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A time-resolved synchrotron X-ray study of a crystalline phase bilayer transition and packing in a saturated monogalactosyldiacylglycerol-water system

Leonard J. Lis [b] and Peter J. Quinn [a]

[a] *Department of Biochemistry, King's College London, London W8 7AH (U.K.)*
and [b] *Department of Physics and Liquid-Crystal Institute, Kent State University, Kent, OH 44242 (U.S.A.)*

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The structural changes associated with a phase transition between the gel-phase bilayer (L_β) in which the acyl chains pack in a hexagonal subcell, and a crystalline bilayer phase (L_{C1}) where the acyl chains are packed in an orthorhombic subcell in a saturated monogalactosyldiacylglycerol-water system are reported. The phase change is cooperative and takes place isothermally after the lamellar-gel phase has been held at 20°C for about 8 min. The transformation of the acyl chain subcell from hexagonal to orthorhombic induces a change in diffraction maxima observed in the region 0.6–0.7 nm which is interpreted as a change in packing of the galactose residues from an orthorhombic to hexagonal subcell. We conclude that the rearrangement of the acyl chains into a more closely packed subcell requires the head groups to reorient to reduce the steric hindrance between the bulky galactose residues.

Introduction

Lyotropic liquid crystals form a variety of phases that differ in the type of three-dimensional mesophase (i.e., hexagonal, cubic, lamellar) and packing of the hydrocarbon chains within two-dimensional subcells (i.e., hexagonal, orthorhombic, monoclinic) depending on water content and temperature [1]. Some phases, such as the hexagonal-II, liquid-crystal lamellar and gel-phase lamellar are believed to be associated with a number of processes mediated by biological membranes [2,3]. Lipids that can undergo phase transitions under physiological conditions are particularly relevant to such events and have been studied in some detail using model membrane systems. One such lipid class is the monogalactosyldiacylglycerols, which represent the major polar lipid component of the photosynthetic membrane of higher plant chloroplasts and photosynthetic microorganisms.

Several studies have focussed attention on the phase behaviour of monogalactolipids under equilibrium thermal conditions when hydrated in excess water [4,5]. Typically, phase transitions have been examined dynamically using differential scanning calorimetry, or statically by a variety of spectroscopic and diffraction methods. It is necessary, however, in order to obtain a complete picture of the thermodynamic changes associated with lipid phase changes to obtain corresponding structural information in real time. The technology for obtaining structural information in the relevant time frame has been developed recently by exploiting the high intensity X-rays from a synchrotron source to produce diffraction patterns of lipid-water systems undergoing phase transitions. Phase transitions driven by temperature jumps or temperature scans of the type used in

Correspondence: Dr. P.J. Quinn, Department of Biochemistry, King's College London, Campden Hill, London, W8 7AH, U.K.

calorimetric studies have been monitored using this technique with a time resolution in the order of hundreds of ms [6–8]. Direct measurements of the kinetics of each of the phase transitions, e.g., lamellar-gel to liquid-crystalline or lamellar to hexagonal-II, have been obtained. From this information it is possible to determine the mechanism(s) involved in the transition process as well as the rate limiting step(s).

In this report, we examine an isothermal phase transition involving the exothermic transition between the L_β and L_{C1} bilayer phases of fully saturated monogalactosyldiacylglycerol in water. The bilayer structural parameters and acyl chain packing are determined for the first time for the short-lived metastable intermediate L_{C1} phase. Diffraction peaks which can be related to the acyl chain and head group subcells are recorded and observed to change during the L_β to L_{C1} phase transition. Specifically, the number of peaks from the acyl chain subcell increase and those from the head group subcell decrease during this transition. It can be inferred that the driving force for the transition is the rearrangement of the acyl chains into a more closely packed configuration. The rearrangement of the head groups into a lower order subcell must then occur because the molecules must adjust to accommodate the steric hindrance between bulky head groups as the acyl chains become more tightly packed.

Materials and Methods

The lipid used in the studies was extracted from fresh market spinach leaves, isolated by preparative thin layer chromatography and hydrogenated in the presence of Adam's catalyst by methods described elsewhere [9]. Dispersions of the saturated lipid, which contained 75% stearoyl; 25% palmitoyl fatty acid substituents, in excess water were examined using the 0.150 nm X-radiation at station 7.25 of the synchrotron radiation source at the SERC Daresbury Laboratory [10]. A cylindrically bent single crystal of Ge [11] and a long float glass mirror were used for monochromatization and horizontal focussing, providing about $2 \cdot 10^9$ photons $\cdot s^{-1}$ 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 sample path of 1 mm. Scattered X-rays were recorded on a linear detector constructed at the Daresbury Laboratory. The dead time between data acquisition frames was 50 μs and the acquisition time for each of 255 consecutive frames of the data set was 6 s. Diffraction spacings were calibrated using Teflon (0.48 nm) [12] and no corrections were applied to path distances from the sample to the detector consequently wide-angle spacings will be slightly longer than measured directly by the detector. All mesophase and subcell spacings were calculated using Bragg's Law [13].

Results

Previous studies of saturated monogalactosyldiacylglycerol-water systems have shown the presence of metastable states and the relationship between the different phases is illustrated in Fig. 1. Four separate phases have been described including three lamellar phases with regularly packed hydrocarbon chains and a high-temperature phase in which the hydrocarbon chains are disordered [5]. According to calorimetric evidence the $L_{C2} \rightarrow L_{C1}$ and $L_\beta \rightarrow L_{C1}$ transitions are both exothermic. Reversal of the two exothermic transitions were found to be relatively show processes and to take place at a rate which depended directly on the temperature. Thus the L_{C1} to L_β transition is accelerated by high temperature and the L_{C1} to L_{C2} transition favoured at lower storage

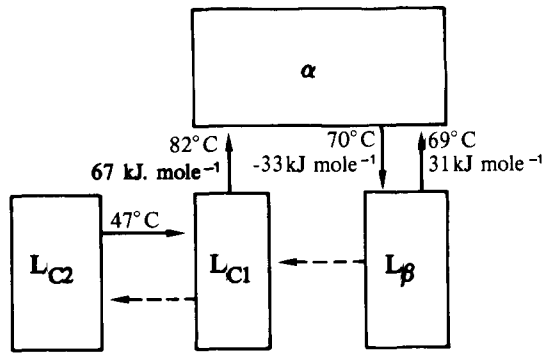

Fig. 1. Thermotropic phases of fully hydrated distearoyl derivative of monogalactosyldiacylglycerol based on calorimetric data [5]. Dashed lines signify apparently non-cooperative transitions.

temperatures. One of the distinguishing features observed for these particular phases is the extent of hydration of the lipid with decreasing hydration in the series $L_\beta > L_{C1} > L_{C2}$. It can be inferred on the basis of calorimetric results that the L_{C1} phase is a more highly ordered phase than either the L_{C2} or L_β phases, and that the L_{C1} and L_β phases are metastable intermediates in the transition between the L_{C2} and the α-phase.

Examination of the transition of saturated monogalactosyldiacylglycerol from the L_β to the L_C phases was performed by time-resolved X-ray diffraction. A lipid dispersion was initially allowed to equilibrate at 20°C for 3 days to form the L_{C2} phase and then heated in the X-ray sample holder to 70°C to induce the α-phase. The lipid was then cooled rapidly (3 Cdeg per s) to 20°C, when it was converted into the L_β phase. Sequential diffraction patterns were recorded each of 6 s duration under isothermal conditions and the results obtained displayed as a three-dimensional plot of diffraction intensity vs. reciprocal spacing for every 10th frame of the data set (Fig. 2). The intense diffraction at low angle (right side of the plot) arises from the three-dimensional structure of the mesophase, while the scattering at high angles (left side of the plot) is a measure of the packing of the acyl chains in a two-dimensional subcell of the methylene chain group. In addition, diffraction peaks are present in intermediate scattering angles located between the usual wide- and low-angle diffraction features characteristic of most membrane lipids. After 8 min at 20°C the L_β phase is transformed isothermally to the L_{C1} phase as monitored by X-ray diffraction profiles. Once nucleated, the L_β phase is almost completely transformed to the L_{C1} phase in about 30 s. Relaxation of the lipid structure continues after formation of the metastable L_{C1} phase and transformation into the stable L_{C2} phase is completed after several days.

Static diffraction patterns of saturated monogalactosyldiacylglycerol recorded in the L_{C1} and L_β phases respectively are presented in Fig. 3. Comparison of these patterns emphasizes the considerable changes in packing of the lipid molecules

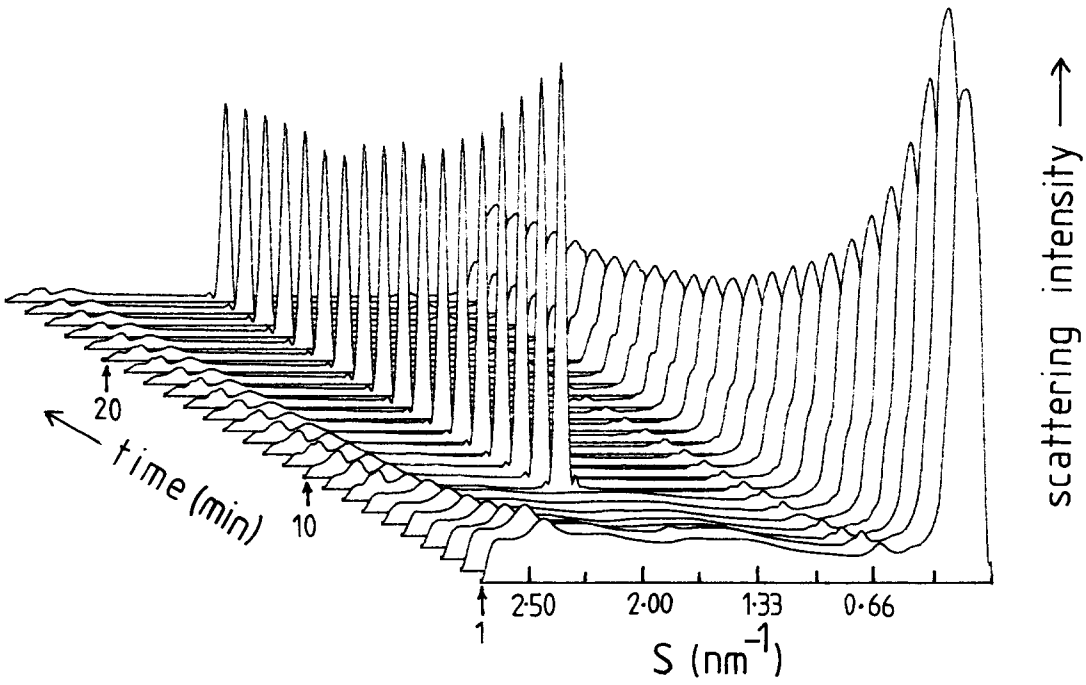

Fig. 2. X-ray scattering intensity as a function of reciprocal spacing for saturated monogalactosyldiacylglycerol in excess water. The hydrated lipid was cooled from the α-phase to 20°C and changes with time recorded. Only every 10th frame (6 s/frame) of the total data set is plotted.

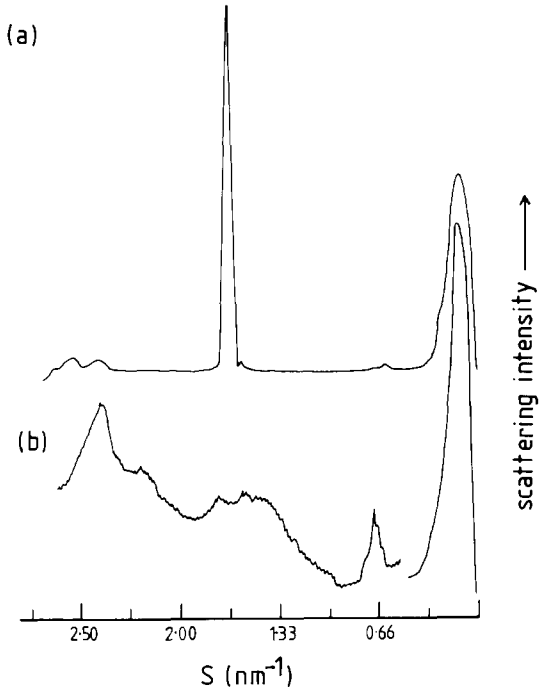

Fig. 3. X-ray diffraction patterns recorded at 20 °C of saturated monogalactosyl diacylglycerol in excess water in the L_{C1} phase (a) and L_{β} phase (b).

in the bilayer phase that occur during the transition. The L_{β} phase has a lamellar *d*-spacing of 6.25 nm indexed by four orders of reflexion; the prominent fourth order is seen in the expanded intensity scale. The scattering from the acyl chain subcell is observed at wide angle with a dominant band centred at a spacing corresponding to 0.41 nm and a broader component at 0.46 nm. The broad diffraction bands at intermediate scattering angles we ascribe to packing of the sugar residues of the head groups of the lipid (see Discussion). Two broad bands can be discerned in the L_{β} phase, one centred at 0.61 nm and the other at 0.69 nm. Transition to the L_{C1} phase involves a simultaneous change in the acyl chain packing and the packing of the sugar groups as seen from the static pattern of this phase (Fig. 3a). The pattern is dominated by an intense diffraction band centred at 0.60 nm and the acyl chain spacings are located in two bands of approximately equal scattering intensity located at 0.38 and 0.40 nm. The phase is again lamellar, with 5 diffraction orders indexing a bilayer repeat of 7.41 nm. As can be seen from Fig. 2, the change of the lamellar lattice is not complete on first appearance of the L_{C1} phase and an analysis of the low-angle reflections of this data set indicates there is some residual L_{β} phase which disappears within about 20 min. Prolonged incubation of the lipid results in a reduction of the lamellar repeat spacings the origin of which can be seen after about 16 min in the data set in Fig. 2 as a splitting of the low-angle first-order lamellar repeat. This spacing reaches a minimum of approx. 6.0 nm after several days and may reflect dehydration of the lattice.

Discussion

Phase changes in lipid-water systems under isothermal conditions have been reported in phosphatidylcholines [14,15], phosphatidylethanolamines [16–18], *N*-methyl derivatives of phosphatidylethanolamines [19] and monogalactosyldiacylglycerols [5]. In general, these transitions are observed during prolonged storage at temperatures around 0 °C when transition from a metastable lamellar gel phase takes place to form a stable crystalline phase. Slow isothermal transitions of DL-dipalmitoylphosphatidylethanolamines at high temperatures (85 °C) have also been observed in which transition from a lamellar crystalline phase to a lamellar α-phase is observed; the rate-limiting step appears to be the rate of hydration of the phospholipid [20]. X-ray diffraction studies of the $L_{\beta'} \rightarrow L_{C}$ phase transition of phosphatidylcholine during storage at −2 °C [15] has indicated a two-step change involving a rapid alteration in packing of the acyl chains from the disordered orthorhombic subcell typical of the $L_{\beta'}$ phase to a regular orthorhombic subcell of the crystalline phase. This is followed by a slower reduction in the lamellar *d*-spacing as water was expelled from the lattice. The crystallization is almost complete after standing for about 12 h.

The formation of the L_{C1} phase of saturated monogalactosyldiacylglycerol from the L_{β} phase under isothermal conditions is clearly a process different from that reported for the equivalent transition in the saturated phosphatidylcholines. Firstly, the transition is relatively fast and is completed within 1 min at 20 °C. Secondly, the transition appears to be highly cooperative, with simul-

taneous changes taking place in the packing of different regions of the molecules oriented in a bilayer configuration. The interpretation of these changes in diffraction maxima depend on the identity of the subcells from which the reflections originate. Single crystal studies of alkylglucopyranosides [21,22] and galactocerebroside [23] have all shown diffraction spacings in the region of 0.6 to 0.7 nm which could represent spacings corresponding to packing of the pyranoside sugar moieties. According to this interpretation, the broad diffraction bands at 0.61 and 0.69 nm of lipid in the L_β phase would represent the spacings corresponding to the packing of the galactose residues, while the major chain spacing is located at 0.41 nm which represents an hexagonal arrangement of the methylene groups of the hydrocarbon chains [24]. The packing, and subcell dimensions for the head groups and acyl chains need not be the same as evident in the case of cerebroside [23] where reorientation of the respective groups serves to minimize steric hindrance between the bulky head groups. By contrast, the acyl chains in the L_{C1} phase can be indexed to an orthorhombic packing, since two diffraction peaks are observed each corresponding to one coordinate of the acyl chain subcell. The main lamellar repeat of the L_{C1} phase is centred at a spacing of 7.41 nm and in Fig. 3a is indexed by 5 diffraction orders. A weaker diffraction centred at 6.29 nm can also be detected in this diffraction pattern suggesting that some residual L_β phase may be present in the dispersion; however, no evidence of diffraction from the head groups or the hexagonal chain packing is evident.

The driving force for the L_β to L_{C1} transition can be determined by comparison of the diffraction data reported in this study with the thermal studies of saturated monogalactosyldiacylglycerol conducted previously [5]. The L_β to L_{C1} transition is an exothermic transition and therefore consistent with an ordering process. The creation of a more ordered acyl chain subcell in the L_{C1} phase indicates that the ordering of the acyl chains is the most probable factor responsible for driving the transition. The concomitant change in the headgroup packing must then be rationalized in the light of the change in acyl chain packing. Since the galactose residues are relatively bulky in comparison to the head groups of many phospholipid classes it may be inferred that the reduction in steric hindrance between head groups is required to accommodate the change in acyl chain packing and this is the reason for the observed change in the spacing between the head groups.

The stability of the L_{C1} phase can be judged from the changes in diffraction spacing observed over the course of the time-resolved X-ray experiment. It is a metastable phase and is transformed ultimately to the stable low-temperature phase designated L_{C2}. The short-lived existence of the phase precludes characterization by conventional X-ray diffraction methods, where recording of diffraction patterns has a time resolution of minutes. It is likely that use of the synchrotron X-ray source will enable the characterization of other transient metastable phases that have been identified in different lipid-water systems using calorimetric methods.

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References

1 Luzzati, V. (1968) in Biological Membranes (Chapman, D., ed.), pp. 71–133, Academic Press, New York
2 Verkleij, A.J. (1984) Biochim. Biophys. Acta 779, 43–63
3 Cullis, P.R., De Kruijff, B., Hope, M.J., Verkleij, A.J., Nayar, R., Farren, S.B., Tilcock, C., Madden, T.D. and Bally, M.B. (1983) in Membrane Fluidity in Biology, Vol. 1, pp. 35–81, Academic Press, New York
4 Sen, A., Williams, W.P. and Quinn, P.J. (1981) Biochim. Biophys. Acta 663, 380–389
5 Sen, A., Mannock, D.A., Collins, D.G., Quinn, P.J. and Williams, W.P. (1983) Proc. R. Soc. Lond. B 218, 349–364
6 Caffrey, M. and Bilderback, D.H. (1983) Nucl. Instr. Methods 208, 495–510
7 Caffrey, M. and Bilderback, D.H. (1984) Biophys. J. 45, 627–631
8 Caffrey, M. (1985) Biochemistry 24, 4826–4844
9 Mansourian, A. and Quinn, P.J. (1986) Biochim. Biophys. Acta 855, 169–178
10 Nave, C., Helliwell, J.R., Moore, P.R., Thompson, A.W., Worgan, J.S., Greenall, R.J., Miller, A., Nurley, S.K., Bradshaw, J., Pigram, W.J., Fuller, W., Siddons, D.P., Deutsch, M. and Tregear, R.T. (1985) J. Appl. Cryst. 18, 396–403

11 Helliwell, J.R., Greenough, T.J., Carr, P.D., Rule, S.A., Moore, P.R., Thompson, A.W. and Worgan, J.S. (1982) J. Phys. E15, 1363–1372
12 Bunn, C.W. and Howells, E.B. (1954) Nature (London) 174, 549–551
13 Levine, Y.K. (1973) Progr. Surface Sci. 3, 279–352
14 Ruocco, M.J. and Shipley, G.G. (1982) Biochim. Biophys. Acta 691, 309–320
15 Ruocco, M.J. and Shipley, G.G. (1982) Biochim. Biophys. Acta 684, 59–66
16 Seddon, J.M., Harlos, K. and Marsh, D. (1983) J. Biol. Chem. 258, 3850–3854
17 Cheng, H. and Epand, R.M. (1983) Biochim. Biophys. Acta 728, 319–324
18 Mantsch, H.H., Hsi, S.C., Butler, K.W. and Cameron, D.G. (1983) Biochim. Biophys. Acta 728, 325–330
19 Mulukutla, S. and Shipley, G.G. (1984) Biochemistry 23, 2514–2519
20 Tenchov, B.G., Boyanov, A.J. and Koynova, R.D. (1984) Biochemistry 23, 3553–3558
21 Moews, P.C. and Knox, J.R. (1976) J. Am. Chem. Soc. 98, 6628–6633
22 Dorset, D.L. and Rosenbusch, J.P. (1981) Chem. Phys. Lipids 29, 299–307
23 Pascher, I. and Sundell, S. (1977) Chem. Phys. Lipids 20, 175–191
24 Persson, P.K.T. (1984) Chem. Phys. Lipids 34, 287–299