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Conformational changes of the lecithin headgroup in monolayers at the air/water interface

A neutron reflection study

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Abstract. The headgroup conformation of the phospholipid dipalmitoyl-glycero-phosphocholine (DPPC) in monolayers at the air/water interface has been studied by neutron reflection in the fluid like liquid-expanded (LE) and in the crystal like solid (S) phase. Information on the headgroup conformation in the two phases has been obtained by scattering contrast variation of the lipid monolayer using four differently deuterated species of DPPC: perdeuterated, chain perdeuterated, choline group perdeuterated and selectively headgroup deuterated. Since the measurements were done mainly on a subphase of null reflecting water (i.e. water scattering contrast matched to the air) there is no subphase contribution to reflectivity and the simplest one layer model can be employed for the data analysis, thus minimising the number of free parameters. A remarkable change of the headgroup orientation was observed between the LE and the S phase. We found that the phosphate-nitrogen dipole of the DPPC headgroup exhibits an in-plane orientation with respect to the monolayer in the LE phase but it assumes a more parallel orientation to the surface normal at lateral pressures above 30 mN/m (S phase). Moreover, this conformational change is accompanied by a significant alteration of the headgroup hydration.

Key words: Lipid monolayer – Lecithin – Headgroup orientation – Phase transition

Abbreviations: DPPC, Dipalmitoyl-Phosphatidylcholine; DMPC, Dimyristoyl-Phosphatidylcholine; DPPE, Dipalmitoyl-Phosphatidylethanolamine; DMPE, Dimyristoyl-Phosphatidylethanolamine; DMPA, Dimyristoyl-Phosphatic Acid; DMPG, Dimyristoyl-Phosphatidylglycerol

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Introduction

Neutron and X-ray reflectivity measurements on phospholipid monolayers at the air/water interface have emerged as powerful tools for the investigation of such soft surfaces on a molecular scale (Penfold and Thomas 1990; Möhwald 1990). In particular, the method provides detailed information about the monolayer thickness and the volume of different interfacial regions as well as about the lattice constant and the chain tilt angle of the monolayer in the liquid condensed phase. Several different phospholipid monolayers have been studied to date and include the zwitterionic lipids DPPC (Helm et al. 1987; Vakinin et al. 1991), DMPC (Bayerl et al. 1990), DMPE and DPPE (Helm et al. 1991; Böhm et al. 1993) as well as the electrically negatively charged lipids DMPA (Helm et al. 1987; Kjaer et al. 1988) and DMPG (Johnson et al. 1991).

An important question which has not so far been addressed is about the conformation of the phospholipid headgroup in the liquid-expanded (LE) phase at low lateral pressure π on one side and the high π phases (liquid condensed (LC) and solid (S) phase) on the other side. There are a number of studies which indicate that the hydration properties of the monolayer headgroup region change significantly with π (Vakinin et al. 1991; Bayerl et al. 1990; Johnson et al. 1991). This suggests that changes of the headgroup conformation could be associated with or could even be the driving force which leads to a partial dehydration of the headgroup in the liquid condensed phase. For phospholipid bilayers in the liquid crystalline state, a correlation between the average orientation of the phosphocholine headgroup with respect to the surface normal and the hydration level has been reported recently on the basis of NMR measurements (Bechinger and Seelig 1991).

In this work, we present neutron specular reflection data obtained from DPPC monolayers at low and high lateral pressure (corresponding to the LE and the S phases (Albrecht et al. 1978) which may provide for the

first time information about changes of the lipid headgroup conformation with π. In order to obtain as many scattering contrasts as possible, our experiments were done by employing four isotopically distinct species of DPPC, abbreviated in the text as follows: 1) DPPC-d75: perdeuterated tail (62 deuterons) and deuterated choline head (13 deuterons), the only protons are located in the glycerol backbone (5 protons). 2) DPPC-d62: perdeuterated tail. 3) DPPC-d13: fully deuterated choline headgroup. 4) DPPC-9: selectively deuterated choline headgroup: the aminotrimethyl group is deuterated.

Neutron reflectivity measurements on monolayers consisting of one of these four DPPC species at high and low lateral pressure π can provide the following information. DPPC-d75 on a subphase of water contrast matched to air (CMA) enables a total thickness determination of the monolayer with highest accuracy due to the large scattering contrast of the lipid to the air and to the subphase. DPPC-d62 will provide essentially the tail region thickness and (if used on a D_2O subphase) the degree of headgroup hydration. For DPPC-d13 and DPPC-d9, the reflectivity will be determined mainly by the headgroup conformation since a significant change of the phosphate-nitrogen (P–N) dipole orientation of the headgroup can be expected to change the thickness and the scattering length density ϱ.

Materials and methods

All lipids were obtained from Avanti Polar Lipids (Alabaster, Al., USA). The purity of the lipids was checked by measuring their main phase transition and its width by DSC. For all lipids a transition width (HWHM) of less than 0.05 °C was obtained, indicating a high purity. A sealed and thermostated Langmuir trough (size 15×80 cm), equipped with computer control for the barrier position and for the Wilhelmy plate pressure detector was used to measure the isotherms. The temperature was adjusted by an external water bath thermostat. The monolayers were prepared on ultrapure water by spreading a chloroform solution of the lipids.

Neutron reflectivity measurements were made on the CRISP Reflectometer (Penfold et al. 1987) of the Rutherford Appleton Laboratories (Didcot, UK). The experimental setup and preparation procedures have been described in detail previously (Johnson et al. 1991). Surface roughness has not been included for data analysis, since its consideration did not improve the quality of the fits to the data. Reflectivity data were analysed by employing the optical matrix method (Penfold and Thomas 1990; Russel 1990). The computer controlled film balance used for these measurements has a trough size of 15×45 cm and the temperature of the measurements was 20 °C. The incoherent background for each sample measured on null reflecting water (8% D_2O, 92% H_2O), which is uniform over the measured momentum transfer range, was obtained from model fitting of the data measured at high lateral pressure (S phase). The background value giving the best fit of the S phase data in terms of a single layer model was used for the analysis of the reflectivity curve of the same sample at low lateral pressure (LE phase). This approach has been chosen for the extreme sensitivity of the model fitting results to the correct background value for low contrast samples (headgroup deuterated lipids at low lateral pressure π). The sample with the lowest sensitivity for background changes is the fully deuterated DPPC-d75, giving the highest scattering contrast of a null reflecting water subphase at high π. Combining the results of single layer fits to DPPC-d75 reflectivity data (d_3 and ϱ_3, cf. Fig. 2) with the theoretical scattering length b_m for this molecule (Table 2), enables the calculation of the area per molecule for the LE and the S phases. We found the molecular area calculated from the reflectivity data in excellent agreement with those experimentally adjusted (A^{exp}) by the barrier position of the film balance. Therefore, we used the experimentally adjusted molecular area values for the calculation of the scattering length of the layer: $b_M = \varrho^{fit} \cdot d^{fit} \cdot A^{exp}$, where ϱ^{fit}, d^{fit} are the results of a single layer fit to the data. We compared these values with DPPC crystal structure data (Pearson and Pascher 1979) to identify the region of highest scattering contrast gradient inside the layer. Attempts to fit the CMA data by the two-layer models did not improve the fitting quality; nor did this procedure provide significant changes in thickness and scattering length density.

Results

Pressure area (π-A) diagrams of the various DPPC species used in this work on CMA as subphase are shown in Fig. 1. It can be clearly observed that the position of the main phase transition is shifted to higher π with increasing degree of deuteration of DPPC. DPPC-d13 has been omitted in Fig. 1 as its isotherm is nearly indistinguishable from that of DPPC-d9. In general, the effect of iso-

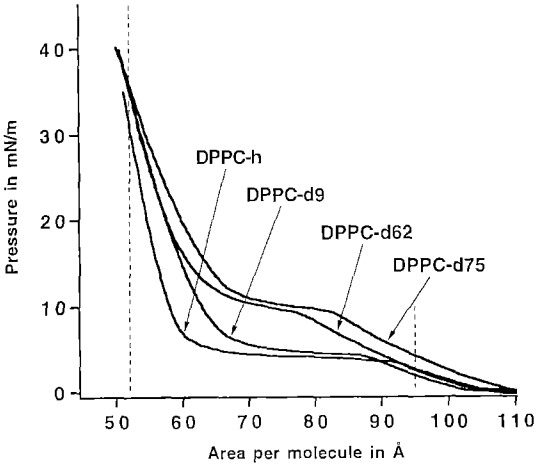

Fig. 1. Pressure-area isotherms of DPPC monolayers with various degrees of selective deuteration (see text for details) measured on a subphase of water contrast matched to air (CMA). For comparison, the isotherm obtained for fully protonated DPPC (DPPC-h) is shown. All measurements were done at 20 °C, the two vertical dotted lines indicate the points at which neutron reflection measurements were performed

topic substitution on the isotherms is most pronounced in the coexistence region. Therefore we performed our measurements at both ends of the isotherms (corresponding to molecular areas of DPPC of 52 Å^2 and 95 Å^2, respectively, as indicated in the figure), where the monolayer is in a well defined phase and the isotope effects are negligible.

All results of our reflectivity measurements, obtained by fitting the data according to one and (for D_2O subphases) two layer models, respectively, are summarized in Table 1, the abbreviations used there for the different thicknesses are explained in Fig. 5.

DPPC-d75 on CMA

Reflectivity curves of DPPC-d75 are shown in Fig. 2 for both phases (LE and S phase), along with the results of a single layer model fit of the data. It can be seen that this simple model is sufficiently to provide satisfactory fits to the data. Note that the bump of the fitted curve for the S phase data at $q \approx 0.3$ Å^{-1} is within the statistical error of the data in this q range. Introduction of 1 Å surface roughness causes the bump to disappear. However, since we found that this procedure does not improve the fitting quality in the statistically relevant q-regions but increases the number of free parameters, we refrained from using surface roughness through this work.

The fitting results are given in Table 1. We obtain at high pressure (S phase) a monolayer thickness $d_{3,S} = 26.5 \pm 0.5$ Å. This value agrees within the experimental errors with the $d_{3,S} = 25.3$ Å reported earlier for DPPC-d62 monolayers using the same technique (Vakinin et al. 1991). At low pressure (liquid expanded phase, LE) a reduction of d_3 by 5.5 Å ($d_{3,LE} = 21.0 \pm 0.5$ Å) is observed. The values obtained for the scattering length densities ϱ_3 imply that the molecular volume V_m is higher in both phases than the one we used on the basis of lipid bilayer data in the literature (Knoll 1981; Nagle and Wilkinson 1978) for the calculation of ϱ_3 (cf. Table 2). The introduction of an excess volume of 17.5% at high- and 29% at low pressure, respectively, accounts for this deviation. There are two explanations for this excess V_m. One is that water may penetrate the headgroup region in a different way in bilayers. On the other hand monolayer defects due to the non-uniformity of the molecular tilt direction in the S phase have to be considered. It has been shown by electron diffraction that small angle grain boundary defects are abundant in the S phase (Fischer and Sackmann 1986). Moreover, local defects are far more likely in monolayers than in multibilayers owing to the lower dimensionality of the former and may significantly contribute to V_m provided that the domain size is smaller than or comparable to the coherence area of the reflectivity experiment.

The drastic increase of ϱ_3 between low and high π by about 30% (Table 1) is consistent with data obtained previously on a DMPC/DMPG monolayer (Bayerl et al. 1990) and indicates a significant reduction of V_m. Taking into account the bulky choline headgroup of DPPC, this reduction of V_m might be an indication of a conformational change of the headgroup in addition to the changes of the tail region in order to accomodate the DPPC molecule for the higher packing density in the liquid condensed phase.

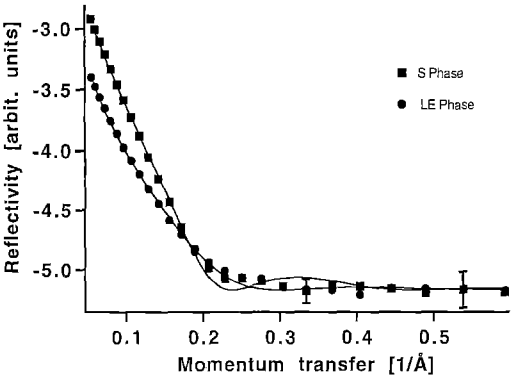

Fig. 2. Reflectivity profiles of nearly fully deuterated DPPC-d75 on CMA for the LE (●) and the S (■) phase. The full lines represent fits to the data according to the most simple single layer model, the fitting results are given in Table 1. The statistical error of the data for $q < 0.2$ Å^{-1} does not exceed the size of the symbols used in the plot, the error bars are representative for higher q values

Table 1. Results of the data analysis of the neutron reflectivity curves for one layer (CMA subphase) and two layer (D_2O subphase) model fits. The meaning of the different thicknesses d_i ($i = 1, 2, 3$) is explained in Fig. 5 and the ϱ_i are the scattering length densities belonging to the layer d_i

Lipid	Phase	Thickness [Å]	Scattering length density [10^{-6} Å^{-2}]
One layer model fits (CMW subphase)			
DPPC-d75	S	$d_3 = 26.6$	$\varrho_3 = 6.1$
DPPC-d13	S	$d_2 = 11.5$	$\varrho_2 = 3.1$
DPPC-d9	S	$d_2 = 11.5$	$\varrho_2 = 2.5$
DPPC-d75	LE	$d_3 = 21.0$	$\varrho_3 = 4.35$
DPPC-d13	LE	$d_2 = 7.7$	$\varrho_2 = 2.5$
DPPC-d9	LE	$d_2 = 6.5$	$\varrho_2 = 2.25$
Two layer model fits (D_2O subphase)			
DPPC-d62	S	$d_1 = 15.2$	$\varrho_1 = 6.75$
DPPC-d62	S	$d_2^* = 16.5$	$\varrho_2^* = 4.85$
DPPC-d62	LE	$d_1 = 13.5$	$\varrho_1 = 5.3$
DPPC-d62	LE	$d_2^* = 9.2$	$\varrho_2^* = 5.15$

DPPC-d13 and DPPC-d9 on CMA

Neutron reflection data and single layer model fits to them for DPPC-d13 are shown in Fig. 3 A for both phases. Since DPPC-d9 gives very similar reflection curves we refrained from showing them here. Both headgroup deuterated DPPC molecules give a thickness of the headgroup of $d_{2,S} = 11.5 \pm 1.5$ Å in the S phase and of $d_{2,LE} = 7.7 \pm 1.5$ Å (DPPC-d13) and $d_{2,LE} = 6.5 \pm 1.5$ Å (DPPC-d9) in the LE phase of the monolayer. This re-

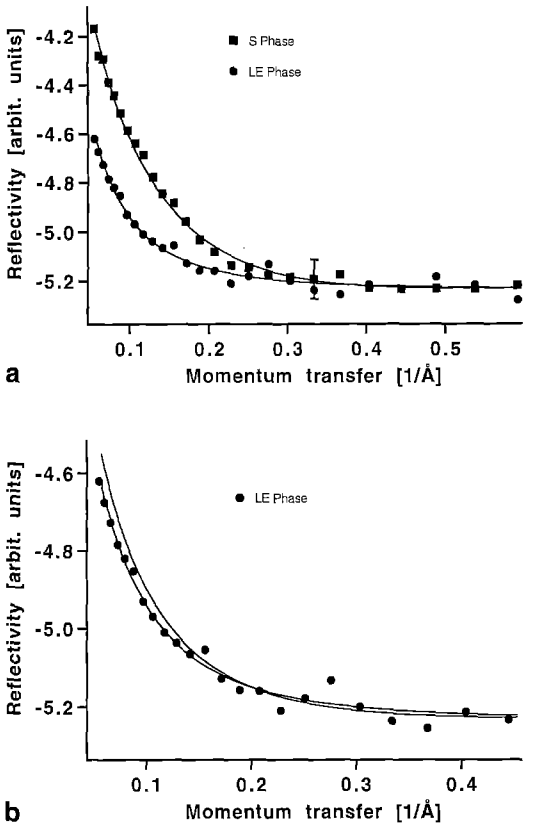

Fig. 3. **A** Reflectivity profiles of headgroup deuterated DPPC-d13 on CMA for the LE (●) and the S (■) phase. The full lines represent fits to the data according to a single layer model. **B** Comparison of the reflectivity data and fit for the LE phase from Fig. 3A with a simulation where it was assumed that the DPPC head group conformation of the S phase is retained in the LE phase (see text for details). The statistical error of the data for $q < 0.2$ Å^{-1} does not exceed the size of the symbols used in the plot

Table 2. Theoretical scattering length (b_M) and scattering length densities (ϱ) of the various selectively deuterated DPPC used in this work. Scattering lengths (Jacrot 1976) and volumes (Knoll 1981; Nagle and Wilkinson 1978) for the calculation of the scattering length densities were taken from the literature

DPPC species	b_M [10^{-4} Å]	ϱ [10^{-6} Å^{-2}]
DPPC-d75		
Total	80.9	6.6
Tail: 2 · [CD_3–$(CD_2)_{14}$]	61.3	7.1
Head: $C_{10}D_{13}H_5O_8PN$	19.6	5.4
DPPC-d62		
Total	67.3	5.6
Tail: 2 · [CD_3–$(CD_2)_{14}$]	61.3	7.1
Head: $C_{10}H_{18}O_8PN$	6.0	1.75
DPPC-d9		
Total	12.1	1.0
Tail: 2 · [CH_3–$(CH_2)_{14}$]	−3.25	−0.4
Head: $C_{10}D_9H_9O_8PN$	15.4	4.4
DPPC-d13		
Total	16.3	1.38
Tail: 2 · [CH_3–$(CH_2)_{14}$]	−3.25	−0.4
Head: $C_{10}D_{13}H_5O_8PN$	19.55	5.4

duction Δd_2 by 4–5 Å in the headgroup thickness at the transition to the LE phase indicates a drastic conformational change of the headgroup. The thickness of the latter, estimated on the basis of the crystal structure of DMPC (Pascher et al. 1987) is $d_{h,\,calc} \approx 9$ Å (starting at the phosphate group) assuming a P–N dipole in the normal direction and $d_{h,\,calc} \approx 5$ Å for the P–N dipole in the plane of the surface. Both values are approximately 2.5 Å below the measured thicknesses. This shows that the scattering contrast boundary for headgroup deuterated DPPC is not located directly below the phosphate group but extends into the glycerol backbone region. There the ester oxygens render the scattering length of this segment ($2.3 \cdot 10^{-4}$ Å) similar to that of the phosphate group ($2.8 \cdot 10^{-4}$ Å). As there are strong indications that the glycerol backbone itself undergoes a conformational change at the transition (Bayerl et al. 1990), the observed thickness change of the headgroup is most likely caused by conformational changes both in the headgroup and in the backbone. The contribution of the latter can be estimated from the length of the glycerol backbone (projected to the normal) of 2–3.5 Å (depending on its conformation), which leads us to expect a contribution to Δd_2 of up to 1.5 Å. This is mainly caused by a modification of the scattering contrast boundary owing to a shift of the carboxyl oxygens coupled to the backbone.

Figure 3B shows a comparison of the data from Fig. 3A for the LE phase with a simulation based on the assumption that the head group conformation of the S phase is retained in the LE phase. This was achieved by keeping the value $d_{2,S} = 11.5$ Å fixed and calculating the scattering length density for this simulation from the molecular area (90 Å^2 as obtained from the film balance measurement) and the theoretical scattering length of the DPPC-d13 headgroup (Table 2). It can be clearly seen from Fig. 3B that under these conditions a satisfactory fit of the LE phase data cannot be achieved.

For fluid-crystalline DPPC multilayers at low (6 wt.-%) hydration and with the P–N dipole oriented perpendicular to the normal, a distance between the carbon at the β position of the chain and the choline methyls (corresponding roughly to d_2) of 6.3 ± 1 Å has been measured by neutron scattering (Büldt et al. 1978). Within the errors of the measurements, this agrees well with our value of d_2 for the LE phase (Table 1). This shows that for the LE phase (but clearly not for the S phase) the P–N dipole is oriented in a similar way as in bilayers.

The ϱ_2 values obtained for the S phase indicates that even in this highly ordered state a significant amount of water must surround the headgroup (see DPPC-d62 data, below). The reduction of ϱ_2 due to the S to LE transition accounts in part for the apparent volume change of the headgroup due to the increased water content of this region in the LE phase (see below). It is interesting to note that this change is nearly twice as high for DPPC-d13 compared to DPPC-d9 (Table 1). This finding can be understood in terms of the anticipated alteration of the P–N dipole orientation: As the P–N dipole changes from parallel (S phase) to in-plane (LE phase) orientation, the region of highest gradient in ϱ is shifted from the glycerol backbone to the phosphate group. Thus the

relative change in scattering length density $\Delta\varrho/\varrho = \Delta b_M/b_M - \Delta V_M/V_M$ between DPPC-d13 and DPPC-d9 is to a large extent caused by $\Delta b_M/b_M$. On the basis of this rather crude model consideration we obtain $\Delta b_M/b_M \approx 17\%$ (DPPC-d13) and $\approx 11\%$ (DPPC-d9) which compares well with the values of $\Delta\varrho/\varrho \approx 20\%$ (DPPC-d13) and $\approx 10\%$ (DCCP-d9) from Table 1.

DPPC-d62 on D_2O and CMA

These measurements were performed in both phases in order to obtain an estimate for (i) the conformational changes in the tail region and (ii) the change of the headgroup hydration. Reflectivity profiles for both phases and D_2O as subphase are given in Fig. 4. The data were fitted by assuming a two layer model. Since the D_2O contributes strongly to the reflectivity and can penetrate into the monolayer down to the chain carbonyls, the location of the scattering contrast boundary will be different to the measurements on CMA. For the D_2O case, this boundary will be rather sharp and located near the carbonyls. Thus a headgroup thickness $d_2^* > d_2$ can be expected while the value d_1 will reflect sensitively the fatty acyl chain thickness in the normal direction.

We obtain $d_{1,S} = 15.2 \pm 1$ Å and $d_{1,LE} = 13.5 \pm 1$ Å for the S and LE phase, respectively. Assuming a tilt angle of the chains of $\beta = 30°$ (Vakinin et al. 1991) a total chain length in the S phase of $l_{chain,S} = 17.6$ Å can be calculated, which is 1.5 Å less than the theoretical lengths of a C-16 chain in all trans conformation (Tanford 1973). There are a number of possible explanations for this discrepancy. 1) There might some gauche kinks left in the S phase. For multibilayers, the presence of gauche kinks in the gel phase has been demonstrated recently (König et al. 1992). 2) Local defects due to the conflict between headgroup packing (preferentially orthorhombic) and chain packing (preferentially triangular lattice) may contribute, as well as small angle grain boundary defects owing to a non-uniformity of the tilt angle direction (Fischer and Sackmann 1986). 3) The mutual displacement of the two chains in the crystalline state by $\Delta_{chain} = 3.7$ Å (Pearson and Pascher 1979) renders the chain/air scattering contrast boundary less sharp (cf. Fig. 5). Considering both the displacement and the chain tilt, a shift of this boundary towards the interface (and consequently a reduction of d_1) by 0.5 $(\Delta_{chain} \cos\beta) = 1.6$ Å can be expected, which is consistent with our result.

The change of d_1 between the two phases by $\Delta d_1 \approx 1.5 \pm 1$ Å is probably caused by two mechanisms. First, there is the formation of gauche kinks along the chain at the transition to the LE phase. Second, the concomitant change of the glycerol backbone conformation (see discussion above) effectively cancels out the chain displacement Δ_{chain}, rendering the scattering contrast of the deuterated chains at both ends less diffuse. Both mechanisms lead to a net reduction of d_1 in spite the loss of chain tilt, where the latter alone would cause an increase of d_1 by 2.4 Å. Hence, the total length reduction of the acyl chain can be estimated to be 2.4 Å + $\Delta d_1 \approx 4$ Å.

For d_2^*, which is d_2 modified by the contribution of the water (D_2O) penetrating the headgroup we obtain $d_2^* = 16.5 \pm 2$ Å (S phase) and $d_2^* = 9.2 \pm 2$ Å (LE phase), respectively. The d_2^* for the S phase is approximately 40% larger than measured with DPPC-d13 owing to the strong contribution of the D_2O in this region to the reflectivity. Thus, d_2^* is not necessarily a measure of the headgroup thickness but rather of the water distribution width in this region. Nevertheless, the ϱ values of this region should be sensitive to changes of the headgroup hydration. The increase of ϱ_2^* by 20% at the transition to the LE phase indicates a drastic increase of water around the DPPC headgroup. Using the known volume of water (30 Å^3), the experimental and theoretical ϱ values (Tables 1 and 2) and the thickness d_2^* we can estimate that the water content of this region changes from 21 water molecules per lipid (LE phase) to 14 in the S phase, i.e. by 30%.

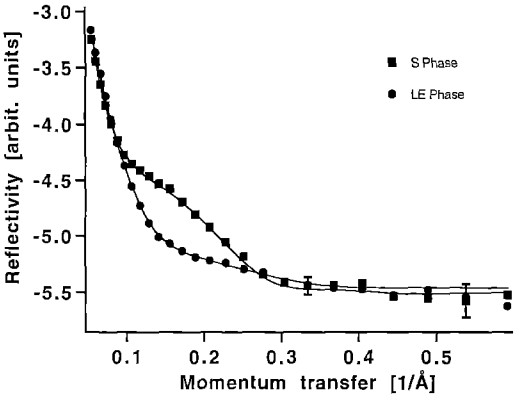

Fig. 4. Reflectivity profiles of chain perdeuterated DPPC-d62 on a D_2O subphase for the LE (•) and the S (■) phase. The full lines represent fits to the data according to a two layer model, the results are given in Table 1. The statistical error of the data for $q < 0.2$ Å^{-1} does not exceed the size of the symbols used in the plot, the error bars are representative for higher q values

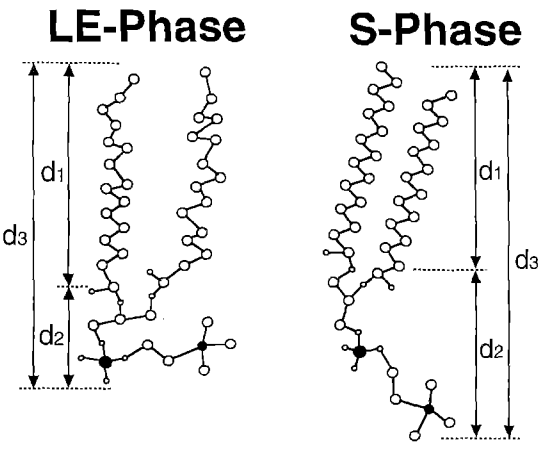

Fig. 5. Schematic drawing of the two suggested conformations of the lipid headgroup in the liquid expanded (LE) phase (*left*) and in the liquid condensed (S) phase (*right*). Protons were omitted in this sketch. The range which corresponds to the thicknesses d_1, d_2 and d_3 as used in the text is indicated, the direction of the arrows corresponds to the normal direction of the monolayer

Discussion

A good confirmatory check of the thickness data is obtained by a comparison of the total monolayer thicknesses (DPPC-d75 results) with the obtained segmental thicknesses. For the S phase, we have $d_3 = 26.5$ Å (DPPC-d75) in excellent agreement with the sum of d_1 (DPPC-d62) and d_2 (DPPC-d13) which gives 26.7 Å. A similar good agreement is obtained for the LE phase.

Combining the above results, we obtain the following picture of the DPPC monolayer structure. The total monolayer thickness (d_3) increases by $\Delta d_3 = 5.5 \pm 1$ A when compressing the monolayer from 95 Å^2 to 52 Å^2. Since the tail thickness (d_1) can account only for a $\Delta d_1 \approx 2$ Å increase, the remaining 3–4 Å must be caused by a conformational change of the DPPC headgroup. The only conformational change of the headgroup which could cause such a thickness increase is associated with a change of the average orientation of the P–N dipole of the phosphocholine group. Our data provide evidence that, in the liquid expanded phase, this orientation is approximately perpendicular to the surface normal while in the S phase the dipole adopts a rather parallel orientation to the normal (as depicted in Fig. 5): This interpretation relies mainly on the comparison of the DPPC-d75 and DPPC-d62 data with the DPPC-d13 and DPPC-d9 results, which enables us to distinguish clearly different conformational changes in both headgroup and tails. Comparing the length of the P–N dipole of 4.5 Å with the observed thickness change of the headgroup of $\Delta d_2 = 3.8$ Å and considering the experimental errors as well as possible contributions from the glycerol backbone conformation, we can conclude that the angle θ of this dipole with the normal undergoes a change $\Delta\theta$ by at least 60° between the two phases. A controversial question is about the contribution of surface roughness to the observed changes between the two phases. For pure water, a roughness amplitude of 3 Å has been measured and ascribed to capillary waves. Since the lipid monolayer exhibits a bending stiffness even in the LE phase, it will damp the thermally excited waves so that amplitudes < 3 Å can be expected. In the S phase, where solid domains with an order of magnitude higher bending stiffness than in the LE phase exist, capillarity roughness of the surface will disappear almost completely. Hence, the maximum contribution to be expected from roughness changes is of the order of 2 Å. However, its contribution could be measured only if the water itself contributes to the reflectivity. This is not the case in our measurements, since null reflecting water was used. Moreover, attempts to fit the data by including a surface roughness of 2 Å for the LE phase didn't change the fit results and didn't improve the fit quality.

Our data do not allow us to assign exactly the site at which a rotation of the chemical bond causes the observed straightening of the headgroup. However, from the x-ray experiments performed on DPPC (Vakinin et al. 1991; Helm et al. 1987) it seems likely that the phosphorus atom itself is not involved in this rotation, since x-ray reflectivity is extremely sensitive to changes at this atom. Hence, the most likely site for this rotation is between the phosphorus and the α-methylene group of the choline, but there might be more than one bond involved.

Our suggested model of DPPC headgroup conformation for monolayers in the S phase is inconsistent with findings observed by small angle neutron scattering (SANS) for phospholipid multilayers, where a perpendicular orientation of the dipole with respect to the normal in both phases, independent of hydration, has been reported (Büldt et al. 1978). However, a comparison between three dimensional multibilayers and strictly two dimensional monolayers regarding their headgroup conformation seems hardly appropriate, since the interbilayer interactions (attractive van der Waals interaction, dipolar interaction and repulsive hydration forces) acting in the third dimension will certainly influence this conformation in bilayers. Moreover, the lateral pressure in the bilayer at which the measurements of Büldt et al. were done is not exactly known and could well be below 30 mN/m where the DPPC monolayer is not in the S phase but in a liquid condensed (LC) phase. Our measurements, however, do not permit any conclusions about the headgroup conformation in this phase.

An interesting question is about the driving force for the suggested conformational change of the DPPC headgroup with increasing π. Since the choline headgroup cross section is larger than that of the tail region, the former is likely to represent the limiting packing constraint. Even in the S phase, the molecular area of DPPC is about 5 Å^2 higher than that of DPPE, which exhibits a significantly lower headgroup volume. Thus, the straightening of the DPPC headgroup parallel to the surface normal at high π is probably a result of an adaptation of headgroup and tail cross sections by steric interactions as a prerequisite of the crystalline lattice formation at high lateral pressure.

Conclusions

A significant change of the DPPC headgroup conformation with lateral pressure π is indicated by comparing neutron reflectivity data which are dominated by contributions from either headgroup, chain or the total molecule reflectivity. It is interpreted as the orientational change of the phosphate-nitrogen dipole of the choline head group with respect to the surface normal by up to 90 degrees between the S and the LE phase. This change coincides with a previously reported alteration of the headgroup hydration. The latter is most likely a result of the headgroup conformational change together with a similar change in the glycerol backbone. At the present state it is not clear whether the headgroup conformation changes at the LE/LC or at the LC/S transition. Comparative measurements in the LC and the S phase would be required to find possible distinguishing features of the DPPC headgroup conformation in the LC and in the S phase.

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