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Phosphorylation of Rhodopsin as a Possible Mechanism of Adaptation*

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Abstract. Light-induced phosphorylation of rhodopsin has been extensively studied by a number of investigators from a biochemical point of view. However, little is known about the physiological function of this reaction. The slow rates measured for phosphorylation and dephosphorylation suggest that it may be involved in visual adaptation rather than in excitation. This paper presents biochemical data obtained from phosphorylation experiments in isolated photoreceptor membranes as well as in the more physiological system of whole retinas and living animals. An attempt is made to compare the phosphorylation reaction with visual adaptation hypotheses taken from the electrophysiological literature. Finally, effects of cyclic nucleotide metabolism on the sensitivity of photoreceptors are presented and discussed.

Key words: Rodopsin – Phosphorylation – Adaptation – Retina – Cyclic nucleotides.

As a late consequence of photon capture, rhodopsin is phosphorylated. The γ-phosphate group from ATP[1] or GTP is transferred enzymatically to the protein moiety of rhodopsin where it is bound to serine and threonine residues (Kühn and Dreyer, 1972). Up to 4 moles of phosphate are incorporated per mole of rhodopsin if crude frog rod outer segment (ROS) suspensions are fully bleached in the presence of AT^{32}P (Miller and Paulsen, 1975, and our observations). This suggests the presence of at least 4 phosphate binding sites in the rhodopsin molecule. With purified cattle ROS, lower phosphorylation yields of 0.3–2 phosphates per rhodopsin are normally obtained. Isoelectric focusing of ^{32}P-phosphorylated rhodopsin shows that the phosphate is highly unhomogeneously distributed in a population of rhodopsin mole-

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[1] Abbreviations used: ATP = adenosine 5′-triphosphate; GTP = guanosine 5′-triphosphate; ROS = rod outer segments; IBMX = isobutylmethylxanthine; cyclic GMP = guanosine 3′, 5′-monophosphate

cules: Some rhodopsins are highly phosphorylated with 4 or perhaps more phosphates bound, whereas a large fraction is not phosphorylated at all. If only small amounts of rhodopsin are bleached, very high phosphate incorporation per rhodopsin bleached has been reported for frog ROS (Bownds et al., 1972; Paulsen and Miller, 1977), namely up to 50 phosphates incorporated per rhodopsin bleached if less than 1% of the rhodopsin was bleached. On the other hand, this "amplification" effect of high phosphorylation at low bleaching levels was not observed for cattle ROS (e.g., Frank et al., 1973). Preliminary experiments with frog ROS in our laboratory also showed an enhanced phosphate incorporation at low bleaching, but much lower than the value of 50. Thus, this important question of a possible amplification mechanism involved in the phosphorylation reaction is still not settled.

Although initiated by bleaching of rhodopsin, the phosphorylation reaction itself is a dark reaction. The phosphorylation activity is greatest shortly after bleaching and then declines with increasing time in the dark (Fig. 1). It can be re-activated by additional bleaching of rhodopsin. Opsin at long times after bleaching is not accessible to phosphorylation, unless it is regenerated to rhodopsin and bleached again. The enzymatic activity (ROS kinase) can be separated from rhodopsin by extraction into

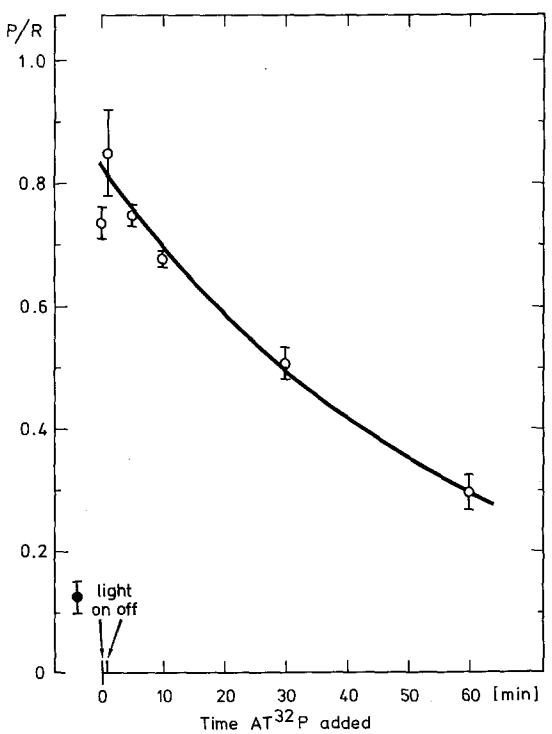

Fig. 1. Decay of phosphorylation activity at 30° C in the dark. "Whole" cattle ROS *containing kinase* were illuminated for 1 min at time zero, and then $AT^{32}P$ was added in the dark at the times indicated in the abscissa. Phosphorylation was allowed to take place for 1 h in the dark after addition of $AT^{32}P$. The solid line is the least squares fit of the data, excluding $t = 0$, to a first order process (half-time 41 min).

Similar results were obtained when *kinase-depleted* ROS membranes were illuminated in the presence of $AT^{32}P$, and then dark-kept kinase was added at various times after bleaching

aqueous buffers, and the phosphorylation ability can be restored by adding the extract back to the kinase-depleted membrane (Kühn et al., 1973). To induce phosphorylation, it is only necessary to illuminate rhodopsin but not the kinase. If kinase-depleted ROS membranes are briefly illuminated in the presence of $AT^{32}P$, and a kinase extract which had never been exposed to light is subsequently added in the dark at various times after bleaching, rhodopsin is phosphorylated and the phosphorylation activity decays slowly in the dark, similar to the experiment shown in Figure 1. Thus light activation as well as the slow inactivation in the dark are properties of the membrane-bound rhodopsin itself; the kinase does not need light to become active.

So far, it has not been possible to show any correlation of rhodopsin phosphorylation with other light-dependent biochemical reactions occurring in the rod outer segments, as for instance rhodopsin regeneration or cyclic nucleotide metabolism. An influence of phosphorylation on the permeability of ROS membranes to Ca^{++} ions has been measured by Weller et al. (1975).

Physiology

Phosphorylation of rhodopsin has been measured under physiological conditions in living frogs (Kühn, 1974), in isolated "intact" retinas (Kühn and Bader, 1976) and in opened eye-cups of frogs, using inorganic ^{32}P-phosphate as nucleotide triphosphate precursor. From these experiments it is obvious that the phosphorylation level of rhodopsin is high in the light-adapted state and low in the dark-adapted state (Fig. 2). The reaction rates of phosphorylation (half-time about 2 min at 21° C) and

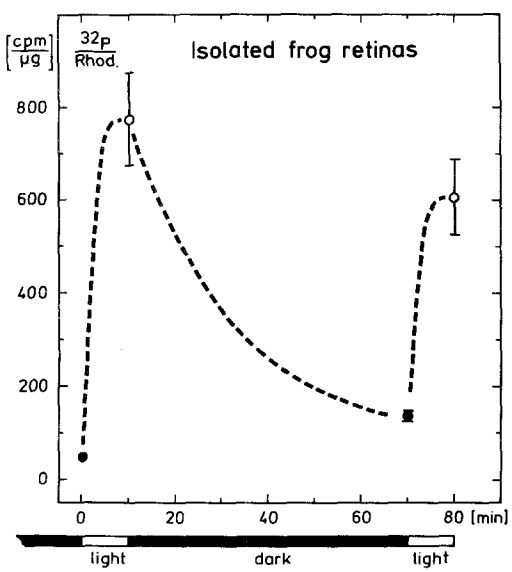

Fig. 2. Phosphorylation and dephosphorylation of rhodopsin in isolated frog retinas (*R. esculenta*). Bleaching was effected with white light of 300 lux which bleached most of the visual pigment (about 80%) within the first minute of illumination. No measurable regeneration of rhodopsin occurred during the 60 min dark period. The ordinate indicates ^{32}P bound per µg of rhodopsin, and the bars indicate standard error for 5 retinas. The time courses (dashed line) were determined in separate experiments under similar conditions

dephosphorylation (half-time about 13 min) are slow as compared to the reactions involved in visual excitation. On the other hand, the time required for dephosphorylation in the dark is roughly similar to the time required for dark adaptation after strong bleaching illumination. This suggests a possible role of the phosphorylation reaction in controlling the light sensitivity of the rods, but at the present time there is no direct proof of this hypothesis. It is interesting to note that dephosphorylation takes place in isolated retinas *in the absence of pigment regeneration* as well as in living animals where regeneration of rhodopsin takes place in the dark together with dephosphorylation. This indicates that dephosphorylation and regeneration are two independent processes. Earlier measurements on isolated ROS have also shown that regeneration of rhodopsin, by addition of 11-cis retinal to opsin or to ^{32}P-opsin, is independent on the phosphorylation state of rhodopsin (Kühn et al., 1973; Miller and Paulsen, 1975).

It is known from the electrophysiological literature that besides the so-called "network" adaptation which takes place in the more proximal network of retinal cells, there is considerable adaptation in the receptor cells themselves (e.g., Green et al., 1975). Two types of adaptation have been distinguished at the receptor level: 1) The so-called "pigment adaptation" or "photochemical adaptation" where the log sensitivity is strictly correlated to the amount of rhodopsin present. The rate of dark adaptation after a bleaching illumination is slow and exactly equals the rate of rhodopsin regeneration (e.g., Rushton and Powell, 1972). 2) The second type of adaptation is not directly correlated to the amount of rhodopsin present and has been termed "receptor adaptation" (Grabowski and Pak, 1975), "fast" or "neural" adaptation (e.g., Dowling, 1963), "intermediate adaptation" (Donner, 1977), or "membrane adaptation" (Stieve, 1977). This type of adaptation is demonstrated by the fact that isolated retinas which have been bleached and light-adapted can dark-adapt over several log units of sensitivity without measurable rhodopsin regeneration. The rate of dark adaptation may be fast or slow (ranging from seconds to more than 30 min), depending on the intensity of the adapting light prior to dark adaptation. The mechanism of this type of adaptation is largely unknown; temporary changes of membrane properties, exhaustion and refilling of ionic pools, active transport, the formation and/or decay of rhodopsin photoproducts, cyclic nucleotide metabolism, and phosphorylation of rhodopsin have been discussed as possible mechanisms. Since dephosphorylation of phosphorylated rhodopsin in the dark is independent of pigment regeneration (see Fig. 2), it is probably not involved in "pigment adaptation". It may, on the other hand, be involved in the slow part of "receptor adaptation" by controlling some properties of the photoreceptor membranes like, for instance, their Ca^{++} permeability. In any event, if phosphorylation of rhodopsin plays a role in adaptation, it is certainly not the only mechanism of sensitivity control in the receptor cells.

It should be noted that the time course of phosphorylation is perhaps too slow to be correlated with light adaptation. It takes less than 2 min to get constant, light-adapted responses from frog retinas after an adapting background light has been turned on (Baumann, 1967). In the living rat, this time is even shorter than 15 s (Dowling, 1963). On the other hand, it takes about 5 min to reach the maximum level of phosphorylation if ^{32}P-labelled retinas are fully bleached (Fig. 2). However, the light intensities used in the electrophysiological experiments cited and in the phosphorylation experiment shown in Figure 2 are different by many orders of magnitude, and it may not be appropriate to compare time courses measured under such different conditions.

Cyclic Nucleotide Metabolism and Adaptation

It is known that light normally causes a decrease in the concentration of cyclic GMP by activating the cyclic GMP phosphodiesterase in ROS (Goridis and Virmaux, 1974; Bitensky et al., 1973). Phosphodiesterase inhibitors, e.g. IBMX, applied externally, are believed to artificially raise the intracellular level of cyclic nucleotides (Lipton, 1976; Lolley et al., 1976). We studied the effect of IBMX on the aspartate-isolated mass receptor response in the frog retina. If dark adapted retinas were treated with 10^{-4} M IBMX, their sensitivity was transiently increased by 0.8 log units beyond the "absolute" dark-adapted threshold, as measured by 10 μV criterion responses. The peak of this hypersensitivity was reached about 3–5 min after addition of IBMX and then declined to a stable value, about 0.5 log units more sensitive than the untreated dark adapted retina. If such IBMX-treated retinas were briefly illuminated with light that bleached 44% of their rhodopsin, their sensitivity was first decreased by 4 log units, but in the following dark period they regained 3 log units of sensitivity within 30–40 min. Comparison with control retinas which were not treated with IBMX showed that the treatment with IBMX had no effect on the relative loss of sensitivity upon light adaptation (4 log units), nor on the relative recovery of sensitivity during dark adaptation (3 log units), and almost no effect on the time course of dark adaptation. However, it shifted most of the threshold measurements by 0.5 log units towards higher sensitivity. Thus, most of the terms which are normally used to describe adaptation phenomena are not affected by this phosphodiesterase inhibitor, but the absolute sensitivity is significantly affected. This again demonstrates the complexity of adaptation phenomena. Preliminary experiments showed no obvious influence of IBMX either on phosphorylation of rhodopsin induced by light or on its dephosphorylation in the dark.

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References

Baumann, Ch.: Sehpurpurbleichung und Stäbchenfunktion in der isolierten Froschnetzhaut. II. Die Begrenzung der Stäbchenfunktion durch Helladaptation. Pflügers Arch. ges. Physiol. **298**, 61–69 (1967)

Bitensky, M. W., Miki, N., Marcus, F. R., Keirns, J. J.: The role of cyclic nucleotides in visual excitation. Life Sci. **13**, 1451–1472 (1973)

Bownds, D., Dawes, J., Miller, J., Stahlman, M.: Phosphorylation of frog photoreceptor membranes induced by light. Nature (Lond.) New Biol. **237**, 125–127 (1972)

Donner, K. O.: Dark adaptation processes in the amphibian rod. Biophys. Struct. Mechanism **3**, 171–173 (1977)

Dowling, J. E.: Neural and photochemical mechanisms of visual adaptation in the rat. J. gen. Physiol. **46**, 1287–1301 (1963)

Frank, R. N., Cavanagh, H. D., Kenyon, K. R.: Light-stimulated phosphorylation of bovine visual pigments by adenosine triphosphate. J. biol. Chem. **248**, 596–609 (1973)

Goridis, C., Virmaux, N.: Light-regulated guanosine 3′, 5′-monophosphate phosphodiesterase of bovine retina. Nature (Lond.) **248**, 57–58 (1974)

Grabowski, S. R., Pak, W. L.: Intracellular recordings of rod responses during dark adaptation. J. Physiol. (Lond.) **247**, 363–391 (1975)

Green, D. E., Dowling, J. E., Siegel, I. M., Ripps, H.: Retinal mechanisms of visual adaptation in the skate. J. gen. Physiol. **65**, 483–502 (1975)

Kühn, H., Dreyer, W. J.: Light dependent phosphorylation of rhodopsin by ATP. FEBS Lett. **20**, 1–6 (1972)

Kühn, H., Cook, J. H., Dreyer, W. J.: Phosphorylation of rhodopsin in bovine photoreceptor membranes. A dark reaction after illumination. Biochemistry **12**, 2495–2502 (1973)

Kühn, H.: Light-dependent phosphorylation of rhodopsin in living frogs. Nature (Lond.) **250**, 588–590 (1974)

Kühn, H., Bader, S.: The rate of rhodopsin phosphorylation in isolated retinas of frog and cattle. Biochim. biophys. Acta (Amst.) **428**, 13–18 (1976)

Lipton, S. A.: Adaptation of vertebrate photoreceptors: Effects of intracellular messengers. ARVO Abstr. **1976**, 3

Lolley, R. N., Farber, D. B., Hollyfield, J. G.: Photoreceptor cell degeneration resulting from drug-induced accumulation of cGMP. ARVO Abstr. **1976**, 101

Miller, J. A., Paulsen, R.: Phosphorylation and dephosphorylation of frog rod outer segment membranes as part of the visual process. J. biol. Chem. **250**, 4427–4432 (1975)

Paulsen, R., Miller, J. A.: Biochemistry (in press) (1977)

Rushton, W. A. H., Powell, D. S.: The rhodopsin content and the visual threshold of human rods. Vision Res. **12**, 1073–1081 (1972)

Stieve, H.: Photorezeption und ihre molekularen Grundlagen. In: Biophysik. Ein Lehrbuch (eds. Hoppe et al.). Berlin-Heidelberg-New York: Springer 1977

Weller, M., Virmaux, N., Mandel, P.: Role of light and rhodopsin phosphorylation in control of permeability of retinal rod outer segment disks to Ca^{2+}. Nature (Lond.) **256**, 68–70 (1975)

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