Molecular Crystals and Liquid Crystals Incorporating Nonlinear Optics

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gmcl17

X-Ray Cinematography of Phospholipid Phase Transformations with Synchrotron Radiation

Peter Laggner [a] , Karl Lohner [a] & Karl Müller [a]

[a] Institute of X-ray Fine-Structure Research, Austrian Academy of Sciences and Research Society Joanneum, Graz, Austria

Version of record first published: 17 Oct 2011.

To cite this article: Peter Laggner , Karl Lohner & Karl Müller (1987): X-Ray Cinematography of Phospholipid Phase Transformations with Synchrotron Radiation, Molecular Crystals and Liquid Crystals Incorporating Nonlinear Optics, 151:1, 373-388

To link to this article: http://dx.doi.org/10.1080/00268948708075343

Mol. Cryst. Liq. Cryst., 1987, Vol. 151, pp. 373-388
Photocopying permitted by license only

Printed in the United States of America

X-RAY CINEMATOGRAPHY OF PHOSPHOLIPID PHASE TRANSFORMATIONS WITH SYNCHROTRON RADIATION

PETER LAGGNER, KARL LOHNER, and KARL MÜLLER
Institute of X-ray Fine-Structure Research, Austrian Academy of Sciences and Research Society Joanneum, Graz, Austria.

Abstract Time-resolved X-ray diffraction methods were used to study the structural mechanisms and kinetics of phospholipid phase transformations, involving (a) thermotropic transitions between different lamellar phases, (b) thermotropic lamellar-hexagonal phase transitions, and (c) lamellar-isotropic micellar transformations induced by natural detergents (bile salts). The potential of these methods to resolve molecular rearrangements in the millisecond time-scale is discussed.

INTRODUCTION

A considerable wealth of knowledge exists on the equilibrium structural properties of phospholipid-water systems[1]. Solid information, from spectroscopic methods, is also available on the molecular dynamics in terms of local order parameters or diffusion constants[2,3]. An information gap, however, exists on the structural mechanisms and kinetics of phase transitions, where mainly the results from optical studies (e. g. turbidity measurements) form the basis of present knowledge, which is, therefore, mainly restricted to rates[4]. The classical techniques of X-ray structure analysis are by several orders of magnitude too slow to follow structural arrangements in the sub-second domain, as would

be required for this purpose. This situation has changed in the past few years with the availability of powerful synchrotron radiation sources, which exceed conventional X-ray sources by at least four orders of magnitude in brillance[5]. In combination with fast, high-capacity position-sensitive detectors[6], these facilities open the field for X-ray structure analysis in the millisecond time-domain, and possibly also below. In the present article we summarize our experience so far gained with this technique on transformations of phospholipid mesophases. In addition to the previously described temperature-jump approach[7] we present first results on chemically induced changes using stopped-flow mixing.

MATERIALS AND METHODS

Phospholipids (≥99% pure) were purchased from Avanti Polar Lipids Inc. (Birmingham, AL, USA) except for 1-hexadecyl-2-oleoyl-sn-phosphoethanolamine-3 (HOPE) which was synthesized at the Institute of Biochemistry, Technical University Graz. Cholesterol and bile salts were obtained from SIGMA (St. Louis, MO, USA). Aqueous dispersions were prepared by vortexing lipids with appropriate amounts of deionized water to give concentrations of about 0.2 g/ml. Time-resolved X-ray small-angle diffraction experiments were performed on the X-33 camera of the European Molecular Biology Laboratory (Hamburg Outstation at DESY) at the storage ring DORIS II[8]. For the temperature-jump experiments a coupled-thermostat system has been used as shown in Figure 1, in which rapid heating from a temperature below the transition (cold bath "C") to above the transition (hot bath "H") is supported by an intermediate pulse from a higher temperature circuit (very hot bath "VH"). The con-

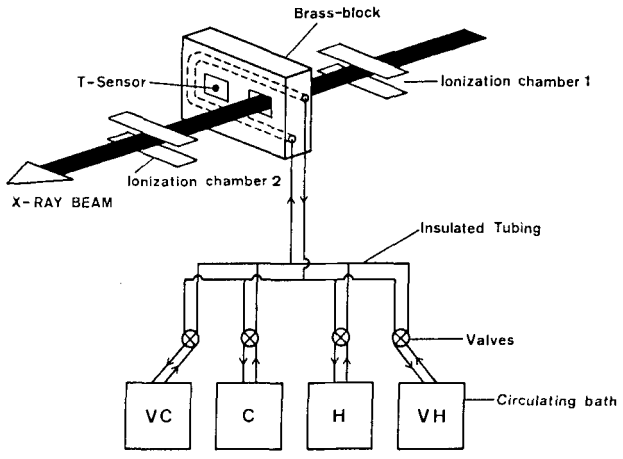

FIGURE 1. Schematic view of T-jump arrangement.

verse procedure, using an intermittent, very cold ("VC") pulse, was used for cooling experiments. The maximum temperature gradients thus achieved were in the order of 6 deg/s. However, owing to the heat conductivity of the sample, the time constant for equilibration is only in the order of 1 s. Temperatures within the sample cell were followed by a fast-response thermocouple.

For rapid mixing experiments, a stopped-flow cell designed by R. Rigler at the Karolinska Institutet, Stockholm,

modified for the optical conditions of X-ray experiments, with 60 micrometer mica windows of 0.8 cm diameter, was used. A schematic view is shown in Figure 2. Blank experiments with coloured solutions have shown that the dead-time is less than 10 ms.

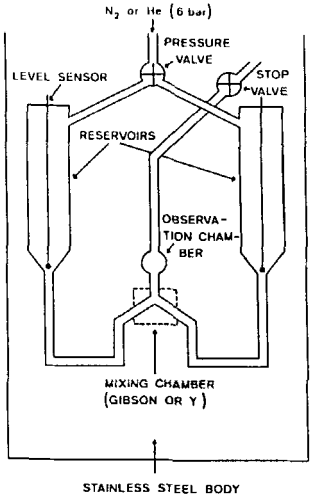

FIGURE 2. Schematic view of stopped-flow cell.

RESULTS AND DISCUSSION

LAMELLAR PHASE TRANSITIONS

Fully hydrated samples of dipalmitoylphosphatidylcholine (DPPC) show a series of three well-described transitions

in the temperature-range between 15 and 45 °C, which are termed sub- (around 17 °C), pre- (around 34 °C), and main transition (41.4 °C)[9,10]. For the main transition characteristic time constants between 1 ms and 1 s have previously been reported[4,11], which were assigned to a sequence of events from fast, noncooperative motional changes of individual molecules to much slower, cooperative processes involving nucleation, growth, and fusion of clusters, as well as changes in solvation. Both the pre- and subtransition are much slower and show a strong hysteresis. Their transition time constants depend on the quenching temperature and vary from minutes to many hours within a few degrees from the equilibrium transition temperature[12,13].

A representative result of a time-resolved temperature-jump experiment on hydrated DPPC is shown in Figure 3. Powder diffraction patterns were sampled in time-slices of 250 ms. The first two orders of the lamellar repeat lattice are clearly resolved at all temperatures, and in the low temperature phase even the third order is visible. The peak intensities in the higher maxima are in the order of 10^4 counts, showing that the intensity conditions would easily allow to extend the experiments down to the millisecond region.

Both the three-dimensional intensity plot and the contour maps show quite clearly that the upper two transitions are not isothermal under these conditions. For the intrinsically faster main transition the patterns indicate a half-width of about 2 s, or 3 °C, where the two phases coexist. The width is considerably larger than expected from precise isothermal diffraction experiments[14], and is in the range of thermal variation within the sample expect-

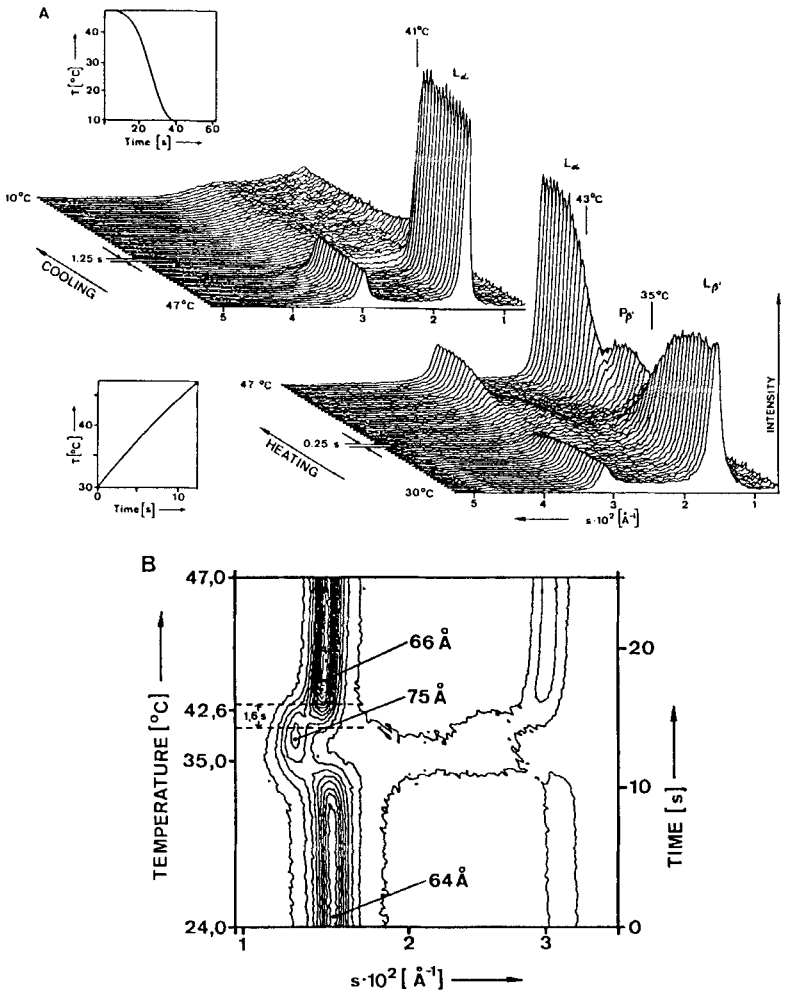

FIGURE 3. a) Time-resolved X-ray small-angle powder diffraction from a multilamellar aqueous dispersion of DPPC during a T-jump and T-drop experiment. b) Contour-line-plot of the intensities in the heating experiment.

ed under the present T-jump conditions. Thus, the effective time-resolution of this experiment is about 1-2 s, a rather disappointing situation if compared to the possibilities given by the intensities and the detector. This limitation is mainly due to the heat conductivity of the sample and can, therefore, hardly be changed.

Nevertheless, the present results show several striking features concerning mainly the pretransition. Upon heating, the pattern indicating a repeat period of about 70Å characteristic of the P'_β phase develops its final intensity only within about 1 h. However, on cooling, the recovery of the ordered, lamellar pattern of the L'_β phase takes even longer, and is easily within the reach of time-resolved experiments on a laboratory X-ray generator. Figure 4 shows the dependence of the recovery rates on the final quenching temperature. This indicates a negative temperature coefficient with the difference between quenching and equilibrium transition temperature, suggesting that the rates are governed by nucleation kinetics[15,16]. It is also possible, that the development of a differently ordered hydration shell is responsible for this behaviour. A complete analysis clearly has to involve the whole range of times between milliseconds and hours, and therefore will require a broad overlap between improved T-jump experiments with synchrotron radiation and long-term conventional diffraction methods.

LAMELLAR-HEXAGONAL TRANSITIONS

The question of potential intermediate structures in this type of transition has caught particular attention, since this involves a major topological change. Micellar intermediates were indeed proposed on the basis of P^{31}-NMR and

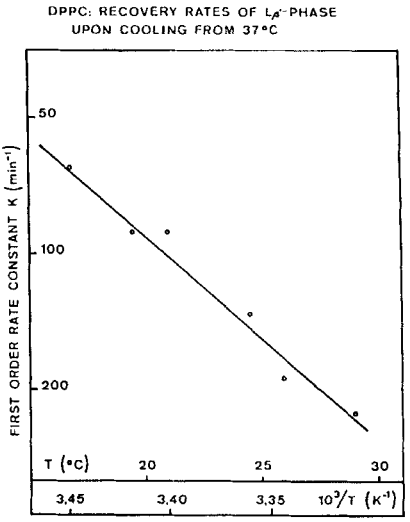

FIGURE 4. Temperature-dependence of the recovery rates of the L_{β}' phase upon cooling from 37 °C.

electron microscopic observations[17,18], and also postulated by a theoretical approach[19]. On the other hand, first results by time-resolved diffraction studies[20] were interpreted in terms of a direct, one-step mechanism involving no micellar intermediates.

Figure 5 shows a pair of heating and cooling scans of HOPE in the fully hydrated state, between 10 and 69 °C. The experiment very clearly resolves the two lamellar phases

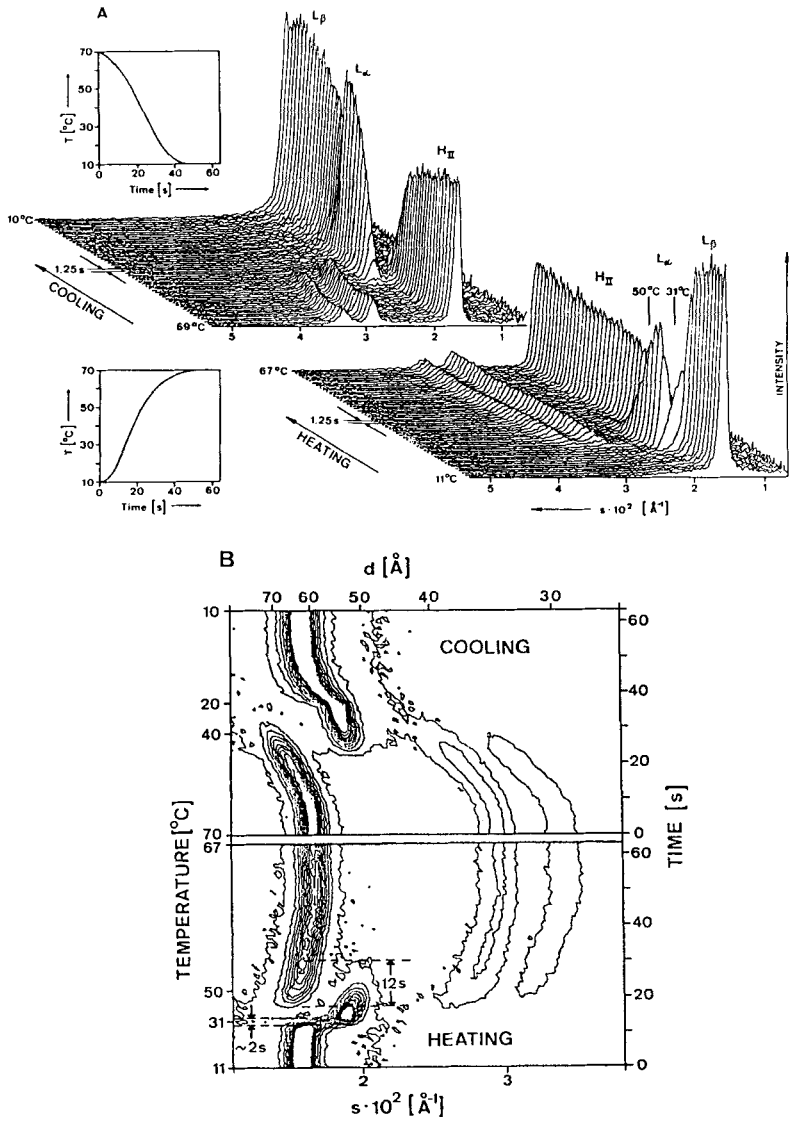

FIGURE 5. a) Time-resolved X-ray small-angle diffraction from an aqueous dispersion of a hexagonal-phase forming lipid (HOPE) b) Intensity contour-line plots.

L_α and L_β at low temperatures, and the hexagonal H_{II} phase at high temperatures. In the heating scan, the L_α - L_β transition is centered around 31 °C and is as sharp as can be expected for an isothermal transition under the conditions of this external heating experiment. The maximum range of coexistence is about 2 s, or 5°C, reflecting again the limit given by inhomogeneous sample temperatures. Very notably, however, the L_α - H_{II} transition is less sharp and involves a broad range of overlap between the two structures: while the onset of H_{II}-formation occurs at 45 °C and coincides with the initial decay of the L_α-structure, the latter coexists with growing amounts of hexagonal structure over more than 12 s, i. e. the temperature range between 50 and 60 °C. It is concluded, therefore, that in the transition range, the lipid-water phase consists of two coexisting structures, corresponding to the two limiting states.

The cooling behaviour is very similar: again, the three phases appear within the time-scale of the experiment. The reciprocal decay and growth of H_{II} and L_α-structures, respectively, is similar to that in the heating experiment. Notably, however, there appears a structural hysteresis: the inflection of the three H_{II}-maxima towards smaller angles during the coexistence range is considerably more pronounced in the cooling experiment. The last coexisting hexagonal structures have a first-order spacing of 73 Å, while on heating, the respective value is 68 Å. This indicates, that the first cylindrical tubes formed on heating are smaller in diameter than the last ones disappearing on cooling.

These results lead to the following conclusions regarding the mechanism of the transition:

a) The L_α - H_{II} transition is a two-structure process, i. e. it proceeds directly from one structure to the other. Neither does it involve detectable amounts of micellar intermediates or particles which would generate a broad, continuous scattering in the transition range, nor does it involve any loss of long range order.

b) The process is readily reversible, with a minor but noticeable hysteresis in the limiting lattice parameters.

c) L_α- and H_{II} structures coexist over a relatively long time and temperature range. This could indicate either, that equilibration is slower than the time constant of the experiment, or that the two structures belong to one coherent phase in equilibrium.

FUSION OF LIPID PHASES

If two hydrated lipid species, one favouring lamellar structures and the other tending to form hexagonal structure, are mixed, their profoundly different diffraction patterns are very well suited to follow the structural changes with time, if fusion occurs. In particular the $(11\bar{2}0)$ reflection of the hexagonal phase, appearing at a spacing of $1.73 \times h_1$, where h_1 is the spacing of the prominent $(10\bar{1}0)$ reflection, is very well suited for this purpose, since it lies well separated from any other, lamellar reflections. In the experiment shown in Figure 6, the system starts from two separate lamellar phases at low temperature. Heating through the L_α-H_{II} transition of the etanolamine lipid component leads to a situation where the hexagonal phase (as clearly seen by the $10\bar{2}0$ reflection) coexists with the lamellar L_α-phase of the choline lipid component. Fusion of the two phases proceeds with a half-time of about 3 minutes and leads to the expected lamellar

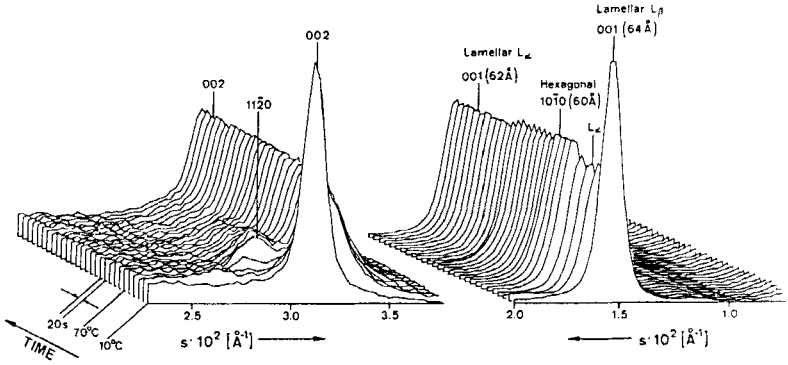

FIGURE 6. Time-resolved X-ray small-angle diffraction from a mixture of POPC and HOPE. Two minutes after mixing of the aqueous dispersions, the temperature was raised from 10 to 70 °C. Fusion of the two phases is evident by the reformation of the lamellar structure.

phase of the equimolar mixture in equilibrium.

The important conclusion to be drawn is, that the fusion process is clearly two-state, i. e. the structures transform directly from one to the other, without involving noticeable amounts of intermediates. Once the local concentration of choline lipids exceeds a critical value,

the entire domain transforms into a well-ordered lamellar state, without loosing long-range coherence.

LAMELLAR-MICELLAR TRANSITIONS

The interaction of natural detergents like bile salts with phospholipids is of considerable interest to the general problem of biomembrane stability and of particular importance to the physiological function of cholesterol solubilization in bile, and its pathological aspect of gallstone formation. Essentially, the reaction can be described as the conversion of lamellar phospholipid bilayer structures into discoidal or spherical mixed micelles with strongly reduced sizes[21]. The details of the mechanism are so far poorly understood.

By X-ray diffraction, this process can be readily monitored since the diffraction patterns of lamellar structures and small isotropic micelles are considerably different.

For the experiments described here (Figure 7), multilayered liposomes of eggyolk lecithin were used in combination with sodiumtaurodeoxycholate. The latter has a c.m.c. of 2.10E-3 mol/l and a partition coefficient between lipid and aqueous phase of ~250, i. e. it strongly prefers the apolar lipid environment. Two sets of experiments were carried out: one using a total lipid/detergent ratio of 1:1 which in equilibrium determines coexistence of discoidal micelles with bilayer structure and globular micelles. The other ratio used was 1:5 for which complete conversion to globular micelles is expected. The results show, that, independent of the initial mixing ratio, always a very fast formation of nonbilayer structures re-

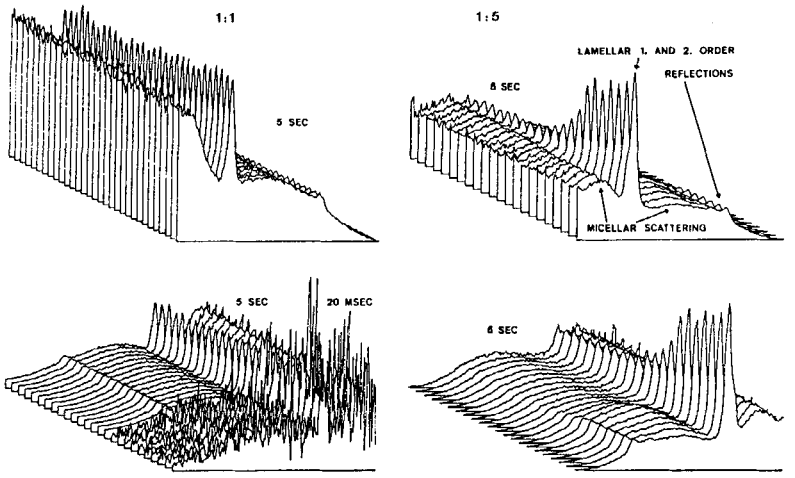

FIGURE 7. Time-resolved diffraction patterns of EYPC/STDC mixing experiments for 1:1 and 1:5 initial molar mixing ratio. The time difference between the individual curves is 0,5 min, the measuring time per curve was 5 sec. The total time span shown by the plots is ~15 min.

flected by a diffuse, low intensity scattering occurs. The diffuse scattering which initially is superimposed by the much stronger diffraction of still dominating stacked lipid bilayers is equivalent to that known for globular mixed micelles. This scattering is present even at the shortest times that can be resolved at the present stage. The first and very fast step of interaction thus seems to be a peeling off mechanism of outer liposome layers, which have reached the critical detergent/lipid ratio by local saturation. The second step is much slower: a redistribution of bile salt from mixed micelles with detergent/lipid ratios much higher than the overall mixing ratio to the remaining liposomes of reduced size. This process depends on the initial relative detergent concentration and can be followed by the steady decrease of Bragg-reflections and an increase of signal-intensity corresponding to the formation of mixed disc and globular micelles, depending on the mixing ratio.

ACKNOWLEDGEMENTS

This work has been supported by grant no. 5264 of the Österreichischer Fonds zur Förderung der wissenschaftlichen Forschung, and by grant no. 2473 from the Österreichische Nationalbank.

REFERENCES

1. D. M. Small, Handbook of Lipid Research, Vol. 4, The Physical Chemistry of Lipids. From Alkanes to Phospholipids (Plenum Press, New York, 1986).
2. R. J. Cherry, in Biological Membranes, edited by D. Chapman and D. F. H. Wallach (Academic Press, New York, 1976) Chap. 2, pp. 48-102.

3. R. M. Clegg and W. L. C. Vaz, in Progr. Protein-Lipid Interactions, Vol. I, edited by A. Watts and J.J.H.H. M. DePont (Elsevier, Amsterdam, 1985), Chap. 5, pp. 173-229.
4. T. J. Tsong and M. I. Kanehisa, Biochemistry, 16, 2674 (1977).
5. G. Materlik, in Uses of Synchrotron Radiation in Biology, edited by H. B. Stuhrmann (Academic Press, New York, 1982) Chap. 1, pp. 1-22.
6. J. Hendrix, in Structural Biological Uses of X-ray Absorption, Scattering and Diffraction, edited by H. D. Bartunik and B. Chance (Academic Press, New York, 1986) pp. 391-407.
7. P. Laggner, ibid. pp. 171-182.
8. C. Boulin, A. Gabriel and J. Hendrix, in Research Report EMBL 1983, p. 135.
9. Y. Inoko and T. Mitsui, J. Phys. Soc. Japan, 6, 1918 (1978).
10. M. J. Ruocco and G. G. Shipley, Biochim. Biophys. Acta, 691, 309 (1982).
11. B. Gruenwald, Biochim. Biophys. Acta, 687, 71 (1982).
12. R. B. Lentz, E. Freire and R. L. Biltonen, Biochemistry, 17, 4475 (1978).
13. K. C. Cho, C. L. Choy and K. Young, Biochim. Biophys. Acta, 663, 14 (1981).
14. M. H. Gottlieb and E. D. Eanes, Biophys. J., 14, 335 (1974).
15. D. Kashchiev, Crystal Res. Technol., 19, 1413 (1984).
16. B. Wunderlich, in Macromolecular Physics, Vol. 2 (Academic Press, New York, 1976).
17. B. De Kruijff, A. J. Verkley, C. J. A. Van Echteld, W. C. Gerritsen, C. Mombers, P. C. Noordam and J. De Gier, Biochim. Biophys. Acta, 555, 200 (1979).
18. S. W. Hui, T. P. Stewart and L. T. Boni, Chem. Phys. Lipids, 33, 113 (1983).
19. D. P. Siegel, Biophys. J., 45, 399 (1984).
20. M. Caffrey, Biochemistry, 24, 4826 (1985).
21. K. Müller, Biochemistry, 20, 404 (1981).