Can zwitterionic amphiphilic bilayers structure water in layers? An experimental study

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Abstract

We have measured with use of a surface forces apparatus the short-range forces between two bilayers for a series of zwitterionic amphiphiles with different headgroup sizes and dipole moments. We found that the compression of two bilayers is discontinuous, revealing 1–3 steps of 0.27 ± 0.05 nm. This corresponds to the characteristic size of water molecules squeezed out layer by layer, suggesting the structuring of water close to the bilayers. Other possibilities are discussed.

Keywords: Amphiphiles; bilayers; surface forces.

Introduction

Understanding the interaction of amphiphilic surfaces is of prime importance for the adhesion and fusion of vesicles and biological membranes, with potential applications to drug delivery systems such as liposomes. The case of lipid bilayers over water has received much attention in relation to biological membranes and surfactant-coated colloidal particles. The major components of the lipid matrices of biological membranes are zwitterionic phospholipids, hence the choice of model zwitterionic amphiphiles for this study.

The interaction of two surfaces over a solvent includes solvent effects [1–4] beyond the contributions from van der Waals and double-layer forces. An exponential short-range repulsion between lipid bilayers has been measured, which cannot be accounted for by DLVO theory [5–8]. The decay length of this short-range repulsion is of the order of 0.2 nm, but the range may be as large as 2.7 nm [8]. Several hypotheses have been explored, from hydration and polarization of the water dipoles by the zwitterions of lecithin [9] to entropic effects including the protrusion of lipid molecules across the plane of the bilayers and other types of thermal movement [10–15]. The subject is still controversial: all the investigated models give an exponential repulsion with a decay length similar to that measured.

In order to investigate the dependence of the decay length on the size and dipole moment of the lipid polar headgroup, we studied a homologous series of zwitterionic amphiphiles in which the two charges are separated by (CH₂)n, where n = 1–10 [16]. We observed that the short-range part of the force could not be fitted to an exponential function because the distance decreased stepwise on compressing the two bilayers together.
Experimental

The forces between bilayers prepared from these amphiphiles were measured with use of a surface forces apparatus [1]. Because the interaction studied is short range, an improved optical set-up was used which also allowed us to detect steps; as in earlier studies, the distance was measured using multiple-beam interferometry [17] which allows the measurement of both distance and refractive index independently and simultaneously. Here, fringes of equal chromatic order were obtained along with the intensity spectra by a home-made cooled charge-coupled device (CCD) linear camera mounted on a vertical translation device and interfaced with a computer. Their wavelengths were determined by computing the position of maximum intensity of the fringes. This increased the resolution, reduced the uncertainty introduced when this is carried out by eye, and also allowed complementary measurements of the distance by following the positions of both fringes of the doublet; since mica is birefringent, the fringes consist of doublets corresponding to the two polarizations of light [17]. This method provided a resolution of the distance between the mica surfaces of 0.02–0.1 nm. The original version of the surface forces apparatus [1] was modified in order to reduce to a minimum any possible shear (to which bilayers may be very sensitive) between the two surfaces. The force-distance profile \( F(D) \) between the curved surfaces was obtained automatically in compression–decompression cycles with controlled timing, and is presented here as \( F(D)/R \) where \( R \) (2 cm) is the surface radius of curvature. The energy per unit area \( E(D) \) between two flat surfaces is deduced from \( F(D)/R \) by Derjaguin’s approximation, \( F(D)/R = 2\pi E(D) \).

The zwitterionic amphiphiles were synthesized by Chevalier et al [16] with a purity better than 99.9%. There is no pre-CMC dip in the surface tension vs In concentration plots. Their chemical formula is \( \text{C}_{12}\text{H}_{25}(\text{CH}_3)_2\text{N}^+\text{(CH}_2)_n\text{COO}^- \) where \( n = 1, 3, 5 \) and 10 are 0.52 nm\(^2\), 0.67 nm\(^2\), 0.68 nm\(^2\) and 0.92 nm\(^2\) respectively. These soluble amphiphiles have CMCs ranging [16] from \( 10^{-4} \) to \( 4.10^{-3} \) \( \text{M} \) and they do not adsorb on bare mica. They were therefore adsorbed from solution on mica surfaces made hydrophobic by the deposition of a monolayer of dioctadecyldimethylammonium bromide (DDOA) [18] by the Langmuir–Blodgett method [19] at a surface pressure maintained at 35 mN m\(^{-1}\) during the deposition. The molecular area of DDOA on mica was measured and was found to be 0.51 nm\(^2\), in agreement with Ref. [18]. Degassed Elga UHQ water was used. The hydrophobized mica surfaces were mounted in the surface forces apparatus, and contact between the two surfaces was made and used as a zero distance reference as indicated in Fig. 1. The surfaces were then separated and immersed in a \( \text{C}_{12}\text{N}_x\text{CO}_2 \) solution at twice the CMC; the forces were measured when adsorption equilibrium was established. Each compression–decompression cycle starting from a distance of 200 nm down to the smallest distance and maximum compression, then back to 200 nm, took 40 min.

Long-range forces

For all the amphiphiles used here, the forces had similar features. Increasing the time for one mea-
measurement cycle to 90 min did not significantly change the data. Below 120 nm, double-layer interactions were measured down to a few nanometres until a van der Waals attraction was obtained, as shown in Fig. 2 for C\textsubscript{12}N\textsubscript{3}CO\textsubscript{2}. The absolute value of the attractive minimum increased with the length of the intercharge arm in agreement with Refs [20,21]; the values were \(-0.6\) mN m\(^{-1}\), \(-1.5\) mN m\(^{-1}\), \(-2.8\) mN m\(^{-1}\) and \(-5\) mN m\(^{-1}\) for C\textsubscript{12}N\textsubscript{n}CO\textsubscript{2} with \(n = 1, 3, 5\) and 10, respectively. Most force-distance profiles showed one or several jumps (3.6 nm for C\textsubscript{12}N\textsubscript{3}CO\textsubscript{2}) just before the attractive minimum occurred (not shown), suggesting the ejection of micelle layers.

**Short-range forces**

On further compression, steep repulsive short-range forces were measured, as shown in Figs 2–6 for the four amphiphiles. The decreasing range of the short-distance repulsions with increasing intercharge distance indicates decreasing adsorption. On a semilogarithmic plot, the slope is large below about 3 mN m\(^{-1}\); above, it is smaller (Figs 3–6), similar to the data given in Refs [5–8]. The compression-decompression plots show a hysteresis; for the same force, the distance on compression is larger than the distance on decompression (up to 0.5 nm for C\textsubscript{12}N\textsubscript{10}CO\textsubscript{2}). This is clearly seen in Figs 5–6. However, the measured forces in the short-distance repulsive regime (including hysteresis) are superimposable in successive cycles. Furthermore, a decrease in the attractive minimum was observed.

Fig. 2. Forces between layers of C\textsubscript{12}N\textsubscript{3}CO\textsubscript{2} over an aqueous solution of this surfactant at twice the CMC, in semilogarithmic and linear coordinates (inset). The distance \(D\) is the smallest distance between the two curved surfaces, and it is, in the subsequent curves, taken from the position of the most intense fringe of the doublet for the least scatter of the data.

Fig. 3. Short-range part of the forces between layers of C\textsubscript{12}N\textsubscript{3}CO\textsubscript{2} over the aqueous solution of this surfactant at twice the CMC, obtained on compression (○) and decompression (●) in linear and semilogarithmic (inset) coordinates. The small arrows indicate the jumps. The solid line is a visual guide.

Fig. 4. Short-range part of the forces obtained for C\textsubscript{12}N\textsubscript{3}CO\textsubscript{2} (and shown in Fig. 2 on a different scale). Same legend as Fig. 3. On most force-distance profiles, there are a few points lying a few angströms outside the curves. They may be due to dynamic effects occurring in the two layers separated by water, or camera parasites. These are easily identified as stray points because they are isolated points off the curve that either do not reappear on repeating the measurement, or reappear elsewhere in the curve. This figure has been chosen among 11 curves which were superposable except for the stray points.
after each measurement cycle, in agreement with data given in Ref. [22]. This suggests that some rearrangement or a slow diffusion of surfactant out of the contact zone may occur, as shown by Helm et al. [22]. Although affected by the compression, the steep short-range repulsion obtained in all the cases indicated that the basic features of our system, i.e. two facing amphiphilic monolayers separated by water with an almost constant density were maintained, even when the compression lasted 1 h.

Discontinuous force-distance profiles were obtained at small distances for all the amphiphiles in the series (Figs 3–6). We also performed one experiment with an amphiphile having a longer alkyl chain, i.e. C_{18}N_{5}CO_{2}, and we observed the same discontinuous behaviour with the same jump distance. In each experiment, continuous force regimes were interrupted by one and up to three 0.27 ± 0.05 nm jumps (Figs 3–6) on both compression and decompression, after the first measurement cycles which were usually continuous. These jumps were observed in both fringes of the doublets when these were separated. Between experiments carried out with the same compound there was some reproducibility, but not a systematic one as regards the number of jumps and the force at which they occurred. Within the same experiment, successive measurement cycles were reproducible. The profiles obtained with C_{12}N_{5}CO_{2} and C_{12}N_{5}CO_{2} showed more jumps (often two) than those obtained with C_{12}N_{4}CO_{2} and C_{12}N_{10}CO_{2} (in general, one).

Discussion and conclusion

The jump distance is similar to the molecular size of water. This suggests that the water between the two bilayers is layer-structured. Other possibilities should also be considered.

Chen et al. [23] have shown that hydrophobic monolayers deposited on mica can swell when exposed to humid air, owing to water penetration into the headgroup region, by amounts that can look like one or two water monolayers. Could any water layers possibly located between the DDOA and the mica be squeezed out during compression and show up as 0.27 nm jumps? This possibility was ruled out by compressing two monolayers of DDOA deposited on mica and immersed 24 h in water; the compression was continuous without jumps in distance.

In work on the forces between surfactant monolayers across hydrocarbon liquids, Gee and Israelachvili [24] reported oscillatory forces for monolayers in the gel state and smooth forces for fluid monolayers. As the amphiphiles used here have a C_{12} alkyl chain, the adsorbed monolayer is certainly in a fluid state at room temperature. Our present work indicates that oscillatory forces may also occur with fluid monolayers.

The possible interdigitation of the C_{12}N_{4}CO_{2} and the DDOA monolayers within each of the
bilayers on the mica surfaces may also provide an explanation for these jumps [25]; two parallel alkyl chains in close-packed layers sliding along each other should move only by jumps of two CH2 groups at a time. The projected length of two CH2 groups on the chain axis is 0.254 nm [26]. However, for such a phenomenon to occur, there must be enough space in the DDOA monolayer to incorporate the C12H25 alkyl chains of the zwitterionic surfactants. The molecular area of the two octadecyl (C18H37) chain amphiphile DDOA on mica is 0.51 nm², and the minimum area of one close-packed C12H25 chain in a monolayer is 0.20 nm² [27]. This leaves an area of 0.11 nm² per DDOA molecule for interdigitation of the adjacent surfactant monolayer. If the minimum area of one close-packed C12H25 chain is also 0.20 nm², there will be space for one chain every 0.93 nm² of the DDOA layer, i.e. interdigitation may be possible only for larger molecular areas of the zwitterionic surfactants. The distances at which the short-range forces start, shown in Figs 3–6, indicate that the adsorbed surfactant molecules are fairly densely packed, with molecular areas unlikely to be larger than 0.70 nm²; even if the hysteresis were entirely attributed to the diffusion of surfactant the increase in molecular area would still be insufficient. We cannot completely rule out interdigitation because we do not know accurately the molecular areas of C12NnCO2 in monolayers under compression, but there are indications that this phenomenon is unlikely to occur in our case.

One more hypothesis is worth mentioning. Monolayers are subject to various types of phase transition. Some monolayers have been found to have transitions in which the alkyl chains go from a perpendicular orientation relative to the interface to a tilted one [28]. In our system, the surfactant layers are in a fluid state. However, when they are compressed together, they may very well pass through a phase transition and become solid. This can lead to tilted monolayers (a physically reasonable tilt of 35° would account for the observed jumps) or alternatively to monolayers in a solid state that may layer structure the water. The tilted transition is not a likely explanation because up to three jumps have been observed, and the jump distance was the same for all the studied amphiphiles, even for C18N2CO2 (one experiment).

This type of discontinuous force has been measured previously between mica surfaces across an electrolyte [3], but not between bilayers [5–8]. These particular surfactants have never been studied. Additionally, we believe that our improved optical method has enabled us to obtain these results.

Layer-structured water between amphiphilic bilayers has also been seen in computer simulations [29–30] and X-ray measurements [31] on multilamellar lipid phases of DPPC (gel state) spread on a solid under controlled humidity. In Ref. [31], phospholipid bilayers were shown to take discrete states of hydration for which the interbilayer distance varies by discrete steps of 0.25 nm.

We have shown that the compression of two amphiphilic bilayers together may be discontinuous, with good indications that this is due to the layer structuring of the water between them. Such structuring has an important bearing on how model biological membranes interact at small distances; the present work should be developed with bilayers for which the lipid density is more accurately known.

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References

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