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HIGH PRESSURE STUDY OF PHASE TRANSITIONS IN DMPC-WATER SYSTEM

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We have carried out a detailed pressure study of the three phase transitions observed in hydrated dimyristoyl phosphatidylcholine (DMPC) containing 95% by weight of water. The P-T diagram shows a Gel III-Gel II-Gel I triple point at 3.5 kbar, 41°C. It is found that the Gel III phase can be obtained by pressure-annealing the sample for about 12 hours at room temperature.

INTRODUCTION

Phase transitions in hydrated dimyristoyl phosphatidyl choline (DMPC) have been investigated in detail.[1-5] In the low hydration regime, there are four distinct transitions whose temperatures vary significantly with water content. However for water concentrations greater than about 30% (by weight), the transitions are independent of the composition: there is a *main* transition at ∿24°C and a *pretransition* at ∿ 14°C.[1] The main transition occurs between a fluid-like liquid crystalline phase and a solid-like gel phase (Gel I) and the pretransition between the Gel I phase and another gel phase (Gel II). The structures of these phases appear

to be well established.[1] The liquid crystalline phase has a lamellar structure with the hydrocarbon chains conformationally disordered (Fig.1a). The Gel I phase has a two-dimensional lattice structure in which the lipid bilayer

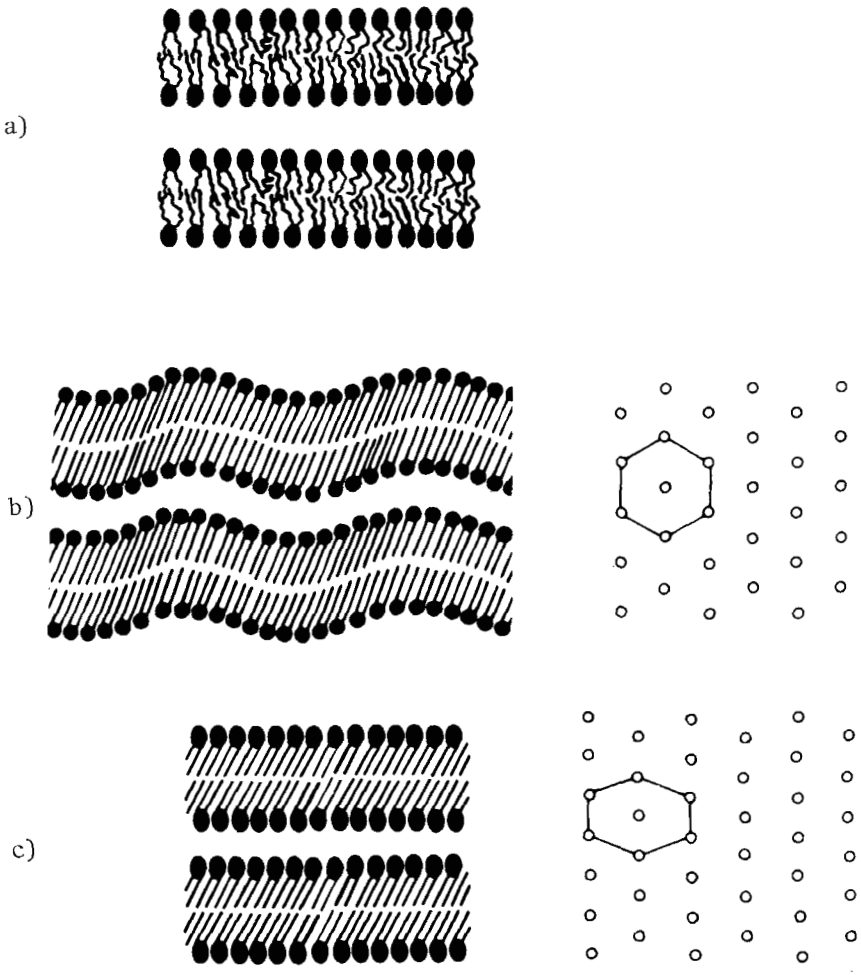

Figure 1 Schematic representation of the structures of (a) Liquid crystal, (b) Gel I, and (c) Gel II phases of hydrated DMPC.

lamellae are distorted by a periodic *ripple* in the plane of the lamellae (Fig.1b); the hydrocarbon chains are in a relatively ordered state and tilted with respect to the bilayer normal and packed in a regular hexagonal lattice. The Gel II phase has a lamellar bilayer arrangement with the hydrocarbon chains fully extended and tilted, but packed in a distorted hexagonal lattice (Fig.1c). Recently, a third transition (Gel II-Gel III) has been observed at -60°C.[5] The structure of the Gel III phase is still to be elucidated.

There have been some pressure experiments on the DMPC-water system,[6-9] but all of them (except that of Wong et al[9]) have been concerned with the main transition only. Wong et al[9] have reported measurements for all three transitions, but the data are not adequate to construct a complete pressure-temperature (P-T) diagram for this system. In this paper we describe a more detailed pressure study up to 4.2 kbar of the transitions in hydrated DMPC (containing 95 wt % of water).

EXPERIMENTAL

A. Sample preparation

DMPC was purchased from Avanti Polar Lipids, Alabama and used without further purification. 50 mg of the phospholipid was dissolved in chloroform. The solvent was removed by drying overnight in vacuum (1 mm). The phospholipid was then dispersed in 1 cc of 0.1 molar KCl in water, warmed to 35°C and agitated gently until the sample became milky white. Differential scanning calorimetry runs, particularly of the pretransition, showed that the sample was well homogenised. Also, the transition temperatures at 1 bar agreed very well

with those reported in the literature.

B. Pressure apparatus

The phase transitions were detected by the optical transmission technique. An optical high pressure cell was used for the experiments. Hydrated DMPC was sandwiched between two optically polished sapphire cylinders which are enclosed in a fluran tube so that the sample is isolated from the pressure transmitting fluid (Plexol). The sample thickness was about 0.5 mm. The experiments were always conducted along isobars and in the heating mode, i.e., the transition temperature at any pressure was determined by raising the temperature at a constant rate of $1^{o}C$/min. The temperature at which there was an abrupt change of transmitted light intensity was taken as the transition temperature. Typical curves for the transmitted light intensity are shown in Fig.2. Pressures were measured to a precision of $\pm$1.5 bars using a Heise Gauge. The temperatures were determined to an accuracy of $\pm 0.05^{o}C$ using a thermocouple whose junction was located in the interior of the pressure cell. Further details of the set up are described elsewhere.[10,11]

RESULTS AND DISCUSSION

Both the pretransition and the main transition could be followed as functions of pressure starting from atmospheric pressure. However, it was not possible to observe the Gel III-Gel II transition at lower pressures. (As already mentioned this transition occurs at $-60^{o}C$ at 1 bar). This was because the pressure cell could not be used below $6^{o}C$, the 'O' rings used in the cell losing their sealing property below this temperature. However, it was found that

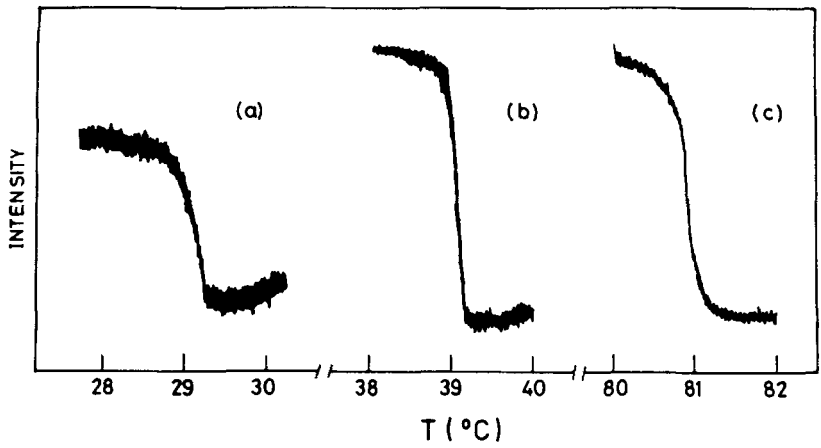

Figure 2 Raw traces showing the change in the transmitted light intensity at the transitions; (a) Gel III-Gel II, 3.10 kbar, (b) Gel II-Gel I, 3.10 kbar, (c) Gel I-Liquid Crystal, 3.12 kbar. The heating rate is 1°C/min. for all the transitions. The intensity scales for (a), (b) and (c) are different.

by keeping the sample at room temperature and at a pressure of 3 kbar for about 12 hours, the material formed the Gel III phase, and the Gel III-Gel II transition could be clearly observed. Having once observed this transition, the sample had to be pressure annealed once again in order to re-observe the transition. Therefore by successive pressure-annealing at different pressures, it was possible to follow the Gel III-Gel II transition for pressures beyond 3 kbar.

The P-T diagram showing all the three phase transitions is given in Figure 3. It is seen that the range of the Gel I phase increases with increasing pressure, showing thereby that the interactions responsible for the formation

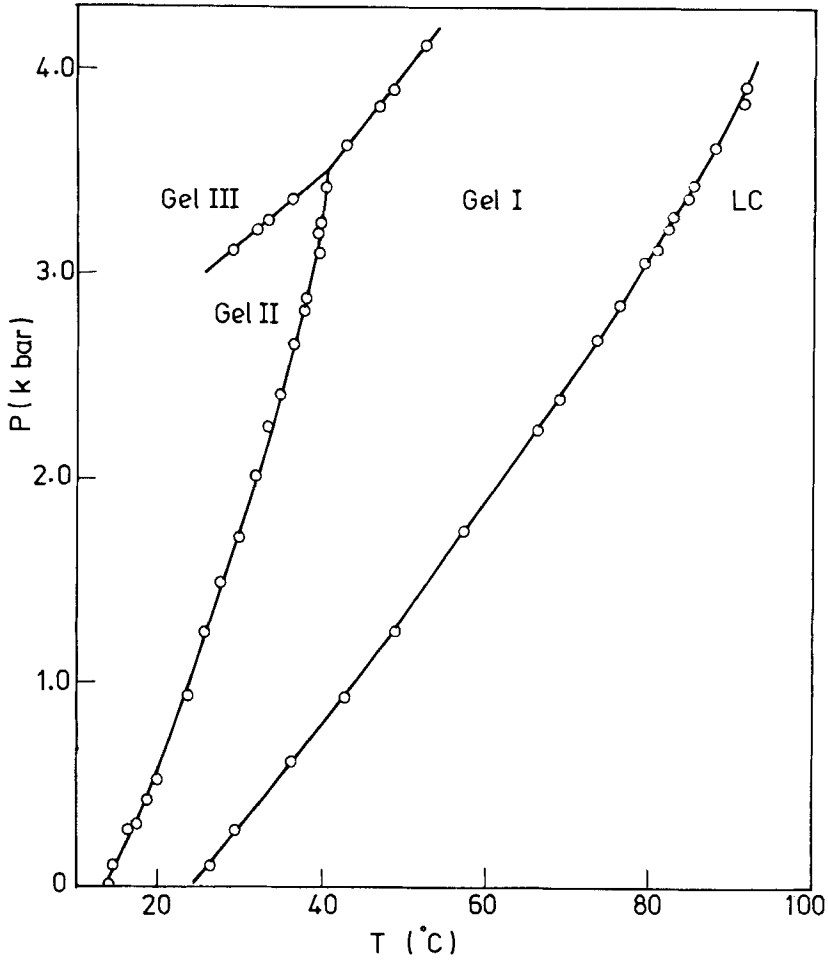

Figure 3 P-T diagram of hydrated DMPC. The Gel III - Gel II - Gel I triple point is at 3.5 kbar, 41°C.

of the hexagonally ordered Gel I phase are probably stabilised at high pressures. The variation of the Gel I-liquid crystal as well as that of the Gel II-Gel I phase boundaries are linear up to about 1.2 kbar beyond which there is a small curvature towards the pressure axis, a feature

which is commonly observed in thermotropic liquid crystal systems. The dT/dP corresponding to the linear portions of the phase boundaries are 21.4°C/kbar and 10.6°C/kbar respectively. Since the volume change at 1 bar for the Gel-liquid crystal transitions is known,[12] $\Delta V = 27 \times 10^{-3}$ cc/gm, dT/dP can be calculated from Clausius-Clapeyron equation using our value of $\Delta H = 6.4$ kcal/mol as measured by DSC. The value comes out to be 21.9°C/kbar which compares very well with the experimental value of 21.4°C/kbar.

The Gel III-Gel II transition line when extrapolated linearly to atmospheric pressure gives a transition temperature of about -60°C, in agreement with the value reportex by Wong and Mantsch.[5] The Gel II phase decreases in range with increasing pressure and ultimately gets bounded at 3.5 kbar; beyond this pressure Gel III transforms directly to Gel I. We thus have a Gel III-Gel II-Gel I *triple point* at 3.5 kbar, 41°C. Table 1 gives the dT/dP values for the three boundaries at the triple point. The dT/dP values derived from the initial linear portions of the Gel II-Gel I and Gel I-liquid crystal boundaries are also given in the same table for comparison.

TABLE I dT/dP values (in °C/kbar) for the various transitions in hydrated DMPC

Transition	dT/dP at 1 bar	dT/dP at the triple point
Gel I - Liquid crystal	21.4	-
Gel II - Gel I	10.6	5.1
Gel III - Gel II	-	29.2
Gel III - Gel I	-	20.0

Experiments on hydrated dipalmitoyl phosphatidyl choline(DPPC) are under way and will be reported elsewhere. It is also proposed to take up high pressure Xray studies to investigate in some detail the structural changes involved in these transitions.

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