Dramatic rigidification of a peptide-decorated lamellar phase

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We have performed small-angle x-ray scattering on a lamellar (L_α) phase made of a nonionic surfactant ($C_{12}E_4$), decane, and water, after the insertion of a triblock peptide. The hydrophilic part of the peptide is rigid and organized in an α helix in the presence of membranes. Surface tension measurements and spectrofluorometry show that the peptide lies on the membrane surface. The Caillé parameter η and the smectic compressibility modulus $\bar{B}$ decrease with peptide concentration, whereas the membrane bending rigidity κ increases threefold for mole ratio of peptide to surfactant as low as 5.2×10^{-4}. The published models for rigid inclusions in membranes cannot account for this dramatic rigidification. However, experimental results are well fitted by a Heuristic renormalization of the membrane thickness.

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INTRODUCTION

Mesophases of surfactants containing host macromolecules have attracted a great deal of attention during the last decade because of their industrial potential, as well as the theoretical problems they address. Studies of the influence of polymers on elastic properties of membranes are relevant, for example, to drug delivery by vesicles. These drugs exist in solution, adsorbed on the membrane of a vesicle, or can protrude into the membrane itself. Several theoretical studies have been directed towards the effect of flexible polymers (adsorbed or end grafted) on the elastic properties of lamellar phases [1–9]. Many experiments have been performed by incorporating flexible polymers into a lamellar (L_α) phase [10–20], but only a few report variations of the membrane bending rigidity or of the smectic compressibility modulus [18–20]. On the other hand, the effect of rigid inclusions has been scarcely theoretically studied for an isolated membrane [21] or a lamellar phase [22]. To the best of our knowledge, very few experiments have been performed with rigid inclusions [41]. Yet, a rigid peptide can be considered as a more realistic object than flexible polymers to simulate peptidic drugs in vesicles. In this paper, we report the extent to which the incorporation of a rigid peptide affects the elastic properties of a L_α phase stabilized by thermal fluctuations.

EXPERIMENTAL RESULTS

Nonionic surfactant membranes have been chosen as an appropriate model for the study of peptide insertion into membranes, in the absence of long range electrostatic interactions. The ternary system tetraethylene glycol monododecyl ether (denoted $C_{12}E_4$), water and decane, which displays a stable L_α phase between 20 and 27 °C and a spongelike phase (L_3) between 27 and 30 °C, has been selected. In such systems (L_α or L_3), a membrane consists of two monolayers of surfactant enclosing decane. The lamellar system is made of periodic stacks of such membranes, separated by water, whereas the L_3 phase consists of a multiconnected membrane separating water into two distinct spaces. Locally, however, the structure of the latter is the same as within a lamellar phase: a membrane surrounded by water. Since the L_3 phase is optically isotropic, circular dichroism, UV spectroscopy and spectrofluorometry experiments have been carried out in this phase. We have worked at a constant volume ratio $V_s/(V_s+V_{\text{decane}})=0.55$ (same membrane thickness $\delta_0=5.6$ nm [23]), where V_s and V_{decane} are, respectively, the volumes of surfactant and decane. We have kept constant the membrane volume fraction $\phi_m\equiv(V_s+V_{\text{decane}})/(V_s+V_{\text{decane}}+V_{\text{water}})=0.38$, where V_{water} is the volume of water. To localize the relatively narrow L_3 phase domain, we have checked that x-ray spectra present a broad correlation peak at q_0', related to the mean diameter of the passages creating the multiconnected topology of the phase, and that the scattered intensity varies as q^{-2} for $q>q_0'$, typical characteristics of a sponge phase [24].

The peptide sequence [25] has been designed to obtain two hydrophobic extremities and a hydrophilic rigid core, organized in α helix. Association between hydrophobic ''ends'' of the peptide and surfactant takes place provided hydrogen bonding or hydrophobic interactions are operative [10]. One hydrophobic part is six residues long (1.8 nm), whereas the second one is nine residues long (2.9 nm). The hydrophilic core is fifteen residues long (2.2 nm) and globally neutral. To check if the peptide was at the hydrophobic-hydrophilic interface, surface tension measurements have been performed, using the drop weight technique at the water-decane interface [26]. In the absence of the peptide, the water-decane surface tension is 48 mN m^{-1}, in good agreement with Goebel and Lunkenheimer [27]. When a small amount of peptide (0.105 mg/ml) is solubilized in

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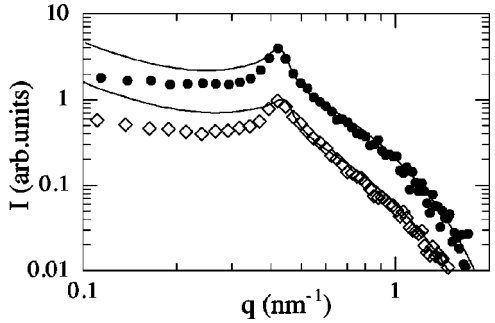

FIG. 1. X-ray spectra of a peptide-free L_α phase ($R=0, \diamond$) and a peptide-doped L_α phase ($R=5.2\times10^{-4}$, ●), both with $\phi_m=0.38$. Lines are the best fits according to Nallet *et al.* To determine η values, the fit is sensitive to the right side of the peak decrease, which is well fitted.

water, the surface tension is decreased to 30 mN m^{-1}, suggesting that the peptide is localized at the water-decane interface. A significant interfacial effect is induced by a very small amount of peptide. The peptide location is further confirmed by spectrofluorometry. In aqueous solution, when excited at 280 nm, the peptide fluorescence presents a broad peak around 350 nm, which originates from the tryptophans (Trp in the peptide sequence). In the sponge phase, this peak is shifted towards smaller wavelengths (340 nm). Together with surface tension measurements, these results confirm the change of environment: the peptide lies on the membrane.

The structure of the peptide has been investigated by circular dichroism in the L_3 phase. The spectrum is characteristic of a well organized α helix, whereas in water, it is rather a random coil [28]. Obviously, the presence of membranes enhances the peptide organization.

The peptide effect on the elastic properties of the L_α phase has been investigated using small-angle x-ray scattering (SAXS). The Caillé parameter η, the membrane bending rigidity κ, and the smectic compressibility modulus $\overline{B}$ have been measured with peptide concentration, and compared with theoretical predictions [21,22]. Small-angle x-ray scattering experiments were performed with a rotating anode (Rigaku) on samples sealed in glass capillaries 1 mm diameter and 10 μm thick (Mark-Röhrchen). Temperature was regulated with an accuracy of $\pm0.1\,^\circ$C. In Fig. 1, x-ray spectra of two lamellar phases are presented. When peptide is added, the position of the first order quasi-Bragg singularity remains constant at $q_0=0.420\pm0.005$ nm^{-1} ($q_0=2\pi/d_B$, d_B being the periodicity of the L_α phase), whereas the width of quasi-Bragg peak decreases. The sharpening of the peak can be quantified by the Caillé parameter

$$\eta=q_0^2\frac{k_BT}{8\pi\sqrt{K\overline{B}}} \tag{1}$$

with $K=\kappa/d_B$. In order to obtain η, the whole x-ray spectrum has been perfectly fitted (except for $q<0.35$ nm^{-1} where the model is not valid) using Nallet *et al.* [29] analytical approach, based on Caillé's model [30]. η variation with

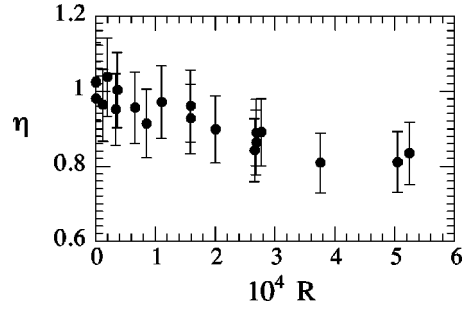

FIG. 2. Variation of the Caillé parameter η obtained from the Nallet's fit of SAXS spectra vs the mole ratio of peptide to surfactant R ($\phi_m=0.38$).

the mole ratio of peptide to surfactant R =[peptide]/[surfactant] is presented in Fig. 2: η decreases as R increases.

We have checked that the sharpening of the quasi-Bragg peak was not a residual electrostatic effect induced by the peptide: η remains identical whether the peptide-containing L_α phase is prepared with pure water or with brine (0.2 M NaCl), which screens electrostatic interactions. Thus the peptide-decorated L_α phase is stabilized by the membrane thermal fluctuations modeled by Helfrich [31] and η can be written as [32][1]

$$\eta=\alpha\left(1-\frac{\delta}{d_B}\right)^2, \tag{2}$$

where δ is the membrane thickness and α a numerical constant. Theoretically $\alpha=\frac{4}{3}$, but other values are found experimentally [34].

DISCUSSION

An analogous decrease of η has been observed when small concentrations of flexible end-grafted polymers are inserted into L_α phases [18,19]. Since in our system, the Bragg distance d_B is independent of peptide concentration, the decrease of η can be understood, according to Castro-Roman *et al.* [19], by considering a renormalized membrane thickness. From the expression 2 of η, one gets

$$\delta_{\rm eff}(R)=d_B[1-\sqrt{\eta(R)/\alpha}]. \tag{3}$$

α has been taken equal to 2.5 to get $\delta_{\rm eff}(R=0)=\delta_0=5.6$ nm, in agreement with the dilution determination. The effective membrane thickness increases with R from 5.6 nm up to 6.6 nm. The linear fit of this variation gives $\delta_{\rm eff}=\delta_0(1+0.34\times10^4R)$ (Fig. 3). This increase has not been observed with SAXS, due to the poor contrast between water and peptide electronic densities. To explain the increase of the membrane thickness, a very naive geometrical model has been developed, with peptides lying on both sides of the

[1]Since decane content is much smaller than water content, this two solvent L_α phase has been considered as a one solvent phase [33].

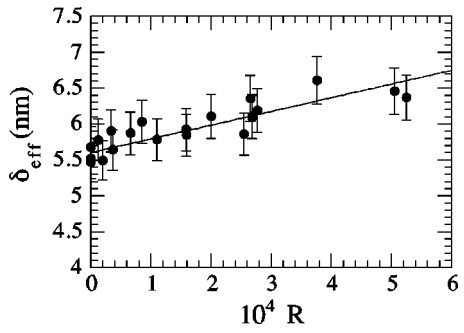

FIG. 3. Variation of the effective membrane thickness δ_{eff} obtained from $\delta_{\text{eff}}(R)=d_B[1-\sqrt{\eta(R)/\alpha}]$ vs the mole ratio of peptide to surfactant R ($\phi_m=0.38$). We choose $\alpha=2.5$ in order to get $\delta_{\text{eff}}(R=0)=\delta_0=5.6$ nm. The line is the best linear fit.

membrane (Fig. 4). This model leads to $\delta_{\text{eff}}=\delta_0+2\Delta h\phi_p$, where Δh is the diameter of the peptide α helix ($\Delta h=1$ nm) and ϕ_p the area fraction of membrane perturbed by the peptide. ϕ_p is related to R by $\phi_p=R(\Sigma/\sigma)$ with Σ the area perturbed by the peptide, and σ the area per polar head of surfactant ($\sigma=0.54$ nm^2 [23]). From the fit of δ_{eff} vs R, Σ has been calculated to be 513 nm^2: the peptide affects the membrane within a radius of $\rho=\sqrt{\Sigma/\pi}\approx 13$ nm, much larger than the length of the peptide α helix. This is in qualitative agreement with Dan *et al.* [35] who have predicted that a membrane inclusion can perturb the membrane within a radius equal to several times the inclusion size.

To estimate the variation of membrane rigidity as a function of peptide concentration, the L_α phase has been studied along a dilution line: in each set of experiments, the bare membrane thickness is kept constant [e.g., constant volume ratio $V_s/(V_s+V_{\text{decane}})=0.55$] as well as the peptide concentration, while the periodicity is increasing with water dilution. Since our L_α phase is stabilized by thermal fluctuations, the projected area of a membrane is smaller than its real area and the dilution law is [36–38]

$$d_B\phi_m=\delta_{\text{eff}}\left[1+\frac{k_BT}{4\pi\kappa}\ln\left(\sqrt{\frac{32\kappa}{3\pi k_BT}}\frac{d_B-\delta_{\text{eff}}}{a}\right)\right] \tag{4}$$

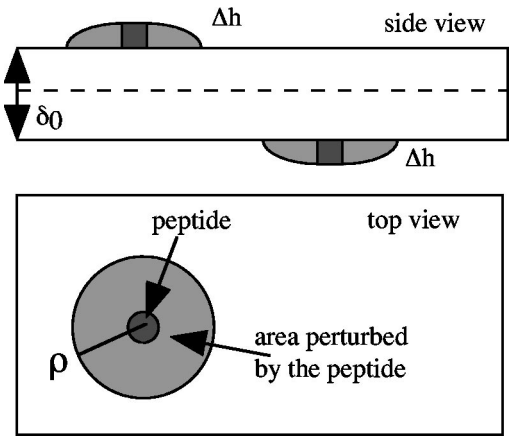

FIG. 4. Schematic geometrical model of a membrane decorated on both sides by peptides. Δh is the thickness of the peptide and ρ the extension of the peptide pertubation on the membrane.

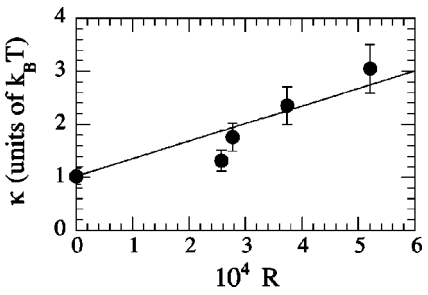

FIG. 5. Variation of the membrane bending rigidity κ vs the mole ratio of peptide to surfactant R. The line is the best linear fit.

which can also be written as

$$d_B\phi_m=V+W\ln(d_B-\delta_{\text{eff}}) \tag{5}$$

with $V=\delta_{\text{eff}}\{1+(k_BT/4\pi\kappa)\ln[\sqrt{32\kappa/3\pi k_BT}(1/a)]\}$ and $W=\delta_{\text{eff}}(k_BT/4\pi\kappa)$, where a is a molecular dimension. κ has been determined either by inserting δ_{eff} value in W, or directly from the ratio V/W, independent of δ_{eff}. Both determinations give the same values with an accuracy of 15%. As shown in Fig. 5, κ increases linearly with peptide concentration. A dramatic effect is induced by a small peptide concentration: the bending rigidity of a bare membrane increases threefold with a mole ratio of peptide to surfactant as low as 5.2×10^{-4}.

Two models exist for rigid inclusions. Chen recently predicted a decrease of the lamellar periodicity and the membrane thickness correlated to an increase of the membrane rigidity, for a cylinder-coated lamellar phase [22]. In our system, d_B remains constant and δ increases. Obviously, Chen's model cannot be applied to our results. The second model was proposed by Netz and Pincus [21]. They showed that, for an isolated membrane with rigid inclusions, for $\Delta\kappa/\kappa_0\ll 1$, the effective rigidity becomes

$$\frac{1}{\kappa}=\frac{1-\phi_p}{\kappa_0}+\frac{\phi_p}{\kappa_0+\Delta\kappa}, \tag{6}$$

where $\Delta\kappa$, the local increase of rigidity, originates from the inclusion. The fit of the above expression to our results leads

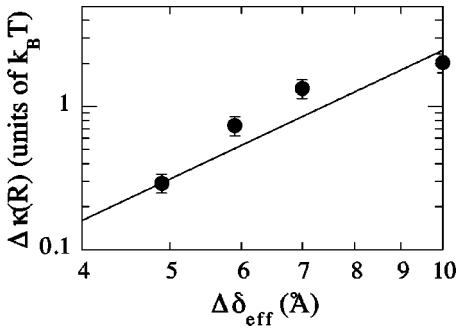

FIG. 6. Variation of the membrane bending rigidity $\Delta\kappa(R)$ vs $\Delta\delta_{\text{eff}}$ [$\Delta\kappa(R)=\kappa(R)-\kappa(0)$ and $\Delta\delta_{\text{eff}}=(\delta_{\text{eff}}-\delta_0)$]. The slope of the straight line, plotted as a comparison with Woo *et al.*, is equal to 3.

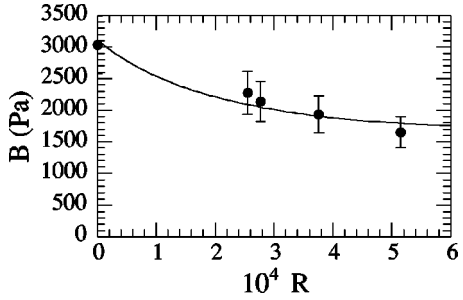

FIG. 7. Variation of the smectic compressibility modulus $\overline{B}$ vs the mole ratio of peptide to surfactant R for $\phi_m = 0.38$. Points are experimental values deduced from the independent determination of η and κ and Caillé formula [Eq. (1)], whereas the line is deduced from the Helfrich expression renormalized by an effective membrane thickness δ_{eff}.

to negative values of $\Delta\kappa/\kappa_0$, which makes it inconsistent. Thus both models fail to explain the important increase of κ we observe.

Since the peptide leads to an effective increase of the membrane thickness, we plotted the variation of $\Delta\kappa(R)$ vs $\Delta\delta_{\text{eff}}$, where $\Delta\kappa(R)=\kappa(R)-\kappa(0)$ and $\Delta\delta_{\text{eff}}=(\delta_{\text{eff}}-\delta_0)$, in log-log representation (Fig. 6). Obviously $\Delta\kappa(R)$ and $\Delta\delta_{\text{eff}}$ are related. This explanation is, of course, very qualitative. However, it is in direct analogy with the prediction of Woo *et al.* [40] who have shown that κ should scale with the membrane thickness to the power 3, for a ''dry'' membrane of surfactants.

Finally, from the independent determination of η and κ, and Caillé's formula, the variation of $\overline{B}$ with R has been calculated. $\overline{B}$ decreases upon peptide concentration: peptide insertion softens interactions between membranes (Fig. 7), whereas, for flexible polymers, all experimental studies have reported the contrary [18,20]. The softening of membrane interactions can be explained by the increase of the membrane rigidity $\kappa(R)$ and the effective thickness $\delta_{\text{eff}}(R)$ originating from the peptide insertion. Since we have checked that our peptide-decorated lamellar phase is stabilized by thermal fluctuations, Helfrich's prediction for $\overline{B}$ is valid:

$$\overline{B}_{\text{Helf}}=\frac{\pi^2}{4\alpha^2}\frac{d_B(k_BT)^2}{\kappa(d_B-\delta_{\text{eff}})^4}, \tag{7}$$

which comes directly by replacing η by expression (2) in Caillé formula [Eq. (1)].

CONCLUSION

In conclusion, a Heuristic renormalization of the membrane thickness accounts well for the decrease of η and $\overline{B}$. The effective membrane thickness increases linearly from 5.6 nm to 6.6 nm for mole ratio of peptide-to-surfactant $R=5.2\times10^{-4}$; and leads also to estimate to 13 nm, the radius of the perturbation induced by the peptide. This extension can be qualitatively explained by the thickness mismatch between the peptide and the membrane. Among published results for flexible end-grafted polymers [18–20,39], only Yang *et al.* [20] observed a doubling of κ, with concentration 30 times higher than our peptide concentration. As far as rigid transmembrane inclusions are concerned, no variation of κ was observed [41]. To the best of our knowledge, existing models cannot shed light on the spectacular rigidification of the membrane induced by such small amounts of rigid objects lying on the membrane. However, a Heuristic model that relates the increase of the effective membrane rigidity to the increase of effective membrane thickness fits well our experimental results.

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