MAGNETIC ORIENTATION OF PURPLE MEMBRANES DEMONSTRATED BY OPTICAL MEASUREMENTS AND NEUTRON SCATTERING

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1. Introduction

The observed magnetic orientation of retinal rod outer segments [1], chloroplasts [2,3] and bacterial chromatophores [4] suggests that this magnetic property may be common to other biological membranes, perhaps even a general feature of membranes. This interesting phenomenon has already found considerable application in optical dichroism, X-ray diffraction and neutron diffraction studies of retinal rod outer segment membranes [5–8] and photosynthetic membranes [2–4, 9–13]. We report here investigations which demonstrate that the relatively planar pieces of purple membranes from *Halobacterium halobium* [14,15] also orient in magnetic fields. For membranes dispersed in water, orientation was first demonstrated by simple optical experiments with linearly polarized light. We observed both prominent birefringence and a degree of optical dichroism. The dichroism result, together with previous work which correlated optical dichroism and X-ray diffraction data from a specimen oriented by drying [15], strongly suggested that the orientation is with the planes of the membranes perpendicular to the magnetic field. Attempts using X-ray diffraction to confirm the sense of the orientation were unsuccessful. However, neutron scattering measurements were technically easier, and they confirmed that the membranes orient perpendicular to the magnetic field. The neutron scattering measurements also determined the degree of orientation, which was found to depend upon the magnetic field strength, the size of the membrane fragments and their state of aggregation. We also found that the degree of orientation has a striking concentration-dependence in the region of 10 mg/ml, which is probably due to a cooperative phenomenon resulting from the membranes' planar dimensions being comparable to the distances between membranes at this concentration. The magnetic orientation apparently results from the diamagnetic anisotropy of the alpha-helices in purple membranes.

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2. Materials, methods and results

2.1. *Optical experiments*

The optical experiments utilized an electromagnet capable of generating a maximum field of over 20 000 G and optical components which were mounted on optical benches at right angles to the magnetic field, one on each side of the magnet. The source of light was a helium–neon laser, $\lambda = 633$ nm. This wavelength

is absorbed fairly strongly by the membranes (λ_{max} = 570 nm). As birefringence is characteristic of the oriented membranes [15] and is a sensitive technique, this was investigated first. A beam of light with the plane of the *E*-field well polarized at 45° to the magnetic field, was provided by a Nicol prism. A second Nicol prism was placed after the specimen, the specimen being in the gap of the magnet. The second prism was rotated so as to minimize the light transmitted when the magnetic field was off. In a fully darkened room, only traces of light were seen on a white screen placed behind the prism. Observations of light intensities were made only by eye.

Purple membranes were prepared according to the procedure of Oesterhelt and Stoeckenius [14] from cultures of *Halobacterium halobium.* The specimen was an aqueous suspension of the membranes in a 1 cm quartz spectrophotometer cell. The concentration was about 5 mg/ml. Appreciable transmitted light was first observed at a field strength of 9000–10 000 G and the intensity increased up to 20 000 G. When the magnet was turned off, the light died away as the field dropped. No lag of the light intensity due to the relaxation time of the membranes was detected, presumably because the magnetic field dropped rather slowly. The observed birefringence indicates that the membranes orient in a strong magnetic field, and they disorient due to Brownian motion when the field is turned off.

An experiment to determine the sense of the orientation by optical dichroism was made (see Discussion). However, the results were not satisfactory and we then tried an X-ray diffraction experiment and finally a neutron scattering experiment, which gave a definitive result (see below).

2.2. *X-ray diffraction*

The optical dichroism observations were difficult to make, and so we attempted to show the sense of the orientation by other means. A smaller electromagnet was used for an X-ray diffraction experiment. Special pole-pieces were needed in order to divert the field slightly eccentric to the normal axis, for fitting on a Franks low-angle diffraction camera [16]. A maximum field of 13 000 G was obtained in this way. A rather concentrated suspension was used (20 mg/ml), but only the strongest in-plane reflections could be seen on the X-ray film after several hours exposure. The sharp rings showed no orientation. Several exposures were made, but all of these failed to detect any orientation. Subsequent neutron scattering measurements suggest that the membranes in the X-ray specimen failed to orient because the suspension was too concentrated.

2.3. *Neutron scattering*

The neutron scattering measurements were made at the Institut Laue-Langevin, Grenoble, on the small-angle scattering instrument D-11 [17]. The main reason for trying neutron scattering is that the contrast between the scattering density of the membrane and that of the dispersing medium is very high when D_2O is used as the dispersing medium [18,19]. Consequently the lamellar scattering intensity is very high and is readily recorded even from very dilute samples. There is also the advantage that the specimen can be exposed in a 5 mm or 10 mm thick spectrophotometer cell, in contrast to a 1 mm thick capillary for the X-ray specimen.

Scattering patterns were obtained from several different specimens of purple membranes dispersed in D_2O. An electromagnet developed for use on the neutron scattering instrument by G. Goeltz provided a magnetic field of up to 17 000 G. Figure 1 shows contour maps of scattering data recorded on the 64 × 64 cm^2 position sensitive detector. In fig.1a the magnetic field is off and the contour map shows unoriented small-angle lamellar scattering (i.e., contours with a diameter larger than the beam stop are concentric circles). The initial specimen, at a concentration of 24 mg/ml, showed no orientation when the field was turned on to 17 000 G (contour map not shown). Gentle stirring of this dispersion while the magnetic field was on did not induce orientation. When the specimen was diluted to 12 mg/ml, the scattering pattern in fig.1b was immediately obtained without stirring. The magnetic field direction is horizontal, and the contours have become elongated in this direction. The intense lamellar scattering along this direction [7,8] therefore demonstrates beyond any doubt that the membranes orient perpendicular to the magnetic field.

If it is assumed that the low-angle lamellar scattering is much more intense than the low-angle in-plane intensity, then the degree of orientation of the specimen can be obtained from the intensities

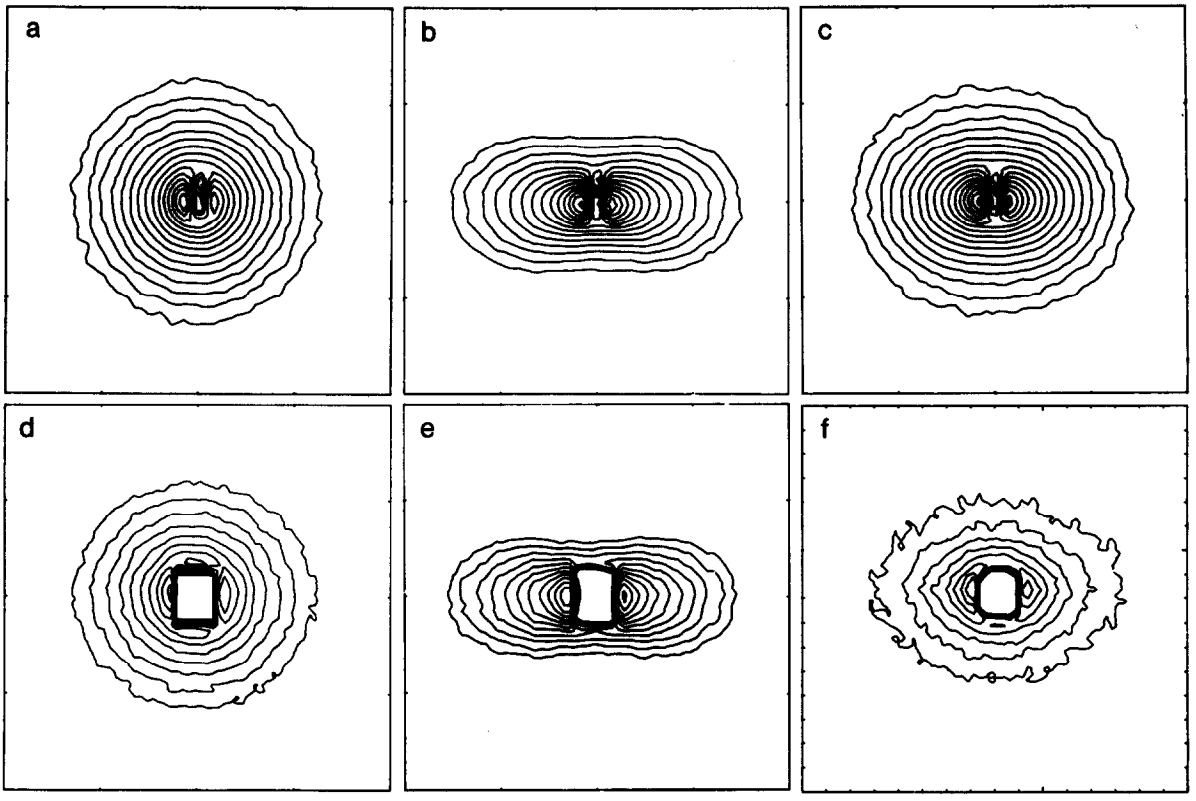

Fig.1. Small-angle neutron scattering data from dispersions of purple membranes in D_2O are shown in these contour maps of data collected with the 64 × 64 cm^2 area detector at the Institut Laue-Langevin, Grenoble. The magnetic field of 17 000 G is horizontal, the wavelength is 10 A and the sample-detector distance, 236 cm. A dispersion of 12 mg/ml gives the scattering in (a) with the field off and (b) with the field on. Dilutions to 6 mg/ml and 2 mg/ml are shown in (c) and (d). Aggregated membranes and reconstituted membranes give the scattering in (e) and (f), respectively. The contours increment by a factor of $\sqrt{2}$ from lowest contours of 100–360 counts/cm^2.

parallel and perpendicular to the field at a fixed scattering angle (fixed radius from the centre of the contour map). For these experiments, the degree of orientation is best defined as the difference of intensities divided by the parallel intensity:

$$R = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel}}$$

The orientation so defined is nearly 90% for the 12 mg/ml sample (fig.1b). For this high orientation, the angular width of the lamellar diffraction also describes the orientation, but is already large for a 90% orientation ratio; 60° full-width at half maximum in fig.1b. The orientation drops to about 40% when the sample is diluted to 6 mg/ml (fig.1c). Other samples at 4 mg/ml and 2 mg/ml (fig.1d) both showed only a small orientation of about 10%. The marked concentration-dependence of the orientation at about 10 mg/ml is probably due to the fact that the membrane diameters (0.5 μm) are comparable to the mean lamellar separation of membranes for concentrations of about 10 mg/ml. Thus the membranes respond cooperatively when the field is turned on, with the orienting motions of each membrane assisting the orientation of its neighbors. This cooperative behavior in the magnetic field is probably closely related to the opalescence of very concentrated membrane dispersions, which is attributed to local cooperative movements of many membranes in the dispersion,

as in 'Swarm Theories' of the effect of magnetic fields on nematic liquid crystals [20]. The membranes were found to disorient only slowly from the high orientation when the magnetic field was turned off (half-life of several minutes). We attribute the lack of orientation in the sample of 24 mg/ml to the membranes not being able to rotate into alignment due to their high concentration. Specimens at this concentration were in the magnetic field for only 10–15 min however, and we cannot rule out the possibility of orientation developing over longer periods of time, or with higher magnetic fields.

High degrees of orientation were also obtained when the membrane fragments were not monodisperse. Neutron scattering from a purple membrane sample aggregated by freezing and thawing an aqueous suspension (2 mg/ml) is shown in fig.1e. Membrane aggregates which were prepared by drying on a glass substrate and then redispersing in water also show this high degree of orientation. For larger aggregates, the best results were obtained if the D_2O density was increased by adding salt (NaCl) in order to prevent rapid sedimentation. Aggregated dispersions were also produced by gently dispersing a centrifuged pellet. This did not give such high orientation however, as samples aggregated by freezing or drying.

The higher orientation obtained for aggregates of membranes compared to monodisperse native membranes demonstrates that the orientation depends on the overall size of the membrane structures. This was also demonstrated by a sample of reconstituted purple membrane (kindly provided by R. Henderson) which consists of sheets about ten times the diameter of the native membranes. The reconstituted membranes are prepared by dissolving native membranes with detergent (Triton X-100) and reassembling by dialyzing out the detergent [27]. For these larger sheets, the orientation of a 1.4 mg/ml dispersion is about 50% (fig.1f) compared to 10% for the smaller native membranes.

3. Discussion

A potentially useful application of the magnetic orientation of purple membranes is for linear dichroism experiments. Using the optical components assembled for the birefringence experiments, we were able to make a qualitative observation of the linear dichroism at 633 nm, which is within the absorption band of the purple membrane chromophore. For this dichroism experiment, the second Nicol prism was placed between the first prism and the specimen, and was rotated to set the plane of polarization either parallel or perpendicular to the magnetic field. The intensity of the light transmitted through the specimen was visually compared by rotating rapidly (roughly one second) between the two positions, and appeared to be about twice as bright in the parallel orientation as in the perpendicular orientation, for two independent observers. From the known dichroism of the chromophore absorption band of purple membranes [15], this result demonstrates that the orientation is with the planes of the membranes perpendicular to the magnetic field, in agreement with the neutron scattering result.

The origin of the magnetic orientation of purple membranes is of some interest. The magnetic orientation of retinal rods has been attributed to the diamagnetic anisotropy of the oriented molecules in the disc membranes of the rods [21]. It was also shown that reasonable inhomogeneity of the magnetic field has too small an effect, due to the shape of a magnetically isotropic membrane, to produce orientation [21]. These calculations were extended and applied to purple membranes [22] using the theory of Peterlin and Stuart [23] for the behavior of a small magnetically isotropic particle of anisotropic shape in homogeneous magnetic or electric fields. In order to evaluate the elliptical integrals in the theory, the shape of the membrane was approximated by an oblate ellipsoid of 0.5 μm major axis and 50 Å minor axis. The calculations showed that the effect due to this form anisotropy is several orders of magnitude too small to account for the observed orientation of purple membranes. We conclude therefore, that anisotropy of the diamagnetic susceptibility is responsible for the orientation.

Diamagnetic anisotropy in purple membranes would result from the oriented molecules of lipid and bacteriorhodopsin [15,16,24]. If the lipids are in a bilayer arrangement and their diamagnetic anisotropy is approximated by that of stearic acid [25], they would tend to orient the membranes parallel to the field [21,30] contrary to the observed orientation. Therefore the contribution from the bacteriorhodopsin must be larger than that of the lipids, and of the

correct sense to produce the observed orientation. Diamagnetic anisotropy in bacteriorhodopsin could result from oriented aromatic groups of the peptide side chains, since aromatic molecules have large diamagnetic anisotropy [26]. The correct orientation of purple membranes would result if there is net orientation of aromatic groups with their planes tending to be perpendicular to the membrane plane. Diamagnetic anisotropy of bacteriorhodopsin also could result from the alpha-helices which are oriented perpendicular to the membrane sheets [16,24]. Other alpha-helical polypeptides (poly-L-glutamic acid, poly-L-lysine hydrobromide) are known to orient with the helix-axis parallel to the magnetic field [28,29] so the alpha-helices in purple membranes have the correct orientation to produce the magnetic orientation. Linear dichroism measurements on dispersions of magnetically oriented purple membranes in the region of tryptophan absorption (280 nm) show that these aromatic groups have net orientation parallel to the membrane sheets (J. Breton and D. L. Worcester, unpublished results) and therefore have the wrong orientation for magnetically orienting the membranes. It is most probable therefore that the alpha-helices of bacteriorhodopsin are responsible for the magnetic orientation of the membranes. In a separate paper (D. L. Worcester, in preparation), the molecular origins of diamagnetic anisotropy in alpha-helices will be clarified.

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