Effect of N-Lauryl-N,N-Dimethylamine N-Oxide on Dimyristoyl Phosphatidylcholine Bilayer Thickness: A Small-Angle Neutron Scattering Study

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Abstract. Small-angle neutron scattering on large extruded unilamellar dimyristoyl phosphatidylcholine (DMPC) liposomes was used to determine the DMPC bilayer thickness d_L and its change in the presence of N-lauryl-N,N-dimethylamine N-oxide (LDAO) At 36 °C, the values of d_L are $d_L = 3\,44 \pm 0\,10$ nm and $d_L = 2\,90 \pm 0\,10$ nm in pure DMPC bilayers and in bilayers at DMPC LDAO = 2 1 molar ratio, respectively Using the specific volumes of DMPC and LDAO and supposing that the molecular volumes and surface areas in the bilayer are additive, the surface areas of DMPC (A_{DMPC}) and of LDAO (A_{LDAO}) were found to be at 36 °C $A_{\mathrm{DMPC}} = 0\,644 \pm 0\,018$ nm^2 and $A_{\mathrm{LDAO}} = 0\,25 \pm 0\,05$ nm^2

Key words: Small-angle neutron scattering — Unilamellar liposomes — Bilayer thickness — Dimyristoyl phosphatidylcholine — N-Lauryl-N,N-dimethylamine N-oxide

Introduction

Non-aromatic amine oxides represent a large group of chemical compounds, derived from tertiary amines and containing a strongly polarized N-O bond These compounds are used successfully in different areas of industry, agriculture, pharmacy and science (see Devínsky 1986, Kopecká-Leitmanová et al 1989) They display a great variety of biological activities For example, amphiphilic N-alkyl-N,N-

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dimethylamine N-oxides (ADAO) were found to possess antimicrobial (Devínsky et al 1990), antiphotosynthetic (Šeršeň et al 1992) and immunomodulatory (Ferenčík et al 1990, Jahnová et al 1994, Bukovský et al 1996) activities ADAOs inhibit also sarcoplasmic reticulum (Ca-Mg)ATPase (Andriamainty et al 1997) The well known N-lauryl-N,N-dimethylamine N-oxide (LDAO) is widely used as a mild biological detergent In particular, it has been used in the preparation of bacterial photosynthetic reaction centers (Loach 1980) It is believed that ADAOs interact with the lipid part of biological membranes (Kopecká-Leitmanová et al 1989 Bukovský et al 1996)

ADAOs penetrate into phospholipid bilayers and affect their fluidity (Balgavy et al 1989, Šeršeň et al 1989, Devínsky et al 1990, Gallová 1993) and stability (Uhríková and Stanovská 1990) ADAO polar group interacts with the phospholipid polar head groups, and the long ADAO alkyl substituent extends into the hydrophobic region of the bilayer Because of this insertion between the phospholipid molecules, the bilayer surface increases, and due to the difference between the lengths of the hydrophobic parts of amphiphile and phospholipid molecules a modulation of trans-gauche isomerization and/or hydrocarbon chain interdigitation occurs in the bilayer hydrophobic region As a result of combination of these effects, one can expect a change in bilayer thickness In the case of biological membrane, the change in bilayer thickness will result in the variation of the balance of hydrophilic and hydrophobic interactions between the bilayer and membrane proteins This effect can influence protein structure and function This might be the primary mechanism of the ADAO inhibition of sarcoplasmic reticulum (Ca-Mg)ATPase activity (Andriamainty et al 1997)

In the present paper we study the influence of LDAO on the thickness of model phospholipid membrane As model membrane we use the bilayer of the 1,2-dimyristoyl-sn-glycero-3-phosphorylcholine (DMPC) unilamellar liposomes prepared by the extrusion method The bilayer thickness is determined using the small-angle neutron scattering (SANS) from the radius of gyration, assuming a constant scattering length density distribution throughout the bilayer The accuracy of the thickness determination using this method is better than 0 1 nm for bilayers with very low neutron scattering length densities such as phosphatidylcholine bilayers in heavy water (Gordely et al 1993)

Materials and Methods

DMPC was obtained from Fluka (Buchs Switzerland) and heavy water (99 98 % ^2H_2O) from Izotop (Moscow, Russia) LDAO was prepared from N-lauryl-N,N-dimethylamine by hydrogen peroxide oxidation as described by Devínsky et al (1978) The other chemicals used were obtained from Lachema (Brno, Czech Republic) DMPC was dispersed in heavy water by hand shaking at a temperature

above 35 °C, and by repeated heating and cooling through the main phase transition temperature of DMPC (Lipka et al. 1984). From this dispersion of multilamellar DMPC liposomes, large unilamellar DMPC liposomes were prepared according to MacDonald et al. (1991) using LiposoFast Basic extruder (Avestin, Ottawa, Canada). The multilamellar DMPC liposomes were extruded through polycarbonate filter (Nucleopore, Plesanton, USA) with pores of diameter 50 nm mounted in the extruder fitted with two 0.5 ml Hamilton syringes (Hamilton, Reno, USA). The sample was subjected to 19 passes through the filter at 50 °C. An odd number of passes was performed to avoid contamination of the sample by large and multilamellar vesicles which might not have passed through the filter. LDAO was added to the liposomes as the heavy water solution. The LDAO:DMPC molar ratio was 1:2 in this sample. The total DMPC concentration in all samples was 1 w/w %. The maximum period between the sample preparation and its first measurement was 3–4 hours. Because of the high partition coefficient and high lipid concentration, practically all the LDAO molecules can be expected to be incorporated in the lipid bilayer in the fluid (liquid crystalline) phase (Devínsky et al. 1990).

The SANS measurements were performed at a small-angle time-of-flight axially symmetric neutron scattering spectrometer MURN (named now YuMO in honor of deceased Yu. M. Ostanevich) at the IBR-2 fast pulsed reactor (2 MW power, 5 Hz frequency, 230 μsec half-width at the half-height of the power output maximum) of the Frank Laboratory of Neutron Physics, Joint Institute for Nuclear Research in Dubna (Vagov et al. 1983; Ostanevich 1988). The data matching and the resolution function of this spectrometer has been described in detail by Ostanevich (1988). According to this paper, the averaged relative Q-variance $\overline{\sigma}_Q/Q$ expressed in per cents decreases non-linearly with the increase of the scattering vector Q (for definition see eqn. 1 below) from about 17–18% at $Q = 0.05$ nm^{-1} to 10% at $Q = 0.5$ nm^{-1} and to about 5% at $Q = 5$ nm^{-1}.

The samples were poured into quartz cells (Hellma, Müllheim, Germany) to provide the 1 mm sample thickness. The sample temperature was controlled electronically with the precision of ± 0.1 °C. Each sample was equilibrated for 1 h at the fixed temperature after each heating step. A typical acquisition time for one sample at one fixed temperature was one hour. The scattering patterns were corrected for background effects and the coherent scattering cross section was obtained by using a vanadium standard scatterer as described in detail by Ostanevich (1988).

Results and Discussion

Fig. 1 shows the plot of the measured scattering intensity $I(Q)$ as a function of the neutron scattering vector Q which is defined as

$$Q = 4\pi \sin \theta/\lambda \tag{1}$$

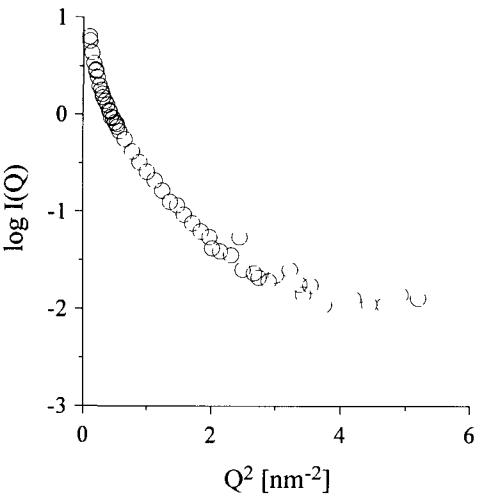

Figure 1. Dependence of small-angle neutron scattering intensity $I(Q)$ on scattering vector Q for unilamellar DMPC liposomes in ^2H_2O at 36 °C

where θ is half the scattering angle and λ is the wavelength of neutrons The data in Fig 1 were obtained for a sample with pure DMPC at 36 °C, which is about 11–12 °C above the gel to liquid crystal phase transition of DMPC in the heavy water (see below) Notable is the absence of the first order Bragg diffraction peak in the region of $Q = 0\ 7-1\ 2\ \mathrm{nm}^{-1}$ This is a control of the sample preparation procedure The first order Bragg diffraction peak was observed in SANS experiments with multilamellar DMPC liposomes (Winter and Pilgrim 1989) and with a mixture of unilamellar and multilamellar DMPC liposomes prepared by sonication in a bath sonicator (Knoll et al 1981) The sharpness of this peak increases above the gel-liquid crystal phase transition temperature of the lipid (Winter and Pilgrim 1989) Unilamellar liposomes are thermodynamically unstable and are known to undergo aggregation and fusion when stored for prolonged periods of time below gel-liquid crystal phase transition temperature or when passed (repeatedly) through this temperature (Suurkuusk et al 1976 Schullery et al 1980, Wong and Thompson 1982, McConnell and Schullery 1985, Hauser 1993) Since our experimental protocol involves passing of the sample through this phase transition temperature minimally twice and a rather prolonged staying below this temperature during measurements, the liposomes could fuse and/or aggregate during the experiment The neutron scattering pattern in Fig 1 was the last one obtained in the whole experiment with this particular sample The absence of the Bragg peak is therefore a strong evidence that the prepared sample did not contain considerable amounts of multilamellar liposomes It is further seen from Fig 1 that the statistical accuracy of $I(Q)$ values is lower at larger Q values This is due to reduction of small-angle neutron scattering with the increase of the scattering angle Similar scattering patterns have been obtained for a LDAO+DMPC sample (not shown)

LDAO molecules could destabilize the DMPC bilayer due to their detergent properties. The resulting LDAO+DMPC particles could be mixed globular, rod-like or sheet-like (discoid) micelles. The results of SANS experiment can be used to discriminate between these three possibilities. The simplest method is the inspection of the scattering intensity $I(Q)$ as a function of the scattering vector Q at very small scattering angles. The neutron scattering intensity can be written as

$$I(Q) = NP(Q)S(Q) \tag{2}$$

where N is the number of scattering particles in unit volume, $P(Q)$ is the particle structure factor, and $S(Q)$ is the size- and orientation-dependent interparticle structure factor. $S(Q)$ approximately equals to 1 for dilute and weakly interacting particles. It has been found experimentally that at the phospholipid concentration (1 wt.%) as used in our experiments, $S(Q) \cong 1$ is a good approximation (Knoll et al. 1981; Komura et al. 1982; Nawroth et al. 1989), and that deviations occur at concentrations > 2 wt.% (Nawroth et al. 1989). According to Guinier approximation for very small scattering angles (Guinier and Fournet 1955; Glatter and Kratky 1982; Kratky and Laggner 1987; Hjelm et al. 1990), one rewrites then equation (2) as

$$I(Q) = I_g(0) \exp(-Q^2 R_g^2/3) \tag{3}$$

for the scattering for a globular object, where $I_g(0)$ is the intensity at zero scattering vector, and R_g is the particle radius of gyration taken about the scattering mass centroid. For a rod-like object of infinite length and uniform cross section the form is

$$I(Q) = I_c(0) \exp(-Q^2 R_c^2/2) Q^{-1} \tag{4}$$

where $I_c(0)$ is the corresponding intensity at zero scattering vector, and R_c is the cross sectional radius of gyration taken about the rod axis. The analogous form for the infinite sheet-like object is

$$I(Q) = I_t(0) \exp(-Q^2 R_t^2) Q^{-2} \tag{5}$$

where $I_t(0)$ is the corresponding intensity at zero scattering vector, and R_t is the radius of gyration taken along a line normal to the sheet surface. Equations (4) and (5) have been shown to be good approximations for rods of finite lengths (Hjelm et al. 1990) and for sheets with lateral dimensions large compared to their thickness (see below), respectively. Fig. 2 shows the modified Guinier analysis of the scattering intensity data $I(Q)$ obtained with unilamellar DMPC liposomes in ^2H_2O in the presence of LDAO at 36 °C in the range of scattering vectors 0.7 $nm^{-2} \geq Q^2 \geq 0.1$ nm^{-2}. Hjelm et al. (1988) have used the same region of Q in their analysis of

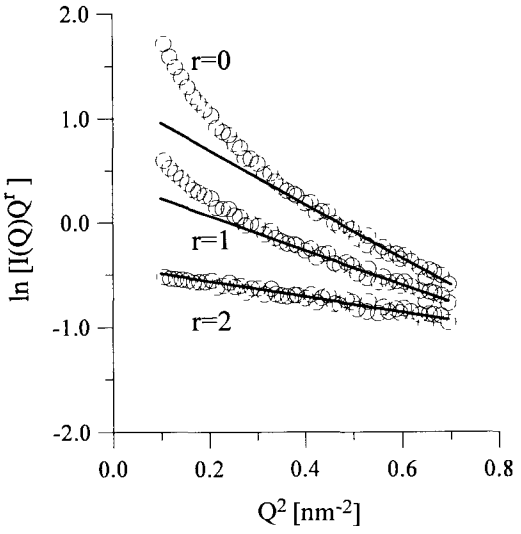

Figure 2. Modified Guinier analysis of small-angle neutron scattering intensity $I(Q)$ data for unilamellar DMPC liposomes in ^2H_2O at 36 °C in the presence of LDAO, for the globular particle ($r = 0$), for the rigid rod ($r = 1$) and for the sheet ($r = 2$).

SANS data for glycocholate+egg yolk phosphatidylcholine system. We have fitted our data using equations (3), (4) and (5) in the range of 0.7 $nm^{-2} \geq Q^2 \geq 0.4$ nm^{-2} by the least-squares method and the fitted functions extrapolated in the interval 0.4 $nm^{-2} > Q^2 \geq 0.1$ nm^{-2} (full line). It is seen that the experimental data deviate from the fitted functions when supposing rods or globules in the DMPC+LDAO system, while all the experimental data are approximated well by the fitted function when supposing the presence of sheets. However, this is not an evidence for the LDAO induced destabilization of DMPC liposomes resulting in the formation of sheet-like objects (e.g. discoid mixed micelles), because the same scattering data can be fitted equally well by using the model of polydisperse hollow spheres (see below). Furthermore, no destabilization of the bilayer structure by LDAO has been observed in multilamellar egg yolk phosphatidylcholine liposomes at LDAO:lipid molar ratio as used in the present study (Uhríková and Stanovská 1990).

Unilamellar liposome is a hollow sphere consisting of a lipid bilayer concentric shell with the aqueous phase inside and outside of this shell. Using the well-known equations for the scattering on hollow sphere (Glatter and Kratky 1982), it can be written for monodisperse unilamellar liposomes with the heavy water inside and outside the bilayer shell at low lipid concentration ($S(Q) \cong 1$) as

$$I(Q) = I_{hs}(0)(1/Q^3)^2(A_2 - A_1)^2 \tag{6}$$

with

$$A_i = \sin(QR_i) - (QR_i)\cos(QR_i) \tag{7}$$

where $I_{hs}(0)$ is the corresponding intensity at zero scattering vector, and R_1 and

R_2 correspond to the inner and outer radius of the liposome, respectively. The bilayer thickness is then $d_L = R_2 - R_1$. Pilz et al. (1970) have suggested that the oscillating scattering function $I(Q)$ vs. Q for monodisperse hollow spheres is smeared by convoluting with the sphere radius distribution function. In the suitable range of the scattering vectors, the scattered intensity should approach that for the two-dimensional sheets having the same thickness as the hollow sphere shell. The extrusion method produces unilamellar liposomes with the mean diameter close to the filter pore diameter and with a broad distribution of diameters. For example, filters with pores of 50 nm gave phosphatidylcholine liposomes with an average diameter of 46 nm and a relatively broad polydispersity: the number distribution of radii derived from light scattering experiments using the Laplace inversion indicates the presence of liposomes with radii from about 15 nm to 60 nm, the number distribution obtained from electron micrographs of freeze-fractured samples is shifted to smaller sizes with radii between 10 nm and 48 nm (Hauser 1993). We have observed recently by evaluating computer simulated scattering patterns (Balgavý et al. 1997) that in the range of bilayer thickness from 1 nm to 6 nm the scattering function is smeared for $Q > 0.3$ nm^{-1} and liposome radii and their polydispersity typical of liposomes prepared by the extrusion through 50 nm and larger filter pores. The thickness of the bilayer can be thus obtained easily by using the Kratky-Porod plot (Glatter and Kratky 1982; Kratky and Laggner 1987):

$$\ln\left(I(Q)Q^2\right) = \ln\, I_t(0) - R_t^2 Q^2 \tag{8}$$

The thickness of the sheet d_S in heavy water can be obtained then from the radius of gyration approximated by

$$d_S = 12^{1/2} R_t \tag{9}$$

Eequations (5), (8) and (9) are valid in the region of scattering vectors

$$2\pi/S^{1/2} \leq Q \leq 1/R_t \tag{10}$$

where S is the total area of the sheet. This approach was used by several authors (Knoll et al. 1981; Nawroth et al. 1989; Sadler et al. 1990; Gordeliy et al. 1993, 1997) to determine the thickness d_L in polydisperse small unilamellar phosphatidylcholine liposomes prepared by sonication. The cited authors simply supposed that $d_L \cong d_S$. Using this approach and supposing that the scattering length density of molecules in the bilayer is homogenous, the accuracy of the thickness determination is better than 0.1 nm for bilayers with very low neutron scattering length density such as DMPC or DMPC+LDAO bilayers in heavy water (Gordeliy et al. 1993). However, this method is sensitive to the presence of multilamellar liposomes in the sample. For example, liposomes with shells composed of two to three bilayers increase the

value of d_L appreciably (Knoll et al 1981), and thus a careful preparation of the sample is a prerequisite to obtain reliable data The second possible method of data evaluation involves calculation of the scattering function for the hollow sphere including its convolution by the known or supposed size distribution function and spectrometer resolution function (Komura et al 1982, Hjelm et al 1988, 1990, Lin et al 1991, Pedersen et al 1995) This method is more involved and does not provide more reliable results for the present experiments especially because of the large experimental error in the high Q value region

The values of Q which fulfill the second condition in (10), $Q \leq 1/R_t$, and which can thus be used in further evaluation of experimental data, can be obtained from expected parameters of our liposome preparations For pure DMPC liposomes, the expected bilayer thickness is less than 4 5 nm (Janiak et al 1976, 1979, Laggner et al 1979 Cornell and Separovic 1983, Lewis and Engelman 1983, Cevc 1993, Gordeliy et al 1997) In the presence of LDAO we expect a decrease in the bilayer thickness because the alkyl (lauryl) chain is shorter than DMPC acyl (myristoyl) chains Substituting $d_L = 4\ 5$ nm into (9) and (10), one gets for the usable scattering vectors in the Kratky-Porod plot the first condition $Q^2 \leq 0\ 619\ \mathrm{nm}^{-2}$ In further evaluation of our experimental data we therefore used the region $0\ 6\ \mathrm{nm}^{-2} \geq Q^2 \geq 0\ 1\ \mathrm{nm}^{-2}$ In this region all the Kratky-Porod plots of experimental data were linear within the experimental error and could be fitted well with straight lines As an illustration, we present in Fig 2 the experimental data for the DMPC+LDAO sample For this sample, the fitting using eqn (8) gives the value of $R_t^2 = 0\ 699\ \mathrm{nm}^2$ with a standard deviation of $0\ 028\ \mathrm{nm}^2$ and the correlation coefficient $r = 0\ 941$ The 90% confidence limits for R_t^2 are $0\ 738\ \mathrm{nm}^2$ and $0\ 644\ \mathrm{nm}^2$ Using eqn (9) one gets from these values $d_L = 2\ 9 \pm 0\ 1$ nm where the $\pm$ range gives the values of the 90% confidence limits for d_L The correlation coefficients of fitting of the other plots were in the range of $r = 0\ 953 - 0\ 985$ and the 90% confidence limits for d_L were $d_L \pm 0\ 08 \div 0\ 11$ nm Taking the 90% confidence limits, the relative statistical accuracies were in the range $2\ 2\% \div 3\ 1\%$ The statistical accuracy was $1\ 3\% \div 1\ 8\%$ when using the values of standard deviation of R_t^2 However, it is important to note that the statistical accuracy represents the errors obtained using the fitting procedure Since the procedure used in the data evaluation is only approximate, the d_L values can be affected by a systematic error

The temperature dependence of d_L for the DMPC sample (open squares) and DMPC+LDAO (full square) obtained as described above is shown in Fig 3 The vertical bars indicate the limits of d_L calculated using the 90% confidence limits of R_t^2 The limits of d_L calculated using the standard deviation of R_t^2 are smaller than the size of symbols The main conclusion is that the addition of LDAO causes a decrease of the DMPC bilayer thickness, d_L This conclusion would hold even in the case of a hypothetical destruction of DMPC liposomes into discoid micelles ("sheet-like objects"), because the disks consisting of DMPC and LDAO would

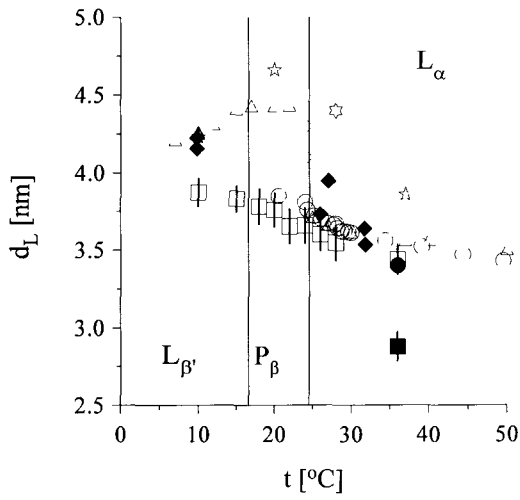

Figure 3. Temperature dependence of the lipid bilayer thickness in unilamellar (× □ ○ ◆ ● ✡) and multilamellar (△ ◇ ☆) DMPC liposomes and the effect of LDAO on lipid bilayer thickness in unilamellar DMPC liposomes (■). Symbols. □ ■ (present work), ○ (Gordeliy et al. 1997), △ (Janiak et al. 1976, 1979), ✡ (Laggner et al. 1979), ◇ (Lemmich et al. 1996), ● (Lewis and Engelman 1983), ☆ (Lvov et al. 1986), × Nawroth et al. (1989), ◆ (Sadler et al. 1990).

have a bilayer structure with the hydrocarbon chains forming a close-packed center region and with the surface region consisting of hydrophilic head groups.

The vertical lines in Fig. 3 indicate the phase transition temperatures for multilamellar DMPC liposomes dispersed in heavy water and measured using adiabatic differential scanning microcalorimetry (Lipka et al. 1984): In the high temperature region above 24.54 °C the lipid bilayer in multilamellar liposomes is in the fluid liquid crystalline L_α phase with melted acyl chains. Between about 16.6 °C and 24.54 °C, the lipid bilayer is rippled and the chains in all-trans configuration are parallel with respect to the bilayer normal (P_β gel phase). Below 16.6 °C the lipid bilayer is flat and the chains in all-trans configuration are tilted with respect to the bilayer normal ($L_{\beta'}$ gel phase). During a long sample incubation at low temperature the crystalline phase L_c can form (Cevc and Marsh 1987), but our experimental protocol excludes this possibility. In small unilamellar phosphatidylcholine liposomes prepared by sonication, the region of the gel – liquid crystal phase transition (main transition) is broadened, and the $L_{\beta'} - P_\beta$ phase transition (pretransition) is absent (Suurkuusk et al. 1976; Inoue et al. 1981; Vojčíková et al. 1989). However, with the fusion and aggregation of small liposomes the pretransition re-appears (Suurkuusk et al. 1976; Inoue et al. 1981). Our data in Fig. 3 indicate that the d_L value in DMPC liposomes does not change appreciably in the $L_{\beta'} \rightarrow P_\beta$ and $P_{\beta'} \rightarrow L_\alpha$ phase transition regions.

We have compared our data for DMPC unilamellar liposomes with selected data from the literature. First of all, our data coincide within experimental error with the data of Gordeliy et al. (1997) obtained using SANS with sonicated (small) unilamellar DMPC liposomes where the temperature regions used overlap. In the L_α phase region, our data coincide within experimental error not only with

the data of Gordeliy et al (1997), but also with the data of Janiak et al (1976, 1979) obtained using small-angle X-ray scattering (SAXS) on fully hydrated multilamellar DMPC liposomes, with the data of Lewis and Engelman (1983) obtained using SAXS on unilamellar sonicated DMPC liposomes, and with three of four data points obtained by Sadler et al (1990) using SANS on large unilamellar DMPC liposomes prepared by reverse phase evaporation method In the L_α phase region, the data of Lvov et al (1986) and Lemmich et al (1996) obtained on fully hydrated multilamellar liposomes using SAXS and SANS, respectively, and three data points of Laggner et al (1979) and Nawroth et al (1989) obtained on sonicated small unilamellar liposomes using SAXS and SANS, respectively, are outside the region of the experimental errors of our and the other authors data With the exception of the data point of Lvov et al (1986), the other data points which deviate from our results, lie within the region of 5 K above the $P_\beta \rightarrow L_\alpha$ phase transition temperature Within this region, an anomalous swelling of DMPC has been observed in multilamellar liposomes connected with the strong lateral density fluctuations (Honger et al 1994, Lemmich et al 1996) This might be an explanation of the difference in data In the $L_{\beta'}$ and P_β phase regions, our results differ significantly from the results of other groups of authors Significantly higher values of thickness were obtained using multilamellar DMPC liposomes and SAXS method by Janiak et al (1976, 1979) and Lvov et al (1986) and SANS method by Lemmich et al (1996), and using large unilamellar liposomes prepared by reverse phase evaporation method and the SANS method by Sadler et al (1990) At present it is not clear to us why our data in the $L_{\beta'}$ and P_β regions deviate from those reported in the literature One possible reason could be that we have assumed the lipid and water to form separated layers However, the water molecules penetrate into the bilayer head group region, the head groups are mobile and the entire bilayer undergoes thermal undulations Consequently, the d_L values obtained using the method described can be smaller than the actual thickness, because the method used takes into account mainly the regions where the neutron scattering density deviates strongly from that of ^2H_2O

From the d_L data, the surface area of the lipid molecule in the bilayer (of unilamellar liposomes or of discoid mixed micelles) can be calculated using the partial specific volumes of DMPC and LDAO The specific volume of DMPC in multilamellar liposomes has been determined by Laggner and Stabinger (1976) and Nagle and Wilkinson (1978) The DMPC specific volume is 0 9840 cm^3/g at 36 °C, which corresponds to 1 1075 nm^3 per DMPC molecule (Nagle and Wilkinson 1978) Using the d_L value obtained at this temperature, the surface area of the lipid molecule in the bilayer is $A_{DMPC} = 0\ 644 \pm 0\ 018\ nm^2$, where the limits are given by the 90% confidence limits of d_L Lewis and Engelman (1983) calculated $A_{DMPC} = 0\ 657 \pm 0\ 030\ nm^2$ from their SAXS data at the same temperature for unilamellar sonicated DMPC liposomes and Cornell and Separovic (1983) evaluated $A_{DMPC} = 0\ 646\ nm^2$

from the SAXD data obtained on fully hydrated diacylphosphatidylcholine multilamellar liposomes at about 20 °C above the $P_\beta \rightarrow L_\alpha$ phase transition temperature by several groups of authors The molecular volume of LDAO has been found to be 0 3872 nm^3 in crystals at 25 °C (Benjamin 1966) Using this value and supposing that the molecular volumes and surface areas of DMPC and LDAO in the bilayer are additive, we obtain for the LDAO surface area $A_{LDAO} = 0\ 25 \pm 0\ 05\ nm^2$ at 36 °C for 1 2 LDAO DMPC molar ratio in the bilayer The values of A_{DMPC} and A_{LDAO} are most probably slightly overestimated because of the underestimation of d_L values discussed above The value of A_{LDAO} can be overestimated also because of lower than 1 2 LDAO DMPC molar ratio in the bilayer due to partial LDAO partition in the aqueous phase In conclusion we observed LDAO to reduce the thickness of DMPC bilayers and to cause their lateral extension

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