



Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

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Reports can now be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

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Deadlines for submission of Experimental Reports

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Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.



Experiment title:

Time Resolved Studies of the Lyotropic Phase Transitions of the Inverse Bicontinuous Cubic Phases in a Monoelaidin/H₂O system

Experiment number:

CH-1898

Beamline:

ID02

Date of experiment:

from: 22nd April 2005 to: 25th April 2005

Date of report:

30/08/06

Shifts:

9

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Received at ESRF:

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Report:

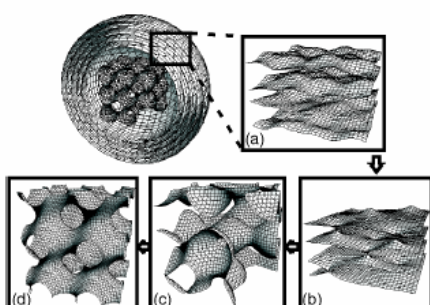
CH-1898

Time Resolved Studies of the Lyotropic Phase Transitions of the Inverse Bicontinuous Cubic Phases in a Monoelaidin/H₂O system

Summary

Our experiments as part of proposal CH-1898 proved extremely successful and have so far resulted in two high impact publications (Ref 1 and Ref 2). Our aim was to investigate the transitions between various lyotropic liquid-crystalline phases formed by the monoelaidin (ME) / water system, including the nature of any intermediates formed. For the first time, we have overcome the variability associated with dynamical measurements of lyotropic phase transitions and this has allowed us to record the dynamical process reproducibly in pressure-jump experiments. We have shown that the liquid crystalline lamellar (L_α) to double-diamond inverse bicontinuous cubic (Q_D^{II}) phase transition resulting from pressure jumps exhibits a remarkable sequence of structural transformations. Our data indicate that the transition dynamics depend on a coupling between changes in molecular shape and the geometrical and topological constraints of domain size. As a result we have been able to propose a qualitative model (Fig 1) for this coupling based on theories of membrane fusion via stalks and an existing knowledge of the structure and energetics of bicontinuous cubic phases.

FIG. 1 In these images we show the structural transformations observed in our experiments by sketching the bilayer ane. The figures are scaled relative to each other to reflect our experimental observations. A spherical onion e consisting of concentric bilayers is shown including the swollen cubic phase present at the center. Thermal ations in the lamellar phase (a) disappear as interlamellar attachments (ILAs) are formed (b). This occurs se the bilayer's area and the volume it circumscribes are fixed in an onion vesicle and hence forming contacts : a lateral tension. When the tension reaches a critical value, ILAs rupture and form a disordered network of ls (c) through which water flows from the center of the vesicle. The disordered sponge structure soon resolves rdered bicontinuous cubic structures such as the swollen P cubic (d).



Results

The dynamics of the L_α to Q_D^{II} phase transition, brought about by rapid changes in pressure have a number of common features, as shown in Fig. 2a. Most importantly, we find the dynamics are only reproducible if samples are composed of topologically distinct, physically separated domains with a narrow size distribution (200+/-20 nm diameter in our case). This reproducibility can be clearly seen in Fig. 2b which plots the lattice parameter as a function of time for the pressure and temperature jumps shown in Fig. 1. A second set of data obtained from a jump of the same amplitude has been added to the plot.

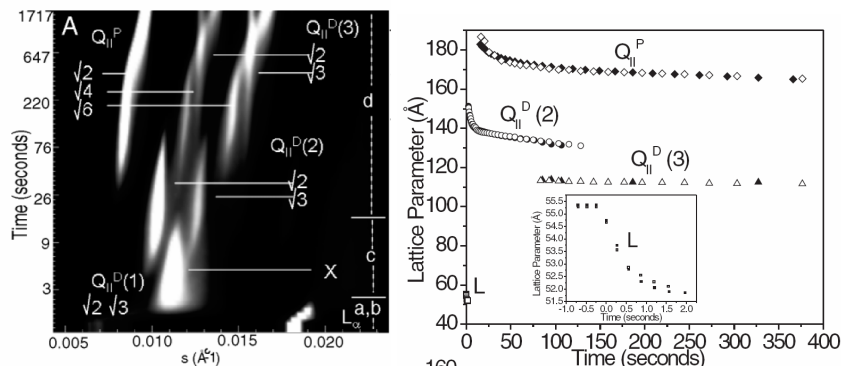


Fig 2- A pressure jump from 1100 to 260 bar, $T = 46.7^\circ\text{C}$. Each 2D image is integrated to produce a 1D plot of intensity versus scattering vector, s , which is then stacked as a function of time. Regions a, b, c, and d correspond to the structural intermediates depicted in Fig. 1. The change in lattice parameter with time following the pressure jump shown in Fig. 1a. Two experiments having the same jump amplitude are plotted. In each case the inset is a close-up view of the behaviour of the lamellar phase immediately following the jump.

Initially, we always observe a weak and previously unreported signal from a highly swollen bicontinuous cubic phase, Q_D^{II} , of maximum spacing 232 Å, in coexistence with the fluid lamellar phase. The lamellar gel phase, which occurs below 15 °C at atmospheric pressure and above 1300 bar at 46.7 °C, also coexists with a swollen primitive Q_P^{II} cubic phase of maximum lattice parameter 311 Å. Following the step change in pressure the lamellar phase always shrinks to a specific threshold interlamellar spacing, constant to within the resolution of our experiments before disappearing. The rate of decrease in interlamellar spacing increases in proportion to the pressure amplitude of the jump. This dependence is not observed during lamellar to nonlamellar transitions which do not involve the formation of stalks. During this phase the highly swollen bicontinuous cubic signal smears out, moves to higher scattering angle, and, coincident with the disappearance of the lamellar Bragg peaks, merges with a broad, featureless ring of scatter (marked X in Fig. 2a). After a period of a few seconds the diffuse scatter resolves itself into a set of peaks from intermediate inverse bicontinuous cubic phases, Q_D^{II} and Q_P^{II} , which replace it. The intermediate Q_D^{II} and Q_P^{II} phases may be used for transport of water; the small curvature elastic energy associated with expanding channel size means this can be an energetically favourable way of increasing water flux through the sample.

The fact that dynamical reproducibility is dependent on uniform domain size is strong evidence for structural changes at the scale of the unit cell being topologically and geometrically coupled to structural constraints at the nanoscale; see Fig. 1. From polarizing microscopy we know that in the lamellar phase our samples consist of closely packed onion vesicles. Using cryo-TEM, we observe that upon transformation into the Q_D^{II} cubic phase, the rounded multilamellar vesicles are transformed into cubosomes of approximately the same dimensions as the onion vesicles. Smooth powder diffraction patterns obtained from these samples set an upper limit of the order of 1 μm on the domain size. These three observations suggest that the phase transitions that we record all occur within the confines of the vesicles we have formed. This sets significant constraints on local composition, diffusion and topology.

The fact that the disappearance of the highly swollen cubic phase after a thermodynamic jump is structurally continuous with the appearance of diffuse scatter is strong evidence that it exists inside the onion vesicle, in coexistence with the lamellar phase. This suggests that its function is to act as a seed for the growth of the bicontinuous cubic phases out of an initially only locally ordered bicontinuous phase (the source of the diffuse scatter). We hypothesize that the swollen cubic is located in the center of the onion vesicle (Fig. 1) removing the high energetic cost of filling the core with spherical bilayers of increasingly higher degrees of mechanically unfavorable curvature. It is interesting to note in this regard that the cubic phase lattice parameter is larger than the upper limit predicted by Bruinsma [Ref 3], who calculated that thermally activated vibrational modes would disorder the structure. However, with the swollen cubic phase anchored to a vesicle wall perhaps the structure becomes stabilized. When we make a thermodynamic jump we are heading in a direction in which the bicontinuous cubic phase will shrink. At the same time the lamellar lattice parameter is shrinking, which indicates that water being expelled from the cubic phase does not pass into or through the lamellar bilayers.

The shrinkage of the lamellar lattice is occurring at the same time that stalks are formed between bilayers. From equilibrium temperature studies one expects a change in lattice parameter of the fluid lamellar phase of $+1.5 \text{ \AA kbar}^{-1}$. In every case the actual changes are significantly greater than this. We hypothesize that this is due to the formation of stalks. This will produce the observed effects only if the bilayers have excess area in the form of thermal undulations and, of course, these undulations are an absolute prerequisite for stalk formation itself (Fig. 1). For fixed water volume between adjacent bilayers in an onion vesicle the formation of stalks will reduce the average bilayer spacing as it irons out undulations, while simultaneously imparting lateral tension in the bilayers. We know that rupture of the stalk to form a fusion pore is dependent on the presence of sufficient lateral tension to open up the pore. Our hypothesis is therefore able to rationalize the very specific and reproducible value of the threshold lamellar lattice parameter prior to the phase's complete disappearance.

The rupture of the fluid lamellar bilayer throughout the sample requires the concomitant movement of the water stored in the central, disordered bicontinuous structure through the pores (Fig. 1). This destroys lamellar symmetry, and with pores distributed over spherical surfaces and the initially rapid flow of both lipid and water there can be no initial cubic symmetry to replace it. Hence the broad unresolved peak during this stage of the process. The appearance of an initially highly swollen cubic phase from this broad peak is explained by the now slower flow of large volumes of water, allowing the reorganization of some parts of the domain into cubic regions. Regular, cubic arrays of pores are at a lower free energy than the random arrangement in phase X and the energetic cost of swelling the *D* and *P* cubic phases is not that great. At fixed lipid mass within the domain the shrinkage of these intermediate cubic phases can only occur via the growth of new unit cells, and hence an increase in topology (density of channels). This, by comparison with maintaining a swollen cubic phase, is clearly energetically costly as can be seen by the slow rate of growth of the equilibrium phase (Fig. 2).

References

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