BRIEF COMMUNICATIONS

DECREASE IN MAGNETIC ANISOTROPY OF EXTERNAL SEGMENTS OF THE RETINAL RODS AFTER A TOTAL PHOTOLYSIS

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ABSTRACT The magnetic anisotropy of a polymembrane cell, such as the external segment of the frog retinal rod, is defined as the difference between the axial magnetic susceptibility (χ_a) and the radial magnetic susceptibility (χ_r), $\chi_a - \chi_r = \Delta\chi$ of the segment. After the total photolysis of the rhodopsin *in situ*, $\Delta\chi$ decreases significantly by 20%. This decrease in magnetic anisotropy should involve a subtle molecular disorder, mostly due to an alteration of the rhodopsin molecule.

In previous work we reported that the external segments of frog retinal rods may orient parallel to the direction of a constant and homogeneous magnetic field (Chalazonitis et al., 1970). A first explanation of this effect has been advanced by Hong et al. (1971): the orientation in the field is due to the difference in magnetic susceptibility $\Delta\chi$ between the axial (χ_a) and the radial (χ_r) principal volume susceptibilities. In addition $\Delta\chi = \chi_a - \chi_r$ may depend on oriented anisotropic molecules in the rod, such as rhodopsin.

From the general equation of motion proposed by Hong et al., neglecting the quadratic terms and integrating, we have:

$$\Delta\chi = \frac{f[\ln\tan\theta_0 - \ln\tan\theta_1]}{VH^2t} \quad (1)$$

where f is the rotatory frictional coefficient, θ the angle between the long axis of the rod and the direction of the magnetic field H, t the time required for the rod to swing from θ_0 to θ_1, and V the volume of the rod. The validity of relation 1 has been experimentally demonstrated by Chagneux and Chalazonitis (1972).

In further work, changes in $\Delta\chi$ have been evaluated after treatment of the external segment with chemicals such as CO_2 (Chalazonitis and Chagneux, 1972); lipophilic agents, which accumulate in phospholipids; and detergents, which attack lipoprotein binding or proteolytic enzymes (Chagneux and Chalazonitis, unpublished work). A decrease in $\Delta\chi$ has been found after application of the above chemical treatments. The origin of such a decrease was related to the disorder or partial disorganization in the molecular framework of the photomembranes effected by the above chemical treatments, and involved mainly rhodopsin.

In the present article, we report new data that reflect subtle modifications of the photomembrane structure after photolysis of the rhodopsin in initially dark-adapted rods.

In a first set of experiments we evaluated the change in measuring the maximum speed $V_m = (d\theta/dt)\,\max$) at $\theta = 45°$, during a strong illumination. The results obtained showed both a decrease and an increase in $\Delta\chi$. As a matter of fact, in this case, we reported that this photolysis did not exceed 40% (Chagneux and Chalazonitis, 1976).

The ambiguity of the results suggested the necessity of further experiments involving total photolysis of the rhodopsin *in situ*, in the segments, to accentuate a possible molecular disorder. The segments were isolated from retinas adapted in darkness for 3 h, and were suspended in a Ringer saline (at 22°C) supplemented with 15% metrizamide. This substance increases the density of the saline and allows the segments to float. The measurement of the viscosity of this solution (at 22°C) allows the evaluation of the rotatory frictional coefficient *f*, taking into account the volume of a given segment rotating in the field.

The disposition of the preparations was the same as described previously (Chagneux and Chalazonitis, 1976). The intensity of the magnetic field was 5 kg in all experiments. In this case, we measured the time elapsed during the rotation of the image of the rod (projected on a television screen) between $\theta_0 = 70°$ and $\theta_1 = 10°$. This rotation time was 30 s for selected segments 45 μm long and 9 μm in diameter. The measurement of the rotation time was made 60 s after bleaching.

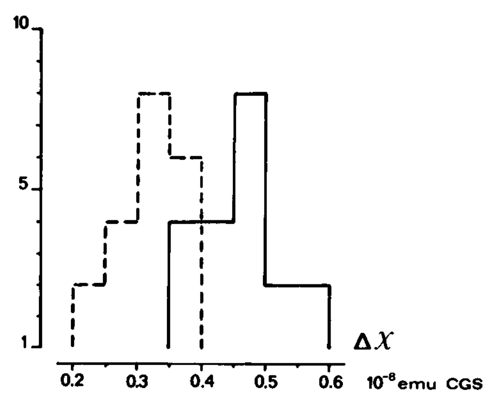

FIGURE 1 Histograms demonstrating the decreased magnetic anisotropy of the rod external segment after the total photolysis of the rhodopsin. Ordinate: numbers of segments. The $\Delta\chi$ values of segments are included in the intervals indicated along the abscissa. Continuous line indicates dark-adapted segments; Interrupted line, completely photolyzed segments.

Total photolysis of the rhodopsin was carried out in two ways: first, on isolated dark-adapted segments, and second, on a whole dark-adapted retina. The photolysis was effected with 600 lux (white light, devoid of infrared radiation) during 10 min of illumination. Difference absorption spectra obtained *in situ* before and after bleaching in the segment (microspectrophotometer, Chagneux and Chalazonitis, 1976) showed a maximum difference at about 500 nm (peak of the photolysed rhodopsin). We calaculated the magnetic anisotropy $\Delta\chi$ by Eq. 1. For several experiments, we compared the mean value of $\Delta\chi$ after photolysis ($\Delta\chi_m^l$) to the mean value of $\Delta\chi$ obtained in the dark ($\Delta\chi_m^d$), by the ratio $\Delta\chi_m^l/\Delta\chi_m^d$.

Irrespective of the kind of the photolysed preparation used (whole retina or isolated segments), the ratio $\Delta\chi_m^l/\Delta\chi_m^d$ was 0.80 (mean value of 40 cases) and the standard error 0.04. Therefore the decrease in magnetic anisotropy is the same, whatever the photolysed preparation.

The 20% decrease of $\Delta\chi$ after total photolysis of the rhodopsin may find a structural basis in the results described by Chabre and Cavaggioni (1975). They reported that the photolysis involves a decrease of the "interdisc" space, i.e. a shortening or shrinkage along the axis of the segment. Analogously, a change in conformational state of the membrane has been described by Ostrovsky (1972) and a protein conformation change by Liebman et al. (1974).

Whatever the molecular or ultrastructural alteration, we suggest as a possible conclusion that total photolysis involves a subtle molecular disorder, detectable by the measurement of the magnetic anisotropy. This molecular disorder is due to an alteration of the rhodopsin molecule.

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