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The combined and separate effects of low temperature and freezing on membrane lipid mesomorphic phase behavior: relevance to cryobiology

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Time-resolved X-ray diffraction is used to demonstrate that for certain lipids low temperature alone may not be sufficient to bring about changes in mesomorphic phase state. However, when combined with slow freezing of the aqueous substrate, the fluid bilayer phase is destabilized as a result of dehydration, and thus more prone to undergoing deleterious thermotropic phase transformations. The cryobiological relevance of these results is discussed.

The systematic study of the physiochemical properties of lipids promises an understanding of lipid phase relations in cellular membranes and in other biological and reconstituted lipid aggregates. The lipid fraction of natural membranes consists of a vast array of different lipid species each with their own unique thermotropic, lyotropic and miscibility properties. The behavior of the native membrane reflects to some extent the behavior of its individual components. To understand how the biomembrane responds to environmental stresses such as extremes of temperature and hydration we must first of all establish the behavior of the individual lipid components in isolation and subsequently in more complex mixtures under similar conditions [1].

In the present study the combined and separate effects of temperature and hydration on the mesomorphic phase properties of membrane lipids is addressed. It is shown that slow freezing causes dehydration and that for phospholipids with suitably poised transition temperatures (T_t), this can effect a direct lyotropic (solvent-induced) lamellar liquid crystal-to-gel phase transformation. Such an effect occurring in a native biological membrane could trigger undesirable, possibly irreversible, lateral phase separations in the plane of the membrane or simply destabilize the bilayer structure leading to loss of trans-membrane electrical potential and eventually to cell death.

In this study time-resolved X-ray diffraction (TRXRD) [2–4] is used and is shown to be an effective and revealing method for monitoring the combined and separate effects of temperature and freezing on the mesomorphic phase properties of hydrated dioleoylphosphatidylserine (DOPS). The method reports directly and continuously on the phase state and degree of hydration of the lipid, and the phase state of the suspending aqueous medium. Thermal events occurring during the heating and cooling scans are monitored simultaneously by recording sample temperature.

Time-resolved X-ray diffraction was used to decipher the structural rearrangements undergone when fully hydrated DOPS is cycled in temperature between 20°C and −25°C. Static measure-

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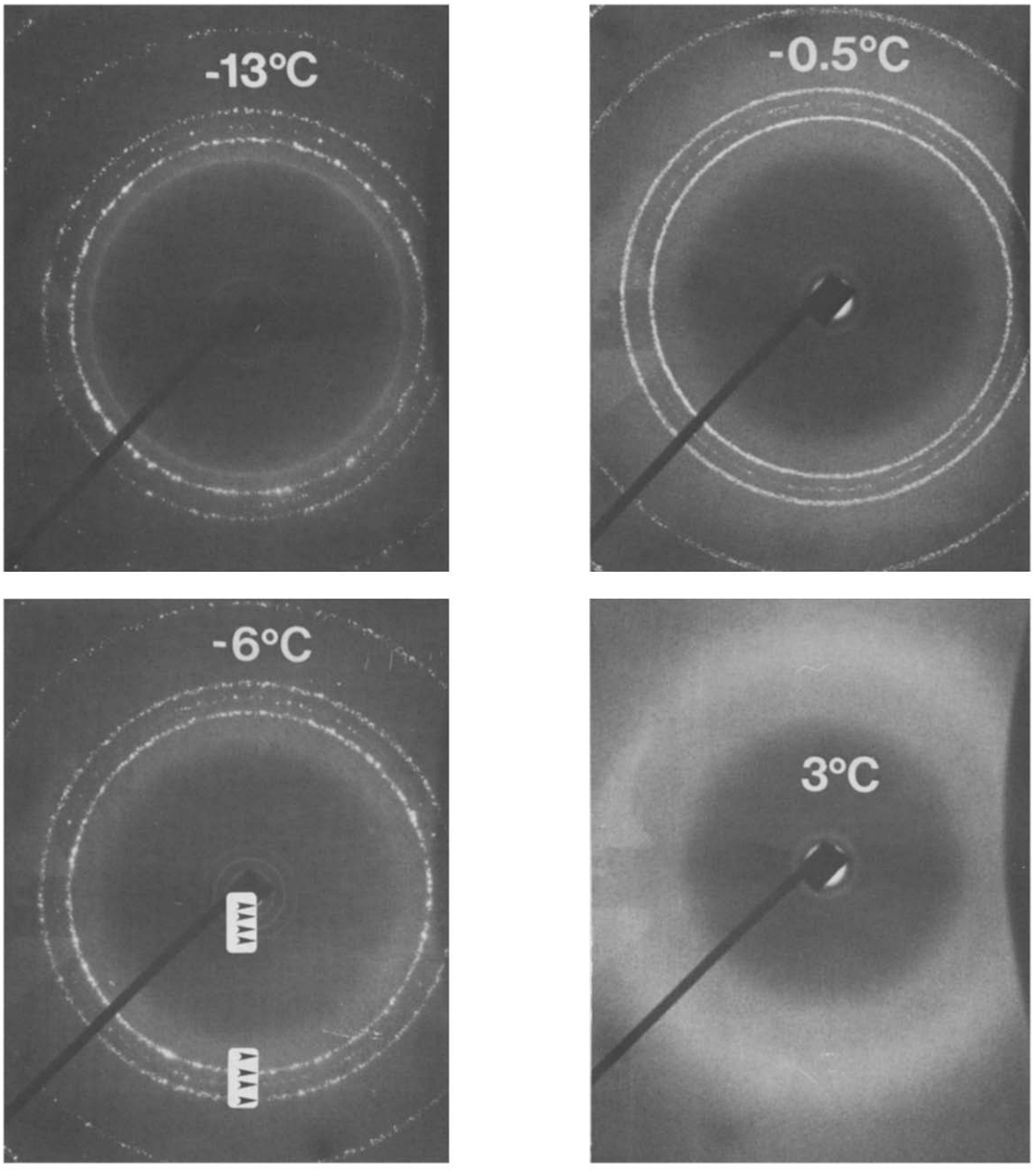

Fig. 1. X-Ray diffraction patterns from fully hydrated dioleoylphosphatidylserine above and below the freezing ($-18°C$) and melting ($-0°C$) temperature of the aqueous phase. Samples were slowly ($\leqq 6$ K/min) cooled to $\leqq -25°C$ and subsequently warmed to the temperature indicated. At $-6°C$, the arrowed reflections correspond to lipid peaks at $(5.5\ nm)^{-1}$, $(2.75\ nm)^{-1}$, $(1.83\ nm)^{-1}$, and about $(0.46\ nm)^{-1}$ and to ice peaks at $(0.39\ nm)^{-1}$, $(0.366\ nm)^{-1}$ and $(0.346\ nm)^{-1}$. The lipid, obtained from Avanti Polar Lipids, Inc., gave a single spot by thin-layer chromatography [2] and was used without further purification. It was dispersed at approx. 20 mM lipid in excess buffer (0.1 M KCl/0.01 M Hepes (pH 7)) by vortexing at room temperature in an argon atmosphere and concentrated by centrifugation [2,5]. The hydrated lipid pellet was transferred to a thin-walled (10 μm) capillary (1 mm internal diameter, Supper), flame-sealed under argon and hermetically sealed with 5-min epoxy. X-Ray diffraction measurements were carried out on the A-1 line at the Cornell High Energy Synchrotron Source as previously described [2–5]. Sample temperature was controlled by using a gas crystal heating/cooling system. A thermocouple positioned outside but touching the capillary next to the X-ray beam was used to record sample temperature [2]. Placing the thermocouple inside the capillary essentially eliminated undercooling and resulted in ice formation upon cooling at close to 0°C. Static diffraction patterns were recorded on X-ray sensitive polaroid film (Polaroid, Type 57) with an exposure time of 2 min, a sample-to-film distance of 46 mm, and a 0.3 mm collimator (Supper).

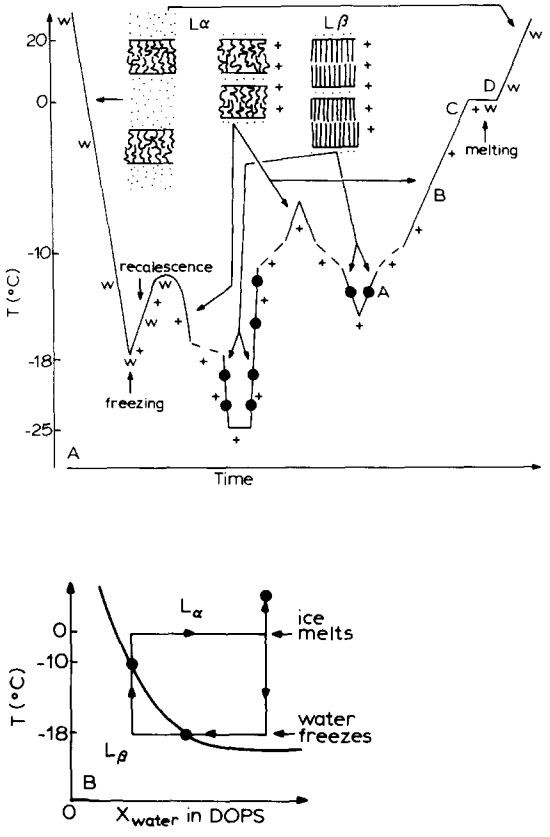

Fig. 2. (A) Heating and cooling curve for hydrated dioleoylphosphatidylserine. A schematic view of phase changes undergone by the lipid and aqueous components of the system is also included. Linearity along the time and temperature axis is not implied. The figure incorporates time-resolved and static X-ray diffraction data along with measurements of elapsed time and sample temperature. The following notation is used: ———, and ●———● denote the lamellar liquid crystal and gel phases, respectively; — — —, gel/liquid crystal phase coexistence; +, ice; w, water. The points labelled A–D correspond to the temperatures at which the diffraction patterns in Fig. 1 were recorded. (B) An interpretation of the effects of freezing and low temperature on the phase properties of dioleoylphosphatidylserine in terms of a typical temperature/composition isobaric phase diagram (see for example, Ref. 7). The sample begins at high temperature in the fully hydrated L_α phase. Cooling procedes down to -18°C at which point water freezes. In so doing it dehydrates the lipid effecting a disorder-to-order transition in the vicinity of −18°C. Warming the sample facilitates an order-to-disorder transition in the presence of ice at −10°C. At 0°C, ice melts and the lipid imbibes water to restore the original fully hydrated L_α phase. The important point to note is that a higher transition temperature is observed in the heating compared to the cooling direction which reflects the lower hydration level of the lipid in the presence of ice.

ments were made using X-ray sensitive polaroid film at intervals during the thermal cycles [5]. The lipid was dispersed in an excess of aqueous buffer (pH 7) as described in the legend to Fig. 1.

A summary of the experimental observations along with an interpretation of the results are presented in Fig. 2. At 20°C and in the presence of excess aqueous substrate electrostatic repulsion causes the liquid crystalline DOPS bilayers to separate and the interbilayer region to imbibe large quantities of fluid. This corresponds to the swollen or fully hydrated lamellar liquid crystal (L_α) phase [7]. The corresponding diffraction pattern from this phase is shown in Fig. 1. It consists of a series of diffuse continuous low-angle peaks accompanied by a broad peak centered at (0.46 nm)$^{-1}$ arising from the disordered acyl chains and a broad water peak at approx. (0.33 nm)$^{-1}$ [8–12]. Upon slowly cooling the sample, the above condition is maintained in that no phase change occurs down to −18°C in the presence of an undercooled aqueous substrate. At −18°C, ice crystals spontaneously form, the system experiences a reduction in available water and the lipid undergoes dehydration. During freezing, the latent heat of crystallization released (recalescence) raises sample temperature and upon continued cooling sample temperature slowly decreases. At about −16°C, the phase state of the lipid undergoes a dramatic change from liquid crystalline-to-gel corresponding to the direct lyotrope-induced transformation. The diffraction pattern below −16°C (cf. Fig. 1) shows a series of sharp reflections in the low-angle region and a sharp line at (0.42 nm)$^{-1}$ characteristic of ordered or gel-like acyl chain packing. Also present in the diffraction pattern are the tell-tale spotty reflections from hexagonal ice at $\geq$ (0.3 nm)$^{-1}$ [13]. Further cooling to −25°C does not noticeably change the system from that of a dehydrated or partially dehydrated (referred to as low moisture) gel phase lipid in the presence of ice.

If the sample is now warmed, very little occurs (see Fig. 1) until the system reaches −10.5°C. At this temperature the lipid undergoes a reversible thermotropic chain 'melting' transition from the low moisture gel to the low moisture L_α phase in the presence of ice. Note that this transition occurs some 5–6 K above the T_t recorded upon

sample cooling, a result which is in agreement with the expected behavior of desiccation raising the chain 'melting' T_t [7–10]. That this difference is due to undercooling of the lipid transition seems unlikely in view of the current evidence. In the vicinity of the T_t, changes are seen to occur in both the low- and wide-angle regions of the diffraction pattern (cf. Fig. 1).

At −6°C in the L_α phase the diffraction pattern reveals a lamellar repeat spacing of 5.50 nm (Fig. 1). The corresponding wide-angle region contains a diffuse liquid crystalline peak centered at approx. $(0.46\ \text{nm})^{-1}$ along with characteristic ice crystal reflections. This suggests that the lipid is only partially hydrated given a bilayer thickness increment of 0.105 nm/methylene group in the L_α phase, a water layer thickness increment of 0.16 nm/water molecule and a glycerophosphorylserine headgroup thickness of about 0.7 nm [7,14].

As sample temperature rises it was observed in the time-resolved diffraction patterns that the ice crystals become noticeably mobile beginning at T_t as evidenced by fluctuations in intensity of the individual ice reflections. These fluctuations become progressively more rapid and dramatic with temperature up to 0°C at which point ice melting is complete. As the ice melts and water becomes available the low moisture L_α phase imbibes water and swells to restore the original condition of the fully hydrated L_α phase.

The temperature of ice crystal formation was repeatedly found to occur in this and similar lipid systems in the vicinity of −18°C (unpublished data). Interestingly, if ice formation is nucleated at −11.1°C, the fluid lipid phase persists for many (up to 15) minutes in the presence of ice. Following this protocol, cooling effects the disorder-to-order transition at approx. −15°C.

These results demonstrate that freezing reduces water activity which in turn can bring about lipid dehydration. It is noted in this regard that ice at −20°C in a one-component system has a vapor pressure equal to that of a 10.8 osmolal solution and is capable of exerting an equivalent osmotic pressure of 244 bars (241 atm, 2.44 dyn/cm^2, ~ 24 MPa) [15]. In the case of certain lipids a freeze-induced dehydration occurring at the appropriate temperature, of and by itself, can induce a liquid crystal-to-gel phase transformation. Worthy of note in this connection is (1) the reversibility of the chain melting transition at −10.5°C in the presence of ice, and (2) the observation that ice crystal formation precedes the appearance of the gel phase lipid upon cooling. This precludes gel phase formation acting to trigger ice crystallization. The latter possibility is not unreasonable given that an organized 'rigid' lattice such as exists at the gel phase lipid/water interface is more likely to act as a heterogeneous ice nucleation site than is the fluid surface that obtains at the lipid/water interface of the L_α phase. This ties in with the fact that heterogeneous nucleation of ice can be prevented by emulsifying water as small droplets in an inert carrier fluid [16].

Throughout these measurements samples were slowly cooled and frozen in contrast to an ultrarapid freezing protocol which would fix the system in the fully hydrated, swollen state. This slow cooling approach facilitates extramembranal ice formation and the local condensation of stacked membranes [17,18].

These results have far reaching implications when considered from a cryobiological perspective. Organisms, subcellular organelles, isolated membranes and other lipid-rich systems that are exposed to low temperatures may be in a position to resist or to tolerate such a stress by virtue of their lipid composition. If, however, in addition to low temperature, a freezing stress is imposed, the lipid components may no longer be able to withstand the low temperatures because of the freeze-induced dehydration which destabilizes the lamellar liquid crystal in favor of the gel phase. A lyotropic bilayer-to-nonbilayer transition is also possible since in a variety of lipid systems low moisture stabilizes the nonbilayer phases [2,8,9,19]. In either case, the lipid mesomorphic phase change is likely to profoundly influence membrane structure and thus function.

The knowledge that water freezing can be effected in the hydrated DOPS system by nucleating at −11°C without an accompanying mesomorphic phase transformation has been put to use in experiments where a freeze-thaw method is employed to equilibrate metal ions in aqueous dispersions of DOPS [20]. Multiple freeze-thaw cycling between 22°C and −11.1°C effectively

equilibrates Ca^{2+} ions without inducing a thermotropic or lyotropic phase transition which could conceivably alter the properties of the PS-Ca^{2+} complex.

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References

1 Caffrey, M. (1986) in Membranes, Metabolism and Dry Organisms (Leopold, A.C., ed.), pp. 242–258, Cornell University Press, New York
2 Caffrey, M. (1985) Biochemistry 24, 4826–4844
3 Caffrey, M. and Bilderback, D.B. (1983) Nucl. Instrum. Methods 208, 495–510
4 Caffrey, M. and Bilderback, D.B. (1984) Biophys. J. 45, 627–631
5 Caffrey, M. and Feigenson, G.W. (1984) Biochemistry 23, 323–331
6 Tsui, F.C., Ojcius, D.M. and Hubbell, W.L. (1986) Biophys. J. 49, 459–468
7 Hauser, H., Paltauf, F. and Shipley, G.G. (1982) Biochemistry 21, 1061–1067
8 Luzzati, V. (1968) in Biological Membranes, Physical Fact and Function (Chapman, D., Ed.), Vol. 1, pp. 71–123, Academic Press, New York
9 Shipley, G.G. (1973) in Biological Membranes (Chapman, D. and Wallach, D.F.H., eds.), Vol. 2, pp. 1–89, Academic Press, New York
10 Levine, Y.K. (1973) Prog. Surf. Sci. 3, 279–352
11 Blaurock, H.E. (1982) Biochim. Biophys. Acta 650, 167–207
12 Engelman, D.M. (1970) J. Mol. Biol. 47, 115–117
13 Dowell, L.G., Moline, S.W. and Rinfret, A.P. (1962) Biochim. Biophys. Acta 59, 158–167
14 Cevc, G., Seddon, J.M. and Marsh, D. (1985) Biochim. Biophys. Acta 814, 141–150
15 Nobel, P.S. (1983) Biophysical Plant Physiology and Ecology, W.H. Freeman and Co., San Francisco
16 Franks, F., Mathias, S.F., Galfe, P., Webster, S.D. and Brown, D. (1983) Cryobiology 20, 298–309
17 Franks, F. (1985) Biophysics and Biochemistry at Low Temperatures, Cambridge University Press, New York
18 Finean, J.B., Bunn, T.K., Hutchinson, A. and Mills, D. (1984) Biochim. Biophys. Acta 777, 140–146
19 Seddon, J.M., Cevc, G., Kaye, R.D. and Marsh, D. (1984) Biochemistry 23, 2634–2644
20 Feigenson, G.W. (1986) Biochemistry 25, 5819–5825