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High-pressure small-angle neutron scattering (SANS) study of 1,2-dielaidoyl-*sn*-glycero-3-phosphocholine bilayers

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Small-angle neutron scattering of the *trans*-unsaturated DEPC has been investigated as a function of pressure at 12, 18.6 and 35 °C. A pressure-induced structural phase transition from a liquid-crystalline state to a gel state is observed at the temperatures studied. The critical pressure of this transition increases with increasing temperature with a $\Delta P/\Delta T$ value of 51 bar/C°. The small-angle neutron scattering results indicate that the effect of the *trans* double bonds in DEPC is to enhance the conformational disorder in the hydrocarbon chains In DEPC bilayers, a pressure-induced conformational ordering process is observed not only in the liquid-crystalline phase but also in the gel phase, which indicates that conformational disorder exists in the liquid-crystalline phase as well as in the gel phase.

Introduction

The increased interest in membrane structure and biological transport has stimulated intensive investigations in the chemistry and physics of model membranes – in particular, the phosphatidylcholine systems [1] A common feature of these membrane phospholipids is the existence of a temperature- and pressure-dependent reversible gel to liquid-crystalline phase transition Below a certain phase transition temperature, the lipid bilayer is an ordered gel, characterized by a rigid packing of its hydrocarbon chains Above the phase-transition temperature, the lipid bilayer forms a disordered fluid-like phase with disordered hydrocarbon chains [1] At temperatures below this first-order phase transition, a broad pretransition has also been observed in several phosphatidylcholine systems [1–3], corresponding to an ordered structure which consists of lipid lamellae distorted by a periodic ripple In addition to these thermotropic phase transitions, pressure-induced phase transitions have been observed using high-pressure infrared and Raman spectroscopy [4–7], light transmission techniques [8], adiabatic compression [9], fluorescence polarization [10,11], X-ray [12] and neutron scattering [13,14], volumetric measurements [15,16], ESR [17], light scattering [18], calorimetry [19] and NMR spectroscopy [20] These pressure-induced structural phase transitions, which have been studied mainly in the saturated phosphatidylcholines, are related to the relative mismatch between the area of the choline headgroup and the cross-section of the acyl chains in the bilayer [4–7] Small modifications in the lipid molecule, like the variation of the headgroup, a change in the hydrocarbon chain-length or in the degree of unsaturation in the chains can drastically change the transition temperature and pressure [1,2,4–7] The effect of unsaturation on the structure and packing of the lipids is of special interest, because most bacterial and mammalian cell membranes contain a high percentage of unsaturated acyl chains. The main point of interest is what modifications take place in the structural arrangement of the lipid bilayer to accommodate the geometrical requirements due to double bonds in the acyl chains

In order to understand the influence of the unsaturation on the structural conformation in detail in the different thermotropic and barotropic phases, ^{2}H-NMR [21,22] and high-pressure infrared spectroscopic studies [23] have been performed on *trans*- and *cis*-unsaturated model membranes However, in elucidating the dimen-

Abbreviations DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine, DEPC, 1,2-dielaidoyl-*sn* glycero-3-phosphocholine, DMPC 1,2 dimyristoyl-*sn*-glycero-3-phosphocholine, DOPC, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine.

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sions and the packing arrangement of lipid bilayers in different phases, diffraction experiments are very much needed, but they have not been performed until now The small-angle neutron scattering (SANS) technique has proved to be a very powerful method for the analysis of high-pressure phase transitions [13,14] The conformational changes in the lipid bilayers can be monitored from changes of the bilayer repeat (d-spacing) which is determined by analyzing the small angle part of the neutron diffraction pattern By measuring the d-spacing as a function of temperature and pressure, its temperature and pressure dependence within various phases can be evaluated and, by recording abrupt changes of the d-spacing, phase transitions can be detected and a phase diagram can be constructed In this study we present the experimental results of SANS of DEPC multi-lamellar vesicles as a function of pressure up to 2 5 kbar at three different temperatures 12, 18 6 and 35 °C

Experimental

High-purity DEPC was obtained from Avanti Polar Lipids (Birmingham, AL) A fully hydrated (50 wt% in D_2O) lipid dispersion was prepared by vortexing the lipid/D_2O mixture in a closed vial at room temperature After immediate freezing of the sample in solid CO_2, the vortex/freeze cycle was then repeated twice, leading to a homogeneous lipid dispersion

The SANS experiments were performed on the SAD instrument at the Intense Pulsed Neutron Source at the Argonne National Laboratory The neutron beam was generated in pulses by spallation due to the deposition of 450 MeV protons on a depleted uranium target, followed by moderation using a solid methane moderator (18 K) The neutron beam had a wavelength of 0.5 to 14 Å The beam diameter at the sample position was about 1 cm and the integrated intensity was about $4\ 10^4$ neutrons/cm^2 per s The total flight path was 9 m and the sample-to-detector distance was 1.5 m The scattering intensity was detected by a 64×64 array area-sensitive, gas-filled proportional counter, while the wavelengths λ of the beam were determined by their time-of-flight The Q range ($Q = (4\pi/\lambda) \sin\theta/2$, θ is the scattering angle) covered in these experiments was from 0 005 to 0 35 Å^{-1}.

Two pressure cells were used for the diffraction experiments, one made from an aluminum alloy of high tensile strength for the lower pressures ($P < 1500$ bar) and another from molybdenum for the higher pressures The inner diameter of the cylindrical pressure cells was 1 cm and 0 5 cm, respectively. Details of the apparatus will be described elsewhere The pressure-transmitting fluid was D_2O, the pressure was applied by means of a hand pump and recorded by a Budenberg gauge Temperature control was achieved by circulating water from a thermostat through two outside jackets, located above and below the neutron beam window The temperature was measured with a thermocouple to within 0 2 C° accuracy.

The lamellar structure of membranes produces Bragg diffraction The low angle diffraction region of our multilamellar vesicles of DEPC features one peak corresponding to the lamellar periodicity, d This repeat unit, d, is made up of the bilayer thickness and the D_2O region around its headgroup The d-spacings were calculated according to the Bragg equation $n\lambda = 2d \sin\theta_n$, where n refers to the diffraction order, λ is the neutron wavelength, and θ_n is the Bragg angle of the nth order, which is half of the scattering angle

Results and Discussion

The multilamellar dispersions of DEPC in D_2O yield one intense first-order Bragg diffraction peak Fig. 1 shows the pressure dependence of the diffraction pattern at $T = 12$ °C The diffraction peak shifts its position as the pressure changes, and this shift in the diffraction peak reflects changes in the lamellar bilayer repeat The d-spacing, which is the lamellar thickness including the water region around the headgroups, is calculated from the Bragg equation and shown as a function of pressure in Fig 2 Inflections in the d curves are observed at about 30 bar for $T = 12$ °C, 350 bar for $T = 18$ 6 °C and at 1230 bar for $T = 35$ °C At these temperatures and pressures, the main phase transition from the liquid-crystalline (LC) state to an ordered

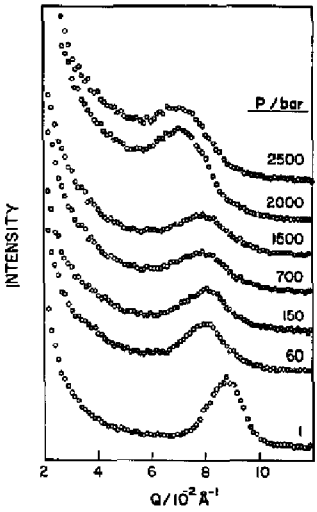

Fig. 1 Examples of scattering patterns $I(Q)$ vs Q for DEPC at $T = 12$ °C and different pressures (1–2500 bar)

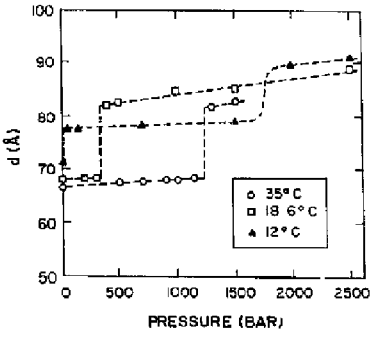

Fig 2 The *d*-spacings of DEPC for different temperatures (12 18 6 35 °C) as a function of pressure

gel state occurs [21–25] At 12°C an additional drastic change in *d*-spacing occurs at $P = 1750 \pm 250$ bar, which indicates a second phase transition at these conditions This second transition may correspond to the G_I/G_{II} transition observed at 5 kbar at 28°C in the high-pressure infrared study [23]

From the inflection points, a phase diagram can be constructed, and the result is shown in Fig 3 Data obtained by other experimental techniques [21,23–25] for the LC-gel transition line are included in this phase diagram Within the experimental errors, these data are in good mutual agreement The experimental data show that the transition temperature, T_m increases linearly with increasing pressure in accordance to the Clausius-Clapeyron equation, giving a value of $\Delta T_m/\Delta P$ of 19 ± 1C°/kbar A linear pressure dependence of T_m has also been observed in saturated lipid bilayers, and the $\Delta T_m/\Delta P$ values for DMPC and DPPC have been found to be 20 1 and 20 8C°/kbar respectively [26–28] $\Delta T_m/\Delta P$ for the *cis*-unsaturated DOPC bilayers, however, has been reported very small, the upper limit being 8 3C°/kbar [23]

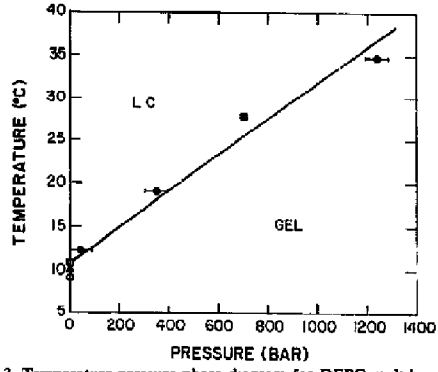

Fig 3 Temperature-pressure phase diagram for DEPC multilamellar vesicles ●, this work △, Ref 21, ■, Ref 23 ○ Ref 24 □, Ref 25

In the liquid-crystalline phase the bilayer thickness decreases as the temperature is raised $(\Delta d/\Delta T)_P = -0\,2$ Å/C° at $P = 1$ bar is estimated from the 35 and 12°C isotherms (see Fig 2) On the other hand, the pressure dependence of the bilayer thickness at constant temperature is of opposite sign, $(\Delta d/\Delta P)_T = 1\,5$ Å/kbar This behavior is typical for a polymer with highly disordered conformation [29] A rise in temperature increases disorder, i e , increases the number of kinks and *gauche* isomers, and thus decreases its elongation A similar mechanism explains the temperature effects observed in the liquid-crystalline phase of lipids An increase in the disorder in the hydrocarbon chains upon raising the temperature enlarges the cross-section of the acyl chains and reduces the elongation. Increasing pressure has the opposite effect, the lipid bilayers in the liquid-crystalline state are more compressible in the lateral than in the transverse direction, which is the result of reduction in the cross-section occupied per hydrocarbon chain due to the pressure-induced conformational ordering. Barotropic studies of DPPC using X-ray diffraction [12] show that in the liquid-crystalline phase, the lateral compressibility of the bilayer exceeds the transverse compression This observation is also in good agreement with the ESR [30] and fluorescence polarization measurements [8,11], which demonstrated that increasing pressure causes progressive ordering of probe molecules located in the hydrocarbon chain region of fluid lipid bilayers and biological membranes

Similar to the behavior observed in the liquid-crystalline phase, the lamellar periodicity of DEPC also increases in the gel phase with increasing pressure $(\Delta d/\Delta P = 2 \pm 1$ Å/kbar at 18 6°C) This implies that a considerable number of *gauche* bonds still remain in the gel phase of DEPC In contrast, X-ray [12] and neutron [13] scattering experiments on DPPC bilayers have shown that the lamellar periodicity in the gel phase decreases slightly as a function of pressure Therefore, the conformationally disordered structure in the gel phase of DEPC must be related to the presence of *trans* double bonds on the hydrocarbon chains The *trans* double bond introduces extra kinks in the chains and thus creates more space adjacent to the double bonds to accommodate more disordered *gauche* bonds.

It is of interest to note in Fig 2 that the lamellar periodicity of the first gel phase (G_I) at 12°C is appreciably smaller than that at 35 and 18 6°C From the temperature effects on the conformational disorder, the *d*-spacing at 12°C in the gel phase is expected to be slightly larger than that of the corresponding phase at higher temperatures Moreover, the bilayer thickness in the G_I gel phase at 12°C is hardly affected by pressure up to 1750 bar, where a second transition occurs, which contrasts with the other two isotherms

The unsaturated structure may be responsible for the relatively small bilayer repeat observed at 12°C As we discussed earlier, the presence of the *trans* double bonds in the hydrocarbon chains of DEPC leads to a more disordered conformational structure in the gel phase Consequently, the cross-section of the two hydrocarbon chains of each phospholipid molecule in the gel phase is larger than that in equivalent saturated phospholipid systems at corresponding temperature and pressure At 18 6 or 35°C, the temperature is high enough to introduce sufficient conformational distortion in the chains that the cross-section of the two hydrocarbon chains is comparable with the headgroup area [31] Under such circumstances, the tilt configuration of the bilayer, which usually exists in the pressure-induced gel phase [5,6,31], does not exist in the G_I gel phase of DEPC at 18 6 or 35°C At 12°C, though, the degree of disorder may not be sufficient to prevent the tilt configuration and therefore the G_I gel phase at 12°C may exist in a tilt configuration This tilt configuration results in a smaller *d*-spacing

In the G_I phase of DEPC at 12°C, lateral compression leads to a conformational ordering process which results in an elongation of the hydrocarbon chains Meanwhile, the conformational ordering process probably leads to a decrease in the effective cross-section of the hydrocarbon chains and a further tilt of the hydrocarbon chains with respect to the bilayer surface The elongation and tilt effects compensate each other and result in a small pressure dependence in the bilayer thickness in the G_I phase The small *d*-spacing and its relatively insensitive pressure dependence in the G_I gel phase at 12°C may suggest the existence of a metastable phase at that temperature which is not present at 18 6 or 35°C

In summary, the experimental SANS results on the DEPC lamellar vesicles show that the presence of the *trans* double bonds in the hydrocarbon chains introduces more kinks and *gauche* conformations in the chains The conformational disorder remains at a relatively high degree in the gel phase, which is in the contrary to the results found for saturated phospholipids

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