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MEASUREMENT OF THE CURVATURE-ELASTIC MODULUS OF EGG LECITHIN BILAYERS

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Summary

The first determination of the curvature-elastic modulus κ of a bilayer is presented. The method is based on the microscopic study of thermally fluctuating bilayer tubes. For egg lecithin at room temperature we obtain $\kappa = (2.3 \pm 0.3) \cdot 10^{-12}$ erg.

Various procedures have been reported to produce lecithin bilayer vesicles in water, large enough to be visible under an optical microscope [1—5]. Some of them start from black films; they require the use of organic solvents which may have the undesirable tendency to be incorporated into the bilayer [3,5]. In the others, dried lecithin is brought into contact with water; they do not guarantee that the membrane of the vesicle is unilamellar [1,2,4]. In our present work we used the second method. Vesicles of large sizes (many μm in diameter) and various shapes were made to swell from lecithin lumps in water at sufficiently elevated temperatures. The materials used were distearoyl, dipalmitoyl, dimyristoyl, and egg lecithin. All observations were made under a phase contrast microscope.

Some of our vesicles had the form of long tubes. If their membranes were thin enough, they displayed wave-like fluctuations as part of their Brownian motion. The strength of these undulations should be related to the curvature-elastic modulus of the membranes. Studying the undulation modes thus opens the possibility to determine the modulus and, by way of a "quantum effect", to discern the number of lamellae making up a given membrane. We note that because of its smallness the curvature-elastic modulus can in general not be derived from the equilibrium shape of vesicles (i.e. the shape of minimum elastic energy). Even though this shape is controlled by curvature elasticity it is a function of membrane area, enclosed volume and spontaneous curvature only. However, the energy required for deformations of any kind depends on the elastic modulus.

In the following we give a theory of the undulation modes of tube-like vesicles and report measurements on egg lecithin demonstrating a "quantum effect" and giving the curvature-elastic modulus of the single bilayer. The curvature-elastic energy per unit area of a fluid membrane may be written as [6]

$$g_c = (1/2)\cdot\kappa\cdot(c_1 + c_2 - c_0)^2 \tag{1}$$

where c_1 and c_2 denote the two principal curvatures, c_0 is the spontaneous curvature, and κ the elastic modulus.

For a slightly bent tube the principal curvatures sufficiently far from its ends can be expressed in terms of the radius r of the tube and the radius R of curvature of the tube as a whole. The curvature $1/R$ will generally vary along the tube. With θ denoting an angle measured around the circumference, we may write

$$g_c = (1/2)\cdot k\cdot \left(\frac{1}{r} + \frac{\cos\theta}{R + r\cdot\cos\theta} - c_0\right)^2 \tag{2}$$

The bending of the tube also increases and decreases the surface area of its outer and inner half, respectively. As a result, we have for the curvature elastic energy per unit length of tube

$$G_c = (1/2)\cdot\kappa\cdot\int_0^{2\pi}\left(\frac{1}{r} + \frac{\cos\theta}{R + r\cdot\cos\theta} - c_0\right)^2 \left(1 + \frac{r}{R}\cdot\cos\theta\right) r\cdot d\theta \tag{3}$$

(The bending may cause a deviation of the cross section from the circular shape. This higher order effect can be neglected.) Expanding $1/(R + r\cdot\cos\theta)$ at $r\cdot\cos\theta = 0$ we obtain up to second order in $(1/R)$

$$G_c = (1/2)\cdot\kappa\cdot\left[\left(\frac{1}{r} - c_0\right)^2\cdot 2\,\pi\, r + \frac{\pi\, r}{R^2}\right] \tag{4}$$

The first term in the bracket represents the curvature elastic energy of the tube when it is not bent. The elastic energy per unit length required by bending is

$$G_c = \frac{1}{2}\cdot\pi\cdot\kappa\cdot r\cdot\frac{1}{R^2} = \frac{1}{2}\cdot K\cdot\frac{1}{R^2} \tag{5}$$

where K denotes the elastic modulus of tube bending.

The undulations of a tube, if they are small enough, may be separated into two components in orthogonal planes. For either component the curvature $C(x)$ of the tube may be represented by a Fourier expansion. Using cosines we have

$$C(x) = C_0 + \sum_{n=1}^{\infty} C_n\cdot\cos\left(n\cdot\frac{\pi}{L}\cdot x\right) \tag{6}$$

where L is the considered length of tube and x the position along the tube $(0 \leqslant x \leqslant L)$. The mean elastic energy per mode as given by the equipartition theorem and Eqn. 5 is

$$\begin{aligned} \frac{1}{2} \cdot K \cdot < C_0{}^2 > \cdot L &= \frac{1}{2} \cdot k_\mathrm{B} \cdot T \\ \frac{1}{4} \cdot K \cdot < C_n{}^2 > \cdot L &= \frac{1}{2} \cdot k_\mathrm{B} \cdot T \end{aligned} \tag{7}$$

where k_B is Boltzmann's constant and T absolute temperature. We are interested in the angle difference $\Delta\varphi = \varphi(L) - \varphi(0)$ between the ends of the tube. Because of $C = \mathrm{d}\,\varphi/\mathrm{d}x$ we have for the angle as a function of x

$$\varphi(x) - \varphi(0) = C_0 x + \sum_{n=1}^{\infty} \frac{C_n}{n(\pi/L)} \sin(n \cdot \frac{\pi}{L} \cdot x) \tag{8}$$

Only the average curvature C_0 contributes to $\Delta\varphi$. Eqns. 5 and 7 lead to

$$< (\Delta\varphi)^2 > = \frac{L \cdot k_\mathrm{B} \cdot T}{\pi \cdot \kappa \cdot r} \tag{9}$$

Egg lecithin purchased from Merck was used without further purification. About 10 mg were suspended in 8 ml of twice-distilled water. Of the optically clear suspension, 10 μl were put between glass slides and sealed to prevent evaporation. Swelling for 1 h or more converted the lecithin lumps almost completely into vesicular structures. All experiments were done at room temperature ((22.0 ± 1.5)°C) well above the transition temperature of the crystalline-fluid phase transition of egg lecithin. The measured pH of the suspension was 5.0 ± 0.3.

Beside other structures each sample showed tubes of varying length, radius and apparent thickness of walls. Most of them were loosely attached at one end to a lump of lecithin while the other end was floating freely in the suspension. Series of at least 20 pictures were taken from some fairly long tubes (11 μm $< L <$ 34 μm: $17 < L/r < 83$). The time intervals were fixed and longer than the apparent decay times of the undulations. Depending on tube length and shape, the intervals ranged between 5 and 30 s. Only those forms were photographed which were in focus over their whole length. The maximum deviation from the plane of observation was 1.3 μm. $\Delta\varphi$ was typically 10—20°, which justifies the assumption of fluctuation smallness. The maximum angle in our measurements was 59°. For each tube we defined a length L in such a way that the tips were omitted. Usually the section cut off was of the order of the tube diameter. The tangents to the centerline of the tube at the end points of L were drawn and the angle $\Delta\varphi$ between the tangents was measured. For each tube we calculated κ. No error was computed for the individual tube, but all tubes with $\Delta\varphi$ strongly deviating from a Gaussian distribution were discarded.

The curvature-elastic moduli calculated for 16 different tubes from Eqn. 9 are shown in Fig. 1. The distinct gap between the first group of values and all the others leads to the conclusion that this group corresponds to

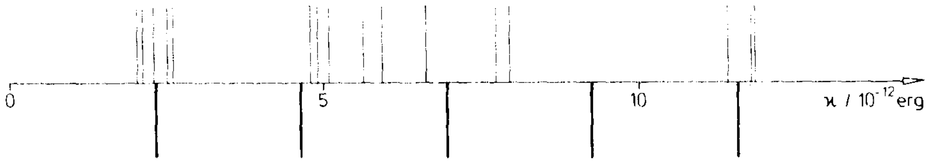

Fig.1. Bars above the axis show the measured values of κ. Heavy bars under the axis indicate the multiples of the mean value of κ obtained from the first group. (The weight attached to the individual values varies with the number of photographs).

tubes with a unilamellar membrane. The larger values of κ scatter too strongly to allow the unambiguous assignment to a number of lamellae. The scatter might be related to the frequent observation that in multilamellar membranes the lamellae are not everywhere in close contact. (Bilayers in immediate contact are likely to influence each other. Therefore, the total curvature-elastic modulus need not be strictly proportional to their number, as long as this number is small.) The result for the single egg lecithin bilayer is

$\kappa = (2.3 \pm 0.3) \cdot 10^{-12}$ erg.

It agrees fairly well with earlier estimates [6,7]. Markedly smaller values, $1.3 \cdot 10^{-13}$ erg $< \kappa < 3 \cdot 10^{-13}$ erg, were obtained from the so-called flicker phenomenon for the human red cell membrane [8].

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