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A Proposed Model for Rhodopsin in Photoreceptor Membranes

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Abstract. Transient electric birefringence studies have been made on bovine rhodopsin solubilized in the detergent lauryldimethylamine oxide from glutaraldehyde fixed rod outer segment (ROS) membranes. It was found that fixation caused no appreciable differences in the measured relaxation times when compared with unfixed ROS. On the basis of these findings a model for the orientation of rhodopsin in photoreceptor membranes is proposed which accounts for translational diffusion and two modes of rotational diffusion. The proposed model is related to a number of experimentally determined biophysical properties reported in the literature.

Key words: Rhodopsin – Glutaraldehyde fixation – Transient birefringence – Diffusion – Photoreceptor – Model.

Introduction

The membrane protein rhodopsin has recently been shown to be highly mobile in rod outer segment membranes (ROS). Brown (1972) demonstrated that dichroism could be photoinduced in frog retina after fixation with glutaraldehyde. He stated that this supports the hypothesis that rhodopsin is free to undergo Brownian rotation in the membrane, and that glutaraldehyde forms crosslinks which prevent the rotation. Such rotational motion of rhodopsin had earlier been suggested by Hagins and Jennings (1959) to explain the absence of dichroism in unfixed retinas. Cone (1972) demonstrated that dichroism could be photoinduced in unfixed frog retina by the use of fast detection apparatus. He stated that the transient photodichroism reveals that rhodopsin has a relaxation time of 20 μs. In some preparations he found that the dichroism decayed in two stages, a rapid initial decay followed by a slower final decay. In an earlier report, Cone (1971) stated that the photodichroism decayed in two approximately equal steps with relaxation times 4 and 50 μs. He further reported that the relaxation times are approximately doubled by soaking the retina in Ringer's solution containing either 30% w/w sucrose, or 3% ethanol, or glycerol. These rea-

gents are assumed to alter the viscosity of the membrane. Finally, the shorter relaxation process survives glutaraldehyde fixation.

In addition to rotational motion, rhodopsin has been demonstrated to undergo lateral diffusion. Poo and Cone (1974) demonstrated this by observing the time distribution of absorbance changes in a single Necturus rod cell after it had been bleached in a localized region. No redistribution of rhodopsin was observed when the rods were fixed with glutaraldehyde.

The shape of rhodopsin has also been the subject of recent investigation. Cone (1972, 1974, 1975) stated that rhodopsin is approximately spherical with an effective radius in the range 22–28 Å. Alternatively, Strackee (1971), Wu and Stryer (1972), W. Wright et al. (1972, 1973), Lewis et al. (1974), and Yeager (1975) have proposed that rhodopsin is an ellipsoid of revolution. A. Wright (1974) reinterpreted the data of Strackee (1971) and Cone (1972) in terms of an ellipsoid of revolution. The dimensions of the semiaxes calculated according to Cone's data were 138 Å and 14.4 Å. It should be pointed out that these dimensions correspond not to rhodopsin but to one of the intermediates in the bleaching sequence, probably metarhodopsin I due to the times involved (Applebury et al., 1974).

In further support of the ellipsoidal shape of rhodopsin, transient birefringence studies of rhodopsin solubilized in two detergents, LDAO (lauryldimethylamine oxide) and digitonin, yield data consistent with a prolate ellipsoid of axial ratio 6.8 ± 0.5 (A. Wright, 1976). In the detergent 0.02% LDAO, the dimensions of the semiaxes for the rhodopsin-detergent micelle are 117 ± 4 Å and 17.1 ± 0.8 Å. Bleaching with white light caused a conformational change with resulting semiaxes 169 ± 4 Å and 15.3 ± 0.6 Å. These values support the above observation that the dimensions calculated from Cone's data correspond to an intermediate in the bleaching sequence. In the detergent 0.045% digitonin, the long birefringence relaxation time for rhodopsin-digitonin micelles was 144 ns. This is in excellent agreement with the value of 148 ns which was reported by Tao (1971) for N-retinyl-opsin solubilized by digitonin. Additionally, Tao reported that a lower limit of 300 ns can be set for the rotational relaxation time of membrane-bound N-retinyl-opsin. This lower limit is consistent with the data reported by Cone (1972, 1971).

Based on the results of the above experiments, it is possible to propose a model for rhodopsin in ROS membranes. Rhodopsin is a prolate ellipsoid imbedded in the lipid bilayer with its long axis normal to the axis of the ROS membrane. Because of the fluidity of the membrane the ellipsoid is able to undergo both translational and rotational motion. The rotational motion is resolved into two modes; rotation of the long axis about the short axis, and rotation about the long axis. These modes correspond to the long and short relaxation times observed by Cone. Such a model is in agreement with that suggested by W. Wright et al. (1973): "rhodopsin molecules float upon the disk membranes like logs on a pond".

Several interesting consequences of this model can be displayed by considering the effects of glutaraldehyde fixation. Since the amino group containing phospholipids, phosphatidyl ethanolamine and phosphatidyl serine, comprise approximately 50% of the lipids of ROS membranes (Daemen, 1973) it is reasonable that glutaraldehyde crosslinks these lipids by a reaction scheme similar to that proposed by Blauer et al. (1975). As such, a cage would be formed about rhodopsin restricting translation motion and one mode of rotational motion, that corresponding to rota-

tion of the long axis about the short axis. Thus, the orientation of rhodopsin is fixed in this case. This supports the observation by Brown that photodichroism can be induced in glutaraldehyde fixed retina. It also supports the observation by Cone that the long relaxation time vanishes on glutaraldehyde fixation, and that the short relaxation time survives such fixation. This is because the cage does not constrain the mode of rotation about the long axis. The existence of such a cage is consistent with the observation of Hall and Nir (1974) that fixation of ROS membranes with glutaraldehyde prevents extraction of phosphatidyl ethanolamine and phosphatidyl serine with prolonged exposure to chloroform-ethanol (2 : 1). Also, Arden et al. (1968) stated "the possibility that rhodopsin is firmly secured within the highly cross-linked structure of the fixed outer segments is unlikely since after thorough fragmentation it is more easily extracted".

Methods, Results and Discussion

In order to test the above model with respect to glutaraldehyde fixation, the following experiment was performed. Purified bovine ROS membranes were incubated for a minimum of 30 min in 4% glutaraldehyde prepared in 5 mM Tris buffer, pH 7.4. These conditions of glutaraldehyde fixation were similar to those adopted by Cone (1972), and were reported to give strong reaction by Brown (1972). After fixation the ROS were washed two times in Tris buffer and then exposed to an excess of sodium borohydride. The ROS were then washed four times and resuspended in 2% LDAO prepared in 0.067 M, pH 7.0 phosphate buffer. This suspension was sonicated three times for 5 min, and then stored in the cold overnight to complete the extraction of rhodopsin. The extracted rhodopsin was purified by chromatography on calcium phosphate. The purity criteria, O.D. 280/O.D. 500, was 3.5. The concentration of rhodopsin was calculated using the molar extinction coefficient at 500 nm of 43,250 M^{-1} cm^{-1} and the molecular weight for rhodopsin was taken to be 35,000 daltons (Lewis et al., 1974; Akhtar et al., 1967). The resulting concentration of rhodopsin extracted from the fixed ROS was 0.7 mg/ml, which was not different from that extracted from unfixed ROS. All procedures were carried out in the dark or under dim red light.

Transient birefringence studies were made on the stock solution which had been dialyzed three times against 0.02% LDAO in distilled water and at 50% and 25% dilutions. Two relaxation times were estimated for each transient birefringence decay curve and the values extrapolated to zero protein concentration were $\tau_s = 263$ ns and $\tau_f = 23.2$ ns. These values differ only slightly from those observed for rhodopsin isolated from unfixed ROS: $\tau_s = 222$ ns and $\tau_f = 20$ ns (A. Wright, 1976). Therefore, it appears that the action of the glutaraldehyde was not to crosslink rhodopsin molecules, since one would expect much larger differences in the relaxation times. Details of the apparatus, experimental measurements, and data analysis have been published elsewhere (A. Wright, et al., 1973, 1975; Williams et al., 1976).

Aliquots of the extracted rhodopsin solutions were made 5% in SDS and 1% in mercaptoethanol and heated at 90° for 20 min. 5% and 1% SDS-rhodopsin samples were subjected to polyacrylamide gel electrophoresis. Rhodopsin extracted from unfixed ROS membranes showed a band corresponding to 40,000 molecular weight as compared to standard proteins, whereas the rhodopsin extracted from fixed ROS

membranes did not migrate and displayed a single dark band at the top of the gel. A possible reason for this observation may be that SDS, Ammonyx LO, glutaraldehyde mixture crosslinks to such a degree that the rhodopsin-detergent micelle is entrapped in the meshwork sufficiently free to display all possible modes of rotation and yet not free to enter the gel. That such a reaction occurs is evidenced by the observation that a mixture of the detergents SDS and Ammonyx LO with glutaraldehyde, at the same concentrations used with the rhodopsin sample, forms a product of dimensions large enough to scatter visible light. Upon standing for several hours a precipitate forms. Additionally, when the rhodopsin samples were bleached with white light, the characteristic red color disappeared only for rhodopsin extracted from unfixed ROS, whereas an orange color developed on bleaching the rhodopsin extracted from fixed ROS. This color remained even after exposure to the white light for 1 h. A similar visible band has been observed by Brown when ROS membranes, which have been fixed with glutaraldehyde, are bleached by white light (P. K. Brown, personal communication). Similarly, colored products of amines and glutaraldehyde have been discussed recently by Swenson et al. (1975). The above observations indicate that glutaraldehyde did react with the ROS membranes.

An additional experiment was conducted using ROS which were initially bleached with white light. No measure of the purity criteria was obtained since the absorption band at 500 nm disappeared. However, the absorbance at 280 nm indicated protein was present in the effluent, in the same region as the unbleached rhodopsin and at the same concentration. The observed relaxation time for bleached-fixed opsin was 42 ns. This value is greatly different than those for the unbleached case and may represent complete unfolding of the peptide chain of the opsin.

Since the above experiments yield results consistent with the proposed model for rhodopsin in ROS membranes and the effects of glutaraldehyde fixation, it is meaningful to consider biological consequences of the proposed model which may lead to additional experiments to test the model.

It is reasonable to suppose that translational motion compensates for localized bleaching of ROS membranes, as in the case of Poo and Cone (1974), and that the rotational mode corresponding to rotation of the long axis about the short axis compensates for bleaching with polarized light. This explains the visual acuity data with polarized light observed by De Vries (1948), who found no differences in visual responses to light polarized in two orthogonal planes when the eye had been adapted to light polarized in one of the planes. He concluded that the visual pigments were free in the photoreceptors. This motion might therefore correspond to biological adaptation in a world of scattered and therefore polarized light.

The most interesting consequence of this model is that associated with the rotational mode of rotation about the long axis. This motion would most likely be associated with facilitated transport across the ROS membrane and therefore responsible for initiation of the visual response. Since the early receptor potential (ERP) is a membrane potential, this model provides an explanation for the observations by Pak and Helmrich (1968) that glutaraldehyde fixation and freezing the retina produced no differences in the ERP for pulses of light parallel and perpendicular to each other. Although Pak and Helmrich were attempting to detect photodichroism, their results were negative, according to the above model, because the ERP is based on a

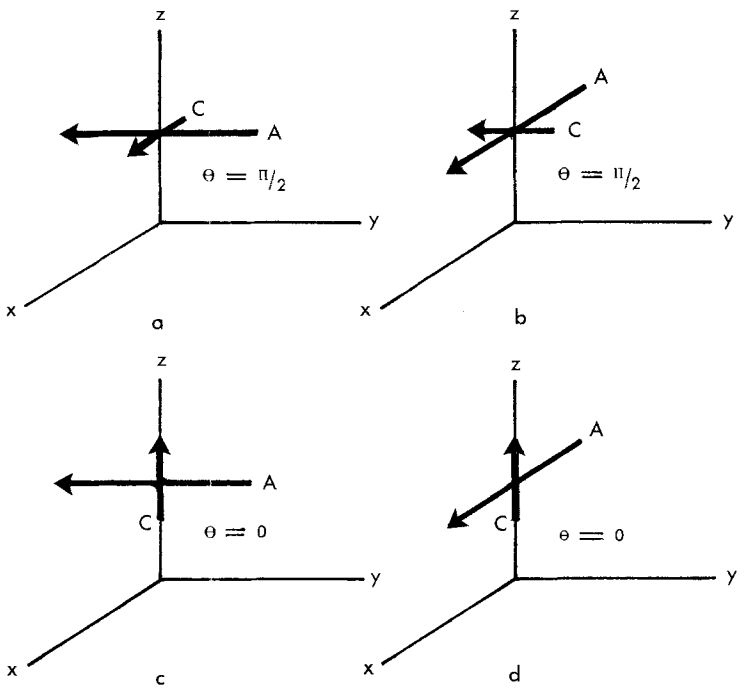

Fig. 1. See text

different rotational mode than that responsible for dichroism. Thus, their results are not inconsistent with those of Brown.

With respect to dichroism of unfixed rods, Liebman (1972, 1975) has reported data which are interpreted by him to rule out rotation of rhodopsin about the chromophore axis. His interpretation is based in part on the data of Sperling and Rafferty (1969) which show that the cis band transition is polarized at right angles to the α band transition in 11-cis retinal. Since two vectors define a plane, together with the observation by Liebman that both the cis band and α band are stronger in transversely polarized light, Liebman (1975) states that such rotation is absolutely ruled out. My inspection of Liebman's data (Fig. 11, 1972) leads me to believe that the dichroic ratio for the cis band transition at 350 nm may in fact be less than that for the α band transition at 500 nm. This would be in accordance with the model presented above, which is explained below in a qualitative argument.

Figure 1 shows four orientations of the α transition moment, denoted by A, and the cis transition moment, denoted by C, for the visual pigment. The coordinate axes are defined in a rod cell such that the z-axis is parallel to the rod axis, and the x-axis is the direction of propagation of plane polarized light. The angle θ denotes the colatitude of the cis transition moment with respect to the z-axis. (a) and (b) correspond to the case discussed by Liebman for two extreme rotations about the rod axis, and only (b) gives rise to transverse cis band absorption. (c) and (d) correspond to rotation about the chromophore axis of rhodopsin, again for two rotations about the rod axis, and both give rise to parallel cis band absorption.

If we now state that rotation about the chromophore axis, in the plane of the disk membrane, is not uniform, and if Probability ($\theta = \pi/2$) $\geq$ 1/2 Probability ($\theta = 0$), then under the equality the cis band would not be dichroic, and under the inequality the cis band would be dichroic with transition absorption greater than parallel absorption. Thus, such non-uniform rotation of rhodopsin about an axis in the plane of the disk membrane would not be inconsistent with the dichroism data of Liebman. Only qualitative statements may be made at present since available data are relatively imprecise. It should further be noted that orientation cannot be followed as above for rhodopsin activated by light, since the first step in the bleaching sequence is isomerization of 11-cis to all trans retinal.

Rotational Brownian motion of the pigment molecules around axes lying in the plane of the disk membrane has been suggested by Gribakin and Govardovskii (1975) as a possible reason that the dichroic ratio of rod disk membranes is experimentally observed to be about 4.5 as opposed to a theoretical value infinitely large. The non-uniform rotational model presented here would also explain this observed discrepancy between experimental data and theory.

A further objection to rotation of rhodopsin about the chromophore axis which needs to be considered is based on the fact that a hydrophilic hexaglycoside is attached to rhodopsin and is exposed to the interdisk space (Steinemann and Stryer, 1973). If such a group were bound to rhodopsin at either end of the rotational axis (parallel to the chromophore axis), then rhodopsin could rotate about the chromophore axis without dragging the hydrophilic moiety through the hydrophobic membrane lipid core of the disk. Free rotation about single bonds readily allows such motion.

The model presented here for rhodopsin in rod outer segment membranes of vertebrates is readily extended to photoreceptor cells of the rhabdomeric type. Goldsmith and Wehner (1976a, 1976b) have recently reported that there is no evidence of translational diffusion of visual pigment in microvilli of retinular cells of isolated crustacian rhabdoms. Also, they report preferential alignment of the chromophores with the axes of the microvilli. Fixation with glutaraldehyde only served to increase the already existing property of photo-induced dichroism. They interpreted their observations as evidence that the rhodopsin molecules are preferentially oriented and are normally free to wobble within some limiting angle with the microvillar axis.

Such restrictions on the orientation of the chromophores of visual pigment molecules represents adaptation enabling the animal to detect orientation of the plane of polarized light. It is well known that the plane of polarization to which cells are maximally sensitive correspond to the microvillar axes of the rhabdomeres (Goldsmith, 1973). The best known example has been presented by von Frisch, who demonstrated that the honey bee detects the polarization pattern of sky light for directional purposes (von Frisch, 1967). Finally, in further support of the model presented in this paper is the observation of Hagins and McGaughy (1967) that glutaraldehyde fixation leaves the fast photoelectric waveforms of squid retina essentially unchanged from those of fixed retinas, which is in agreement with the observations of Pak and Helmrich for vertebrate photoreceptors.

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