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X-Ray Diffraction Measurements of Dipalmitoylphosphatidylcholine as a Function of Pressure

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Summary

Results of the first X-ray diffraction measurements of the lamellar periodicity of dipalmitoylphosphatidylcholine as a function of pressure are presented. Below the gel transition the periodicity decreases with increasing pressure. Above the transition, the periodicity increases with increasing pressure at a rate ~10 times that below the gel transition. This discovery is interpreted in terms of the relative compressibility within and perpendicular to the bilayer plane.

We find the gel transition temperature, T_m, is elevated with pressure. We determine $\frac{dP_m}{dT_m} \cong 48\ atm./^oC$ and is constant with pressure. In addition, we find coexistance of gel and liquid crystalline phases near the transition as a function of pressure. We believe these measurements to represent the first observation of the gel transition as a function of pressure using a structural technique.

Introduction

The biological implications of lipid phase transitions are both exciting and not well understood. Detailed investigation of these transitions in non-biological settings hopefully will clarify their mechanisms and give physical insight into possible biological roles.

Dipalmitoylphosphatidylcholine (DPPC) has been studied in great detail by many investigators who share the above philosophy. Theoretical models[1] and thermodynamic studies[2-7] have clarified the physical nature of the gel transition. Below this transition the hydrocarbon chains of this bilayer forming molecule are rigid. Above the transition, the chains are mobile and fluid. The resulting bilayer then is liquid crystalline and resembles a single smectic A bilayer.

We have investigated the phase behavior of DPPC multilayers as a function of temperature and pressure using X-ray diffraction methods. We find that the compressibility is larger

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within the bilayer plane than perpendicular to the plane above the gel transition. Below the transition, the compressibility is diminished and is larger perpendicular to the plane than within the plane. As in previous studies,[2-6] we find the phase transition temperature is elevated with increasing pressure. Our preliminary measurements indicate that $dP_m/dT_m = 48 atm/o_C$ at 13% water and is constant with pressure. This is in agreement with previous volumetric work[5] and spin label studies.[6]

Previous small angle X-ray diffraction studies as a function of temperature have shown the coexistance of gel and liquid crystalline phases about the transition point at lower water content $(<25\% H_2O)$.[8] This macroscopic separation of phases is consistent with phase diagrams as a function of temperature and water content.[9] Our results show the expected macroscopic equilibrium of phases as a function of pressure.

Materials and Methods

DPPC was purchased from Sigma and used without further purification. Mixtures of DPPC in water were prepared gravimetrically. X-rays were obtained from a bright $(70 KW/mm^2)$ rotating anode generator. Copper $K\alpha_1$ were selected and focused by a two element mirror - bent quartz monochromator system. Diffracted X-rays were detected with a stable position sensitive detector filled with $Ar-CH_4$ *at* 100 lbs/in^2 using a nichrome wire anode. The detector is ~90% efficient. Charge division position encoding was used.

Samples were contained in a pressure temperature cell designed for X-ray measurements. DPPC samples were placed a inside a beryllium tube with an outside diameter of 0.25" and an inside diameter of 0.040". Temperature could be adjusted from ambient to 120°*C* with an accuracy of ± 0.5°*C*. Pressure could be varied up to 3K bars ± 5 bars.

Results:

Below 20% water, the gel and liquid crystalline phases could be distinguished easily. The gel phase produced Bragg peaks with lamellar periodicities >55Å. The liquid crystalline phase produced Bragg peaks with lamellar periodicities <53Å.

Fig. 1 shows the variation of the lamellar periodicity of the gel phase for three different temperatures as a function of pressure. The water content of this sample was 13% by weight. The transition to the liquid crystalline phase was found at 60.5°*C* at 1 bar, 64.5°*C* at 200 bars and 78.5°*C* at 875 bars. A plot of the transition temperature as a function of pressure is given in Fig. 2.

We find coexistance of phases over a narrow pressure interval about the transition point. In Fig. 3 we display this equilibrium between the two macroscopic phases as a function of pressure. Finally, in Fig. 4 we show the variation of lamellar periodicity with pressure in the liquid crystalline phase.

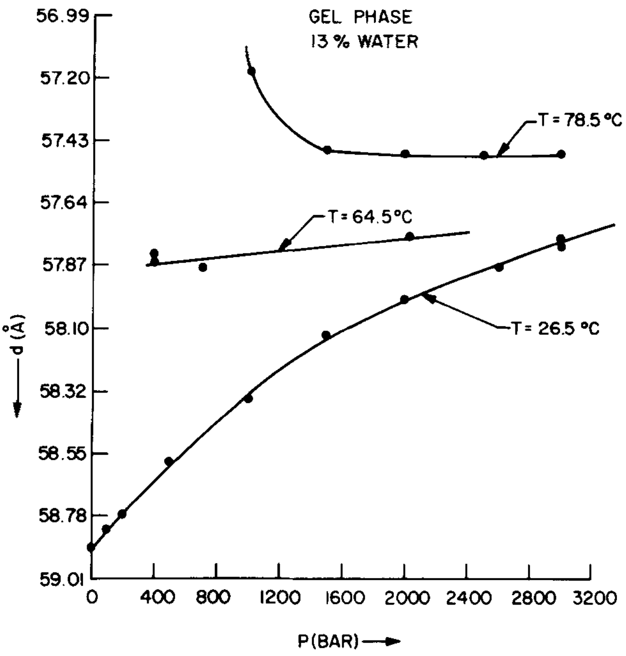

Fig. 1 The isothermal variation of the lamellar periodicity of the gel phase as a function of pressure. The water content was 13% by weight. The results for three different temperatures are given.

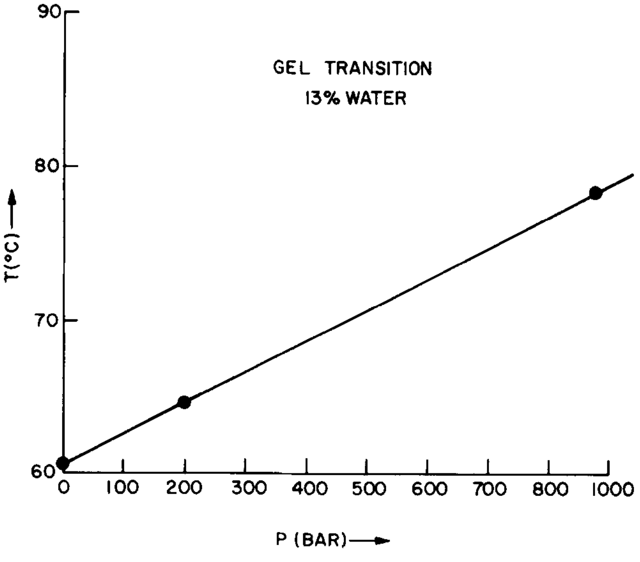

Fig. 2 The pressure and temperature dependence of the transition temperature. The slope of this line gives $\frac{dP_m}{dT_m} \cong 48 \frac{atm}{^oC}$.

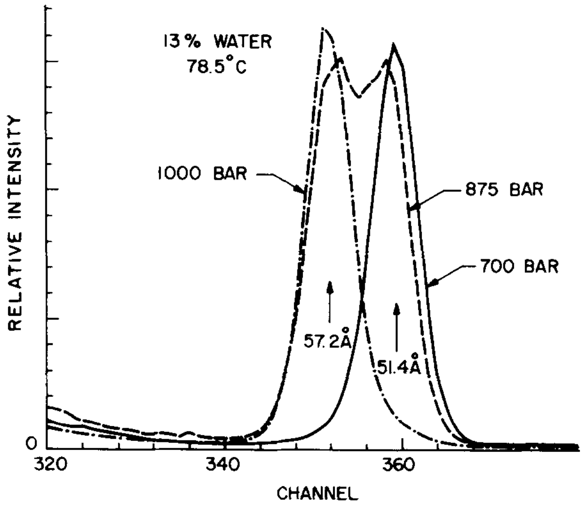

Fig. 3 X-ray diffraction patterns of the first diffraction order as a function of pressure near the transition point. The two peaks are the first orders for the gel and liquid crystalline phases. This data shows the pressure dependence of the coexistance of phases.

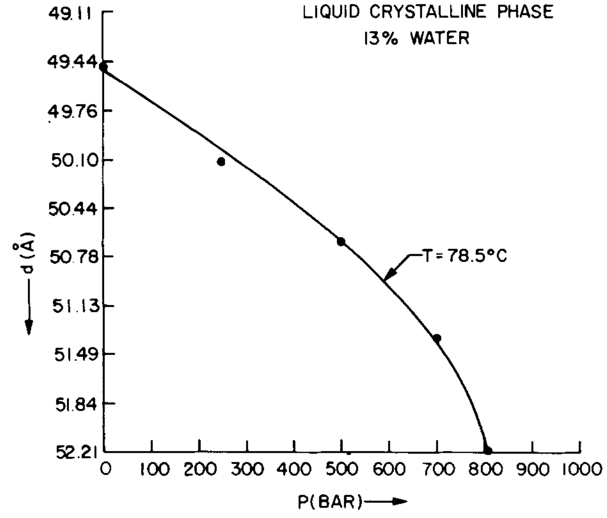

Fig. 4 The isothermal variation of the lamellar periodicity of the liquid crystalline phase as a function of pressure. The water content was 13% by weight.

Discussion

As a function of increasing pressure we find that the lamellar periodicity, d, decreases in the gel phase. We find that $\Delta d/d \cong -0.020$ from 1 bar to 3K bar. For a water layer of 10Å between the lipid bilayers, the isotropic compressibility of water would account for only $\Delta d/d \simeq 0.005$

In the liquid crystalline phase we find not only that $\Delta d/d$ is larger but of the opposite sign. As a function of increasing pressure, the lamellar periodicity increases. We find that $\Delta d/d = +0.057$ from 1 bar to 800 bar. Thus, $\Delta d/d$ is ~9 times larger per kbar above the gel transition. The increasing periodicity indicates that the lateral compressibility within the bilayer plane exceeds the compressibility perpendicular to the bilayer plane. This strongly suggests that the the melted hydrocarbon chains, which produce an increased surface area per molecule, are straightening and therefore extending upon increasing pressure.

Our value of dP_m/dT_m appears to be constant with pressure. It is remarkably similar to the value determined previously by volumetric[5] and spin label[6] studies even though the water content is considerably different.

Our demonstration of coexistance of macroscopic phases as a function of pressure is expected from previous phase diagrams.[9] It is the pressure analog of the results showing coexistance as a function of temperature.[8] This coexistance region for these water contents preclude detailed measurements of gel transition widths.[7] We believe these measurements to be the first demonstration of the gel transition as a function of pressure using a structural technique.

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