Photoreceptor Membrane Properties and Visual Excitation

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The rod photoreceptor cell in the vertebrate retina is an elongated cell, consisting of an inner and an outer segment, connected by a narrow cilium. The inner segment contains nucleus, mitochondria and endoplasmic reticulum. The outer segment contains 1000-2000 neatly stacked flat sacs and serves as the photoreceptor unit. The major component of the sac membrane is the visual pigment rhodopsin, which consists of a protein, opsin, and a chromophoric group, the 11-cis isomer of vitamin A aldehyde or retinaldehyde (see review by Bonting, 1969).

Rhodopsin is the key substance of the visual process; absorption of a light quantum by a visual-pigment molecule leads through a series of chemical and physical processes in a few milliseconds to stimulation of the synapse at the end of the inner segment. This generates a nerve impulse, which is conducted through the optic nerve to the brain.

The two most characteristic properties of rhodopsin are its absorption spectrum and its photosensitivity. The absorption spectrum has two major peaks, the \( \alpha \) band at 500nm and the \( \gamma \) band at 278nm, and a minor band at 340nm. Illumination causes a disappearance of the \( \alpha \) band and the appearance of a 380nm peak, but the \( \gamma \) band remains unchanged. This spectral change is an important analytical criterion guiding the isolation of the visual pigment. The process reflected by this spectral change is called 'bleaching', since it is accompanied by a change in colour from purple to light yellow.

Isolation of rod photoreceptor membranes is carried out by mild homogenization of the retina followed by sucrose-gradient centrifugation. Any bleached rhodopsin is regenerated by treatment with 11-cis retinaldehyde, which readily reacts with free opsin. This yields an aqueous suspension of photoreceptor membranes with a maximum and constant rhodopsin content without the use of detergents (de Grip et al., 1972). Although it is possible to solubilize rhodopsin from its membrane suspension by means of detergents, this causes more or less drastic changes in the pigment (Bonting et al., 1974). Thus the appearance of two thiol groups during bleaching of rhodopsin in digitonin solution has been shown to be a detergent artifact (de Grip et al., 1973c).

In enriched isolated cattle photoreceptor membranes rhodopsin represents 87% of the total membrane protein (Daemen et al., 1972). Cattle rhodopsin has a molecular weight of 38900 and a molar absorbance at 500nm of 40300 (Daemen et al., 1970, 1972). The main phospholipids present in the photoreceptor membrane are phosphatidylethanolamine, phosphatidylcholine and phosphatidylserine; it has an abnormally high unsaturated fatty acid content (34% \( \text{C}_{22:6} \)), but the cholesterol content is rather low (Borggreven et al., 1970). This means that the photoreceptor membrane has a highly fluid lipid phase, in which the rhodopsin molecules can freely rotate and diffuse. The phospholipids present in the membranes can be removed nearly quantitatively by means of enzymic delipidation without affecting the rhodopsin spectrum (Borggreven et al., 1971, 1972). These and other experiments (Daemen et al., 1971) have excluded phospholipid amino groups as the binding site of the chromophoric retinaldehyde group.

Definite proof for the earlier conclusion that the chromophoric retinaldehyde in native rhodopsin has the 11-cis conformation, which changes to the all-trans conformation on bleaching, has been obtained by cautious extraction of the chromophore in organic solvent and its identification through t.l.c. and the spectral change on iodine-catalysed photoisomerization (Rotmans et al., 1972). This means that bleaching of rhodopsin involves isomerization of the chromophore.

The bleaching process of rhodopsin involves several spectrally identifiable intermediates: rhodopsin (500nm) \( \rightarrow \) prelumirhodopsin (543nm) \( \rightarrow \) lumirhodopsin (497nm) \( \rightarrow \) metarhodopsin I (478nm) \( \rightarrow \) metarhodopsin II (380nm) \( \rightarrow \) metarhodopsin III (470nm) \( \rightarrow \) retinol + opsin. The initial steps are very fast. Metarhodopsin II is formed in about 1ms after illumination at body temperature, whereas its decay is slow, taking
minutes. The chromophore is isomerized from the 11-cis to the all-trans form in the first step (rhodopsin → prelumirhodopsin), which is the only one requiring light (see Bonting, 1969).

In rhodopsin, retinaldehyde is bound by means of an aldimine bond to the ε-amino group of a lysine residue of opsin. This has been established by blocking all free amino groups by amidination with methylacetimidate without removal of the chromophore and subsequent determination and identification of the non-amidinated amino groups with dansyl reagent (de Grip et al., 1973a,b). Addition of NaBH₄, which fixes the aldimine bond by reduction, during illumination of rhodopsin, followed by treatment with 11-cis-retinaldehyde, does not form any light-sensitive pigment. This shows that the chromophore remains bound to the same lysine residue up to the formation of metarhodopsin I₁₁. When NaBH₄ is added at increasing time-intervals after illumination, followed again by treatment with ¹₁₁-cis-retinaldehyde to 'probe' the chromophore binding site, increasing amounts of photopigment are formed. This shows that during the decay of metarhodopsin I₁₁ the chromophore migrates by means of transiminization to other amino groups (Bonting et al., 1973; Rotmans et al., 1974).

One of the amino groups, to which the chromophore can migrate, belongs to the active centre of the retinol dehydrogenase, which is present in the photoreceptor membrane (de Pont et al., 1970a,b). In the presence of NADPH this enzyme reduces the all-trans retinaldehyde to all-trans retinol, which is then released. In darkness an ionic current, carried by Na⁺, runs from the inner segment along the rod to the outer segment. There Na⁺ enters passively across the outer membrane. Synaptic stimulation results from a sudden lowering of the dark current, caused by a light-induced decrease in the Na⁺ permeability of this membrane. The latter effect may be due to a release of Ca²⁺ from the rod sac on bleaching of rhodopsin, evidence of which has been obtained from chemical studies (Hendriks et al., 1974).