Molecular Origin of Model Membrane Bending Rigidity

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The behavior of the bending modulus κ of bilayers in lamellar phases was studied by Small Angle X-ray Scattering technique for various nonionic C_iE_j surfactants. The bilayers are either unswollen and dispersed in water or swollen by water and dispersed in dodecane. For unswollen bilayers, the values of κ decrease with both an increase in the area per surfactant molecule and in the polar head length. They increase when the aliphatic chain length increases at constant area per surfactant molecule. Whereas for water-swollen membranes, the values of κ decrease as the content of water increases converging to the value of the single monolayer bending modulus. Such a behavior results from the decoupling of the fluctuations of the two surfactant membrane monolayers. Our results emphasize the determinant contribution of the surfactant conformation to κ

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Membranes are assemblages of amphiphilic molecules into bilayers. In biomembranes, the amphiphilic molecules are phospholipids while in model membranes there are surfactants. We usually use the latter as they provide a simpler and an easier system to deal with. Model membranes exhibit various structures: vesicles, stack of membranes, sponge phases, etc. Whatever the type of membrane, the bending rigidity modulus represents a key property that can determine the shape of the membrane assemblage as well as processes such as membrane fusion, protein adhesion, and interactions between membrane proteins. For these reasons, studies have been performed to investigate the dependencee of the bending rigidity on salt [1], inclusion adhesion or insertion [2,3], and membrane structure [4–9]. It is still unclear how the molecular properties of a membrane determine its rigidity, although some of these studies have demonstrated that properties such as membrane thickness or electrostatics contribute to it.

Few theoretical studies attempted to model the bending rigidity modulus from the membrane molecular properties [10–13]. Among them, Würger [12] has developed a model of a film where the surfactant hydrophobic tails were modeled by dangling rods. In this model, the bending rigidity represents the interplay between two contributions. The first one, κ_a, is proportional to the surface area per surfactant head and originates from entropy. The second contribution, κ_v, represents the interaction between the surfactant molecules. Würger predicted a decrease of the bending modulus with either an increase of area per surfactant molecule or a decrease in the hydrophobic surfactant chain length. To our knowledge, there are no systematic study that can be used to verify this prediction. Some of them [8,9] report the bending rigidity of nonionic Tetraethylene glycol monodecyl ether surfactant C_iE_j monolayers, but none provide at the same time the values of area per polar head and the length of the hydrophobic tail that is needed for the model predictions of bending rigidity.

In this Letter, we investigate the variation of the bending rigidity of stacked bilayers when changing its molecular properties. Specifically, we used a series of C_iE_j surfactants (of general formula CH_3-$(CH_2)_{(i-2)}$-CH_2-$(O$-CH_2-$CH_2)_j$-OH) where the polar head and the aliphatic chain lengths can be varied at will. Changes in these parameters are reflected by a change in membrane thickness as well as in the membrane surface area, that can be both experimentally measured.

Materials and methods.—Water was purified using a Purelab Prima system from Elga. The C_iE_j surfactants (purchased from Nikko) and dodecane (from VWR International), ''Rectapur'' grade, were used without further purification. In our Small Angle X-ray Scattering (SAXS) experimental set up, the x-ray detector resolution is better than 17×10^{-4} Å^{-1}. We checked the smectic nature of C_iE_j phases and measured their periodicity by SAXS [14]. The spectrum of nonionic lamellar phase exhibits an important scattering for $q \rightarrow 0$, a broad first quasi Bragg peak at position q_o, and no higher order Bragg singularities [15]. The first-order Bragg peak position, q_o, provides the lamellar spacing $d_B = 2\pi/q_o$. Density measurements were performed for pure surfactant solutions using a 2.00 cm^3 picnometer and a balance with a precision of 0.01 mg.

Theoretical background.—Helfrich [16] showed that highly flexible and nonionic staked membranes are stabilized by a steric long-range repulsion, which originates from the thermally induced out of plane membrane fluctu-

ations. In this case, the steric interaction overcome both the van der Waals attraction and the electrostatic repulsion, so that the latter interactions can be considered negligible. Helfrich derived a swelling law [16,17] that relates the membrane volume fraction, ϕ, to the membrane thickness, δ, and the membrane bending rigidity, κ:

$$\phi = \frac{\delta}{d_B}\left[1 + \frac{k_B T}{4\pi\kappa}\ln\left((d_B - \delta)\sqrt{\frac{32\kappa}{3\pi k_B T a}}\right)\right] \quad (1)$$

where d_B is the interlamellar distance. At small d_B values (<100 Å in our case), the above equation can be simplified to an "ideal swelling law" $\phi = \frac{\delta}{d_B}$, that allows us to derive the membrane thickness, δ, with high accuracy. The membrane volume fraction ϕ is known from the amount of material added to the solution. The bending rigidity κ is derived using Eq. (1) to fit the values of $(d_B\phi)$ versus $\ln(d_B - \delta)$.

For a bilayer dispersed in water, the area per surfactant molecule, a, is easily deduced from a simple geometrical model [18] $a = (2M)/(\rho N_A \delta)$, where N_A is the Avogadro number, M is the surfactant molar mass, and ρ is the surfactant density.

Assuming that the hydrophobic chains of C_iE_j do not overlap inside the bilayer, we can also derive the hydrophobic chain length, L, from low dilution experiments using the relation $L = \phi_c \times d_B$, where ϕ_c is the volume fraction of the hydrophobic chain. This fraction is easily derived from $\phi_c = \frac{m}{M}\frac{\bar{V}_c}{V_T}$, where m represents the surfactant mass in the sample, M_c and $\bar{V}_c$ are, respectively, the molar mass and the specific volume ($=1.25$ cm^3 g^{-1} as given by [18]) of the hydrophobic chain, and V_T is the sample volume.

Binary System C_iE_j *-water.*—SAXS measurements have been performed on solutions containing different water-diluted lamellar phases, where each membrane is composed of a C_iE_j bilayer (Fig. 1). For each C_iE_j used, we measure the surfactant density ρ, the area per surfactant polar head a, the membrane thickness δ, the membrane bending rigidity κ, and the surfactant hydrophobic chain length L. All the experimental values are presented in Table I and are in a good agreement with literature [18–22].

For $C_{10}E_4$ and $C_{12}E_5$ lamellar phases at high dilution, the interlamellar distance d_B does not follow the dilution law represented by Eq. (1). This anomalous behavior could be the result of a slight phase transition or be due to the overlap of the hydrophobic chains between the two monolayers of a membrane [23]. Therefore, the hydrophobic chain length and the bending rigidity could not be determined for these two lamellar phases.

To interpret and characterize the contribution from the membrane molecular properties to the bending modulus, we decided to use the model of Würger as a guide. In this model, two main structural properties influence dramatically the bending modulus: the hydrophobic chain and the polar head surface area of the surfactant. Using Würger's notations, the bending rigidity, κ, is expressed as

$$\frac{\kappa}{k_B T} = \frac{L^2}{a} + \frac{L^3}{24ad}\frac{\epsilon}{k_B T}\left[\left(\frac{\rho_o}{\sigma}\right)^2 - 1\right]^{-1}\left\{\frac{156}{5}\left[1 - \left(\frac{\sigma}{\rho_o}\right)^{10}\right] - 42\left[1 - \left(\frac{\sigma}{\rho_o}\right)^4\right]\right\} \quad (2)$$

where d is the length of a methyl group ($= 1.27$ Å), i is the number of methyl groups, $L = id$, ρ_o is the nearest-neighbor distance between surfactants (with the dense-packing approximation $\rho_o^2 = \frac{2}{\sqrt{3}}a$), ϵ and σ are the Lennard-Jones parameters of a surfactant methyl group that are equal to $\epsilon = 4.3$ meV and $\sigma = 3.9$ Å, respectively, according to the Gromos 96 force field [24].

At first, let us consider an increase of the hydrophobic chain length from 12 to 14 methyl groups, with a constant number of ethylene glycol units equal to 4 (see Table I).

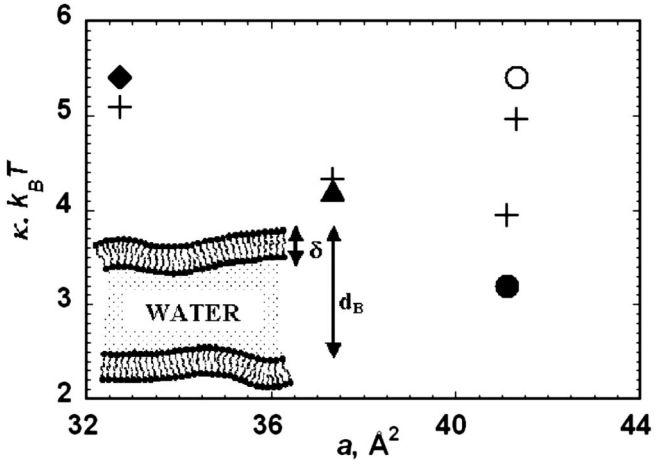

FIG. 1. C_iE_j-water binary systems. Influence of the area per molecule on the bending modulus κ for $C_{14}E_4$ (○), $C_{12}E_4$ (●), $C_{12}E_3$ (▲), $C_{12}E_2$ (◆), and the corresponding κ theoretical values from Würger's model (+). The inset represents the schematic structure of the corresponding lamellar phase.

TABLE I. Experimental properties obtained for C_iE_j-water lamellar phases. The density ρ, area per polar head a, bilayer thickness δ, bending modulus κ, and aliphatic chain lengths L are in good agreement with literature.

C_iE_j	ρ (g/cm^3) ±0.003	a (Å^2) ±0.3	δ (Å) ±0.1	L (Å) ±0.1	$\kappa(k_BT)$ ±0.1
$C_{10}E_4$	0.965	40.3	28.6	···	···
$C_{12}E_2$	0.913	32.7	30.5	10.8	5.4
$C_{12}E_3$	0.923	37.3	30.8	9.5	4.2
$C_{12}E_4$	0.942	41.1	31.1	8.6	3.2
$C_{12}E_5$	0.967	42.9	32.6	···	···
$C_{14}E_4$	0.945	41.3	33.1	10.1	5.4

This change induces no significant variation of the area per polar head ($a \approx 42$ Å^2), an increase of the membrane thickness of ~2 Å, and a substantial increase in the bending modulus from ~$3.2k_BT$ to ~$5.4k_BT$. The Eq. (2) predicts that the observed variation in the membrane thickness should induce an increase of the bending rigidity from $3.9k_BT$ and $4.9k_BT$, in fair agreement with our data as shown in Fig. 1.

Now, we consider an increase of the length of the surfactant polar head from 2 to 4 ethylene glycol units, while the length of the hydrophobic chain is kept constant to 12 methyl groups. In this case, we observe a slight increase in the membrane thickness of ~0.6 Å, a substantial increase in the area per polar head of ~9 Å^2, and a decrease in the bending modulus from ~$5.4k_BT$ to ~$3.2k_BT$.

Würger's model does not take explicitly into account the length of the surfactant polar head. Nevertheless, experimentally we observed that an increase in this length induces mainly a change in the area per polar head, that is defined in the Würger's model. In this case, Eq. (2) predicts a decrease of the bending modulus from ~$5.0k_BT$ to $3.9k_BT$ (Fig. 1).

From the Fig. 1, we conclude that Würger's model coupled with the provided values of ϵ, σ, and d successfully describes the amplitude and variation of the experimental bending rigidity. This suggests that the surfactant hydrophobic chain length and the surface area per surfactant polar head are effectively two major contributions to the bending rigidity. The observed discrepancy between the theoretical and experimental data probably originates from the fact that the model does not take into account the *gauche* conformation experienced by both the surfactant polar head and hydrophobic chain. This observation is emphasized by the negligible increase in the membrane thickness (~ 0.6 Å) upon the addition of two ethylene glycol units.

Ternary System C_iE_j *-water-dodecane.*—We now consider a stack of membranes diluted in dodecane where the membranes are composed of two surfactant monolayers swollen by water (see Fig. 2). Dodecane dilution experiments first provide the swollen membrane thickness using the ideal dilution law. It is then possible to evaluate the water thickness, δ_w, by subtracting the unswollen membrane thickness (given in Table I) from that of the swollen membrane. Indeed, it has been shown that inside a membrane, the surfactant length is independent of the water content [19,25]. The bending rigidity, κ, of the swollen membrane is derived using Eq. (1). For these systems, there is no model that allows the accurate determination of the surface area per surfactant polar head and the surfactant hydrophobic chain length from our measurements. For the $C_{10}E_4$ and $C_{12}E_5$ surfactants, a maximum water thickness of 14 Å could be achieved before destabilization of the membrane. Larger values could be reached for $C_{12}E_4$ and $C_{14}E_4$ as shown in Fig. 2.

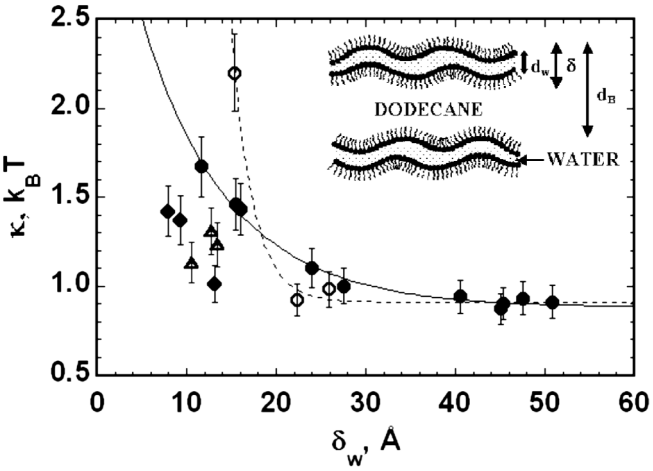

FIG. 2. Evolution of the bending moduli for C_iE_j-water-dodecane ternary systems: $C_{12}E_4$ (●), $C_{14}E_4$ (○), $C_{10}E_4$ (△), $C_{12}E_5$ (◆). The continuous curves are just guides for the eye. The inset represents the schematic structure of the corresponding swollen lamellar phase.

As previously, we start by investigating the effect of the variation in the number of methyl groups for a constant number of ethylene glycol units. At low water concentration (for $\delta_w = 13$ Å), an increase of the hydrophobic chain from $C_{10}E_4$ to $C_{14}E_4$ induces an increase of ~$0.8k_BT$ of the bending rigidity (Fig. 2). Since in the binary system a variation of ~$2.2k_BT$ has been observed from $C_{12}E_4$ to $C_{14}E_4$, we conclude that the effect is somehow damped by the presence of water. This effect even vanishes at a high water content (for $\delta_w > 22$ Å).

We now consider a variation in the number of ethylene glycol units for a constant number of methyl units. From Fig. 2, at $\delta_w = 12$ Å, we observe a decrease of ~$0.7k_BT$ of the bending rigidity from $C_{12}E_4$ to $C_{12}E_5$. The same effect on the bending rigidity was observed in the binary system when changing the number of ethylene glycol unit from 2 to 4.

In conclusion, the effect of the variation of the surfactant molecular structure on the bending rigidity observed for the binary system is still present for the ternary system, but damped when slightly swollen with water ($\delta_w < 22$ Å), and it seems to disappear for a higher water content ($\delta_w >$ 22 Å).

Now we consider the effect of the water thickness on the membrane bending rigidity for the $C_{12}E_4$ and $C_{14}E_4$ surfactants. For both surfactants, we observe a decrease of the bending rigidity when increasing the water thickness until $\delta_w \approx 25$ Å, at which point κ remains constant and equal to ~$0.9k_BT$. A similar behavior has been observed when swelling with water, a membrane made of a mixture of SDS and pentanol [4]. However, the authors have not provided a satisfactory explanation of the phenomenon.

We suggest that when the water thickness is small enough, the fluctuations of the two monolayers are syn-

chronized: the two monolayers act then as a single thick membrane. For larger values, the two monolayers start to be less coupled and will eventually fluctuate independently for a large water content. This could explain why the bending rigidity first decreases and then converges to a value corresponding to a single monolayer κ_{mono}.

However, we should expect the κ_{mono} values to depend on the surfactant molecular structure as described by Würger's model, but in our case, $\kappa_{\text{mono}} \approx 0.9 k_B T$ whatever the surfactant used. An inspection of the same model reveals that, whatever the surfactant used, the bending rigidity of a monolayer will decrease and converge to $1 k_B T$, if the surface area per surfactant molecules grows to become larger than 50 Å^2. In such case, the monolayer behaves rather as a free water-dodecane interface. We have no way to extract the surfactant surface area of a decoupled monolayer from our data, but we can estimate it from the value of the same surfactant within a monolayer at an air-water interface. For instance, Persson *et al.* [26] have found a surface area of about 60 Å^2 for $C_{12}E_4$ at the air-water interface.

This result suggests that the $C_{12}E_4$ surface area could have increased from 42 to 60 Å^2 from an unswollen bilayer to two decoupled monolayers.

Conclusion.—We provide experimental evidence that the bending rigidity of a bilayer depends mainly on two membrane molecular properties: the length of the surfactant molecule and the surface area per surfactant polar head. In a good agreement with the model of Würger, we describe in detail the dramatic variation of the bending modulus accompanying these two properties by using a series of nonionic surfactants C_iE_j. We also explain the observed decrease in the bending rigidity accompanying the swelling of a membrane with water. At low water content, the bending rigidity is the same as for a swollen bilayer. When the water content increases, the two monolayers of the swollen bilayer start to be decoupled, while the surface area per surfactant increases in both monolayers. Consequently, the bending rigidity converges to that of a monolayer acting as a free interface, independent of the surfactant structural properties.

Finally, our results indicate that Würger's model is missing some parameters that may add a minor but non-negligible contribution. Among them are the *gauche* conformations experienced by the surfactant hydrophobic tail and polar head. The evaluation of the contribution to rigidity by these parameters could be achieved in the future by molecular dynamic (MD) simulations [11,25,27]. Besides, our results may also provide information about the correct scale used in coarse-grain simulations.

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