Magnetic Anisotropy of the Visual Pigment Rhodopsin

ABSTRACT A new estimate of diamagnetic anisotropy of the frog rhodopsin is reported. The estimate is obtained by combining the data of magnetic field induced orientation of isolated frog rod outer segments as measured by Chagneux and Chalazonitis (1972) and the data of diamagnetic anisotropy of lecithin membranes as recently reported by Boroske and Helfrich (1978). The anisotropy of the volume susceptibilities of frog rhodopsin is calculated to be 4.4×10^{-8} cgs unit/cm^3, which corresponds to 1.5×10^{-27} cgs unit/molecule, or 9.0×10^{-4} cgs unit/mol.

Dear Sir:

In 1970, Chalazonitis and co-workers reported that isolated frog rod outer segments in aqueous suspension can be oriented with a homogeneous magnetic field of 10 kG. Hong et al. (1971) proposed that this orientation effect is due to macroscopic magnetic anisotropy of the rod. The predicted kinematic behavior of rod outer segments in a homogeneous magnetic field was subsequently verified experimentally by Chagneux and Chalazonitis (1972). Hong et al. (1971) further theorized that the major contribution to macroscopic magnetic anisotropy is from the visual pigment rhodopsin rather than from phospholipid in the membranes. This latter assertion is supported by data of Becker et al. (1978) and Chabre and Breton (1979). Still, the magnetic anisotropy of the rhodopsin molecules cannot be ascertained from data of magnetic field induced orientation of isolated rod outer segments, since the anisotropy value so obtained contains contribution from rhodopsin as well as from phospholipid molecules. If one neglects the contribution from phospholipid, the anisotropic susceptibility of rhodopsin can be estimated to be 1.2×10^{-8} cgs unit/cm^3 (Hong, 1977, 1979, using the data of Chagneux and Chalazonitis [1972]), or 7.6×10^{-4} cgs unit/mol (Chabre, 1978). Since phospholipid outnumbers rhodopsin in a retinal rod (there are $\sim 4 \times 10^9$ molecules of rhodopsin and 1×10^{13} molecules of phospholipid), the total anisotropy of rhodopsin may be partially cancelled by that of phospholipid, rendering the above values underestimated (Hong, 1979). A recent report of the diamagnetic anisotropy of phospholipid by Boroske and Helfrich (1978) makes it now possible to present a new estimation of the diamagnetic anisotropy of rhodopsin[1] which takes into account the contribution of phospholipid. Here, we shall use the kinematic data of Chagneux and Chalazonitis (1972) to evaluate the combined contribution of anisotropy from rhodopsin and phospholipid.

The time-course of angular orientation of rod outer segments reported by Chagneux and Chalazonitis (1972) is reproduced in Fig. 1 *a*. The corresponding time-course as predicted by a mechanism of diamagnetic anisotropy is given by (Eq. 15 in Hong, 1977)

$$\ln \tan \theta = \ln \tan \theta_0 - \frac{H^2 \sum_i V_i(\chi_{ai} - \chi_{ri})}{\zeta} t, \tag{1}$$

where θ and θ_0 are the acute angle between the rod axis and the direction of magnetic field at time t and 0, respectively, H is the magnetic field strength, ζ is the rotational frictional coefficient, χ_{ai} and χ_{ri} are the axial and radial principal volume susceptibilities, respectively, of the species i of oriented molecules, and

[1]The diamagnetic anisotropy of rhodopsin has been attributed to either (*a*) oriented α-helical segments in the peptide backbone (Chabre, 1978; Worcester, 1978), or (*b*) oriented aromatic amino acid residues in the protein (Becker et al., 1978). Recent results by Chabre and Breton (1979) suggest that the former is the major origin of diamagnetic anisotropy of rhodopsin, while the latter may be responsible for the light-induced change of diamagnetic anisotropy.

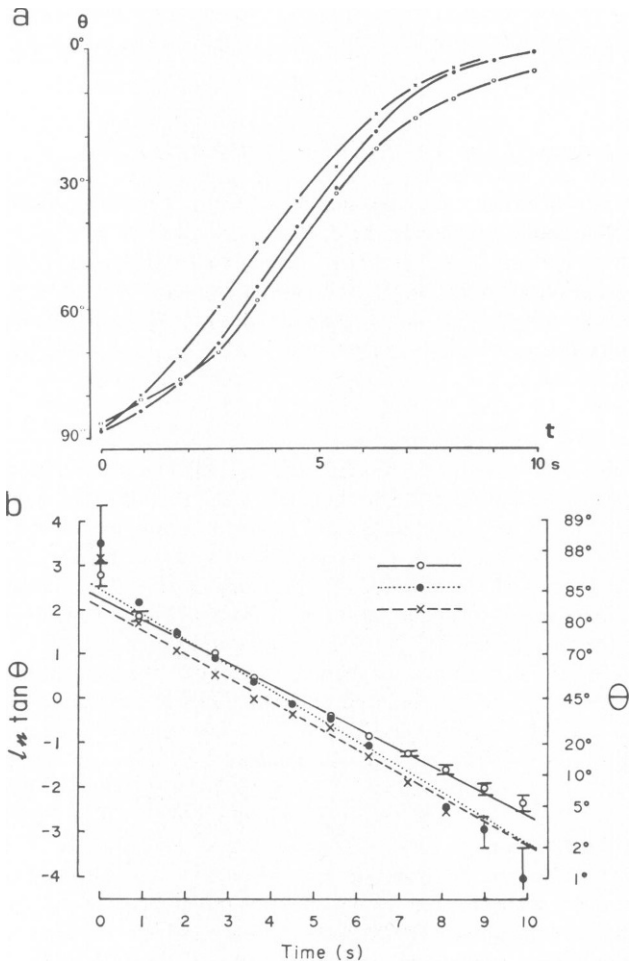

FIGURE 1 The angular position of three isolated frog rod outer segments of comparable size in Ringer's solution as a function of time under a homogeneous magnetic field of 10 kG. In *a*, the data are displayed as originally reported by Chagneux and Chalazonitis (1972), except that the ordinate has been relabeled to conform to our present definition of the angle θ, which is complementary to the angle defined in the report of Chagneux and Chalazonitis (1972). (Reproduced from Chagneux and Chalazonitis [1972]). In *b*, the data from *a* are replotted as ln tan θ vs. time. The angle θ is indicated on the right ordinate. The straight line is least-square fit on the original scale of *a* rather than on the transformed scale to avoid bias due to points at the two extreme positions, where the error is aggravated. The error bars correspond to an error of 1° in reading the angular position of the rods. Given $H = 10^4$ G and $\zeta = 3.1 \times 10^{-10}$ g-cm²/s (Chagneux and Chalazonitis, 1972), the slopes of the straight lines give values of summed anisotropy of 1.5×10^{-18} cgs unit (○), 1.8×10^{-18} cgs unit (●), and 1.7×10^{-18} cgs unit (x), respectively (average, 1.7×10^{-18} cgs unit). Reproduced from Hong (1977).

V_i is the corresponding volume. Here the axial direction refers to the axis of the rod as well as the axis of the majority of phospholipid molecules. The term $\Sigma_i V_i(\chi_{ai} - \chi_{ri})$, or simply $\Sigma_i V_i \Delta\chi_i$, is the summed anisotropy, which is the linear sum of anisotropy contributions from all oriented molecules. (Randomly oriented molecules do not contribute to a macroscopic anisotropy.) When data of Chagneux and Chalazonitis (1972) are replotted as ln tan θ vs. time (Fig. 1 *b*), least-square fit with a straight line gives

1.7×10^{-18} cgs unit as a value of summed anisotropy of a retinal rod, according to Eq. 1 (Hong, 1977). (Using a method of rotating magnetic field, Chabre [1978] obtained an average value of 2.4×10^{-18} cgs unit for rods 50 μm long.) Thus,

$$V_{rh}\Delta\chi_{rh} + V_{pl}\Delta\chi_{pl} = 1.7 \times 10^{-18} \text{ cgs unit}, \quad (2)$$

where the subscripts rh and pl refer to rhodopsin and phospholipid, respectively. The volume of phospholipid per rod (V_{pl}) is taken to be 12.7×10^{-10} cm^3, the value estimated by Chagneux and Chalazonitis (1972). The volume of rhodopsin per rod (V_{rh}) is estimated to be 1.2×10^{-10} cm^3 (corresponding to 3.5×10^9 molecules), based on the known concentration of rhodopsin (2.5 mM) (Liebman, 1962), the known size of a rhodopsin molecule (40–50 Å) (Blasie et al., 1969), and the reported dimensions of the rods (Nilsson, 1965). An alternative estimate, based on the size of unit cells of the square arrays of rhodopsin (Blasie et al., 1969) and the known surface area (Nilsson, 1965), gives approximately the same value (1.4×10^{-10} cm^3) for V_{rh}. Inserting the value of $\Delta\chi_{pl}$ (-2.8×10^{-9} cgs unit/cm^3) from Boroske and Helfrich's (1978) measurements, we obtain a new estimated value for the volume anisotropic susceptibility of rhodopsin:

$$\Delta\chi_{rh} = 4.4 \times 10^{-8} \text{ cgs unit/cm}^3. \quad (3)$$

This value corresponds to 1.5×10^{-27} cgs unit/molecule, or 9.0×10^{-4} cgs unit/mol. The sign of $\Delta\chi_{rh}$ is in agreement with the theoretical prediction of Hong et al. (1971). The magnitude of $\Delta\chi_{rh}$ is also within the estimated theoretical maximum of 7×10^{-8} cgs unit/cm^3, reported by Becker et al. (1978).

As for the accuracy of this new estimation, several possible sources of errors can be considered. For example, Eq. 1 assumes an idealized cylindrical symmetry (which neglects the contribution from the edge of disk membranes as well as the plasma membrane envelope) and assumes also a perfect orientation of phospholipid and rhodopsin molecules. Furthermore, the anisotropy of the phospholipid is taken as to be that of egg lecithin. However, the most serious source of errors arises from the estimated values of the rotational frictional coefficient (Chabre, 1978) and the effective volumes of phospholipid and rhodopsin. Nevertheless, it is to be pointed out that the estimate of the summed anisotropy by means of fitting the data to Eq. 1, being the average over the entire time course of orientation (Fig. 1), gives better accuracy than the estimate based on measured time intervals between the initial and endpoint orientation.

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