32}P was subsequently found to be present in the GTP chromatographic fraction, 4% with inorganic phosphate and no significant radioactivity in the ATP fraction. This indicates the probable absence of Pi exchange from GTP to ATP in rod outer segments under the incubation conditions described above.

It thus appears that rod outer segments exhibit an active phosphorylation system utilizing GTP that is stimulated by light rather than by cyclic nucleotides. In other systems (11, 12) it has been found that phosphorylation by GTP involved prior transfer of γ-^{32}P from GTP-γ-^{32}P to ADP with subsequent phosphorylation by the ATP. This does not seem to be the case in rod outer segments. First, no transfer of ^{32}P from GTP-γ-^{32}P to ATP was observed in our incubations as assessed by high pressure anion exchange chromatography. Secondly, light induced phosphorylation

by GTP is greater than that with ATP making it unlikely that ATP acts as an intermediate in the phosphate transfer. The rate of phosphorylation is linear for at least 40 seconds with either GTP or ATP under the present conditions (unpublished observations) thus the time of incubation (30 seconds) in the present study was short enough to measure initial rates of reaction.

Although the physiological role of phosphorylation in rod outer segments is presently unknown, the presence of significant kinase activity and its light sensitivity make it likely that phosphate transfer is involved in photoreceptor functioning. The rapid activation of GTP phosphorylation by light and its higher activity in comparison to ATP kinase would seem to indicate a special role for this activity in visual or neural processes.

References

1). Goridis, C., Virmaux, N., Urban, P. and Mandel, P. (1973). FEBS Letters 30, 163-166.

2). Pannbacker, R. (1973). Science 182, 1138-1140.

3). Bensinger, R., Fletcher, R. and Chader, G. (1974). Science 183, 86-87.

4). Chader, G., Johnson, M., Fletcher, R. and Bensinger, R. (1974). J. Neurochem. 22, 93-99.

5). Chader, G., Fletcher, R., Johnson, M. and Bensinger, R. (1974). Exp. Eye Res. 18, 509-515.

6). Krishna, G., Krishnan, N., Fletcher, R. and Chader, G. (1973). Abstract, 2nd Internat. Conf. on Cyclic Nucleotides, Vancouver, B.C. Canada, p. 54.

7). Krishna, G., Krishnan, N., Fletcher, R. and Chader, G. (1974). Submitted for publication.

8). Kuhn, H. and Dreyer, W. (1972). FEBS Letters 20, 1-6.

9). Bownds, D., Dawes, J., Miller, J. and Stahlman, M. (1972). Nature, New Biology 237, 125-127.

10). Frank, R., Cavanagh, H. and Kenyon, K. (1973). J. Biol. Chem. 248, 596-609.

11). Rodbell, M. and Salomon, M. (1973). J. Biol. Chem. 248, 3363-3365.

12). Kuo, J. (1974). J. Biol. Chem. 249, 1755-1759.