Giant Vesicles

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Chapter 14

Mechanical Properties of Lipid Bilayers Containing Grafted Lipids

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1. INTRODUCTION

One of the most promising applications of the liposomes is their use as drug carriers [2–4]. Usually, when drug carrying liposomes circulate in a blood stream, they are taken up by the mononuclear phagocitic system in several minutes. Their circulation time could be increased by adding modified (PEGylated, grafted, stealth – named after the military plane because the supplied vesicles, circulating in the blood stream, are invisible to the immune system) lipids to their lipid membrane. These are ordinary lipids with a polymer chain (usually poly(ethylene glycol), PEG), cova-

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lently attached to their hydrophilic heads. The polymer layer screens the vesicles and increases their average lifetime up to several days. The modified lipids change the mechanical properties of the membranes. The aim of the present work is to investigate both theoretically and experimentally the mechanical properties of a lipid bilayer with modified lipids. To achieve this aim, phenomena related to the out-of-plane fluctuations of the membranes of giant vesicles were exploited.

In what follows, a lipid bilayer is discussed, with or without modified lipids in its matrix and only in its liquid crystal state, so that it can be considered as a two-dimensional liquid. The mechanical properties can thus be characterized by stretching and bending elastic moduli. Let S_0 be the area of a flat tension-free membrane. If ΔS is the change of S_0, the density of the stretching elastic energy g_s and its tension $\sigma(\Delta S)$ is then defined as

$$\begin{aligned} \sigma(\Delta S) &= k_s \frac{\Delta S}{S_0} \\ g_s &= \frac{1}{2} k_s \times \left(\frac{\Delta S}{S_0}\right)^2 \end{aligned} \tag{1}$$

where k_s is the stretching elasticity modulus of the membrane.

If the tension-free membrane is bent, its shape can be characterized locally by its principal curvatures, c_1 and c_2. Then the density of the curvature elastic energy g_c is given by [5]

$$g_c = \frac{k_c}{2}(c_1 + c_2 - c_0)^2 + \bar{k}_c c_1 c_2 \tag{2}$$

where k_c and $\bar{k}_c$ are the bending and saddle bending elastic moduli and c_0 is the spontaneous curvature of the bilayer. Symmetrical membranes with $c_0 = 0$ are considered later.

Two different bending elastic moduli exist: k_c^{fr}, when the exchange of lipid molecules between the monolayers of the bilayer is free, and k_c^{bl}, when it is blocked. When the exchange is forbidden, the number of the molecules in each monolayer of the bilayer is constant. At free flip-flop, the bending elasticity energy is lower because it has been minimized with respect to the difference between the number of molecules in each monolayer and, consequently, $k_c^{fr} < k_c^{bl}$. For all phenomena related to the out-of-plane fluctuations of membranes, the relevant quantity is k_c^{fr} [6–8]. These phenomena include the thermal fluctuations of quasispherical vesicles [9,10], as well as vesicle suction in micropipettes at very low suction pressures [1].

2. THEORETICAL DESCRIPTION OF THE MECHANICAL PROPERTIES OF LIPID BILAYERS CONTAINING GRAFTED LIPIDS

For the theoretical description of the free energy of the PEG-chains of any modified lipid, the results of Milner *et al.* [11,12] were used. Assume that identical polymer chains are grafted on a flat surface, and let $f_p(s_p, N)$ be the free energy per chain in the brush regime at mean surface area s_p and the number of segments per chain be N. If k_B denotes the Boltzmann constant, T is the absolute temperature, a is the length of a single segment of the polymer chain, and ξ is the persistence length of the polymer chain, the free energy $f_p(s_p, N)$ can be expressed in the following way [11,12]

$$f_p(s_p, N) = \frac{9}{10}\left(\frac{\pi^2}{12}\right)^{1/3} N k_B T \frac{a^2 \xi^{-2/3}}{(s_p)^{2/3}} \tag{3}$$

If a layer containing grafted polymers is curved as a part of a cylindrical surface with curvature c, the free energy per chain $F_p(s_p, c, N)$ is

$$F_p(s_p, c, N) = f_p(s_p, N)\left\{1 - \frac{5}{24}h(s_p, N)c + \frac{5}{64}[h(s_p, N)]^2 c^2 + \cdots\right\} \tag{4}$$

where $h(s_p, N)$ is the thickness of the polymer layer, given by

$$h(s_p, N) = \left(\frac{12}{\pi}\right)^{1/3} N \frac{a\xi^{2/3}}{(s_p)^{1/3}} \tag{5}$$

As previously shown [13,14], the results of Milner *et al.* [11,12] can be used for calculating the stretching $K_s(C, N)$, bending $K_c^{fr}(C, N)$, and saddle bending elasticities $\bar{K}_c(C, N)$ of a lipid bilayer, containing a modified lipid with molar concentration C and number of monomers per polymer chain N. In these theoretical investigations it was assumed that the lipid part of the modified lipid molecules is identical to that of the basic lipid. It was also supposed that the effects of protrusion of the molecules of the modified lipid are negligible [15]. Retain the symbols k_s, k_c^{bl}, and k_c^{fr} for the elastic moduli of the lipid bilayer without modified lipid. Let s_0 be the mean area per molecule in the monolayer of a flat tension-free bilayer without PEGylated lipid and $s(C, N)$ – the mean area per lipid molecule (including modified lipid) in the decorated bilayer. Then $K_s(C, N)$ and $s(C, N)$ satisfy the equations

$$\left[\frac{K_s(C, N)}{k_s} - 1\right]\left[\frac{3}{8}\frac{K_s(C, N)}{k_s} + \frac{5}{8}\right]^{5/3} = \frac{16}{5}\left(\frac{\pi^2}{12}\right)^{1/3} \frac{k_B T N a^2 \xi^{-2/3}}{k_s (s_0)^{5/3}} C^{5/3} \tag{6}$$

$$\frac{s(C, N) - s_0}{s_0} = \frac{3}{8}\left[\frac{K_s(C, N)}{k_s} - 1\right] \tag{7}$$

The bending elastic modulus at free flip-flop, $K_c^{fr}(C,N)$, depends on the elastic moduli k_c^{fr}, k_c^{bl} and k_s of the lipid bilayer without modified lipid and on the thickness d_{ch} of the hydrophobic core of the monolayer of the flat tension-free bilayer without modified lipid:

$$K_c^{fr}(C,N) = \frac{s_0}{s(C,N)} k_c^{bl} + \left[\frac{s_0}{s(C,N)}\right]^3 (k_c^{fr} - k_c^{bl}) + k_s \frac{[s(C,N) - s_0](s_0)^2}{[s(C,N)]^3} d_{ch}\left[d_{ch} - 2\varepsilon\sqrt{\left(\frac{k_c^{fr} - k_c^{bl}}{k_s}\right)}\right] \tag{8}$$

where ε is equal to $+1$ or -1 depending on the transport direction of the molecules between the monolayers of a cylindrically curved bilayer when flip-flop is permitted. If $\varepsilon = +1$, the molecules move from the monolayer whose hydrophobic heads are nearer to the center of the curvature to the other monolayer (equivalently, they move within one monolayer from the higher-density to the lower-density polymer chain regions). On the contrary, when $\varepsilon = -1$, the molecular flow between the monolayers is in the opposite direction.

The saddle-bending elasticity is [14,16]

$$\bar{K}_c(C,N) = \frac{s_0}{s(C,N)} \bar{k}_c - \frac{6}{35}\left(\frac{12}{\pi^2}\right)^{1/3} k_B T \frac{a^4 \xi^{2/3} N^3}{[s(C,N)]^{7/3}} C^{7/3} \tag{9}$$

where $K_s(C,N)$ and K_c^{fr} depend only on the product $CN^{3/5}$. At a given value of C, $\bar{K}_c(C,N)$ is proportional to N^3, which could lead to negative values of great magnitude. One of the criteria for stability of the lamellar phase is

$$2K_c^{fr}(C,N) + \bar{K}_c(C,N) \geq 0 \tag{10}$$

which means that the flat membrane is energetically more favorable in comparison to a vesicles' suspension. For high enough values of C and/or N this inequality is not valid and the lamellar phase, built of such a lipid system, will be destabilized.

3. SOME LIMITATIONS OF THE MICROPIPETTE METHOD FOR DETERMINATION OF THE ELASTIC MODULI OF A LIPID BILAYER

There are several methods for the experimental measurement of k_c^{fr}. One of these, [1] consists of determination of the effective stretching modulus of a membrane fluctuating at very low tensions. The principles of the method are shown on Figure 14.1.

A vesicle of radius R_v is sucked in a micropipette with inner radius $R_p (R_v > R_p)$. The hydrostatic pressures inside and outside the pipette and inside the vesicle are p^{in},

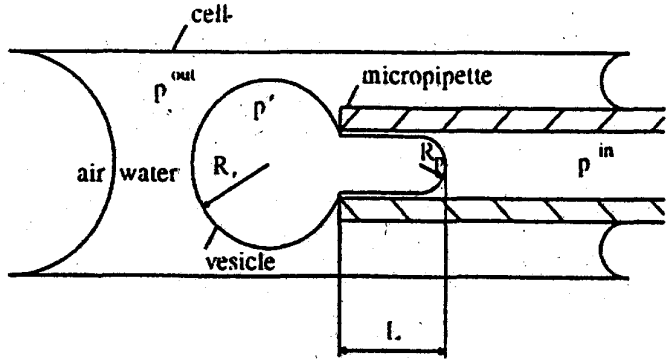

Figure 14.1 Experimental set-up for bending elasticity k_c^{fr} measurements. A vesicle with a radius R_v is sucked in a micropipette with a radius $R_p < R_v$. The hydrostatic pressures inside and outside the micropipette and inside the vesicle are p^{in}, p^{out}, and p'. The length of the membrane sucked in the micropipette is L. The bending elasticity is determined from the dependence of L on $\Delta p = p^{out} - p^{in}$.

p^{out}, and p'. Denote Δp as $p^{out} - p^{in}$. Let L be the length of the membrane sucked in the micropipette. For low enough Δp, the following relationship between L and Δp exists [1]:

$$\frac{1}{2}\left[\left(\frac{R_p}{R_v}\right)^2 - \left(\frac{R_p}{R_v}\right)^3\right]\frac{L}{R_p} = \frac{k_B T}{8\pi k_c^{fr}}\ln(\sigma) + \frac{\sigma}{k_s} + \text{constant} \tag{11}$$

where σ is related to Δp via the Laplace law:

$$\sigma = \frac{\Delta p R_v \cdot R_p}{2(R_v - R_p)} \tag{11a}$$

These relationships permit the measurement of k_c^{fr} when σ is low enough, and k_s when σ is high enough.

The following investigates some limitations of the micropipette method for determination of the elastic moduli. Equation (11) is obtained assuming that the volume of the vesicle and the number of molecules in its membrane do not change. The volume of the vesicle remains constant only if the membrane is completely impermeable, which is not exactly the case. Taking into account the permeability of the membrane and assuming that it is permeable only for the water and not for the solute, there will be a flow of water from the cell to the inner part of the vesicle, as well as from the inner part of the vesicle to the canal of the micropipette. The vesicle will be in equilibrium if the quantity of water, penetrating into the vesicle, is equal to the quantity leaving it. The equilibrium state of the vesicle does not depend on the permeability of the membrane. This quantity determines only the period of time for which the equilibrium state at given value of Δp is reached. Let C^a be the concentration of admixtures in the water. Assuming that the solution is ideal

(which is true when the activity of dissolved admixtures is equal to 1), the following dependency is obtained as a generalization of eq. (11):

$$\frac{1}{2}\left[\left(\frac{R_p}{R_v}\right)^2-\left(\frac{R_p}{R_v}\right)^3\right]\frac{L}{R_p}=\frac{k_BT}{8\pi k_c^{fr}}\ln\sigma+\frac{\sigma}{k_s}+\frac{2}{3}\frac{\Delta p}{C^a R^{gas}T}\left[\frac{R_p}{R_p-R_v}+\frac{1}{2}\frac{R_p^2}{R_v^2}\right]+\text{constant} \tag{12}$$

where R^{gas} is the gas constant and the relationship between σ and Δp is given by eq. (11a).

In such experiments the accuracy of measurement is usually of the order of 10%. Using the estimations given below, the conditions are now specified for when the error in the calculation of k_c^{fr}, due to the permeability of the membrane and its stretching elasticity, is negligible. The stretching elasticity effects are negligible if the following inequality is satisfied ($k_s \sim 200\,\text{dyn cm}^{-1}$, $k_c^{fr} \sim 20k_BT$):

$$\sigma^{max} < 0.1\frac{k_BT}{8\pi k_c}k_s \sim 0.05\ \text{dyn cm}^{-1} \tag{13}$$

The effects with respect to volume changes are negligible if:

$$C^a > \frac{320\pi}{3}\frac{k_c}{(k_BT)^2}\frac{1}{R_vA}\sigma^{max} \sim 10^{-2}\ \text{mol l}^{-1} \tag{14}$$

where A is Avogadro's number. To estimate eq. (14) take R_v as 10^{-3} cm and σ^{max} as $0.05\,\text{dyn cm}^{-1}$.

When k_s is measured the effects due to the permeability of the membrane can be disregarded when

$$C^a > \frac{40}{3}\frac{k_s}{Ak_BTR_v} \tag{15}$$

If $k_s \sim 200\,\text{dyn cm}^{-1}$ C^a has to be greater than $0.1\,\text{mol l}^{-1}$. If $k_s \sim 1500\,\text{dyn cm}^{-1}$ (e.g. for membranes containing cholesterol) C^a has to be of the order of $0.7\,\text{mol l}^{-1}$. These estimations must be taken into account in the measurements.

4. MATERIALS AND METHODS

Liposomes were made from 1-stearoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (SOPC, Avanti Polar Lipids Inc., USA). The modified lipid was 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[poly(ethylene glycol)-2000] (PEG2000 lipid, Avanti Polar Lipids Inc., USA). The average molecular weight of the polymer chain was 2000 Da. This corresponds to a mean number of N of 45 monomers per chain. Fluorescent dye, 1.5 mol % (lyssamine® rhodamine B 1,2-dihexadecanoyl-

sn-glycero-3-phospho-ethanolamine, triethylammonium salt (rhodamine DHPE, L-1392), (Molecular Probes Inc., USA)) was added together with SOPC and PEG2000 lipid. The quantity of the PEG2000 lipid in the aqueous phase was controlled to be much less than this in the lamellar phase, as described in the next section. Liposomes with 5, 10, and 15 mol % PEG2000 lipid in their membrane were prepared. To obtain giant vesicles, about 3–7 mg of SOPC/PEG2000 lipid mixture (depending on the molar concentration of the PEG2000 lipid) were dissolved into 1 ml of 2 : 1 (v : v) chloroform : methanol solvent. The organic solution was placed in a 10 ml flask and the solvent was removed by vacuum for about 5 h. The flask was then filled with solution of 0.18 mol l^{-1} sucrose and 2.5 mmol l^{-1} imidazol (to prevent proliferation of bacteria) into deionized water. The sample was kept at room temperature for 72 h. Probes were taken from the flask, taking care not to disturb the solution. Vesicles were chosen as a unilamellar objects without any visible defects on the bilayer, their diameter being of the order of 15–20 μm.

The experimental set-up was similar to that of Evans and Rawicz [1]. An inverted fluorescent microscope *Axiovert* 100 (Zeiss, Germany) was used. The pressure transducers (Sedeme Kistler, France) that measure the difference between hydrostatic pressure inside and outside the micropipette have a precision better than 0.1 N m^{-2}, corresponding to a water column height of 10 μm. The CCD camera was a C2400-77 (Hamamatsu Photonics K.K., Japan). The resolution of the whole optical system was 0.17 μm per pixel. The micropipettes were prepared from a borosilicate glass using a puller (Narishige, Japan). They had an inside diameter of the order of 6 μm.

The vesicle membrane containing the fluorescent marker, was observed under the microscope, by working in a fluorescent regime.

The experimental results show that under the conditions described above the vesicle membrane sucked into the micropipette attains its equilibrium position very quickly (5 min after the pressure was applied there was no change in the position of the membrane inside the micropipette). All the reported measurements were performed using the same micropipette.

5. EXPERIMENTAL RESULTS AND DISCUSSION

The modified PEG2000 lipid used is significantly more soluble in water than the ordinary lipids and, consequently, its quantity in the aqueous phase is not negligible. An estimation was therefore needed of the maximal concentration of the PEG2000 lipid in micellar solution in equilibrium with liquid crystal phase. Note that the swelling of the vesicles is maintained under these conditions. In fact, this is a ternary system, comprising water–lipid (SOPC)–PEG2000 lipid. The boundaries of the micellar region in the phase diagram of this system depend on the ratio PEG2000 lipid : lipid. The reasonable assumption was made that the quantity of PEG2000 lipid in the micellar phase will attain its maximal value in the system pure PEG2000

lipid–water. The light absorption of water solutions with various concentrations of PEG2000 lipid was investigated. The absorption maximum is at $\lambda = 200$ nm (Figure 14.2).

The concentration of $\sim 4 \times 10^{-6}$ mol l^{-1} is the cross-point of the linear fit of the absorptions at low and high concentrations of the PEG2000 lipid. This concentration is assumed to be the boundary of the micellar phase. Consequently, the concentration of the PEG2000 lipid in the water subphase will not exceed 4×10^{-6} mol l^{-1}.

It was noted that in each sample (see Materials and Methods) the quantity of the PEG2000 lipid in the water subphase was 10 times lower than the maximum quantity of the PEG2000 lipid in the entire volume of the sample. This guaranteed a better than 10% precision of the molar ratio between the lipid and the PEG2000 lipid in the membranes.

Most of the vesicles in the sample had defects inside and/or outside. These defects were either thin tubes (some having a length of tens of microns) or bright packages, connected to the membrane by such tubes. The defects were often absorbed by the membrane when a vesicle was sucked up with a high enough pressure into the micropipette. When decreasing the pressure, package-type defects almost always appeared (usually inside the vesicle). When a vesicle without observable defects was sucked into the micropipette, in the process of the decreasing of the pressure defects (packages of lipid) appeared. This meant we could not do any measurements by decreasing the pressure.

Only unilamellar vesicles without observable defects and diameters between 15 and 20 μm were studied. The criterion for the estimation of the number of bilayers of the vesicle was the brightness of its image observed under a microscope working in a

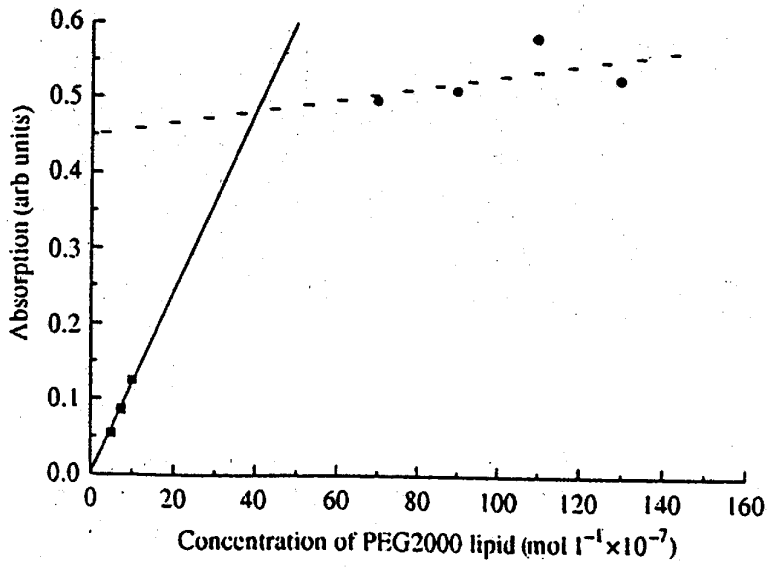

Figure 14.2 Absorption of light with $\lambda = 200$ nm as a function of the PEG2000 lipid concentration in water: (■) experimental points at low concentrations; (——) linear fit of low concentrations; (●) experimental points at high concentrations; (– – –) linear fit of high concentrations.

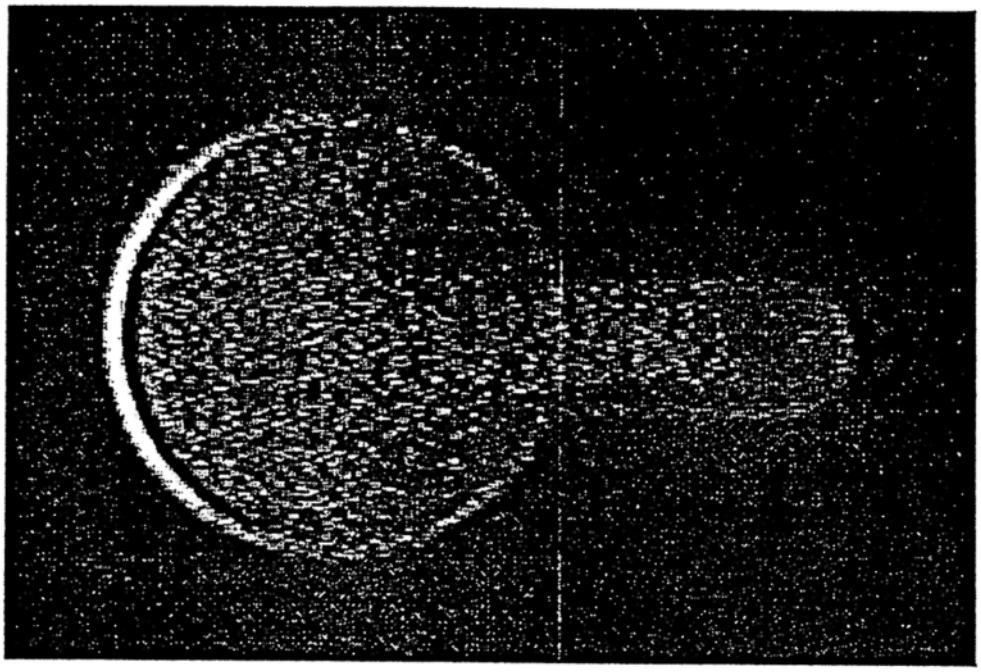

Figure 14.3 A vesicle, sucked up in a pipette, as observed under a microscope working in a fluorescence regime. The diameter of the vesicle is $\sim 20\,\mu m$, while the inner diameter of the pipette is $\sim 6\,\mu m$. The vesicle matrix is SOPC, with 5 mol % PEG2000 lipid and 1.5 mol % rhodamine DHPE.

fluorescent regime [17]. Figure 14.3 shows a vesicle, sucked into the micropipette, as observed under the microscope.

Three molar concentrations C of the PEG2000 lipid in the membrane were studied, namely 0.05, 0.10, and 0.15. Measurements of the length of the membrane sucked into the micropipette were made only for the cases when the applied pressure was consequently increased. The pressures were in the range from $20\,dyn\,cm^{-2}$ to $300\,dyn\,cm^{-2}$, corresponding to tensions from $\sim 5 \times 10^{-3}\,dyn\,cm^{-1}$ to $\sim 7.5 \times 10^{-2}\,dyn\,cm^{-1}$. The results are presented in Figure 14.4. The bending elasticity increases with $\sim k_B T$ each time the concentration of the modified lipid is increased with 5 mol %. The same dependence was measured by Evans and Rawicz [18] for a membrane consisting of the same PEG2000 lipid in a matrix of DGDG. They obtained the same change of K_c with the concentration, but the value of K_c for the pure lipid, measured by them [1], is about four times greater than the extrapolated value obtained from the results. The bending elasticity of a pure SOPC membrane, measured by analyzing the thermal form fluctuations of the vesicle, is up to six times greater than ours [19]. The following discussion presents a possible explanation for this discrepancy.

As previously mentioned, the experimental data were analyzed under the assumption that the stretching elasticity of the membrane is linear and of the order of $200\,dyn\,cm^{-1}$. Another assumption used implicitly is that for the studied ratios of lipid : PEG2000 lipid the membrane is a homogeneous planar structure. There is

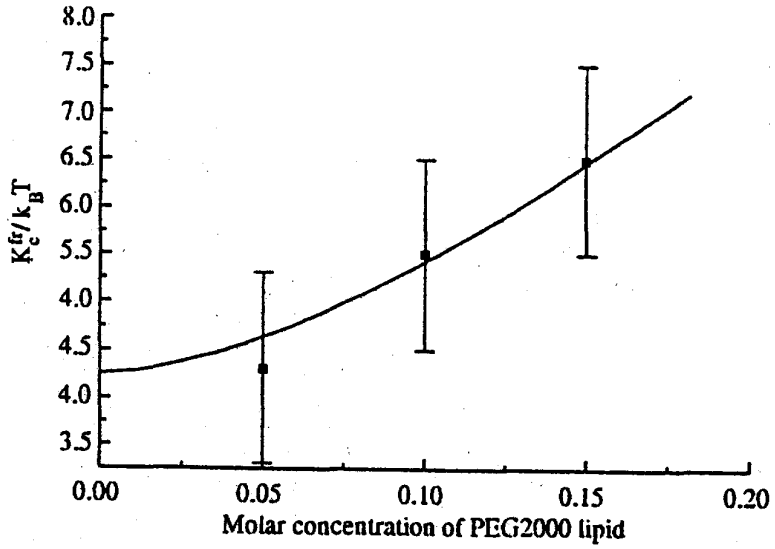

Figure 14.4 Dependence of the bending elasticity of a bilayer of SOPC containing molar concentrations 0.05, 0.10, and 0.15 of the PEG2000 lipid. The measured bending elasticities are $(4.3 \pm 1)k_BT$, $(5.5 \pm 1)k_BT$, and $(6.5 \pm 1)k_BT$, respectively. The continuous line is the theoretical curve, calculated with eq. (9). The parameter values are $k_c^{fr} = 4.25k_BT$, $k_c^{bl}/k_c^{fr} = 15$, $d_{ch} = 15$ Å, $s_0 = 70$ Å ^{2}, $a = 3.5$ Å, $\xi = a$.

evidence that these two assumptions might not be fulfilled. In such a case the theory has to be modified.

Consider a flat tension-free membrane of SOPC and a cylindrical micelle consisting of SOPC, with PEG2000 lipid molar concentrations of $C1$ and $C2$ respectively. Let $\mu_l^1(C1)$, $\mu_p^1(C1)$, $\mu_l^2(C2)$ and $\mu_p^2(C2)$ be the chemical potentials of one lipid and PEG2000 lipid molecule in the membrane and in the micelle, respectively. The expressions for these quantities are:

$$\mu_l^1(C1) = 0.56\frac{a^2\xi^{-2/3}Nk_BT}{[s_1(C1)]^{2/3}}(C1)^{5/3} \tag{16a}$$

$$\mu_p^1(C1) = 0.84\frac{a^2\xi^{-2/3}Nk_BT}{[s_1(C1)]^{2/3}}\left(\frac{5}{3} - \frac{2}{3}C1\right)(C1)^{2/3} \tag{16b}$$

$$\mu_l^2(C2) = f^{mic} - 0.56\frac{a^2\xi^{-2/3}Nk_BT}{[s_2(C2)]^{2/3}}\left\{1 - \frac{5}{24}\frac{h\left(\frac{s_2}{C2}, N\right)}{R^{out}} + \frac{5}{64}\left[\frac{h\left(\frac{s_2}{C2}, N\right)}{R^{out}}\right]^2\right\}(C2)^{5/3} \tag{16c}$$

$$\mu_p^2(C2) = f^{mic} + 0.84\frac{a^2\xi^{-2/3}Nk_BT}{[s_2(C2)]^{2/3}} \times \left\{1 - \frac{5}{24}\frac{h\left(\frac{s_2}{C2}, N\right)}{R^{out}} + \frac{5}{64}\left[\frac{h\left(\frac{s_2}{C2}, N\right)}{R^{out}}\right]^2\right\} \times \left(\frac{5}{3} - \frac{2}{3}C2\right)(C2)^{2/3} \tag{16d}$$

In eqs (16), f^{mic} is the free energy of a lipid molecule in the cylindrical micelle, $s_1(C1)$ and $s_2(C2)$ are the mean areas per lipid molecule in the flat membrane and in the cylindrical micelle at its outer surface (where the polymer chains are grafted), and R^{out} is the radius of curvature of this surface. All other quantities are those defined in the section on theory. To determine the value of f^{mic}, use the fact that the cylindrical micelle can be considered as two hydrophilic edges of a flat membrane glued together. Consequently, the free energy per unit length of the micelle is twice the edge energy γ. The edge energy of a membrane, made of egg-yolk lecithin was measured by Harbich and Helfrich [20]. They obtained $\gamma = 2.1 \times 10^{-6}$ dyn, assuming that k_c for the membrane is 2.1×10^{-12} erg. Taking for this membrane the more realistic value of $k_c = 0.55 \times 10^{-12}$ erg [9], and using their approach, gave $\gamma = 0.5 \times 10^{-6}$ dyn. Assuming the edge energy of the SOPC membrane to be the same. Then $f^{mic} = 2\gamma/M$, where M is the number of the molecules per unit length of the micelle. To determine M, it was supposed that the volume of the hydrophobic part of one molecule remains unchanged, the radius of the core of the cylindrical micelle is equal to the length l^{at} of the all-*trans* conformation of the hydrophobic chain, and that it is 50% greater than the thickness d_{ch} of the hydrophobic part of a monolayer of the tension-free bilayer. The last assumption stems from the fact that at the main gel–liquid crystal transition of a lipid bilayer, its area increases by about 50% while the volume of the hydrophobic part remains practically constant [21]. A value of 10Å was used for the height of the hydrophilic head of the SOPC. Taking $d_{ch} = 15$Å, as the radius of the hydrophobic core of the vesicle gave $l^{at} = 22.5$Å, and the radius of the outer surface of the micelle as $R^{out} = 32.5$Å. The number of molecules M per unit length of the micelle is then $\pi(l^{at})^2/(s_0 d_{ch})$. The condition for equilibrium between the flat membrane and the rod-like micelle is

$$\mu_l^1(C1) = \mu_l^2(C2)$$
$$\mu_p^1(C1) = \mu_p^2(C2)$$

This is a system of equations for the quantities $C1$ and $C2$. With the parameters values given above, the solution is $C1 \approx 0.1$, and $C2 \approx 0.4$. The exact values of $C1$ and $C2$ are not so important, because the numbers used are estimations, and not exact values. For example, if the value of the correlation length ξ is assumed to be equal to $4a$ (a being the length of the monomer unit) instead of a, $C1$ becomes of the order of 0.05. More important is the fact that $C1$ and $C2$ exist. If the total concentration of the PEG2000 lipid is less than $C1$, the planar membrane is stable. Only in this case are the theoretical results described in section Theory relevant. When the total concentration of the PEG2000 lipid is between $C1$ and $C2$, the membrane consists of planar parts with attached rod-like micelles. In the planar parts the concentration of the modified lipid is $C1$, whereas in the rod-like micelles it is $C2$. When planar parts and rod-like micelles coexist, the stretching elasticity of the membrane becomes very low. It is possible that the concentration range of PEG2000 lipids studied are within this domain. If this were the case, the studied vesicles, even without any observable defects, should contain some microscopic rod-like micelles,

in equilibrium with the planar membrane. When the suction pressure increases, the membrane enters in the micropipette not only because the amplitudes of the fluctuation modes decrease, but because part of the rod-like micelle is transformed into planar membrane. This could be the reason why the analysis gave very low value of bending elasticity, and would also explain the observations previously described in this section. The theory presented in the beginning of the paper is developed under the assumption that the bilayer, containing modified lipids, can be considered as a relatively regular surface, whereas the last theoretical results show that it could be surface bearing whiskers of cylindrical micelles. In the latter case, the quantity of the material stored in the whiskers will depend on the concentration of the modified lipid and, therefore, on the tension of the bilayer. The theoretical description of the mechanical properties of such a complicated system have yet to be elaborated.

Some of these difficulties should be partially avoidable if the bending elasticity determined from the analysis is of the form fluctuations of a quasispherical vesicle.

Our theoretical predictions show, that $C1^{max}$ (the maximal concentration of the PEG2000 lipid in the planar part of the membrane) decreases when the number of segments N in the polymer chain increases and becomes zero at sufficiently high N. The theory of elasticity developed by the authors is valid only for the brush regime, where the surface concentration of the grafted chains is high and the statistical coils interact. For low enough C1 the brush regime is no longer valid and the mushroom regime with practically independent statistical coils becomes effective. Evidently, for high enough N, the maximal concentration of the PEGylated lipid that the membrane can absorb is of the order of $s_0/(a^2N^{6/5})$.

6. ACKNOWLEDGMENTS

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7. REFERENCES

1. E. A. Evans and W. Rawicz, *Phys. Rev. Lett.*, 64, 2094 (1990).
2. G. Blume and G. Cevc, *Biochim. Biophys. Acta*, 1029, 91 (1990).
3. D. Lasic, F. J. Marin, A. Gabizon, S. K. Huang and D. Papahadjopoulos, *Biochim. Biophys. Acta*, 1070, 187 (1991).
4. D. Needham, K. Hristova, T. McIntosh, M. Dewhirst, N. Wu and D. Lasic, *J. Liposome Res.*, 2, 411 (1992).
5. W. Helfrich, *Z. Naturforsch. C*, 29, 693 (1973).

6. I. Bivas, P. Hanusse, P. Bothorel, J. Lalanne and O. Aguerre-Chariol, *J. Phys. France*, **48**, 855 (1987).
7. A. Yeung and E. Evans, *J. Phys. II*, **5**, 1501 (1995).
8. I. Bivas, P. Méléard, I. Mirtcheva and P. Bothorel, *Colloids and Surfaces A* (in press).
9. J.-F. Faucon, M. D. Mitov, P. Méléard, I. Bivas and P. Bothorel, *J. Phys. France*, **50**, 2389 (1989).
10. M. D. Mitov, J.-F. Faucon, P. Méléard and P. Bothorel, in *Advances in Supramolecular Chemistry*, (ed.) G. W. Gokel, Jai Press Inc., Greenwich, 1992, vol. II, p. 93.
11. S. T. Milner, T. A. Witten and M. E. Cates, *Macromolecules*, **21**, 2610 (1988).
12. S. T. Milner and T. A. Witten, *J. Phys. France*, **49**, 1951 (1988).
13. I. Bivas, D. Georgescauld, N. Jeandaine, M. Winterhalter, P. Méléard, G. Marinov and P. Bothorel, *Progr. Colloid Polym. Sci.*, **105**, 197 (1997).
14. I. Bivas, M. Winterhalter, P. Méléard and P. Bothorel, *Europhys. Lett.*, **41**, 261 (1998).
15. J. Majewski, T. L. Kuhl, M. C. Gerstenberg, J. N. Israelachvili and G. S. Smith, *J. Phys. Chem. B*, **101**, 3122 (1997).
16. C. Hiergeist, V. A. Indrani and R. Lipowsky, *J. Phys. II*, **6**, 1465 (1996).
17. K. Akashi, H. Miyata, H. Itoh and K. Kinosita Jr., *Biophys. J.*, **71**, 3242 (1996).
18. E. Evans and W. Rawicz, *Phys. Rev. Lett.*, **79**, 2379 (1997).
19. P. Méléard, C. Gerbeaud, P. Bardusco, N. Jeandaine, M. D. Mitov and L. Fernandez-Puente, *Biochimie (France)*, **80**, 401 (1998).
20. W. Harbich and W. Helfrich, *Z. Naturforsch.*, **34a**, 1063 (1979).
21. V. Luzzati and A. Tardieu, *Ann. Rev. Phys. Chem.*, **25**, 79 (1974).