MAGNETIC ANISOTROPY OF LECITHIN MEMBRANES

A New Anisotropy Susceptometer

FRIEDHELM SCHOLZ, EDWIN BOROSKE, AND WOLFGANG HELFRICH
Fachbereich Physik, Freie Universität Berlin, D-1000 Berlin 33, Federal Republic of Germany

ABSTRACT Cylindrical giant vesicles prepared from egg lecithin and 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) are oriented in an external magnetic field and observed by phase contrast microscopy. The anisotropic part of the diamagnetic susceptibility of the lecithin membrane is determined from the distribution of angles between the magnetic field and the long cylinder axis due to thermal fluctuations. The anisotropy of DMPC is found to be larger by a factor of 2 than that of egg lecithin. This is attributed to the presence of unsaturated acyl chains in egg lecithin.

INTRODUCTION

Magnetic field alignment of biological membranes prepared as cell fragments (1) and of artifical vesicles (2) in aqueous solutions has been used to determine the magnetic anisotropy of membrane constituents such as phospholipids and various proteins. A necessary condition for orientation in a homogeneous magnetic field is a sufficient anisotropy of membrane polarizability. Chagneux et al. (1) determined the magnetic anisotropy of retinal rods, using the method introduced by Hong et al. (3, 4). They measured the variation with time of the angle between the rod axis and the field direction. The frictional constant was obtained from independent measurements of the viscosity of the solution and from the geometry of the rods. A similar procedure was applied by Kawamura et al. (5) who determined the anisotropy of microcrystals of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and polyethylene grown and suspended in xylene. However, calculating rotational friction from the membrane shape seems somewhat questionable as exact solutions are available only for spheres and ellipsoids.

Studying cylindrical egg lecithin vesicles that align parallel to the magnetic field, Boroske and Helfrich (2) avoided this drawback by determining the rotational diffusion constant from the Brownian motion of the vesicle. The velocity of rotation in a magnetic field was also measured, and the magnetic anisotropy was derived from the experimental data by means of an Einstein relation. The method appears to be accurate, but measuring dynamical quantities is rather cumbersome.

Therefore, it seems attractive to determine the magnetic anisotropy from purely static experiments. In the present paper we use a novel method, studying cylindrical vesicles of egg lecithin (EL) and 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC). We simply measure a large number of uncorrelated angles between the projection of the cylinder axis on the plane of observation and the field direction. The magnetic anisotropy of the cylinder is determined from the mean square fluctuation angle; it serves to calculate that of the membrane.

The magnetic anisotropy obtained for EL is comparable with the earlier result (2). The value for DMPC, determined for the first time in the present work, is about twice as large. This is qualitatively discussed on the basis of molecular and membrane structure. Furthermore, we show that oxygen molecules dissolved in the membranes have little or no influence on their magnetic anisotropy.

MATERIALS AND METHODS

EL was purchased from Merck (Darmstadt FRG), DMPC from Serva (Heidelberg, FRG), and both materials were used without further purification. Cylindrical vesicles, whose lengths and diameters were ~30 and ~10 μm, respectively, were prepared by spontaneous swelling (6, 7). For the magnetic alignment we selected thin-walled (i.e., uni- or bilamellar) cylinders by means of the optical method of Servuss and Boroske (8).

The sample chamber consisted of two parallel glass slides 8 mm in diameter, held ~0.1 mm apart by a rubber ring, and a tightly screwed brass holder. It rested in a water-thermostated brass plate brought between the pole caps of the magnet. The plate could be moved along all directions and the chamber could be rotated about an axis perpendicular to its faces and to the field. Homogeneous magnetic fields up to $H = 19$ kG were applied parallel to the slides. The vesicles were observed with a phase contrast microscope extended by a Grundig video recording system. The lengths and angles were measured on the screen of the monitor with a drawing apparatus having the necessary scales (Fig. 1). The experiments were done in a temperature range from 23° to 26°C so that EL and DMPC were in the fluid L_α phase.

THEORY

The diamagnetic polarizability of lecithin molecules is anisotropic. In a fluid bilayer the molecules are aligned in a certain way, the hydrocarbon chains being preferentially parallel to the layer normal. The resulting anisotropy of the membrane susceptibility per volume is defined as

$$\chi_a = \chi_\parallel - \chi_\perp \qquad (1)$$

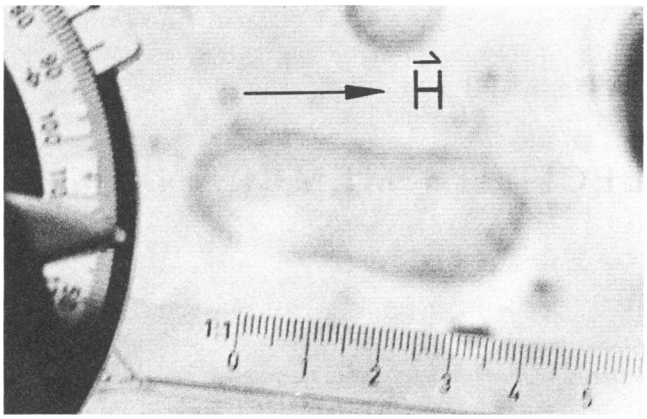

FIGURE 1 Television monitor displaying cylindrical vesicle. Also seen is the drawing apparatus with measuring scales. The angle between long cylinder axis and magnetic field is nearly five degrees. One centimeter on the scale corresponds to 6.7 μm of the object.

$\chi_\parallel$ and $\chi_\perp$ being the susceptibilities parallel and perpendicular, respectively, to the layer normal. As a consequence, cylindrical vesicles in water are aligned in a homogeneous magnetic field with the cylinder axis parallel to the magnetic field if $\chi_a < 0$ and perpendicular if $\chi_a > 0$. The magnetic part of the free energy per unit volume of membrane material is

$$F = -\frac{1}{2}\chi_\perp H^2 - \frac{1}{2}\chi_a (\mathbf{n} \cdot \mathbf{H})^2 \tag{2}$$

where $\mathbf{n}$ is the layer normal and $\mathbf{H}$ is the magnetic field. Only the second term, which depends on the orientation of the cylinder, is of interest here. Integrating over the volume of the cylinder shell we obtain the orientation-dependent part of the total magnetic energy. It is for a unilamellar vesicle

$$E(\theta) = \frac{1}{2}\pi l r b \chi_a H^2 \sin^2\theta. \tag{3}$$

Here l is the length and r the radius of the cylinder, b the membrane thickness, and θ the angle between cylinder axis and magnetic field. The two hemispheres capping the cylinder do not contribute to E. Cylinders of a fairly small length-to-diameter ratio (~3) were selected to minimize bending fluctuations (6). The actual shape of the vesicles deviated slightly from the ideal assumed here; this leads to a small systematic error. Because of thermal motion the long axis of the cylinder will fluctuate around the direction of the magnetic field, provided $\chi_a < 0$, as in our case. The energy states are occupied according to a Boltzmann distribution depending only on the polar angle θ but not on the azimuthal angle φ:

$$w(\theta, \varphi) \sin\theta \, d\varphi d\theta \sim e^{-a\sin^2\theta} \sin\theta \, d\varphi d\theta \tag{4}$$

with

$$a = \frac{\pi l r b \chi_a H^2}{2kT}. \tag{5}$$

Under the microscope we see only the fluctuations of angle ψ within the plane of observation, while the out-of-plane fluctuations of angle ζ are hardly detectable. Splitting the field into components parallel and perpendicular to the in-plane direction denoted by ψ, we have the distribution function

$$f(\psi) \, d\psi \sim e^{-a\sin^2\psi} \left(\int_{-\pi/2}^{\pi/2} e^{-a\cos^2\psi \sin^2\zeta} \cos\zeta d\zeta \right) d\psi. \tag{6}$$

For weak fluctuations ($|\psi| < 10°$) the limits of integration over $\sin\zeta$ can be shifted from ∓ 1 to $\mp\infty$. Integration then leads to the relation

$$f(\psi) \, d\psi \sim \frac{1}{\cos\psi} e^{-a\sin^2\psi} \, d\psi. \tag{7}$$

In all our experiments the magnitude of ψ rarely exceeded 10°. Therefore, Eq. 7 could be replaced by the simpler form

$$f(\psi) \, d\psi \sim e^{-a\psi^2} \, d\psi \tag{8}$$

within the accuracy of the measurements. The second moment of this Gaussian distribution is

$$\langle \psi^2 \rangle = 1/2a. \tag{9}$$

Insertion in Eq. 5 yields for the polarizability per unit area of the membrane

$$b\chi_a = \frac{kT}{\pi l r \langle \psi^2 \rangle H^2}. \tag{10}$$

The error introduced by using Eq. 8 rather than Eq. 7 was found to be no more than 2%.

RESULTS AND DISCUSSION

Sufficiently well-defined Gaussian distributions were obtained by taking a large number of values of ψ. To avoid correlation between the measured angles, different time intervals between the successive measurements were tested. 1 min turned out to be appropriate. After each 10 values of ψ the distribution was calculated. The variation of the second moment became very small after 50 measurements. Increasing the number of angles from 50 to 100 changed $\langle \psi^2 \rangle$ by <1%. The data were then taken to calculate $b\chi_a$ (Fig. 2). The anisotropies given in Table I are the averages of measurements on 8 and 12 thin-walled cylinders prepared from EL and DMPC, respectively. The error given in the last column refers to a single measurement and takes into account the uncertainties of the single measurements of l, r, $\langle \psi^2 \rangle$, and H, as well as the deviation from the ideal vesicle shape and the use of the simplified

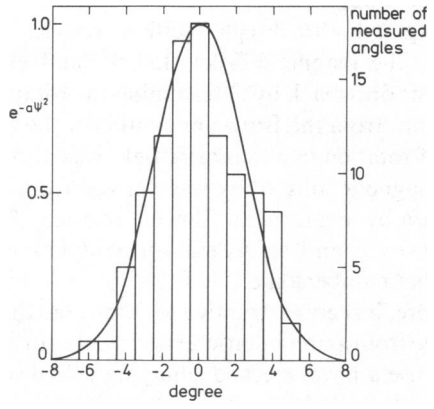

FIGURE 2 Typical distribution of measured angles as obtained by assigning them to one-degree compartments. The smooth curve is the calculated Gaussian distribution.

TABLE I
MAGNETIC POLARIZABILITY PER UNIT AREA OF BILAYER

Material	Total number of cylinders	Average value $b\chi_a$/cm	Error $b\chi_a$/cm
EL	8	$-0.28 \cdot 10^{-14}$	$0.04 \cdot 10^{-14}$
DMPC	12	$-0.58 \cdot 10^{-14}$	$0.06 \cdot 10^{-14}$

distribution (8). The root-mean-square deviations of the single values $b\chi_a$ from the averages are very similar.

The anisotropy of EL measured in this work is by a factor of 1.7 larger than the value $b\chi_a = -0.17 \cdot 10^{-14}$ cm reported by Boroske and Helfrich (2). Although the disagreement is considerable, we think that it may be caused by a difference in composition of the egg lecithins used. The content of ethanolamine, as checked by thin layer chromatography (R. M. Servuss, personal communication) and the concentration of (singly) unsaturated acyl chains (9) appear to vary substantially in commercial egg lecithins.

To understand why the magnetic anisotropy of DMPC is stronger than that of EL, let us briefly discuss the role of a double bond in an otherwise unsaturated chain. A saturated acyl chain has the lowest free energy in a magnetic field when it is oriented perpendicular to it. In a double bond, the two electrons are delocalized, forming orbitals comparable with the delocalization orbitals in an aromatic ring that has the lowest free energy in a magnetic field when oriented parallel to it. Seelig and Waespe-Šarčevič (10) concluded from NMR measurements on 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine dispersions at $T = 27°C$ that the orientation of the double bond is roughly parallel to the bilayer normal with small deviations up to ~9°. Therefore, the double bond is expected to weaken the susceptibility anisotropy of the lecithin membrane.

The value of the anisotropy of DMPC, $b\chi_a = -0.58 \cdot 10^{-14}$ cm, is by a factor of two larger than that of EL found in this work. Although it is not possible to compare directly our results to those of Kawamura et al. (5), their values may be regarded as consistent with ours. They found $\chi_a = -9 \cdot 10^{-8}$ for DPPC crystals at $T = 22°C$, which gives an anisotropy of the polarizability per unit area $b\chi_a = -4.5 \cdot 10^{-14}$ cm on the assumption of a membrane thickness of 50 Å. The lower value which we find for DMPC membranes seems mainly caused by the lower molecular order in the L_α phase.

The effect of dissolved oxygen in the DMPC membrane was checked with samples prepared and studied under nitrogen. Oxygen is paramagnetic and could, therefore, lead to a decrease in diamagnetism of the membrane. Its influence may be estimated from the solubility in water and in the lecithin membrane. The concentration of oxygen in air-saturated water at $T = 25°C$ is $c = 0.26$ mmol per liter (11) or $N = 1.57 \cdot 10^{17}$ molecules per cubic centimeter. Peters and Kimmich (12) determined the concentration of oxygen in lipid bilayers in aqueous dispersions by NMR to be three times the concentration in water, i.e., $N = 4.7 \cdot 10^{17}$ molecules per cubic centimeter. The isotropic paramagnetic susceptibility contribution of the O_2-molecules can be deduced from the well-known Curie law (13)

$$\chi_{O_2} = \frac{N\mu^2}{3kT}. \tag{11}$$

N is the number of molecules per cubic centimeter, $\mu^2 = g^2J(J+1)\mu_B^2 = 6.88 \cdot 10^{-40}$ (erg/G)^2, the square of the magnetic moment of the O_2-molecule ($g = 2$, the electronic g factor; $J = 1$ the total angular momentum; $\mu_B = 0.92731 \cdot 10^{-20}$ erg/G, the Bohr magneton). With a membrane thickness of $b = 50$ Å we get $b\chi_{O_2} = 0.13 \cdot 10^{-14}$ cm. To estimate an upper limit of the effective paramagnetic anisotropy contribution we assume that the paramagnetic anisotropy of the susceptibility equals the susceptibility itself and that the order parameter of the oxygen molecule imbedded in the membrane is the same as found for the CH_2-segments of the acyl chain by Seelig and Seelig (14), i.e., $S_{mol} = 0.3$. With these assumptions the effective paramagnetic anisotropy is estimated to be $b\chi_{O_2,eff} = S_{mol} \cdot b\chi_{O_2} = 0.04 \cdot 10^{-14}$ cm. This is an upper limit and the actual paramagnetic contribution due to dissolved oxygen is probably much smaller, thus lying far below the experimental error. In fact, in our measurements we could not find any differences between samples under nitrogen and those in air.

CONCLUSION

It was shown that the measurement of the angular distribution of cylindrical vesicles fluctuating with their long axes around the direction of a homogeneous magnetic field is a convenient method to determine the magnetic anisotropy of lipid membranes. The method may easily be applied to other nonspherical artificial or biological vesicular structures. It is quite sensitive and may thus be used to determine the anisotropy of polarizability of proteins once the anisotropy of susceptibility of the membrane without them is known (15).

Received for publication 4 February 1983 and in final form 8 September 1983

REFERENCES

1. Chagneux, R., H. Chagneux, and N. Chalazonitis. 1977. Decrease in magnetic anisotropy of external segments of the retinal rods after total photolysis. *Biophys. J.* 18:125–127.
2. Boroske, E., and W. Helfrich. 1978. Magnetic anisotropy of egg lecithin membranes. *Biophys. J.* 24:863–868.
3. Hong, F. T., D. Mauzerall, and A. Mauro. 1971. Magnetic anisotropy and the orientation of retinal rods in a homogeneous magnetic field. *Proc. Natl. Acad. Sci. USA.* 68:1283–1285.
4. Hong, F. T. 1977. Photoelectric and magneto-orientation effects in pigmented biological membranes. *J. Colloid Interface Sci.* 58:471–497.

5. Kawamura, Y., I. Sakurai, A. Ikegami, and S. Iwanayagi. 1981. Magneto-Orientation of phospholipids. *Mol. Cryst. Liq. Cryst.* 67:77–88.
6. Servuss, R. M., W. Harbich, and W. Helfrich. 1976. Measurement of the curvature-elastic modulus of egg lecithin bilayers. *Biochim. Biophys. Acta.* 436:900–903.
7. Harbich, W., R. M. Servuss, and W. Helfrich. 1976. Optical studies of lecithin membrane melting. *Phys. Lett. A.* 57:294–296.
8. Servuss, R. M., and E. Boroske. 1980. Dependence of the optical contrast of vesicle walls on lamellarity and curvature. *Chem. Phys. Lipids.* 27:57–69.
9. Tattrie, N. H., J. R. Bennett, and R. Cyr. 1968. Maximum and minimum values for lecithin classes from various biological sources. *Can. J. Biochem.* 46:819–824.
10. Seelig, J., and N. Waespe-Šarčevič. 1978. Molecular order in *cis* and *trans* unsaturated phospholipid bilayers. *Biochemistry.* 17:3310–3315.
11. Adam, G., P. Läuger, and G. Stark. 1977. Physikalische Chemie und Biophysik. Springer-Verlag, Berlin. 99.
12. Peters, A., and R. Kimmich. 1978. The heterogeneous solubility of oxygen in aqueous lecithin dispersions and its relation to chain mobility. *Biophys. Struct. Mech.* 4:67–85.
13. Ashcroft, N. W., and N. D. Nermin. 1976. Solid State Physics. Holt, Rinehart & Winston, New York. 656.
14. Seelig, A., and J. Seelig. 1977. Effect of a single *cis* double bond on the structure of a phospholipid bilayer. *Biochemistry.* 16:45–50.
15. Hong, F. T. 1980. Magnetic anisotropy of the visual pigment rhodopsin. *Biophys. J.* 29:343–346.