Molecular Crystals and Liquid Crystals
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gmcl16

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Version of record first published: 17 Oct 2011.

To cite this article: E.L Chang & P. Yager (1983): Effect of High Pressure on a Lipid Non-Bilayer Phase, Molecular Crystals and Liquid Crystals, 98:1, 125-129

To link to this article: http://dx.doi.org/10.1080/00268948308073467

Mol. Cryst. Liq. Cryst., 1983, Vol. 98, pp. 125–129
0026-8941/83/9804–0125/$18.50/0

Printed in the United States of America

Effect of High Pressure on a Lipid Non-Bilayer Phase

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(Received January 31, 1983)

We have developed a new optical method to monitor the lamellar to hexagonal$_{II}$ phase transition of lipids under high pressure. This method depends on the changes in the transmittance property of a pelleted sample inside a windowed pressure cell. The pelleted sample is opaque to a focused He-Ne laser beam below the phase transition whereas the compacted lipid becomes translucent above the phase transition temperature. The change in transmitted light intensity is about an order of magnitude and allows us to identify the onset of the phase transition to within 0.1°C. We have found a large hysteresis in the heating and cooling scans of egg phosphatidylethanolamine that is independent of the rate of scan. In addition, pressure increases the phase transition temperature of the lamellar to hexagonal$_{II}$ phase indicating that the hexagonal$_{II}$ phase is destabilized with respect to the lamellar phase under pressure.

Most lipids in excess water exist in the bilayer phase, although certain lipids such as phosphatidylethanolamines, cardiolipin, and phosphatidic acid can form a non-bilayer hexagonal$_{II}$ (H_{II}) phase. There is currently much interest in the possible role that non-bilayer phases may play in controlling fusion-related events in biological membranes such as endocytosis and exocytosis.[1–4] While much work has been done on the effects of the environment on the relative stabilities of the different lipid bilayer phases, there is little known about the stability of non-bilayer states with respect to the lamellar state.

The H_{II} phase is an inverted hexagonal phase with the polar headgroups lining the inside of infinitely long cylinders and the tails facing outwards. Such a packing structure is naturally very hydrophobic and requires a drastic reorganization of the bilayer geometry for conversion from lamellar to hexagonal phase (L-H_{II}). In fact, it is most likely that, in excess water, the boundaries of lipids in the H_{II} state will have a "protective" sheathing

of bilayers in order to interface with the outside water. In view of the large structural changes that have to take place during the $L\text{-}H_{II}$ transition, it is surprising that while differential scanning calorimetry shows a distinct peak for the $L\text{-}H_{II}$ transition, the coexistence region is rather broad (2-3°C) and the enthalpy is significantly lower than that of the main transition (gel to liquid crystal). In fact, turbidity measurements, which have been shown to be sensitive to the bilayer phase transitions, show no noticeable change during the $L\text{-}H_{II}$ phase transition. We have therefore developed a new optical method to observe the $L\text{-}H_{II}$ transition at both atmospheric and high pressure.

Our method for examining the influence of hydrostatic pressure on the $L\text{-}H_{II}$ transition, although simple, is sufficiently novel to merit description. Samples were rotovaporized to dryness, with subsequent high-vacuum evaporation for at least 12 hours. These lipid samples were then hydrated with buffer (20mM NaCl, 2mM HEPES, .1mM EDTA at pH 7.4) by gentle sonication at room temperature, resulting in a white flocculent precipitate. An aliquot of this dispersion was transferred to a stainless steel pan and calorimetry was performed using a Perkin Elmer DSC-2 equipped with a Bascom-Turner digital plotter. Another aliquot was transferred into a capillary then pelleted down. The capillaries were sealed with Seal-Ease hematocrit sealer (Clay Adams, Inc) and placed into a custom designed capillary holder inside a high pressure cell (Nova Swiss) with sapphire windows. The temperature of the cell was controlled by a circulating water bath (Neslab) and measured with a thermocouple inside the cell (Autoclave Engineers). Temperature readings were converted by a digital thermometer (Omega, Inc.) to binary coded decimal. A He-Ne laser beam was focused onto the capillary through one of the sapphire windows. A depolarized, magnified image of the capillary was projected (zero angle) onto the plane of a diaphragm which masked out all of the image except for the spot where the laser beam penetrates the lipid sample above the $L\text{-}H_{II}$ temperature. The transmitted light was then measured by the usual photon counting method. The light level measured therefore was the depolarized component of almost randomly polarized "transmitted" light. The photon count rate from a ratemeter and the temperature were recorded with an Apple II microcomputer at 0.1°C intervals. The results were displayed in real time. Pressure, applied with a hand pump (Enerpac) and measured with a calibrated gauge (Aminco), was held constant during the temperature scans. Heating scans were at the rate of about 1°C/min. The transition temperature was determined by picking the first large change in light transmission on either heating or cooling. The cooling curves were made at greater speed, particularly at high pressures.

We found that both phase transitions (the gel-liquid crystal and the L-H_{II}) were accompanied by large changes in the light level, but the increase at the L-H_{II} transition was by far the larger of the two, giving greater than a tenfold increase in intensity. The magnitude and the abruptness of the change in our method allowed us, while scanning the temperature of the cell, to optically ascertain the initiation of phase transition to within 0.1°C.

Our calorimetry data on egg phosphatidylethanolamine (egg PE) made by transphosphatidylating egg lecithin (Figure 1) shows two transitions corresponding to the gel-liquid crystal and L-H_{II} phase transitions. The heating and cooling scans show hysteresis with respect to one another and there appears to be a change in heat capacity, C_p, in the gel-liquid crystal transition (heating scan). A study of the dependence of the hysteresis of the two transition temperatures on the scanning rate is presented in Figure 2. It is seen that the lower temperature transition has no real hysteresis whereas the L-H_{II} transition has a definite and large hysteresis of approximately 9°C. The hysteresis is presumably due to a fairly large energy barrier to conversion from one state to another, as might be expected for so large a topological change as must occur. Also, the differing water contents of the two phases may necessitate bulk water flow through the liposomes during the transition.

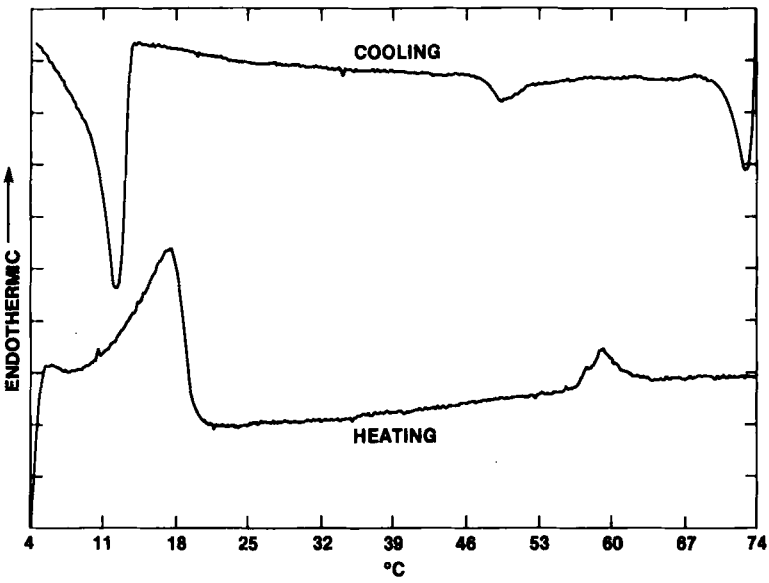

FIGURE 1 Differential scanning calorimetry of egg phosphatidylethanolamine at 5°C/min.

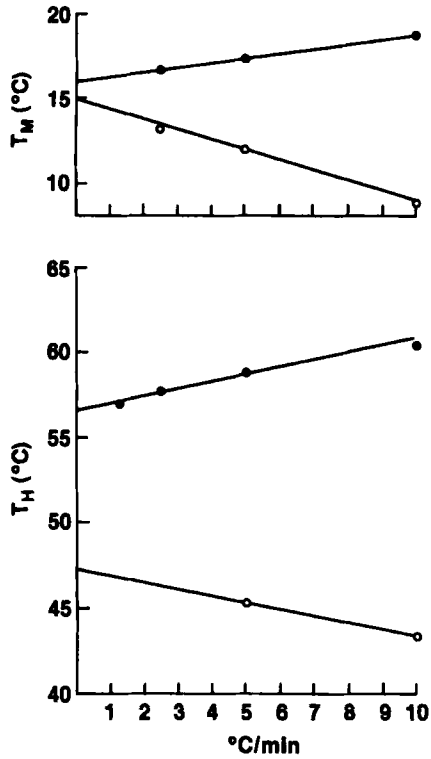

FIGURE 2 The dependence of the calorimetrically-measured transition temperatures of egg PE in excess water on the rates of heating (●) and cooling (○) are plotted above. The changes in transition temperatures were monitored by observing the shifts in the calorimetric peaks as a function of scanning rate. Extrapolation of the transition temperatures to zero rate of change gives the following: T_m(heating) = 16°C, T_m(cooling) = 15°C, T_h(heating) = 56°C and T_h(cooling) = 47°C. The small difference in the T_m for heating and cooling is probably not significant, whereas the 9°C gap between the heating and cooling values of T_h is clearly due to an instrumentally-independent hysteresis.

Figure 3 shows the data taken of egg PE at two different pressures. Each data point represents 0.1°C. The low level oscillatory noise is most likely due to convection within the cell. The relative displacement of the 220 bars data when compared to the 50 bars data shows that pressure destabilizes the H_{II} phase with respect to the lamellar liquid crystal state. In other words, the H_{II} phase has a higher molar volume than the lamellar phase. This result implies that processes involving the H_{II} phase as an intermediate[4] should be slowed or stopped by application of hydrostatic pressure. The demonstrated pressure sensitivity of the H_{II} phase provides a basis for testing the role of non-bilayer phases in biological phenomena such as membrane fusion.

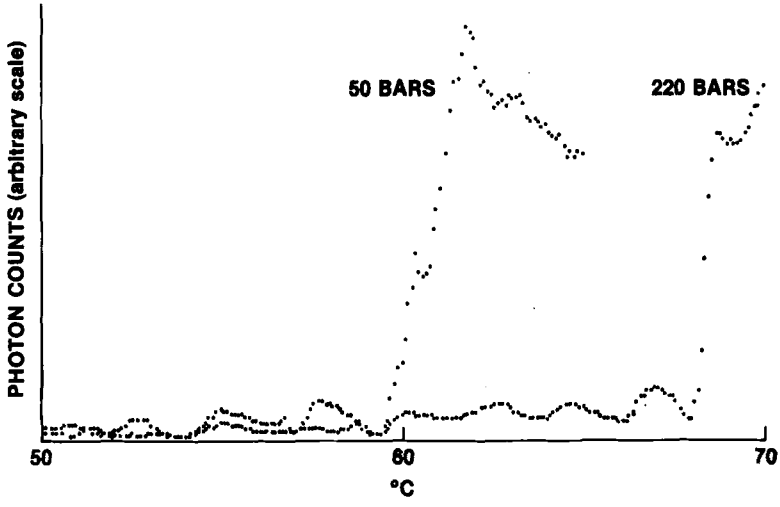

FIGURE 3 Transmission of laser beam through pelleted sample of egg PE at 50 and 220 bars. Each data point is 0.1°C.

Sensitivity of membrane fusion to pressure may well prove to underlie the effects of hydrostatic pressure on the nervous system, as well as on other organs which perform secretory functions.

This study was supported by the Office of Naval Research. One of us (P. Y.) was the recipient of a National Research Council/Naval Research Laboratory Resident Research Associateship during the period of this research.

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