BRIEF COMMUNICATION

MAGNETIC ANISOTROPY OF EGG LECITHIN MEMBRANES

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ABSTRACT Magnetic realignment and rotational diffusion of cylindrical egg lecithin vesicles were measured under a phase contrast microscope. The anisotropy of magnetic susceptibility times membrane thickness was calculated from the data for several thin-walled vesicles. The resulting values were assigned to discrete numbers of bilayers. The difference between the susceptibilities parallel and perpendicular to the long axes of the lecithin molecules is deduced to be $\chi_{\parallel} - \chi_{\perp} = -(0.28 \pm 0.02) \cdot 10^{-8}$ cgs at 23°C, if a bilayer thickness of 60 Å is assumed.

INTRODUCTION

Lecithin vesicles of some microns in size may be regarded as the simplest possible model system for biological cells, lecithins being essential ingredients of biological membranes. Various procedures have been reported to produce such vesicles (1–5). Reeves and Dowben (3) proposed a method where vesicles are prepared by evaporating the organic solvent, exposing the resultant lecithin film to a water-saturated nitrogen atmosphere, and finally covering it with water. Servuss et al. (4) and Harbich et al. (5) discovered that very large vesicles of up to 30 μm and only few bilayers in the membrane are formed by spontaneous swelling when a small quantity of lecithin is brought into water. Vesicles of egg lecithin as well as dimyristoyl, dipalmitoyl, and distearoyl lecithin could be obtained by this method. A number of membrane properties were studied under a phase contrast microscope. The curvature elasticity modulus of egg lecithin was determined from the bend angle distribution of long tubular vesicles (4). A dependence of the main transition temperature of dimyristoyl, dipalmitoyl, and distearoyl lecithin on the membrane thickness was also found (5). Furthermore, contours of rotationally symmetric vesicles were photographed and theoretical shapes adjusted to them (6). The good agreement obtained, with spontaneous curvature as the only adjustable parameter, confirmed earlier theoretical work on vesicle shapes (7) and fluid membrane elasticity (8).

It is the purpose of this work to determine the anisotropic part of the magnetic

polarizability of lecithin bilayers by studying field-induced alignment and rotational diffusion of nonspherical vesicles. To simplify evaluation, all experiments were performed on cylindrical vesicles.

EXPERIMENTAL

Egg lecithin was purchased from Merck (AG Merck, Darmstadt, W. Germany) and used without further purification. Large vesicles were prepared by spontaneous swelling (4, 5). The sample cells consisted of two parallel slides 15-mm wide and about 100 μm apart. To avoid water evaporation, the cells were sealed with an insoluble adhesive that had no effect on the swelling process. The vesicles thus prepared were stable for weeks.

To measure the field-induced alignment of cylindrical vesicles, a homogeneous field of 15 kG was applied parallel to the slides. Simultaneously, the sample was observed under a phase contrast microscope, the optical axis being normal to the slides. The vesicle movements, translational and rotational, could be observed by eye or photographed with a time-controlled motor-driven camera. Frequencies from 1 picture/2 min up to 5 pictures/s were possible. The cell could be moved parallel and perpendicular to the magnetic field and rotated around the microscope axis so that the initial angle of orientation made with the field was variable.

THEORY

The origin of magnetic alignment of nonspherical vesicles is the interaction of the magnetically anisotropic bilayer with the magnetic field. The total torque $\boldsymbol{M}$ exerted by the field $\boldsymbol{H}$ on a vesicle is given by the integral

$$\boldsymbol{M} = \delta(\chi_{\parallel} - \chi_{\perp}) \int \mathrm{d}F(\boldsymbol{H} \cdot \boldsymbol{n})(\boldsymbol{n} \times \boldsymbol{H}) \tag{1}$$

over the surface area F, where δ is the membrane thickness, and $\chi_{\parallel}$ and $\chi_{\perp}$ are the average susceptibilities parallel and perpendicular to the layer normal $\boldsymbol{n}$, i.e., to the long molecular axes of the lecithin molecules. For a cylindrical vesicle the two ends can be approximated by two half-spheres which do not contribute to the aligning torque. $\boldsymbol{M}$ is perpendicular to the cylinder axis and the field $\boldsymbol{H}$. Its magnitude is

$$M = \tfrac{1}{2}(\chi_{\parallel} - \chi_{\perp}) H^2 \, \delta r l \omega \sin 2\theta, \tag{2}$$

where l is the cylinder length, r its radius, and θ the angle between the cylinder axis and the field. Obviously, the aligning torque is zero for the angles $\theta = 0$ and $\theta = \pi/2$ and largest for $\theta = \pi/4$. Starting with an angle $0 < \theta < \pi/2$, the cylinder will align its axis either parallel or perpendicular to the field, depending on whether the sign of anisotropy is negative or positive.

An analytical expression for the time dependence of the orientation angle $\theta(t)$ during the field-induced alignment process can be derived under the assumption of dynamic equilibrium

$$\boldsymbol{M} = -\boldsymbol{M}_{\mathrm{F}} \tag{3}$$

between the aligning torque $\boldsymbol{M}$ and a frictional torque $\boldsymbol{M}_{\mathrm{F}}$ due to the viscosity of the surrounding medium. This assumption is allowed if the relaxation time of the rational motion as given by the ratio $\tau_D = I/f$ of the moment of inertia of the body I and the

frictional constant f of stationary rotation is much smaller than the reciprocal angular velocity of the cylinder axis. Using spherical vesicle forms for a rough estimate of the order of τ_D, one has (9)

$$\tau_D = \frac{m}{20\,\pi\eta a}, \tag{4}$$

where m is the vesicle mass, a its radius, and η the viscosity of the medium. With $a = 5\,\mu$m and $\eta = 1$ cP, τ_D becomes about $2 \cdot 10^{-6}$ s, in fact, eight orders smaller than the observed reciprocal angular velocities of about 10^2 s. Inserting

$$M_F = -f \cdot \frac{d\theta}{dt} \tag{5}$$

In Eq. 3 and solving the differential equation for $\theta(t)$, one finds

$$\theta(t) = \arctan(\tan\theta_o \exp[-At]) \tag{6a}$$

or, written in a form linear in t,

$$\ln\tan\theta(t) = \ln\tan\theta_0 - At, \tag{6b}$$

where θ_0 is the initial orientation of the cylinder axis and

$$A = \frac{(\chi_\parallel - \chi_\perp)\delta}{f} \cdot r \cdot l \cdot \pi \cdot H^2 \ . \tag{7}$$

Upon measuring the time dependence of the orientation angle $\theta(t)$, the constant A is determined from Eq. 6. The remaining unknown parameters in Eq. 7 are $(\chi_\parallel - \chi_\perp)$, δ, and f.

The frictional constant f can be determined independently by observing the Brownian rotational motion of the cylinder axis without field. The mean square deviation $\overline{\theta^2(\tau)}$ of the orientation from its initial value after a time interval τ is given by

$$\overline{\theta^2(\tau)} = 2\,D_R\tau \tag{8}$$

where D_R is the rotational diffusion constant and the factor of 2 indicates that only one degree of rotational freedom is observed. Making use of Einstein's relation $D_R = kT/f$ and combining Eq. 7 and 8 yields

$$(\chi_\parallel - \chi_\perp)\delta = \frac{AkT}{D_R \cdot r \cdot l \cdot \pi H^2}, \tag{9}$$

which permits the determination of the product of susceptibility anisotropy times membrane thickness.

RESULTS AND DISCUSSION

The vesicle alignment was measured at a temperature of 23°C. It was found that a field of 15 kG was sufficient for practically complete alignment (order parameter ~ 1)

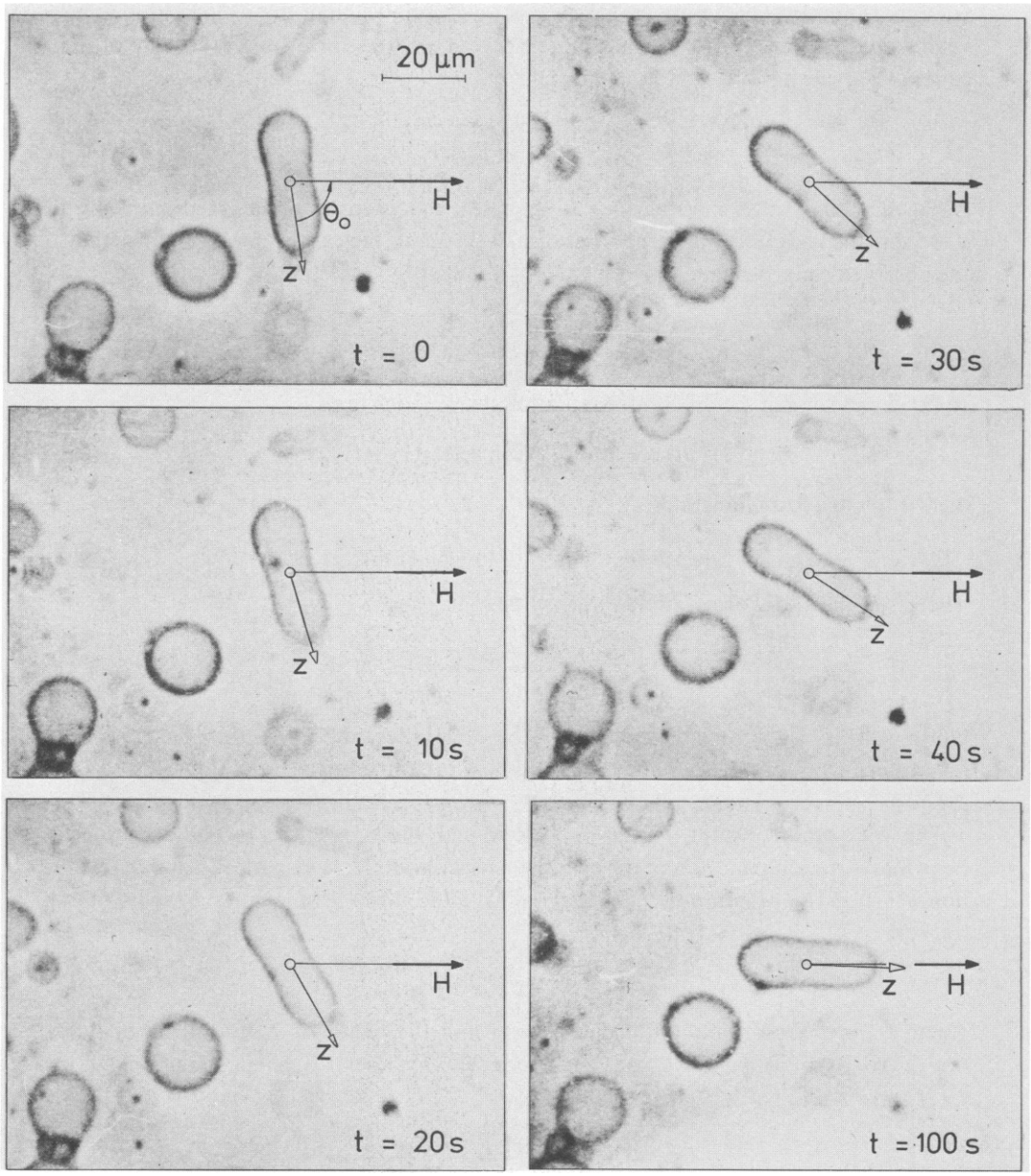

FIGURE 1 Time sequence of alignments of a cylindrical vesicle in a magnetic field, H.

of cylindrical vesicles that had only few lamellae and were of the size $r \sim 3$ μm and $1 \sim 20$ μm. At this field strength, no significant field-induced vesicle shape deformation was observed during the experiments.

All cylinders aligned parallel to the field. This proves that the lecithin molecules have a negative susceptibility anisotropy $\chi_\parallel - \chi_\perp < 0$ with respect to their long

molecular axis. Fig. 1 shows a sequence of orientations of a typical vesicle observed in a horizontal magnetic field, starting at $\theta_0 \sim 81°$. The aligning constant A was determined from the measured time dependence of the cylinder axis orientation $\theta(t)$. 10 alignment processes were registered for each vesicle studied to reduce the experimental error of A. The constant was then computed by means of a least square fit from about 300 data points. The resultant statistical error was <4%. Depending on the vesicle dimensions and membrane thickness, the constant A ranged from 10^{-2} to $5 \cdot 10^{-4}\ s^{-1}$. The rotational diffusion constant D_R without field was obtained by measuring the alignment change of the cylinder axis within a time interval τ. For each vesicle studied the range of time intervals was chosen to satisfy the following conditions: (*a*) The largest thermal reorientations were limited to some degree to avoid errors due to movements out of the plane of observation and to the spherical rather than planar geometry. The series of photographs was interrupted whenever the cylindrical vesicles were not normal to the viewing direction. (*b*) The magnitude of the mean reorientations had to be at least 1 degree to keep reading errors small. It was checked that in the range of time intervals employed no noticeable change in the proportionality $\overline{\theta^2(\tau)} \propto \tau^2$ occurred. The shortest and longest intervals in all the experiments were 2 and 60 s. At least four different time intervals were taken for each vesicle, using integral manifolds of the smallest interval to simplify data analysis. D_R was computed by a least square fit from about 500 data points. The statistical error was between 5 and 7%.

The product of susceptibility anisotropy times membrane thickness as determined for 12 observed cylindrical vesicles is listed in the second column of Table I. The error in determining the vesicle dimensions $l \cdot r$ was assumed to be 5%. Fig. 2 indicates a discrete distribution of the observed values $(\chi_\parallel - \chi_\perp) \cdot \delta$. The nine smallest values can be assigned without overlap of error bars to three different numbers of lamellae,

TABLE I

EXPERIMENTAL VALUES $(\chi_\parallel - \chi_\perp) \cdot \delta$ AND LAMELLARITIES n OF THE VESICLE MEMBRANES STUDIED

No	$10^{14}(\chi_\parallel - \chi_\perp)\delta$/cm	Lamellarity	$10^{14}(\chi_\parallel - \chi_\perp)\delta_1$/cm
		n	
1	0.340 ± 0.025	2	0.170
2	0.363 ± 0.026	2	0.181
3	0.484 ± 0.036	3	0.161
4	0.477 ± 0.035	3	0.159
5	0.506 ± 0.030	3	0.169
6	0.514 ± 0.039	3	0.171
7	0.643 ± 0.051	4	0.161
8	0.649 ± 0.046	4	0.162
9	0.638 ± 0.045	4	0.159
10	1.129 ± 0.079	7	—
11	1.53 ± 0.15	—	—
12	1.89 ± 0.20	—	—

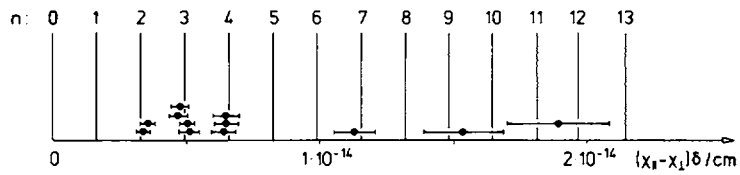

FIGURE 2 Assignment of the product of magnetic susceptibility anisotropy times membrane thickness to the number n of lamellae in the membrane.

n being 2, 3, and 4. On the basis of this assignment, the corresponding values of the product $(\chi_\parallel - \chi_\perp)\delta_1$ of a unilamellar membrane were calculated as listed in the last column of Table I. The resulting mean value is $(\chi_\parallel - \chi_\perp)\delta_1 = -(0.166 \pm 0.0007) \cdot 10^{-14}$ cm. Unfortunately, no unilamellar vesicle could be studied. Although unilamellar membranes are observable under the phase contrast microscope, such vesicles are not easy to photograph and their shapes flucutate quite strongly. Nevertheless, even without experimental values of unilamellar vesicles, an unambiguous determination of the lamellarity appears possible up to $n = 7$.

Assuming a bilayer period of 60 Å we have $\chi_\parallel - \chi_\perp = -(0.28 \pm 0.02) \cdot 10^{-8}$ cgs for the volume susceptibility anisotropy of egg lecithin membranes in multilayer systems.

The present results show that microscopic observation of the alignment of nonspherical vesicles can be used to determine the susceptibility anisotropy of membranes. Once the anisotropy per bilayer is known, a method is available to determine the number of lamellae in the membrane in a nondestructive way, of importance for any study of membrane properties. The alignment of biological particles in magnetic fields has also been used in other studies. Recently, Chagneux et al. (10) oriented frog retinal rods. In contrast to our procedure, they did not use the Einstein relation but estimated the frictional constant from the particle volume and the viscosity of the medium. Magnetic alignment of biological membranes has recently been reviewed by Hong (11).

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