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B. A. Cunningham [a] , L. J. Lis [a] & P. J. Quinn [a b]

[a] Department of Physics, and Liquid Crystal Institute, Kent State University, Kent, OH 44242, U.S.A.

[b] Department of Biochemistry, King's College London, Campden Hill, London, W8 7AH, U.K.

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Influence of Monovalent Cations on the Dipalmitoylphosphatidylcholine Phase Transitions

B. A. CUNNINGHAM, L. J. LIS and P. J. QUINN†

Department of Physics, and Liquid Crystal Institute, Kent State University, Kent, OH 44242, U.S.A.

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The effect of different monovalent cations on the packing arrangements and phase behavior of dipalmitoylphosphatidylcholine (DPPC) in excess water and on the transition of the lamellar phase from the gel to liquid crystalline configuration has been investigated using differential scanning calorimetry and synchrotron radiation at the Daresbury Laboratory. Bilayers of DPPC in 1M KCl or water appear to undergo the same transition sequences without any change in the kinetics of phase induction. It was also observed that bilayers of DPPC in the presence of 1M LiCl produced no small angle x-ray scattering peaks characteristic of the $P_{\beta'}$ phase.

Keywords: phospholipids, phase transitions, dipalmitoyllecithin, x-ray diffraction, lipid-water systems, cation binding

INTRODUCTION

There is conclusive evidence from a number of studies that monovalent ions influence the structure of zwitterionic lipid bilayers by binding directly to the lipid headgroup,[1–5] influencing the binding of another ion to the headgroup[6] or changing the polarization of the water surrounding the bilayer.[7–11] Monovalent cations, in general, bind to phosphatidylcholine headgroups and change the thermodynamic properties[12–14] and interactive forces[9,10] of the bilayers. However, there is little information on how ions affect the dynamic struc-

†Department of Biochemistry, King's College London, Campden Hill, London W8 7AH, U.K.

tural rearrangements that occur in processes involving ions and lipids. Of fundamental interest is the characterization of lipid structures involved in thermotropic phase transitions in the presence of ions. Recently, time resolved studies using high intensity x-rays from a synchrotron source[16–21] have been used to examine phase transitions in phospholipids and galactolipids. These studies provide information on the kinetics of the transition, and identify transient intermediate phases that may appear during the transition. This information is essential to understand the mechanism(s) involved in these processes.

We have used this time resolved structural technique to monitor the small angle x-ray scattering from the three dimensional molecular packing array, and the wide angle x-ray scattering of the two dimensional packing subcell of the acyl chains as a function of time (temperature) for fully hydrated dipalmitoylphosphatidylcholine bilayers in water and 1M LiCl or KCl. Phase transitions can be observed in bilayers of dipalmitoylphosphatidylcholine dispersed in excess water and 1M KCl, but bilayers in 1M LiCl produce x-ray patterns with no diffraction maxima in the wide angle scattering region of the profile, and it is difficult to determine when phase transitions occur. The change in small-angle and wide-angle scattering for dipalmitoylphosphatidylcholine in water and 1M KCl coincide as the transitions from $L_{\beta'}$ to $P_{\beta'}$ to L_{α} proceed. At a high rate of temperature change, the usual $L_{\beta'}$ to $P_{\beta'}$ to L_{α} phase sequence is observed for DPPC in water and 1M KCl with no apparent change in the time required to induce the phase transition after the transition temperature is anchored. Thus, the interaction of K^+ with the DPPC bilayer interface is not significant enough to hinder the transition kinetics.

MATERIALS AND METHODS

Dipalmitoylphosphatidylcholine was obtained from Sigma Chemical Co. (St. Louis, MO) and used without further purification. Salt solutions were made with reagent grade salts and distilled water. X-ray samples were prepared by mixing dry dipalmitoylphosphatidylcholine with a large excess (90%, v/w) of water. The samples were equilibrated by heating to 60°C for one hour and cooling to room temperature. The samples were then mounted between mica sheets 1 mm apart in an x-ray sample holder.

The x-ray experiments were carried out by using a monochromatic (0.15 nm) focused x-ray beam at station 7.3 of the Daresbury Synchrotron Laboratory as previously described.[22] A cylindrically bent

single crystal of Ge[23] and a long float glass mirror were used for monochromatization and horizontal focusing, providing 2.10^9 photons·s^{-1} down a 0.2 mm colimator at 2.0 GeV and 100 to 200 mA of electron beam current. A Keele flat plate camera was used with a linear detector constructed at the Daresbury Laboratory. The deadtime between frames was 50 μs, with the temporal resolution of each frame varying from 100 to 700 ms for temperature jumps or temperature scans. X-ray scattering has been plotted as a function of reciprocal spacing using Teflon™ as a calibration standard.[24] No corrections were applied to path distances from the sample to the linear detector, consequently, wide-angle spacings will be slightly longer than measured directly by the detector. All data analysis used the OTOKO program at the Daresbury Lab.

The temperature scan and jumps were produced by water baths connected internally to the sample mount of the x-ray camera. The rate of change for the temperature jumps was about 1.0°·s^{-1} in both heating and cooling modes. The temperature of the sample was monitored internally using a thermocouple placed adjacent to the sample region of the x-ray sample holder. We expect that the thermal diffusion through our samples was approximately the same as that observed in experiments reported by Caffrey[20] since our sample thickness was also 1 mm.

Samples for calorimetry were prepared initially by pipetting known weights of dipalmitoylphosphatidylcholine dissolved in chloroform in a culture tube and evaporating the solvent under a stream of dry nitrogen. During this process, the culture tube was rotated to produce a thin layer of lipid on the bottom of the tube. The lipid was then dried under vacuum for one hour to completely remove the remaining solvent. An appropriate amount of salt solution was added to the lipid to make a dispersion of 5 mg lipid per ml salt solution. Dispersion was accomplished by sonicating the lipid salt solutions in a bath sonicator (Bransonic 220) for approximately one minute. The samples were incubated in an oven at 60°C for one hour with additional sonication for a few minutes to completely disperse the lipid. High sensitivity differential scanning calorimetry was performed using a MicroCal MC-2 instrument with a scan rate of 10°·$hour^{-1}$. After the first scan, the sample and reference were cooled in the calorimeter cells and rescanned to check reproducibility. All samples were run under a nitrogen pressure of 15 psi to minimize bubble formation in the specimens. Thermograms were analyzed with the MicroCal DA2 software package using an IBM PC interfaced to the calorimeter. Enthalpy values were calculated on the basis of an internal electronic

calibration. The onset temperature of the thermogram was used as the transition temperature.

RESULTS AND DISCUSSION

Dipalmitoylphosphatidylcholine dispersed in excess water undergoes two major thermotropic phase transitions. The pretransition observed at approximately 37°C involves a change from the lamellar gel ($L_{\beta'}$) to rippled ($P_{\beta'}$) phase. Static x-ray diffraction measurements have shown that the characteristic signature of this transition is a change in relative intensities for several peaks and the addition of peaks representative of the bilayer ripple period in the small angle x-ray scattering region which represents the unit cell spacings for the bilayers in the mesophase.[25–28] The main phase transition occurring at approximately 41°C is characterized again by changes in the small-angle scattering region and a change in the hexagonal subcell packing of the acyl chains from 0.42 to 0.475 nm.[26–28]

Samples of dipalmitoylphosphatidylcholine in excess solutions of water, 1M LiCl and 1M KCl were subjected to a temperature scan of $10°\cdot min^{-1}$. In water and 1M KCl, the initial mesophase structure of dipalmitoylphosphatidylcholine at approximately 25°C was a gel-state bilayer ($L_{\beta'}$) with repeat spacing of about 6.4 nm and an acyl chain pseudo hexagonal subcell with a spacing of 0.42 nm. These results agree with structural parameters reported previously from our laboratory.[15] The final structure was a liquid crystal state bilayer with a repeat spacing of approximately 6.2 nm and an acyl chain hexagonal subcell with a spacing of 0.46 nm. The mesophase structure is similar for dipalmitoylphosphatidylcholine bilayers in 1M LiCl, but no information on the acyl chain subcell was observed.

The data for the phase transitions of dipalmitoylphosphatidylcholine in water (Figure 1) indicated that the bilayer underwent the pre- and main-phase transitions at approximately the temperatures obtained by calorimetry (Figure 4a and Refs. 11–14) and produced the expected changes in the small-angle and wide-angle scattering patterns.[25] However, the data for dipalmitoylphosphatidylcholine in 1M LiCl (Figure 2) indicate that the presence of LiCl has affected the appearance of the wide-angle scattering pattern, and eliminated the small-angle scattering pattern that characterizes the presence of the $P_{\beta'}$ phase. At least two small angle diffraction orders were observed in each pattern obtained for DPPC in 1M LiCl indicating that a lamellar structure was present at each temperature studied. These

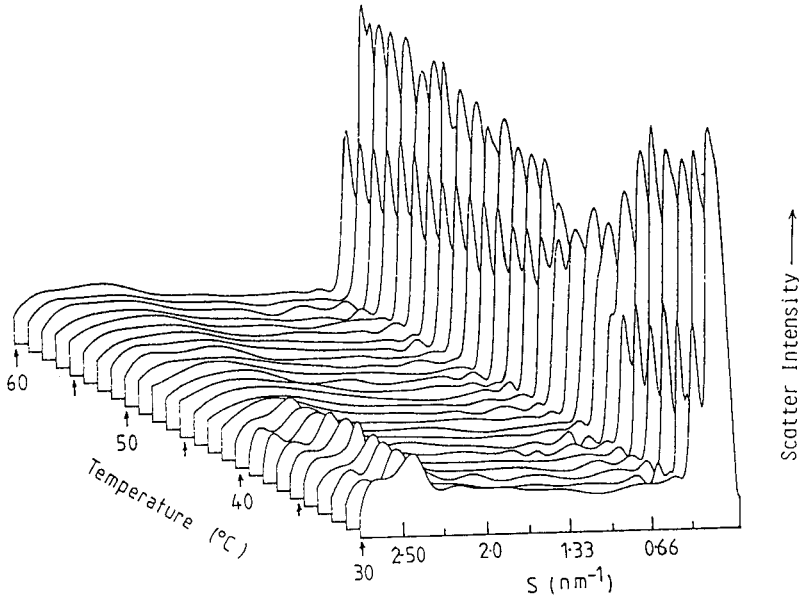

FIGURE 1 Three dimensional plot of scattered x-ray intensity, reciprocal spacing and temperature for dipalmitoylphosphatidylcholine in water. The rate of change in temperature was 10°·min^{-1} between 30 and 60°C. A total of 255 frames each of 700 ms duration were collected. Every tenth frame of the data set is presented in the figure. The patterns are characteristic of the transitions from the gel bilayer ($L_{\beta'}$) to rippled bilayer ($P_{\beta'}$) to liquid crystal bilayer (L_{α}) phases.

higher order reflections were significantly weaker in intensity than the first order diffraction peak resulting in an apparent narrower first order peak width than observed for DPPC in water where there is some overlap in intensity between the first and higher order peaks. Our previous x-ray diffraction study using film methods,[11] also indicated a very low intensity in the wide angle scattering region relative to those in the small angle region of the diffraction pattern. In order to determine the acyl chain subcell, films were typically overexposed in the small angle scattering region. The lack of the wide angle scattering peaks precludes the unambiguous assignment of the thermal phase sequence in this system. However, it is clear that the sequence observed for DPPC in water ($L_{\beta'} \rightarrow P_{\beta'} \rightarrow L_{\alpha}$) is not observed for DPPC in 1M LiCl in the real time x-ray diffraction patterns, even though low[11-14] and high resolution calorimetry (Figure 4) indicate that two thermal transitions are observed for DPPC in water and 1M LiCl. Specifically, the intermediate phase is not the $P_{\beta'}$ phase when DPPC is equilibriated in 1M LiCl. The binding of Li^+ to DPPC has

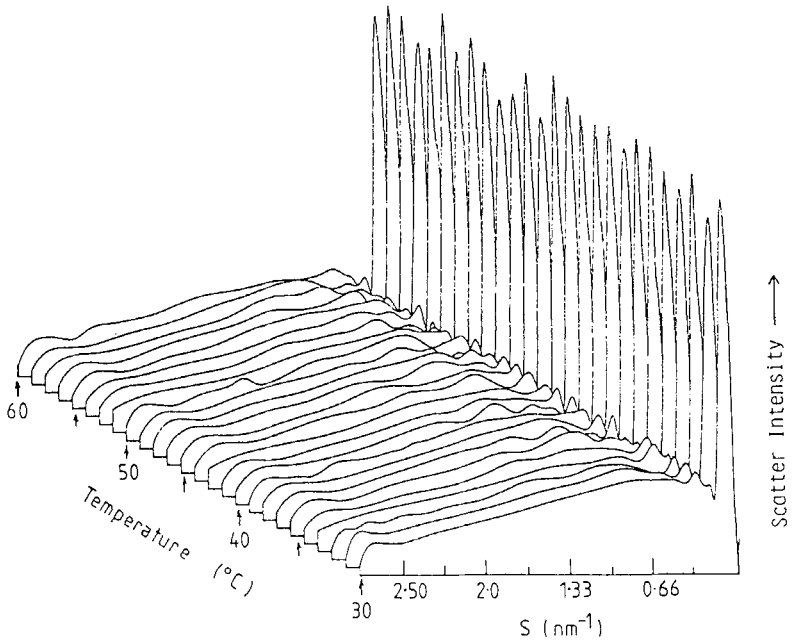

FIGURE 2 Three dimensional plot of scattered x-ray intensity, reciprocal spacing and temperature for dipalmitoylphosphatidylcholine in 1M LiCl. The rate of change in temperature was 10°·min^{-1} between 30 and 60°C. A total of 255 frames each of 700 ms duration were collected. Every tenth frame of the data set is presented in the figure.

been previously inferred by calorimetry[11] and force measurements.[10] Thus, the most plausible explanation of a weak scattering peak involves small scattering units. It can be proposed that any intralamellar binding of Li^+ to the dipalmitoylphosphatidylcholine head groups in a *cis* configuration would lead to a disordering of the long range order between acyl chains. Small domains of varying acyl chain orientations

TABLE I

Thermodynamic parameters for dipalmitoylphosphatidylcholine bilayers in ionic solution obtained at a scan rate of 10°·hour^{-1}.

	Pretransition		Main Transition	
Solution	T_p(°C)	ΔH_p (kJ·mole^{-1})	T_m(°C)	ΔH_m (kJ·mole^{-1})
water	33.5	4.01	40.1	32.61
1M LiCl	38.7	3.93	41.9	26.88
1M KCl	34.0	2.93	41.0	25.30

would then produce a broadening of the characteristic scattering peaks. This explanation is also consistent with the observed reduction in the enthalpy of the $P_{\beta'}$ to L_α transition when LiCl is present in the solvent (Figure 4b and Table I). Extensive ordering in a single orientation of the acyl chain subcells would tend to increase, rather than decrease, the enthalpy of the transitions.

Data for dipalmitoylphosphatidylcholine bilayers in 1M KCl (Figure 3) undergoing a temperature scan of 10°/min indicate a single acyl chain transition, as determined by changes in the wide-angle scattering pattern, taking place at about 41°C. This transition can be correlated to the $P_{\beta'}$ to L_α transition observed by calorimetry. Low[11–14] and high resolution calorimetry (Figure 4c) indicate that the pre- and main phase transitions occur for DPPC bilayers in 1M KCl at 34.0°C and 41.0°C respectively. In addition a rate of temperature change of $1.5°\cdot s^{-1}$ was used to drive the transitions in dipalmitoylphosphatidylcholine dispersions. The time-resolved x-ray diffraction

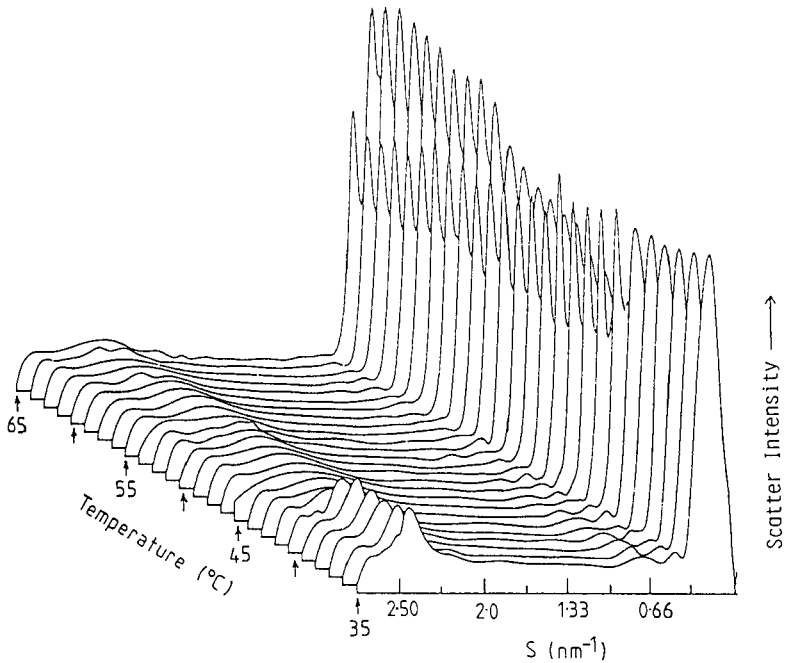

FIGURE 3 Three dimensional plot of scattered x-ray intensity, reciprocal spacing and temperature for dipalmitoylphosphatidylcholine in 1M KCl. The rate of change in temperature was $10°\cdot min^{-1}$ between 35 and 65°C. A total of 255 frames each of 700 ms duration were collected. Every tenth frame of the data set is presented in the figure.

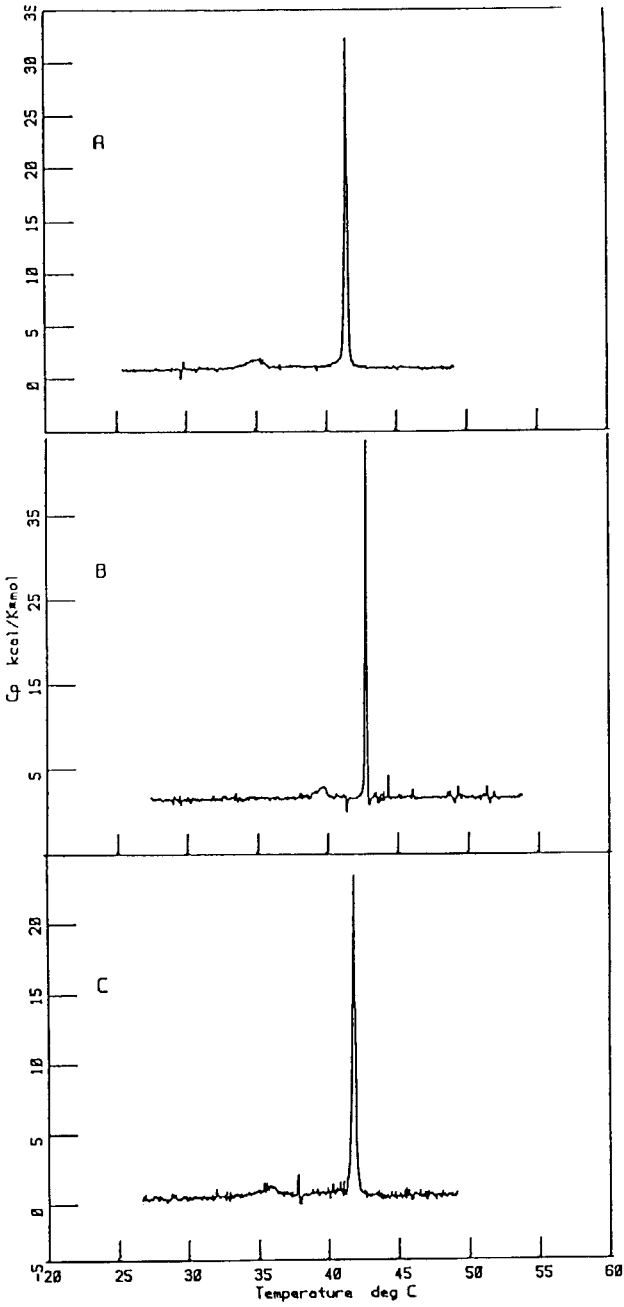

FIGURE 4 Differential scanning calorimetric thermograms of dispersions of dipalmitoylphosphatidylcholine in (a) water, (b) 1M LiCl, (c) 1M KCl. The thermograms were obtained at a scan rate of $10°\cdot hour^{-1}$.

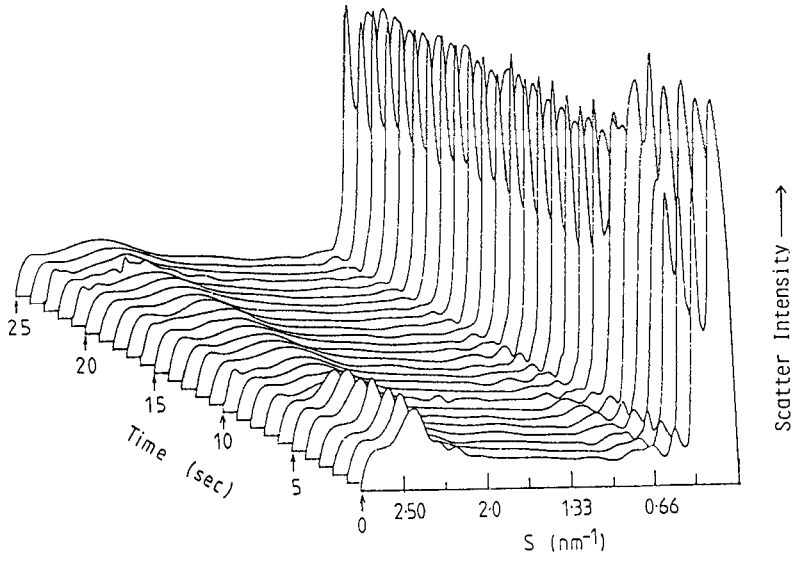

FIGURE 5 Three dimensional plot of a temperature jump experiment showing scattered x-ray intensity *vs.* reciprocal spacing as a function of time for dipalmitoylphosphatidylcholine in 1M KCl. The rate of temperature change from 30 to 60°C was $1.5°\cdot s^{-1}$. A total of 255 frames each of 100 ms duration were collected. Every tenth frame of the data set is presented in the figure.

data are presented in Figure 5 and show that the appropriate phase sequence at this rate of temperature change is $L_{\beta'} \rightarrow P_{\beta'} \rightarrow L_{\alpha}$. The transit time for the pre- and main transitions are less than one second and are no different from that observed for dipalmitoylphosphatidylcholine bilayers in water. Thus there is no indication of ion binding to the DPPC bilayer interface extensive enough to modify the transition kinetics of either transition.

It can be concluded that K^+ interacts with bilayers of dipalmitoylphosphatidylcholine without affecting the structures involved in the pre- and main-phase transitions, or the kinetics of phase induction. The influence of LiCl on both wide-angle and low-angle x-ray scattering can be ascribed to more extensive Li^+ binding at the dipalmitoylphosphatidylcholine-water interface.

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References

1. T. Cserhati and M. Szogyi, *Chem. Phys. Lipids*, **34**, 93–99 (1983).
2. S. A. Tatulian, *Biochim. Biophys. Acta*, **736**, 189–195 (1983).
3. G. L. Jendrasiak, *Chem. Phys. Lipids*, **9**, 133–146 (1972).
4. L. J. Lis, J. W. Kauffman and D. F. Shriver, *Biochim. Biophys. Acta*, **406**, 453–464 (1975).
5. S. McLaughlin, A. Bruder, S. Chen and C. Moser, *Biochim. Biophys. Acta*, **394**, 304–313 (1975).
6. J. F. Hahn, J. M. Collins and L. J. Lis, *Biochim. Biophys. Acta*, **736**, 235–240 (1983).
7. E. Loschchilova and B. Karvaly, *Biochim. Biophys. Acta*, **514**, 274–285 (1978).
8. B. Karvaly and E. Loshchilova, *Biochim. Biophys. Acta*, **470**, 492–496 (1977).
9. S. Afzal, W. J. Tesler, S. K. Blessing, J. M. Collins and L. J. Lis, *J. Coll. Interface Sci.*, **97**, 303–307 (1984).
10. W.-M. Kwok, J. E. Shimotake and L. J. Lis, *Mol. Cryst. Liq. Cryst.*, **132**, 181–188 (1985).
11. B. A. Cunningham and L. J. Lis, *Biochim. Biophys. Acta*, **861**, 237–242 (1986).
12. D. Chapman, J. Urbina and K. M. Keogh, *J. Biol. Chem.*, **249**, 2512–2521 (1974).
13. S. A. Simon, L. J. Lis, J. W. Kauffman and R. C. MacDonald, *Biochim. Biophys. Acta*, **375**, 317–326 (1975).
14. D. Chapman, W. E. Peel, B. Kingston and T. H. Lilley, *Biochim. Biophys. Acta*, **464**, 260–275 (1977).
15. B. A. Cunningham, J. E. Shimotake, W. Tamura-Lis, T. Mastran, W.-M. Kwok, J. W. Kauffman and L. J. Lis, *Chem. Phys. Lipids*, **39**, 135–143 (1986).
16. M. Caffrey and B. H. Bilderbach, *Nucl. Inst. Meth.*, **208**, 495–510 (1983).
17. J. L. Ranck, *Chem. Phys. Lipids*, **32**, 251–270 (1983).
18. J. L. Ranck, L. Letellier, E. Schechter, B. Krop, P. Pernot and A. Tardieu, *Biochemistry*, **23**, 4955–4961 (1984).
19. M. Caffrey and D. H. Bilderbach, *Biophys. J*, **45**, 627–631 (1984).
20. M. Caffrey, *Biochemistry*, **24**, 4826–4844 (1985).
21. P. J. Quinn and L. J. Lis, *Biochim. Biophys. Acta*, **862**, 81–86 (1986).
22. C. Nave, J. R. Helliwell, P. R. Moore, A. W. Thompson, J. S. Worgan, R. J. Greenall, A. Miller, S. K. Burley, J. Bradshaw, W. J. Pigram, W. Fuller, D. P. Siddons, M. Deutsch and R. T. Tregear, *J. Appl. Cryst.*, **18**, 396–403 (1985).
23. R. Helliwell, T. J. Greenough, P. D. Carr, S. A. Rule, P. R. Moore, A. W. Thompson and J. S. Worgan, *J. Phys. E.*, **15**, 1363 (1982).
24. C. W. Bunn and E. R. Howells, *Nature* (*London*), **174**, 549–551 (1954).
25. M. J. Janiak, D. M. Small and G. G. Shipley, *Biochemistry*, **15**, 4575–4580 (1976).
26. A. Tardieu, V. Luzzati and R. C. Reman, *J. Mol. Biol.*, **75**, 1117–1124 (1975).
27. L. J. Lis and P. J. Quinn, *Mol. Cryst. Liq. Cryst.*, **146**, 35–39 (1987).
28. B. Tenchov, L. J. Lis and P. J. Quinn, *Biochim. Biophys. Acta*, **897**, 143–151 (1987).