Specular reflection of neutrons at phospholipid monolayers

Changes of monolayer structure and headgroup hydration at the transition from the expanded to the condensed phase state

T. M. Bayerl,* R. K. Thomas,‡ J. Penfold,§ A. Rennie,‖ and E. Sackmann*

*Technische Universität München, Physik Department E22, D8046 Garching, FRG; ‡Physical Chemistry Laboratory, University of Oxford, South Parks Road, Oxford, OX1 3QZ, England; §Rutherford-Appleton Laboratory, Oxford, OX1 3QZ, England; and ‖Institut Laue-Langevin, Centre de Tri, Grenoble, Cedex, France

ABSTRACT The specular neutron reflection technique has been applied for the first time to study the structure and head group hydration of a phospholipid monolayer (dimyristoylphosphatidylcholine containing negatively charged phospholipids) in the condensed and expanded state. By variation of the contrast of the subphase to that of the air it is shown that at the transition from the condensed to the expanded state the carboxyl bonds of the glycerol backbone are hydrated leading to a strong structural change of the headgroup. The total monolayer thickness in the condensed state is 22.5 ± 1 Å (tilt angle 32 ± 6°) and decreases to 19.5 ± 1 Å in the expanded state. The mean molecular volume increases from 1190 ± 50 Å^3 to 1250 ± 50 Å^3.

INTRODUCTION

The structure of pure monolayers of dimyristoylphosphatidic acid (Kjaer et al., 1987) and of dipalmitoylphosphatidylcholine (Helm, 1988) have been recently studied by x-ray reflection methods as a function of the lateral pressure π. In the present study specular reflection of neutrons is applied for the first time to study the structure of phospholipid monolayers at the air-water interface at various lateral pressures π. The particular advantage of neutrons over x-rays for such measurements is the higher sensitivity of the former to the distribution of protons and the applicability of contrast variation techniques to evaluate different layers of the monomolecular leaflet separately.

To get information on the headgroup hydration as a function of the lateral pressure and of the lipid phase state, two situations are considered. In one series of experiments the scattering length density of the subphase is matched to that of the air (contrast matched water [CMW]) whereas in a second series pure D_2O as subphase is used, yielding a strong contrast to the air.

A binary mixture of negatively charged phospholipids (dimyristoylphosphatidylglycerol [DMPG]) with noncharged phospholipids (dimyristoylphosphatidylcholine [DMPC]) was used. This mixture was chosen to study the adsorption of water soluble proteins to phospholipid monolayers containing charged lipids as nonspecific receptors by this method in subsequent experiments.

MATERIALS AND METHODS

L_α-perdeuterio dimyristoyl phosphatidylcholine (DMPC-d_{54}) and L_α-DMPG were obtained from Avanti Polar Lipids, Inc. (Birmingham, AL). Monolayers of a 7:3 DMPC-d_{54}/DMPG mixture were prepared by spreading a chloroform solution of the mixture (1 mg/ml) on a specially designed film balance (Lee et al., 1989) in an airtight container attached to the CRISP spectrometer at the ISIS neutron spallation source of the Rutherford Appleton Laboratory, England (Penfold et al., 1987).

The subphase buffer was a 30-mM $Na_2^2HPO_4/K^2H_2PO_4$ buffer, pH = 7.0, which contained 1 mM EDTA. The lateral pressure of the monolayer was varied for the reflection measurements in an ascending manner by adjusting the barrier position via a stepping motor. The pressure was constant within 6% over the time of the experiment (~3 h).

On the CRISP reflectometer a polychromatic neutron beam (wavelength from 0.5 to 6.5 Å) is incident on the monolayer at a fixed glancing angle of incidence of 1.5°. Neutron reflection from the monolayer is measured at the specular angle by a single detector. The wave vector dependence of the reflected neutron intensity is obtained by a time of flight analysis. The reflected intensity is converted to reflectivity by removal of the incident spectral shape (using an incident beam monitor), correction for detector efficiency, and reference to a standard scatterer (D_2O). Further experimental details are given by Lee et al. (1989).

The calculation of the specular reflectivity profiles can be done exactly for any model profile using the optical matrix method (Born and Wolf, 1970). In this paper we use the optical matrix method and optimize fits to the data using a nonlinear least squares routine (Penfold, 1989). All the reflectivity profiles reach a flat background at high momentum transfer Q (which corresponds to the incoherent scattering from the aqueous subphase); this flat background has been included in the model refinements.

For a rigorous analysis the monolayer would have to be divided into at least three layers of substantial different scattering length densities n_b: the hydrocarbon chain region, the glycerol backbone region, and the phosphatidyl headgroup. However, the comparison of one layer fits with multilayer fits has shown that the one layer model approximates well the specular reflection data over the whole Q range. Therefore the data of both series of experiments (for CMW and D_2O subphases) were analyzed in terms of this simple one layer model implying only two fitting parameters: the thickness d_{fit} of the total monolayer and the mean scattering length density n_b of the monolayer (Fig. 1). The introduction of surface roughness as a third parameter did not improve the quality of the one and two layer fits of the raw data and therefore has not been considered.

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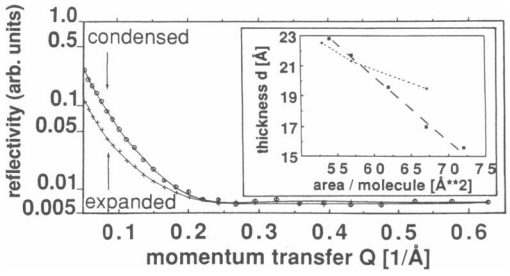

FIGURE 1 Reflectivity profile of a (7:3) DMPC-d_{54}/DMPG monolayer on a subphase of contrast matched water (CMW) in the condensed (*open circles;* π = 31 mN/m) and in the expanded phase state (+; π = 11 nM/m) at T = 20°C and pH = 7.0. The drawn lines represent the fits of the data to a single layer model. The inset shows d_{fit} vs. the molecular area (obtained from Fig. 2) for D_2O as subphase (*squares*) and for CMW as subphase (+).

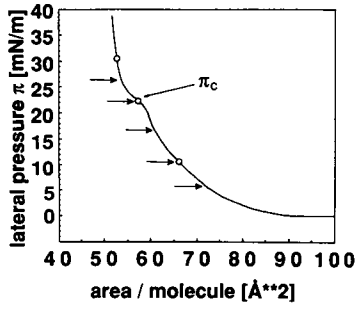

FIGURE 2 Pressure-area phase diagram of a (7:3) DMPC-d_{54}/DMPG monolayer on contrast matched water (T = 20°C, pH = 7.0). The arrows indicate the points of the isotherm at which specular reflection measurements with a D_2O subphase are performed whereas the open circles indicate those points where contrast matched water as subphase is used.

For the two-parameter fitting procedure (one layer model) initial values of the parameters were d = 20 Å and $n_b = 2 \cdot 10^{-5}$ Å^{-2}. The latter value is an estimate obtained by using the known values of the scattering length densities of the atoms (Jacrot, 1976) and an average number density of 0.95.

RESULTS AND DISCUSSION

The values obtained for d_{fit} and $n_{b,fit}$ by the one layer model are given in Table 1. Taking the area/molecule $A_{(\pi)}$ at the surface pressure π from the pressure area diagram of this binary mixture given in Fig. 2, the molecular volume V_m can be calculated as

$$V_m = d_{fit} A_{(\pi)} . \quad (1)$$

For CMW a decrease of V_m by 7% between the expanded (fluid) state and the condensed (solid) state is observed (cf. Table 1). This agrees well with the V_m changes observed for the L_α to L_β transition of bilayers of 5.5% (Knoll et al., 1985).

In contrast, for D_2O one observes an increase of V_m of 8% at the expanded to condensed transition. However, the V_m values obtained for the condensed state are in excellent agreement for both subphases (D_2O and CMW). This suggests that the one layer fits for the D_2O subphase are appropriate only at high surface pressures π (condensed state) whereas in the expanded (fluid) state physically unreasonable results are obtained. The invalidity of the one layer model for the expanded state with a D_2O subphase becomes also evident from the difference of the monolayer thicknesses d_{fit} obtained for CMW (19.5 Å) and D_2O (15.5 Å) at low π (Table 1). The much smaller value of d_{fit} for D_2O can be explained in terms of a deeper penetration of the phospholipid headgroups (phosphoryl headgroup and glycerol backbone) into the water subphase in the expanded (liquid) state. The penetration of D_2O into the headgroup region at low π is also suggested by the ~7% higher value of $n_{b,fit}$ obtained for the expanded (fluid) state (Table 1). In the case of the CMW as subphase, this effect does not contribute to the reflectivity at low π due to the zero contrast between the subphase and the air. As expected, for CMW the value of n_b decreases with π.

It is interesting to compare our values of d_{fit} with those

TABLE 1 Results of one layer fits of the neutron specular reflection data of DMPC/DMPG (7:3) monolayers on a contrast matched water (CMW) subphase and on a D_2O subphase

Phase state	Subphase	Lateral pressure π	Scattering length density	Monolayer thickness	Molecular area*	Molecular vol
		mN/m	$n_{b,fit}$ 10^6Å^{-2}	d_{fit} Å	$A_{(\pi)}$ Å^2	$V_m = d_{fit}A_\pi$ Å^3
expanded	CMW	11	2.38 ± 0.4	19.5 ± 1	66.0	1280
condensed	CMW	31	3.24 ± 0.4	22.5 ± 1	53.0	1192
expanded	D_2O	6	3.42 ± 0.4	15.5 ± 1	72.0	1101
condensed	D_2O	27	3.18 ± 0.4	22.8 ± 1	53.0	1208

*Obtained from Fig. 2.

obtained for oriented multilayers measured by neutron diffraction by Büldt et al. (1978). According to these authors, the thickness of one monolayer leaflet decreases from 22.0 to 19.5 Å at the transition from L_β, to the L_α phase which is in good agreement with the values d_{fit} obtained for CMW (Table 1). However, in the case of D_2O only the value of d_{fit} in the condensed state agrees well with the multilayer data whereas d_{fit} in the expanded state is ~5 Å smaller than the expected value of d = 19.5 Å.

To account for the penetration of water into the monolayer a two layer model was applied to fit the data of the D_2O subphase in the expanded state. Because this fit requires at least four adjustable parameters, two are chosen from the data for CMW in Table 1 and from the results of bilayer studies. The thickness of the chain region is taken as d_{tail} = 12 Å as suggested by Büldt et al. (1978) for the L_α phase and the average scattering length density of the fatty acyl chains ($n_b^{tail} = 0.35 \cdot 10^{-5}$ Å^{-2}) is calculated using the data of Jacrot (1976). Because the total thickness of the monolayer in the expanded state is d = 19.5 Å (Table 1), the thickness of the headgroup region is $d_{head} \sim 8$ Å.

With the above assumptions the only adjustable parameter is the average scattering length density n_b^{head} of the headgroup region. It was found that a two layer model considering these parameters fits well the experimental data for D_2O as subphase in the expanded (fluid) state. If α is the volume fraction of water in the headgroup region in the expanded state, it follows that

$$\langle n_{b,fit}^{head} \rangle = \alpha\, n_b^{D_2O} + (1 - \alpha) n_{b,calc}^{head}, \qquad (2)$$

where $n_{b,calc}^{head} = 0.1 \cdot 10^{-5}$ Å^{-2} is the calculated value of the average scattering length density of the headgroup region without water and $n_b^{D_2O} = 0.635 \cdot 10^{-5}$ Å^{-2} is that of pure D_2O. The best two layer fit (without additional surface roughness) yields for $n_{b,fit}^{head}$ $0.507 \cdot 10^{-5}$ Å^{-2} thus α = 0.76. This considerable amount of water strongly suggests that in the expanded phase state of the monolayer the carboxyl bonds of the glycerol backbone are also hydrated. This implies that the monolayer phase transition from the expanded to the condensed state is associated with a drastic change of the headgroup configuration in the glycerol backbone region, in addition to the chain melting which is known to give rise for the increase of the headgroup area. A possible model which illustrates these changes is shown in Fig. 3. For simplicity, this model considers only changes in the conformation of the glycerol backbone but not the chain tilt in the condensed state or the decreased acyl chain disorder in the expanded state.

The tilt of the fatty acyl chains in the condensed state can be estimated using the known length of a myristoyl chain in an all trans conformation of d_{tail}^{max} = 16.8 Å and the length of the chain region d_{tail} of the monolayer obtained by a two layer fit of the data for the D_2O subphase at highest π (condensed state) as d_{tail} = 14.2 ± 1 Å (d_{head} = 8 ± 1 Å). The tilt angle β can be calculated according to

$$\cos \beta = d_{tail}/d_{tail}^{max}, \qquad (3)$$

which yields b = 32° ± 6°. This value is in good agreement with those obtained by Helm (1988) for DPPC monolayers using x-ray reflection methods.

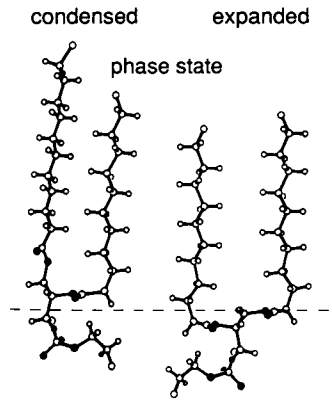

FIGURE 3 Sketch of conformational changes in the glycerol backbone region of the phospholipids in a monolayer at the transition from the expanded (fluid) to the condensed (solid) phase state. The dotted line indicates the border up to which water from the subphase can penetrate into the headgroup region.

CONCLUSIONS

The present results demonstrate that specular reflection of neutrons in combination with contrast variation can provide valuable information about the structure and the degree of hydration of monolayers at the air-water interface. In addition to the variation of the subphase contrast, the use of phospholipids with deuterated headgroups would provide more detailed information about the structure and changes of the degree of hydration at the monolayer phase transitions (Albrecht et al., 1978; Fischer and Sackmann, 1984).

The present study provides the basis for current studies of the adsorption of proteins such as spectrin to phospholipid monolayers containing charged phospholipids as nonspecific receptors.

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