Deformation of giant lipid vesicles by electric fields

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We deform spherical giant vesicles made of lipid bilayers, egg yolk phosphatidylcholine (EYPC), 1,2-dilaureoyl-*sn*-3-phosphatidylcholine (DLPC), 1-palmitoyl-2-oleoyl-*sn*-3-phosphatidylcholine (POPC), and digalactosyldiacylglycerol (DGDG), into prolate ellipsoids of revolution by means of alternating electric fields. The lateral tensions stretching the vesicle membrane are calculated from the Maxwell stress tensor and the eccentricity of the ellipsoid. The apparent increase of membrane area with tension, being due to a flattening of thermal undulations, permits us to determine the bending rigidities. We obtain 2.47×10^{-20} J for EYPC, 3.37×10^{-20} J for DLPC, 2.46×10^{-20} J for POPC, and 1.01×10^{-20} J for DGDG, with errors of up to 20%. These values are slightly smaller than those reported to date for the same materials.

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Biological model membranes such as bilayers of phospholipids and glycolipids have been studied extensively for many years. If exposed to excess water these lipids tend to swell indefinitely, giving rise to a large variety of structures which can be seen by light microscopy. For swelling to occur, the bilayers have to be in the fluid state (L_α phase), but they need not be electrically charged. The simplest form emerging is the single-walled vesicle which most often is spherical. The shape fluctuations of giant flaccid spheres are well visible under a phase contrast microscope.

The key parameter governing the strength of vesicle shape fluctuations, and thermal membrane undulations in general, is the bending rigidity of the membrane [1]. Being of the order of $10k_BT$, it can hardly be measured directly. Instead, the bending rigidity has been computed from the mean square amplitudes of undulation modes [2,3]. Lateral tension of the membrane suppresses undulations, beginning with long waves, so that vesicles look rigid at tensions above 10^{-3} mN/m. Very recently, Evans and Rawicz [4] obtained bending rigidities from the apparent dilation of the membrane that arises from the progressive suppression. In those experiments the tensions were produced by sucking a small part of the vesicle into a micropipet. The measurements started at the limit of sensitivity of 4×10^{-4} mN/m and showed a transition from apparent to real stretching at ca. 0.5 mN/m, the elastic modulus of real stretching being typically near 200 mN/m.

In the following, we describe a procedure of stretching vesicle membranes which consists in subjecting flaccid vesicles to an alternating electric field. The method is very gentle, setting no lower limit to the tension. The field transforms the initial sphere into a prolate ellipsoid of revolution. The electric stresses and force densities are calculated by means of the Maxwell stress tensor. Those acting in the external water produce a homogeneous lateral tension in the membrane which also depends on the eccentricity of the ellipsoid. The electric stresses acting within the membrane are locally neutralized by elastic stresses, thus having no effect on the undulations. The relative apparent dilation of membrane area as measured is plotted versus the logarithm of the homogeneous lateral tension as calculated. We obtain the bending rigidities from the slope of the linear fits to these plots.

To calculate the lateral tensions produced by the Maxwell stresses we begin with the sphere as the deviations from this shape will be small. The membrane may be regarded as an insulating shell since the conductivity of the lipid bilayer, about $10^{-14}\ \Omega^{-1}\ \mathrm{m}^{-1}$ [5], is much less than that of water. Therefore, an external electric field does not enter the vesicle but produces a screening field in the membrane. The latter is larger by roughly a factor R/d than the external field, where R is the vesicle radius and d the membrane thickness. Outside the sphere a dipole field of negative polarization is superimposed on the homogeneous field. Its strength is such that the total field on the surface of the spherical vesicle is everywhere tangential to it [6].

The stresses produced by the field are computed from the well-known Maxwell stress tensor

$$T_{ik}^{M}=\frac{\epsilon_0}{2}\left[\rho\frac{d\epsilon}{d\rho}-\epsilon\right]E^2\delta_{ik}+\epsilon_0\epsilon E_iE_k\ , \tag{1}$$

where ϵ_0 is the permittivity of vacuum, ϵ and ρ the dielectric constant and the mass density, respectively, of the medium, and **E** the electric field strength. In addition to the stresses accounting for charge and polarization forces, the tensor (1) contains the stresses of electrostriction [7]

$$\frac{\epsilon_0}{2}\left[\rho\frac{d\epsilon}{d\rho}-(\epsilon-1)\right]E^2\delta_{ik}\ . \tag{2}$$

Being isotropic, they may be thought to be locally balanced by a variable elastic pressure. Estimates show the pressure to be so weak as to allow the neglect of any volume and concomitant area changes. In the following we will omit the electrostrictive stresses since they gen-

erate no lateral tensions. Accordingly, we will use the modified Maxwell tensor that results from subtracting (2) from (1).

Let us introduce polar coordinates with the polar axis parallel to the applied homogeneous field $\mathbf{E}_0$ and distinguish the dielectric constants ϵ_W and ϵ_L of water and lipid, respectively. It is then easy to write down the normal stresses that act on the surface of an imaginary box tightly enclosing a piece of vesicle membrane. The stress from the outside of the sphere is

$$T_{rr} = -\tfrac{9}{8}\epsilon_0\epsilon_W E_0^2 \sin^2\vartheta \ . \tag{3}$$

It consists of a part $\propto \epsilon_0$ as obtained from the modified Maxwell tensor and another part $\propto \epsilon_0(\epsilon_W - 1)$ due to the pressure produced by the bulk polarization force density

$$\tfrac{1}{2}\epsilon_0(\epsilon_W - 1)\mathrm{grad}E^2 \tag{4}$$

present in the outer water [8]. There is no stress from the inside of the vesicle. The modified Maxwell stress exerted on the small faces which are taken to be normal cuts through the membrane is

$$T_{\vartheta\vartheta} = -\frac{9}{8}\epsilon_0 E_0^2 \frac{R^2}{d^2}\cos^2\vartheta \tag{5}$$

(and the same for $T_{\varphi\varphi}$). It can be shown to be the sum of two opposite contributions accounting for the forces that pull the free charges on the outer surface toward the equator and the polarizable lipid material toward the poles. We omit here the lateral tensions derivable from $T^M_{r\vartheta}$ as this Maxwell stress is smaller than (5) by d/R and should be balanced by elastic torque stresses. In deriving Eqs. (3) and (5) we have assumed $\lambda_D/d \ll \epsilon_W/\epsilon_L$ where λ_D is the Debye screening length. This is to ensure that the electric double layers associated with the transmembrane potential are of no importance.

The two normal electric stresses (3) and (5) produce mechanical lateral tensions in the vesicle membrane. The stress $T_{\vartheta\vartheta}$ is neutralized by an inhomogeneous elastic lateral tension

$$\sigma_i = -T_{\vartheta\vartheta}d = \frac{9}{8}\epsilon_0 E_0^2 \frac{R^2}{d}\cos^2\vartheta \ . \tag{6}$$

The elastic tension is associated with a stretching of the real membrane area. However, as the electric and elastic lateral tensions cancel each other for all ϑ, there is no effect on the undulations, at least not in the absence of electric double layers. A further increase of the real membrane area results from the modified Maxwell stress

$$T_{rr}^{\text{inside}} = -\frac{9}{8}\epsilon_0(2\epsilon_L - 1)E_0^2 \frac{R^2}{d^2}\cos^2\vartheta \tag{7}$$

inside the membrane which tends to reduce the membrane thickness. On the usual assumption of constant bulk density of the lipid, the bulk elastic moduli of membrane stretching and squeezing are the same. Accordingly, the stress (7) should stretch the bilayer $(2\epsilon_L - 1)$ times as much as the stress (5) so that the combined effect is proportional to $2\epsilon_L$.

The stress T_{rr} exerted on the outer surface of the vesicle gives rise to the ellipsoidal deformation and the flattening of the undulations. In the new equilibrium shape there will be a homogeneous tension σ_h and an extra pressure p inside the vesicle such that for all ϑ

$$(c_1 + c_2)\sigma_h - T_{rr} = p \ ,$$

where c_1 and c_2 are the principal curvatures of the membrane. Taking these surface force densities at the equator (eq) and the poles of the ellipsoid of revolution, we can compute σ_h from

$$(c_1 + c_2)_{\text{eq}}\sigma_h - (T_{rr})_{\text{eq}} = (c_1 + c_2)_{\text{pole}}\sigma_h \ . \tag{8}$$

An increase of the lateral tension from an initial value $\sigma_0 > 0$ to the final value σ_h results in the relative increment

$$\frac{k_B T}{8\pi k_c}\ln\frac{\sigma_h}{\sigma_0} \tag{9}$$

of the apparent membrane area through the flattening of undulations [1]. Multiplication by the area of the initial sphere yields the total increase of the apparent membrane area

$$\Delta A = \frac{k_B T}{8\pi k_c}\ln\frac{\sigma_h}{\sigma_0} \times 4\pi R^2 \ . \tag{10}$$

The tension σ_h increases, of course, also the real area of the membrane. It is interesting to note that σ_h diverges for the sphere while σ_i is larger than σ_h, except in a vicinity of the equator, even for very small eccentricities (in fact the smallest in our experiments).

The transformation of the initial sphere into a prolate ellipsoid of revolution weakens the electric field along the vesicle surface [7]. To lowest order in the eccentricity, the resulting reduction factor for T_{rr} is

$$\frac{2}{5} + \frac{3}{5}\frac{b^2}{a^2}$$

at the equator, a and b being the lengths of the principal axes with $a > b$. Simultaneously, the screening field inside the membrane becomes stronger. Both corrections have been taken into account in our calculations.

The materials studied were three phosphatidylcholines, one natural (EYPC = egg yolk phosphatidylcholine) and two synthetic (DLPC = 1,2-dilaureoyl-*sn*-3-phosphatidylcholine, and POPC = 1-palmitoyl-2-oleoyl-*sn*-3-phosphatidylcholine) as well as a natural digalactosyldiacylglycerol (DGDG) from wheat flour. The lipids were purchased from Sigma Chemical, St. Louis, MO, and used without further purification. Giant unilamellar vesicles were prepared by a method similar to that of Reeves and Dowben [9]. The electrically neutral lipids swelled in doubly distilled water to which 50–200 μM of NaN_3 had been added to prevent bacterial contamination. A droplet of the vesicle suspension was transferred into a drop of water in the sample cell which after mounting the cover glass was 0.25 mm high. The electric field was applied horizontally between parallel platinum wires 5 mm apart. Because of $\mathrm{div}\mathbf{j} = 0$, $\mathbf{j}$ being the electric current density, the field inside the cell was

practically uniform between the wires as proved by the absence of any dielectrophoretic movements.

For the experiments only unattached undulating spherical vesicles of diameters larger than 20 μm were selected. They were also unilamellar as inferred from a comparison with other vesicles (and confirmed by the consistency of the measured bending rigidities). The electric field lifted them from the bottom of the cell where they rested in its absence. This seems to be a consequence of the increase of the field strength in a gap between glass slide and vesicle if the gap is not much larger than the vesicle. The vesicles, made visible by a phase contrast microscope, were watched and photographed from above.

The strength of the applied electric field ranged up to 100 V/cm and its frequency was usually 1–3 kHz. (Sometimes the frequency was varied from 300 Hz to 20 kHz without noticeable effect on the shape of the electrically deformed vesicle.) When the voltage was changed the vesicle assumed its new shape within less than 1 s. The effect of the electric field is illustrated in Fig. 1, which shows the same vesicle without field and at 100 V/cm. The shape transformations were reversible with practically no difference in shape between rising and falling voltage. In the few cases where reversibility was missing the vesicles were discarded. Inspection showed such vesicles to be connected to others.

Near the maximum field strength of 100 V/cm the potential drop at the poles is of the order of the electric breakdown voltage of lipid bilayers, which is about 200 mV [10]. Although the electric field seemed to penetrate some of the vesicles at the highest voltages, as indicated by the motion of small enclosed vesicles, the consistency of our data suggests that the penetrating fraction of the field was very small. Also, the maximum possible electric contribution to the bending rigidity resulting from the strong field inside the membrane was estimated to be insignificant.

For each of the photographed deformations of a vesicle we determined the lengths of the principal axes which are needed to calculate $\Delta A/A$ and σ_h for each voltage. The volume as derived from the two lengths did not depend on voltage but displayed some scatter. In order to improve accuracy, we used its mean value and the measured ratio of the principle axes in calculating $\Delta A/A$ and σ_h. The Maxwell stresses were computed with $\epsilon_W = 81$, $\epsilon_L = 2.5$, and $d = 4$ nm [11]. Before plotting the experimental data, we subtracted from the measured increase of area the contribution of real stretching associated with σ_h and σ_i, the latter multiplied by $2\epsilon_L$ to include the effect of (7). Assuming a stretching modulus of 150 mN/m, we found real stretching to account for no more than 1% of the total increase of area at the highest voltage applied. The lateral tension σ_h giving rise to apparent stretching was corrected for the effects of the ellipsoidal deformation. We found the vesicle contours to be strictly elliptic within the limit of microscopic resolution. The correction of σ_h was never more than 30%. The total effect of both corrections on the computed bending rigidity of a given vesicle was 5% or less. We also checked that the bending energy of the ellipsoidal deformation [6] was negligible compared to its stretching energy.

Using the corrected data, we plotted for each vesicle the relative increase of apparent membrane area versus the logarithm of the lateral tension σ_h. An example furnished by the vesicle of Fig. 1 is shown in Fig. 2. A linear least-squares fit yields k_c and σ_0 according to Eq. (10). The bending rigidities thus obtained are listed in Table I. The statistical error of k_c for a particular vesicle did not exceed 10% while the scatter among vesicles of the same lipid was up to 20%. The extrapolated initial tensions σ_0 ranged from 1×10^{-6} to 5×10^{-4} mN/m.

Some of the experiments were carried out with 100 μM NaCl ($\lambda_D = 30$ nm) in addition to NaN_3 without resulting in different values of k_c. This implies that the Debye screening length was generally small enough to rule out an appreciable effect of the electric double layers. The Debye screening length can also be estimated from the specific conductivity of the aqueous medium in the sample cell. The latter was of the order of $10^{-2}\ \Omega^{-1}\ m^{-1}$ as

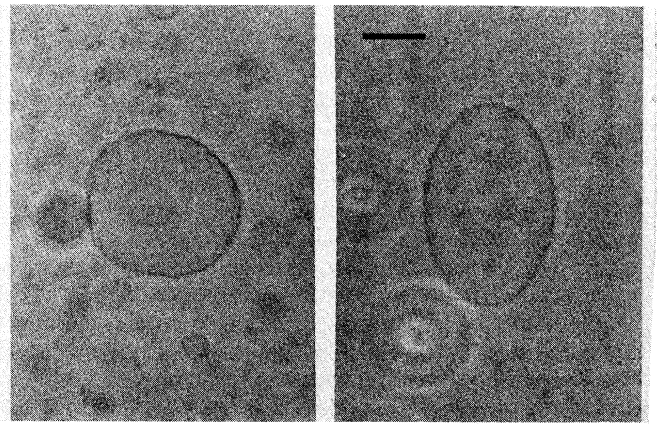

FIG. 1. EYPC vesicle at zero electric field strength (left) and at 100 V/cm (right). The bar represents 20 μm.

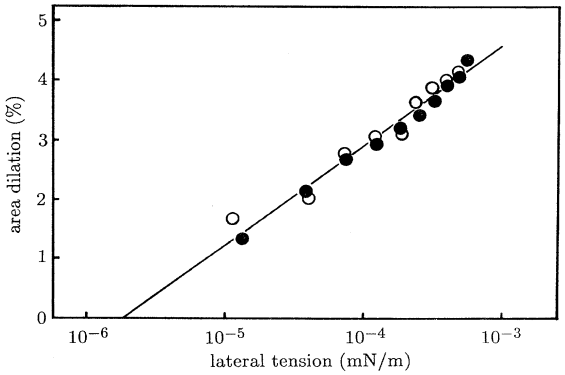

FIG. 2. Apparent relative dilation of membrane area vs the logarithm of the homogeneous lateral tension σ_h for the EYPC vesicle of Fig. 1. The diameter of the sphere is 50 μm. The measurements were made in steps of 10 V/cm for rising (●) and falling (○) voltage. The straight line is a least-squares fit yielding $k_c = 2.28\times10^{-20}$ J and $\sigma_0 = 1.79\times10^{-6}$ mN/m.

TABLE I. List of bending rigidities obtained in the present study. The mean values are given. Their errors are up to 20%.

Lipid material	Bending rigidity k_c in units of 10^{-20} J	Number of vesicles
EYPC	2.47	7
DLPC	3.37	5
POPC	2.46	4
DGDG	1.01	6

computed from ac and dc measurements. This suggests that the Debye screening length was about 10 nm, i.e., only a few times the membrane thickness.

Interestingly, in the case of POPC we found a bending rigidity near 2.5×10^{-20} J with only four out of seven vesicles. The three others displayed surprisingly large apparent increases (up to 17%) of membrane area with rising voltage. One vesicle of this type was also found with EYPC. The relaxation times of these changes, again reversible, reached up to 5 s. If plotted versus the logarithm of the homogeneous lateral tension the relative increase in apparent area has a very steep portion where it actually is S-like. This shape, which wrongly suggests hysteresis, can be avoided by plotting $\Delta A/A$ versus the logarithm of E_0^2. Such a plot is shown in Fig. 3.

The inhomogeneous lateral tension as well as its logarithmic average $\exp\langle \ln\sigma_i \rangle$, are proportional to E_0^2. Let us suppose for a moment that σ_i (or a multiple of it such as the square of the potential drop across the membrane) acts on some new membrane roughness like a lateral tension on undulations. Using (10), we then obtain apparent bending rigidities in the order of $\frac{1}{3}k_BT$ from the straight and steep part of Fig. 3 and correspondingly for the other vesicles of large $\Delta A/A$. Of course, the straightness alone does not prove any particular mechanism.

The enormous area dilation, too large to originate from the flattening of undulations, and the very small apparent bending rigidities would be in line with conclusions drawn from studies of the mutual adhesion induced by lateral tension [12]. Induced mutual adhesion was found with biological model membranes, among them EYPC and DGDG. Specifically, area reservoirs of up to 50% of the visible area and apparent bending rigidities smaller than k_BT were inferred from the large contact angles of

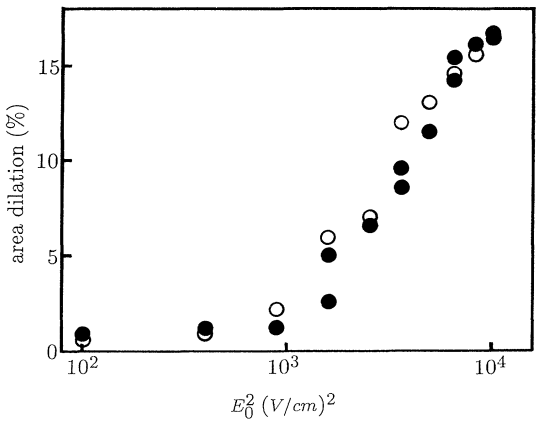

FIG. 3. Apparent relative dilation of membrane area vs the logarithm of the square of the external field strength for a POPC vesicle with a spherical diameter of 23 μm. The measurements were made in steps of 10V/cm for rising (●) and falling (○) voltage.

induced adhesion. These and other inconsistencies led to the speculation that biological model membranes possess a variable superstructure making them sensitive to minute changes of environment and, perhaps, the history of a given vesicle [13].

It is also remarkable that the higher bending rigidities extracted from the majority of plots with a uniform gradient such as Fig. 2 are slightly below all the values measured previously. The earlier results were between 2.3×10^{-19} J [14] and $(4-5)\times10^{-20}$ J [2] for EYPC, the most frequently studied material, and between 4.4×10^{-20} J [4] and $(1.2-2.7)\times10^{-20}$ J [3] for DGDG. No data for comparison are available for the two other lipids of our study. The wide spread of the bending rigidity in the cases of EYPC and DGDG seems to exceed experimental error. It might be another consequence of the suspected variable superstructure. Direct evidence for the latter is presently being searched for by transmission electron microscopy [15].

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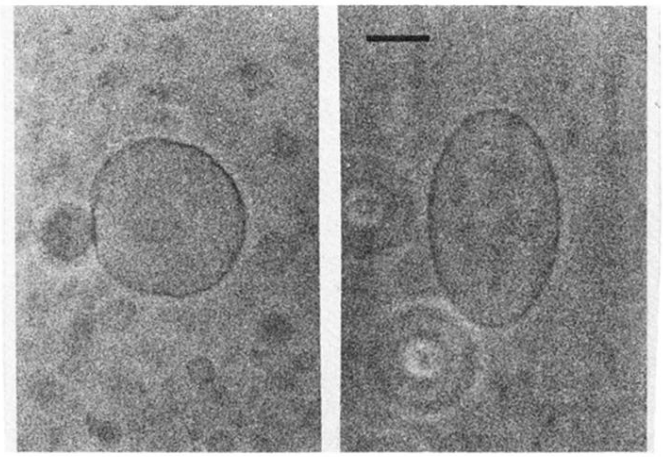

FIG. 1. EYPC vesicle at zero electric field strength (left) and at 100 V/cm (right). The bar represents 20 μm.