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Formation of supported phospholipid bilayers via co-adsorption with β-D-dodecyl maltoside

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Abstract

We have investigated the formation of supported model membranes via the adsorption of phospholipid–surfactant mixtures at the Si–water interface by specular neutron reflection. The adsorption of mixed micelles of the nonionic surfactant β-D-dodecyl maltoside and DOPC or POPC was determined as a function of bulk concentration, and using d_{25}-β-D-dodecyl maltoside, the composition of DOPC and POPC bilayers was determined. Bilayer thicknesses of 39±3 Å for DOPC and 41±3 Å for POPC agree well with data from bulk lamellar phases for both lipids, and the average area per lipid molecule can be varied from 62 to 115 $Å^2$ by varying the bulk concentrations used. The amount of surfactant in the bilayer is very sensitive to the bulk volume-to-surface area ratio, but it can be fully eliminated by ensuring a sufficiently large dilution/rinsing volume of the solution.

Keywords: Phospholipid bilayer; DOPC; Dodecyl maltoside; Micelle; Adsorption; Neutron reflection

1. Introduction

The field of biological interfaces continues to attract the attention of scientists from disciplines as varied as physics, chemistry, molecular biology, biotechnology, and medicine, because even after more than half a century of intense development, the rigorous treatment of such complex supramolecular assemblies remains a challenge, and methods for adapting experimental investigations to fragile and complex biological systems are still largely on the drawing board.

In this paper, we describe a new method for the formation of supported model membranes using a surfactant as a lipid solubilising agent. Accurate in situ determination of interfacial composition is a prerequisite in controlling membrane properties and understanding how they influence membrane interactions with interfacially active biomolecules. We have used neutron reflection to determine the structures and compositions of mixed phospholipid–surfactant bilayers.

In order to study the interaction of phospholipid membranes with biological molecules, single membranes are desirable, because the composition and structure of adsorbed bilayers can be determined by neutron reflection. Several possibilities exist for creating single membranes, the simplest method being the formation of a Langmuir monolayer at the air–water interface [1–3]. Such monolayers allow the investigation of phase behaviour and adsorption as a function of lateral pressure on a film balance, but the absence of a second membrane leaflet means the absence of van der Waals interactions, which may be very important in natural membranes [4]. At the same time, the air–water interface itself is hydrophobic, which may lead to competitive adsorption of amphipathic proteins with lipids and surfactants.

Flat bilayers are useful membrane models due to their physical properties and simple geometry, but their formation is not necessarily simple. Stacks of bilayers can be spin-

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coated onto solid supports and have been investigated by neutron and X-ray reflection [5,6], but inter-bilayer interactions in the stacked structure may interfere with protein adsorption experiments. Langmuir–Blodgett deposition and adsorption of vesicles on solid surfaces [7] can both produce single bilayers. Langmuir monolayers can be formed from practically all insoluble amphiphiles, but their successful transfer onto a solid surface requires the monolayer to be in a condensed state, while deposition of fluid bilayers leads to incomplete surface coverage [8]. Langmuir–Shaefer deposition [9] offers the ability to form floating bilayers that are more mobile than a bilayer directly adsorbed to a solid surface [10], but the method still suffers from the same limitations as Langmuir–Blodgett deposition.

Adsorption of vesicles allows the deposition of bilayers on hydrophilic or hydrophobic surfaces but the results vary, with sometimes adsorption of intact vesicles accompanying bilayer formation [11]. It has been shown by neutron reflection that bilayer coverages up to 80% can be achieved by this method [12], but in the case of amphiphile mixtures the relationship between bulk and surface composition has not been studied.

Micellar adsorption to solid–liquid interfaces is a central theme in surfactant science. Non-ionic surfactant mixtures have been studied extensively, but only recently has this approach been extended to phospholipid–surfactant mixtures. Single surfactants adsorb on hydrophilic surfaces as flattened micelles or spread to form a bilayer depending on their structure [13]. In a binary mixture of surfactants, both the concentration and the composition of micelles determine the surface excess [14]. A stagnant layer (see Fig. 1) exists near the surface, within which the adsorption of micelles and monomers is diffusion controlled. The adsorption of micelles within the diffusion layer leads to a reduction in the concentration relative to the bulk solution, and this leads to the solubilisation of monomers from the adsorbed layer upon rinsing or dilution of the bulk phase. Provided that one of the components is insoluble, this solubilisation happens only to the soluble surfactant. Introducing a more dilute bulk solution to a partially covered surface leads to the adsorption of micelles with a higher content of the insoluble component and gives rise to the enrichment of the surface aggregates in the more insoluble component [15].

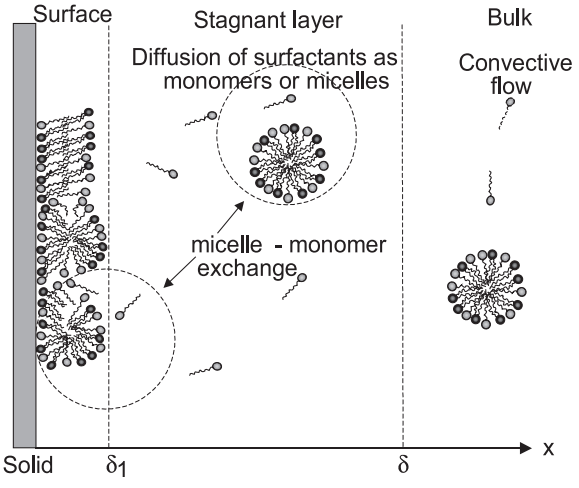

Fig. 1. Adsorption of mixed micelles of insoluble phospholipids (dark grey) and a nonionic surfactant (pale grey). δ denotes the thickness of the stagnant layer within which the bulk concentration is reduced due to adsorption at the surface, and δ_1 defines the thickness of the surface region.

This principle can be used for depositing phospholipids on surfaces. Grant and Tiberg [16] have previously used atomic force microscopy, surface force measurements and ellipsometry to investigate the adsorption of *n*-β-D-dodecyl maltoside and DOPC, and found that the adsorbed layer had characteristics very similar to the bulk lamellar structure of DOPC [17]. More importantly, dilution of the bulk solution or rinsing with water led to desorption in a manner predicted by their work on surfactant mixtures, and the surface excess could be increased in a way which suggested that the surface layer was being enriched in DOPC with a gradual removal of the surfactant. In the present work, neutron reflection was used to monitor the changes in fractional surface coverages of both surfactant and phospholipid using contrast variation.

2. Materials and methods

Ultra-high quality water (Ω=18.2 ohm) was used in all experiments. D_2O for experiments at ISIS, UK was purchased from Sigma-Aldrich, and at ILL, Grenoble, was provided from the reactor. The phospholipids, Tris-buffer and β-D-dodecyl-maltoside were purchased from Sigma-Aldrich at 99% purity and used without further purification. Chain-deuterated d_{25}-β-D-dodecyl-maltoside was synthesised in our laboratory as described by Hines et al. [18]. The solid supports for neutron reflection were polished in house and cleaned for 15 min in a mixture of 1:4:5 $H_2O_2/H_2SO_4/H_2O$ at 80–85 °C, followed by ozonolysis [19]. This treatment leaves a natural oxide layer of 7–10-Å thickness and 3–5-Å roughness with 5–10% water.

Cryo-TEM measurements were performed at the Biomicroscopy Unit of the University of Lund, Sweden. Imaging with a (Philips CM120 BioTWIN Cryo) in energy filtering mode (Gatan GIF 100) was performed by Dr. Gunnel Karlsson. The supports for ice films were carbon-coated copper grids (2–3-mm diameter) with hole size of 1–6 μm and thickness up to 500 nm and made hydrophilic by glow discharge.

Neutron reflection experiments were carried out on the SURF reflectometer at the ISIS facility of the Rutherford-Appleton Laboratory in Didcot, UK and on the D17 reflectometer at the Institute Laue-Langevin in Grenoble, France. All measurements were done in the time-of-flight mode, using wavelengths of 0.5–6 Å on SURF and 2.2–19 Å on D17. The sample solution is contained in a Teflon trough clamped against the Si surface with hollow aluminium plates that allow temperature equilibration by a circulating water

bath to an accuracy of ±0.3 °C. The cell has an inlet and outlet allowing the change of contents without exposing the surface to air, and a cavity for a magnetic flea for stirring the bulk solution during adsorption.

A typical experimental procedure for bilayer formation contains seven stages: adsorption from a 6:1 (w/w%) mixture of β-D-dodecyl-maltoside and the requisite phospholipid at a concentration of 0.114–0.123 g/l, followed by rinsing with D_2O, and readsorption from 10 to 100 times more dilute solutions, each followed by rinsing, and finally, changing to a buffer solution (10 mM Tris–HCl at pH 7.4). All manipulations are performed in situ in the neutron reflectometer, with reflectivity recorded at each stage after an equilibrium is reached at the surface. Prior to adsorption of surfactants, the Si–SiO_2 surface is characterised in D_2O as a reference for data fitting.

3. Neutron reflection

Neutron reflection measures the structure and composition of an adsorbed thin layer perpendicular to the interface, and experiments can be performed at either air–liquid or liquid–solid interfaces. The sensitivity of neutron reflectivity to the layer structure depends on its scattering contrast to the surrounding media, which is a function of nuclear composition and density and may be changed via isotopic substitution of deuterium for hydrogen without altering the chemical properties of a system. The reflection of neutrons from a thin film gives rise to interference fringes, whose thickness is related to the fringe separation.

An adsorbed film is characterised in terms of its neutron scattering length density profile $\rho(z)$, defined in Eq. (1),

$$\rho(z) = \sum_j n_j(z) b_j \tag{1}$$

where b_j is the neutron scattering length of nucleus j and $n_j(z)$ the number density of nuclei in the direction normal to the interface. The reflectivity $R(\rho)$ is a function of the neutron refractive index n of the system,

$$n = 1 - \frac{\lambda^2}{2\pi}\rho \tag{2}$$

and can be calculated using an optical matrix model [20] and then fitted to experimental data. In the case of a mixed layer of two or more components, the resultant scattering length density will be a sum of the individual scattering length densities of all the components weighted by the volume fractions at which they are present in the layer. For example, in a mixture of two components, A and B, and water, the scattering length density will be:

$$\rho_{\text{layer}} = \phi_A \rho_A + \phi_B \rho_B + \phi_w \rho_w \tag{3}$$

By deuterating components A and B in turn, or changing the water contrast, it is possible to define all three volume fractions of a mixed layer.

The thickness resolution of neutron reflection can be of the order of 1 Å at high contrast, and in particular it is increased by the use of contrast variation [21], i.e., altering the scattering length density profile via deuteration of the solvent and/or film, which provides several different reflectivity profiles from the same chemical situation. By constraining the isotopically different situations to the same physical structure, the overall layer thickness and hydration should be the same (to within the reproducibility of bilayer formation) in a mixture of hydrogenated lipid and deuterated surfactant as in a mixture of hydrogenous lipid and surfactant, but at the Si–D_2O interface each measurement will give the surface excess of the hydrogenous component(s) in the bilayer. Data fitting was performed using the Afit (v. 3.1) program [22], which uses the optical matrix method and allows the simulation of reflectivity profiles for layered interfaces by characterising each layer by its thickness, scattering length density, solvent volume fraction and roughness.

In order to construct a bilayer model for fitting, we used phospholipid volume fractions from molecular dynamics simulations [23], which have been shown to agree excellently with experimental data [24–27] on DOPC and POPC. The component volumes and scattering length densities are shown in Table 1.

In the AFit program, the reflectivity profiles of the bilayers were analysed by using a three-layer model, assuming that the headgroups and hydrocarbon chain regions could be modelled as non-interpenetrating homogeneous slabs. The thickness and solvent content of the hydrocarbon region (which are the most sensitive parameters of the bilayer in a D_2O contrast) were fitted to give a coarse fit to the observed reflectivity, and the thickness and solvent fraction in the headgroup region were fitted using a stoichiometric constraint, i.e., that the area per molecule must remain constant to within ±1 $Å^2$ throughout the layers. The fit was refined by adjusting the sublayer parameters while maintaining the stoichiometry until a satisfactory agreement with data was reached. It was not necessary to incorporate any interfacial roughness into the bilayer model nor was a solvent layer found to be present between the headgroups and the SiO_2 surface.

The structure determination of neutron reflectivity is based on measuring the scattering length averaged over the

Table 1
Scattering length densities (ρ) of phospholipids obtained from bilayer molecular dynamics simulations [23]

	DOPC	POPC	h-DDM	d_{25}-DDM
V_{head} ($Å^3$)[a]	337.2	322.1	497.4	497.4
ρ_{head} ($\times 10^{-6}$ $Å^{-2}$)	1.78	1.86	1.31	1.31
V_{chains} ($Å^3$)[b]	985.0	933.7	351.0	351.0
ρ_{chains} ($\times 10^{-6}$ $Å^{-2}$)	−0.21	−0.29	−0.39	7.02

For the surfactant, bulk density was used.
[a] Headgroup=carbonyl, glycerol, phosphate and choline.
[b] Calculated chain volume.

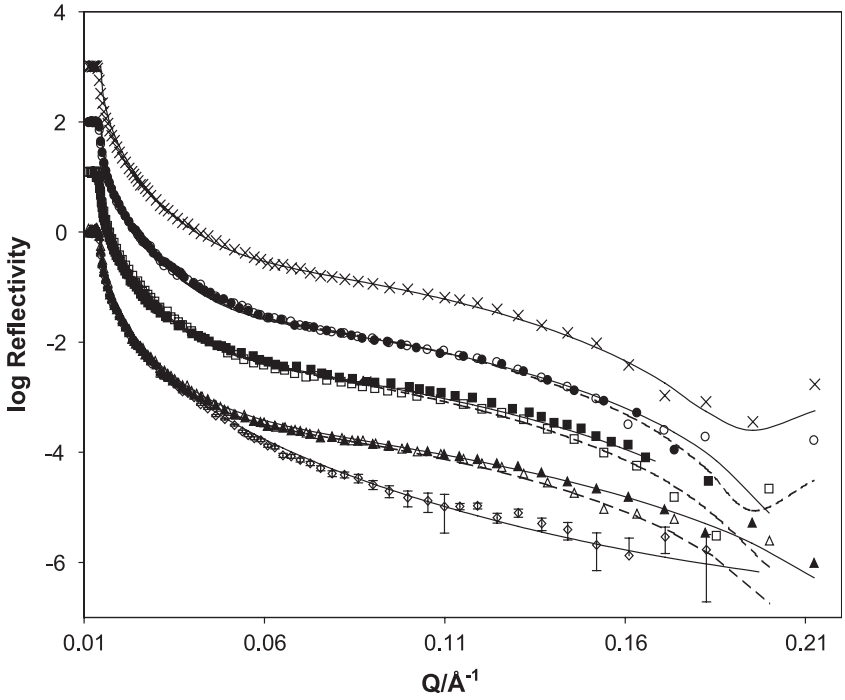

Fig. 2. Adsorption of 1:6 L-α-DOPC and β-D-Dodecyl maltoside mixtures at the Si/SiO_2–D_2O interface. (open diamonds) Substrate reflectivity, (filled triangles) 0.114 g/l, (open triangles) D_2O rinse after 0.114 g/l, (filled squares) 0.0114 g/l, (open squares) D_2O rinse after 0.0114 g/l, (filled circles) 0.00114 g/l, (open circles) D_2O rinse after 0.00114 g/l and (crosses) DOPC bilayer in 10 mM Tris–HCl at pH 7.4. Lines represent the reflectivity calculated using the parameters in Tables 1 and 2, and the markers are experimental data points. Representative error bars for the data points are shown on the substrate reflectivity profile.

entire illuminated area (typically 65×30 mm^2), and hence it is only possible to determine the average area available per phospholipid molecule on the surface, and it should be noted that this does not correspond to the actual volume occupied by each individual molecule. In a situation when the surface coverage is incomplete, we envisage (as has

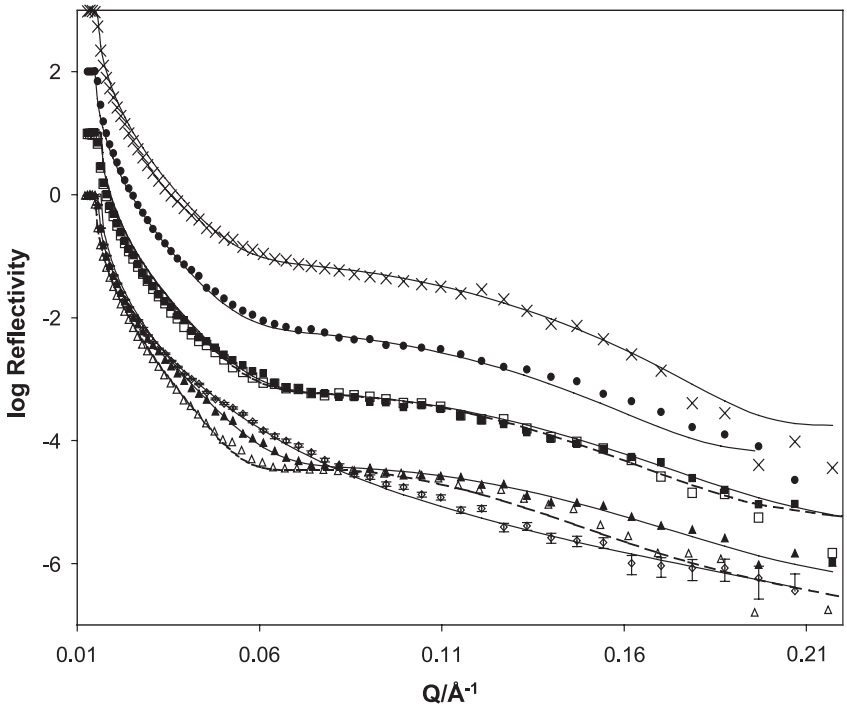

Fig. 3. Adsorption of 1:6 L-α-DOPC and d_{25}-β-D-Dodecyl maltoside mixtures at the Si/SiO_2–D_2O interface. (open diamonds) Substrate reflectivity, (filled triangles) 0.114 g/l, (open triangles) D_2O rinse after 0.114 g/l, (filled squares) 0.0114 g/l, (open squares) D_2O rinse after 0.0114 g/l, (filled circles) 0.00114 g/l and (crosses) DOPC bilayer in 10 mM Tris–HCl at pH 7.4. Lines represent the reflectivity calculated using the parameters in Tables 1 and 3, and the markers are experimental data points. Representative error bars for the data points are shown on the substrate reflectivity profile.

Table 2
Fitted parameters and calculated bilayer properties for DOPC–DDM mixtures

Concentration (mg ml^{-1})	Layer	ρ_α ($\times 10^{-6}$ Å^{-2})	d (Å)	ϕ	A$_a$ (Å^2)	Γ (mg m^{-2})
0.114	head	3.52±0.25	6±1	0.62±0.05	91±26	2.89±0.5
	chains	0.38±0.15	24±2	0.91±0.02	90±10	
D_2O rinse	head	3.79±0.25	7±1	0.56±0.05	86±23	3.00±0.5
	chains	0.91±0.15	27±2	0.83±0.02	88±9	
0.0114	head	3.56±0.25	7±1	0.61±0.05	79±20	3.30±0.5
	chains	0.05±0.15	26±2	0.96±0.02	79±8	
D_2O rinse	head	3.65±0.25	7±1	0.59±0.05	82±21	3.19±0.5
	chains	0.71±0.15	28±2	0.86±0.02	82±8	
0.00114	head	3.97±0.25	7±1	0.52±0.05	93±25	2.82±0.4
	chains	1.36±0.15	28±2	0.76±0.02	93±10	
D_2O rinse	head	3.88±0.25	7±1	0.54±0.05	89±24	2.92±0.5
	chains	1.36±0.15	29±2	0.76±0.02	89±9	
Tris–HCl 10 mM	head	3.47±0.25	6±1	0.63±0.05	89±26	2.92±0.5
pH 7.4 in D_2O	chains	1.36±0.15	29±2	0.76±0.02	89±9	

d=thickness, ρ_a=scattering length density of layer a with its associated solvent, ϕ=volume fraction of molecule, A_a=area per molecule, Γ=surface excess. Errors denoted are those derived from fitting errors in d and ϕ.

often been found to be the case by atomic force microscopy [28]) that the phospholipids occupy the surface in islands or as a continuous bilayer with defects. The lateral arrangement in such cases is known to be dependent on the lipid chain melting temperature, i.e., that lipids in a fluid bilayer are to some extent able to accommodate larger molecular areas by spreading uniformly over the available area, whereas lipids below their melting point are not. In using neutron reflection, the main point of our focus has been to determine the chemical composition of bilayers and to determine the surface coverage. Controlling the surface coverage of an adsorbed layer is of key interest in studying the association of proteins with supported bilayers, as many lipid–protein interactions are thought to be intimately governed by the lateral pressure profile in a bilayer [29–32].

4. Results

The adsorption of DOPC and POPC was studied in two contrast environments, with hydrogenated DDM in D_2O, and with chain-deuterated d_{25}-DDM in D_2O to determine the composition of the mixed bilayers. All experiments were performed at 25 °C and concentrations of 0.114–0.123, 0.0114–0.0123 and 0.00114–0.00123 g/l were used.

Fig. 2. shows the neutron reflectivity profiles of DOPC–DDM bilayers at all adsorption and rinsing stages, and Fig. 3 the same profiles for DOPC–d_{25}-DDM mixtures. In both cases some of the reflectivity profiles have been displaced by factors of 10, 100 and 1000 for clarity of the graphs. The general changes in reflectivity for each concentration and rinsing are similar, but the contrast in Fig. 3 is considerably lower as is expected for the DOPC–d_{25}-DDM bilayer. The scattering length density of d_{25}-DDM is 3.76×10^{-6} Å^{-2}, and its removal appears as a further lowering of contrast as it is replaced by D_2O. The changes in surface coverage become smaller with decreasing concentration reflecting a decreased surfactant content of the bilayer. Bilayer properties derived from the thickness and lipid volume fractions used to fit the reflectivity data are presented in Tables 2 and 3. Essentially, the main requirement used in fitting was that both data sets should conform to a similar surface coverage to within the reproducibility of bilayer formation, and as a result the average molecular area was found to be 90±5 Å^2 in both cases. The scattering length density of the hydrophobic chain region of DOPC–d_{25}-DDM was used to

Table 3
Fitted and calculated bilayer properties for DOPC–d_{25}-DDM mixtures

Concentration (mg ml^{-1})	Layer	ϕ(d_{25}-DDM)	ρ_a ($\times 10^{-6}$ Å^{-2})	d (Å)	ϕ	A (Å^2)	Γ (mg m^{-2})
0.123	head	0.345	3.61±0.25	6±1	0.60±0.05	94±29	2.78±0.8
	chains	0.345	2.49±0.1	22±2	0.95±0.02	94±12	
D_2O rinse	head	0.291	4.07±0.25	6±1	0.50±0.05	112±38	2.31±0.7
	chains	0.291	3.36±0.1	26±2	0.67±0.02	113±13	
0.0123	head	0.335	3.70±0.25	6±1	0.58±0.05	97±30	2.70±0.7
	chains	0.335	2.96±0.1	25±2	0.82±0.02	96±11	
D_2O rinse	head	0.322	3.70±0.25	6±1	0.58±0.05	97±30	2.70±0.7
	chains	0.322	3.11±0.1	27±2	0.76±0.02	96±11	
0.00123	head	0.317	3.15±0.25	5±1	0.70±0.05	96±33	2.71±0.8
	chains	0.317	2.98±0.1	26±2	0.79±0.02	96±11	
Tris–HCl 100 mM	head	0.275	3.52±0.25	6±1	0.62±0.05	91±28	2.87±0.8
pH 8 in D_2O	chains	0.275	2.69±0.1	27±2	0.80±0.02	91±10	

ϕ(d_{25}-DDM)=volume fraction of phospholipid occupied by DDM estimated from the scattering length density of the chain region, using Eq. (3).

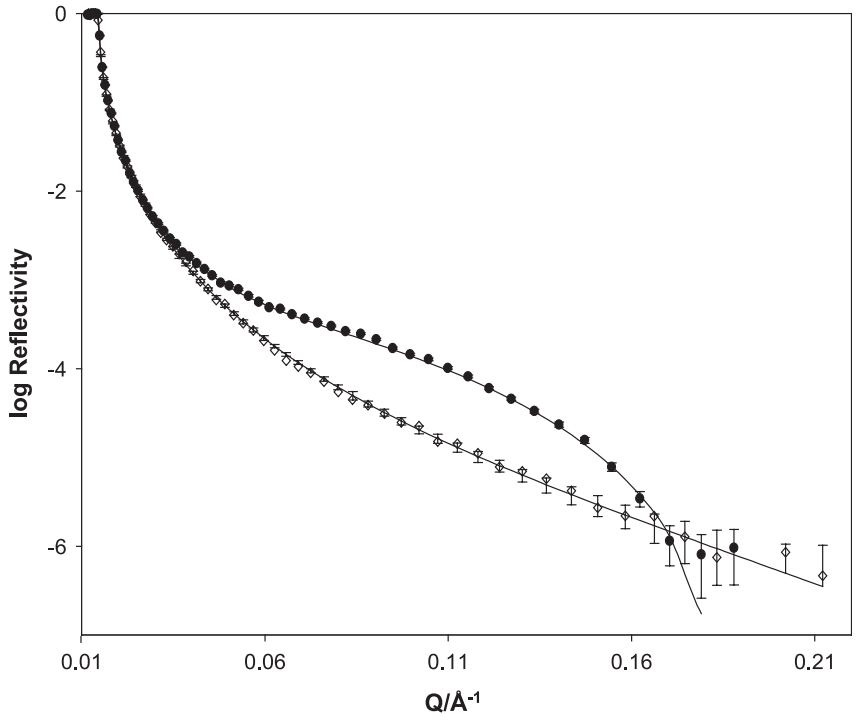

Fig. 4. Reflectivity from a DOPC bilayer at the Si–water interface, after the removal of all DDM. (open diamonds) Si–SiO_2 substrate in D_2O and (filled circles) DOPC bilayer in 10 mM Tris–HCl 2 mM $CaCl_2$ at pH 7.4. Lines represent the reflectivity calculated using the parameters in Tables 1 and 4, and the markers are experimental data points. Representative error bars for the data points are shown on the substrate reflectivity profile.

estimate the volume fraction of DDM present by using the relation in Eq. (3). The surfactant volume fractions listed in Table 3 are presented as the volume fraction of phospholipid occupied by the maltoside.

Although the composition of the mixed bilayer varies qualitatively in the same way as expected from ellipsometry experiments [17], it is obvious from this set of neutron data that not all the surfactant is removed, but that a volume fraction of 28% of the surfactant is present in the bilayer.

We found that the amount of surfactant in the bilayer and the phospholipid surface coverage are very sensitive to the volume-to-surface ratio of bulk solution used in adsorption and rinsing. In order to create a situation more similar to the ellipsometric experiments, where the surface to volume ratio is typically 20 times smaller, we doubled the volume used to exchange the sample cell contents at each stage. After giving a minimum of 1.5-h equilibration time at each adsorption step, the surface coverage of DOPC was found to be increased to 91%. By fitting the scattering length density of the hydrocarbon region, we found that in order to preserve the bilayer stoichiometry, there could be no observable trace of the deuterated surfactant present, as the scattering length density of the chain region was -0.28×10^{-6} Å^{-2}. The DOPC bilayer had a thickness of 44±2 Å, with a 72±7 Å area per DOPC molecule, which is in excellent agreement on the measurements of DOPC in its fully hydrated bulk phase [27]. The reflectivity of such a pure DOPC bilayer is shown in Fig. 4 and the properties derived from the fitting variables are summarised in Table 4.

Cryo-TEM was used to investigate the effect of concentration on the structure of the lipid–surfactant aggregates in the bulk solution. Fig. 5 shows representative Cryo-TEM micrographs of DOPC–DDM solutions at 0.114 and 5.814 g/l.

The dark beams in Fig. 5 are strands of the support grid, and the pale areas the frozen sample film in its holes. At

Table 4
Bilayer properties for DOPC–DDM mixture upon extended adsorption and rinsing

Concentration (g/l)	Layer	ρ_a (Å^{-2})	d (Å)	ϕ	A (Å^2)	Γ (mg m^{-2})
0.123	head	3.38±0.25	6±1	0.65±0.05	86±26	3.00±0.8
	chains	2.88±0.1	24±2	0.94±0.02	87±10	
0.00123	head	3.15±0.25	7±1	0.70±0.05	69±18	3.82±0.8
	chains	−0.7±0.2	29±2	1.00±0.02	68±7	
Tris–HCl 10 mM	head	3.15±0.25	7±1	0.67±0.05	72±19	3.62±0.8
pH 7.4 2 mM $CaCl_2$	chains	−0.28±0.1	30±2	0.91±0.02	72±7	

The cell volume was exchanged five times at each stage.

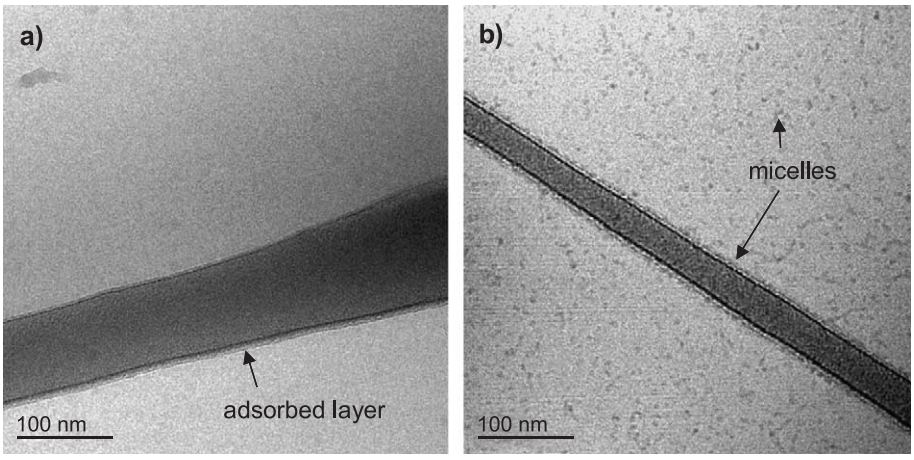

Fig. 5. Cryo-TEM micrographs of DOPC-β-D-dodecyl maltoside solutions vitrified on hydrophilic carbon supports. (a) 0.114 g/l and (b) 5.814 g/l. The adsorbed layer on a carbon grid beam and an adsorbed layer on micelles are indicated by the arrows in (a) and (b), respectively.

0.114 g/l, no micellar or any other bulk structures can be seen and only a 4–5-nm-thick "fuzzy" coating appears on the support surface in Fig. 5a). In contrast, at 5.814 g/l, the frozen solution has a uniform distribution of spherical micelles of 4–5-nm diameter, and some micelle-like aggregates are also clearly seen at the support–ice interface.

The adsorption of POPC with DDM differs from DOPC only in that a slightly thicker layer of 41±3 Å is formed, and the molecular areas are slightly larger at 102±5 Å. Reflectivity profiles of POPC–DDM and mixtures POPC–d_{25}-DDM were recorded at 0.00114–0.114 g/l, and as in the case of DOPC, without increasing the exchange volume of solution, a significant proportion (24%) of surfactant remained in the bilayer.

5. Discussion

The neutron reflection results on DOPC and POPC demonstrate that the composition and bilayer surface coverage are very sensitive to the concentrations and volumes of surfactant–lipid solutions used, and the volume-to-surface ratio of the experiment. The Cryo-TEM results indicate that at a large surface-to-volume ratio and low concentration, all phospholipids are adsorbed at the interface and any excess surfactant exists in solution as monomers. In such a situation, the composition at the surface will be different due to the depletion of micelles, compared to a situation where there is an equilibrium between the surface and bulk aggregates. At high concentration, however, adsorption of micellar aggregates was observed, demonstrating clearly that increasing the bulk concentration changes the structure the adsorbed layer. These observations are consistent with the fact that the surface composition in a binary surfactant mixture approaches the bulk micellar composition with increasing concentration [33]. It also indicates that as the surfactant content in the adsorbed layer increases, the aggregate structure also begins to resemble that in the bulk solution. As DDM has no affinity for the SiO_2 surface on its own, the bilayer stability decreases with increasing DDM content, and this is reflected in the neutron experiments, where a high residual DDM content was always observed simultaneously with a poor bilayer coverage.

These results complete an investigation of surface supported lipid bilayers by four independent techniques (ellipsometry, neutron reflection, Cryo-TEM and AFM), which together have yielded a consistent picture about the surface association of lipids and dodecyl maltoside. We have demonstrated that, using neutron reflection, it is possible to determine the composition of mixed bilayers to a high accuracy (±3%), giving the method confidence as a means of depositing surfactant-free phospholipid bilayers with tailored properties. It is evident that adsorption of micellar lipid–surfactant mixtures offers a simple and elegant method of varying bilayer composition and surface coverage quite precisely by varying the bulk concentration and exploiting monomer solubility differences between the two components. To be able to make supported bilayers with controlled properties by using simple self-assembly is highly interesting as these could be exploited as biological membrane models in surface biochemistry as well as substrates in practical applications, notably in biosensing.

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