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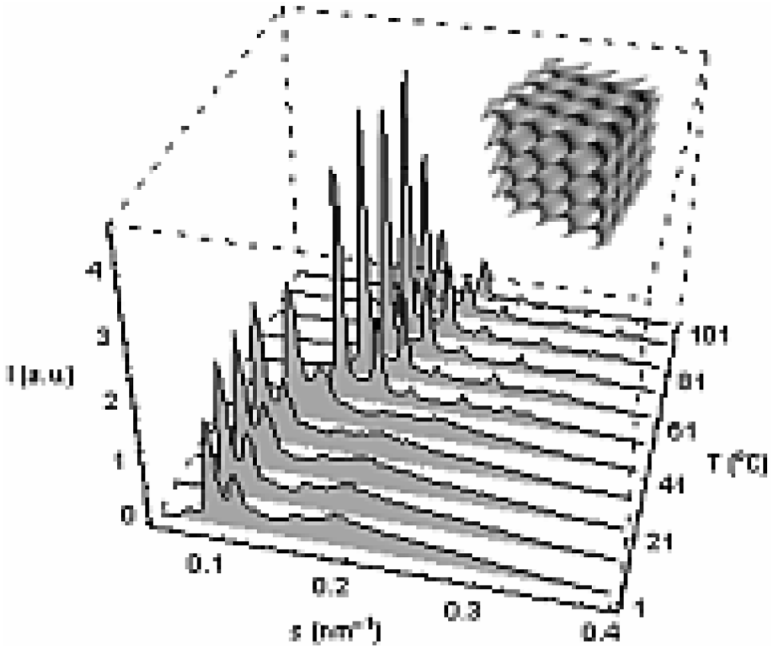

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Diamond-Type Lipid Cubic Phase with Large Water Channels

Borislav Angelov,[†] Angelina Angelova,[‡] Michel Ollivon,*[,§] Claudie Bourgaux,[⊥] and Andrew Campitelli[‡]

Institute of Biophysics, Bulgarian Academy of Sciences, Acad. G.Bonchev Str. Bl.21, BG-1113 Sofia, Bulgaria, Biosensors group, IMEC, Kapeldreef 75, B-3001 Leuven, Belgium, CNRS UMR 8612, 92296 Châtenay, France, and LURE, Université Paris-Sud, F-91898 Orsay, France

Received February 9, 2003; E-mail: michel.ollivon@cep.u-psud.fr

This paper describes a diamond cubic phase with large water channels and determines the temperature dependence of the bilayer thickness in the cubic monoolein/octylglucoside/water system based on time-resolved synchrotron X-ray diffraction data. The X-ray diffraction study evidences a diamond-type lipid cubic phase, D_{large}, with large water channels (6.98 nm water core diameter at 20 °C), which has not been previously reported. It is a distinct phase, different from the diamond cubic phase D_{normal} with normal water channels (2.96 nm water core at 70 °C). The larger channels might allow an enhanced entrapment efficiency of biomolecules in lipid cubic phases. The X-ray diffraction patterns recorded during a thermal scan showed a cubic−cubic structural transition from D_{large} to D_{normal}. The obtained cubic phases displayed much larger lattice spacings (a = 15.3 nm at 20 °C) as compared to those of pure monoolein at full hydration (a = 10.7 nm at 20 °C).

Following the pioneering works on crystallography,[1] mathematical description,[2] and a physical model[3] of lipid cubic phases, it has been found that membrane proteins can be effectively crystallized in such nonlamellar media.[4] Bicontinuous cubic phases have significant features favoring their growing use in biophysical and biomedical fields, and in food and cosmetics industries. Structural and chemical advantages of the lipid cubic phases include highly ordered periodic structures, a large surface area of the lipid/water interface (on the order of 400 m^2/g),[5a] bicontinuous or cubosome textures, and biocompatible media for entrapment of proteins, peptides, and other biomolecules. To utilize these properties, various applications are being developed such as controlled drug delivery systems, biosensors,[5b] and nanostructured composite biomaterials.

Monoolein (MO)/detergent systems may form stable cubic phases for membrane protein crystallization.[4] To understand the structural stability of nonlamellar amphiphilic systems[6,7] as influenced by detergents,[8,9] the lipid bilayer thickness in the cubic phase[10] should be determined. The latter would allow one to estimate the sizes of the water compartments accommodated inside the cubic unit cell. The bilayer and the water channel thicknesses are structural parameters of great practical importance for protein entrapment and membrane protein reconstitution in lipid cubic phases.

Previously reported X-ray diffractograms of cubic lipid/water systems have been mostly explored for symmetry characterization. The structural complexity of the underlying minimal surface and the large number of molecules per unit cell (about 500 molecules for the case of MO)[11] have hampered the in-depth study of the X-ray data. A simplified model for analysis of scattering patterns of self-assembled cubic phases has been recently proposed by Garstecki and Holyst[10] (GH model). It is an insightful continuation of the works of Clerc and Dubois-Violette[12] and Harper and Gruner[13] and requires fitting of model intensity curves to experimental ones.

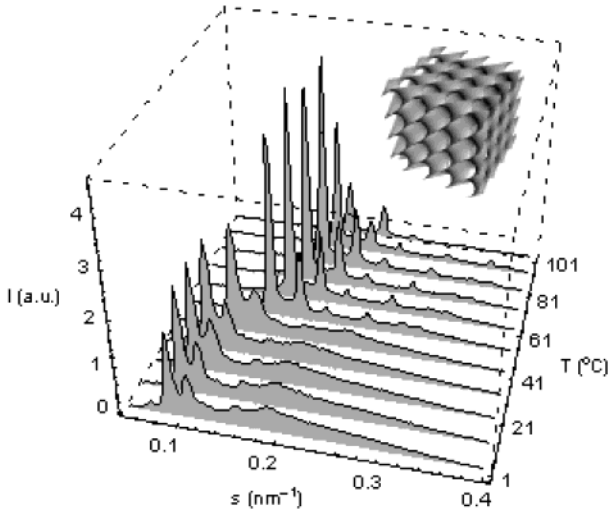

Figure 1. Time-resolved X-ray diffraction patterns of hydrated MO/OG mixture (90/10, mol/mol) showing a transition between diamond cubic phases ($D_{large} \rightarrow D_{normal}$) upon heating. Inset: *Pn*3*m* lattice.

Here we investigate the temperature dependence of the lipid bilayer thickness in a MO cubic phase incorporating a small amount of octylglucoside (OG). A powder of 1-monooleoyl-*rac*-glycerol (purity >99.5%) was hydrated and dispersed in excess aqueous phase containing 0.095 M *n*-octyl β-D-glucopyranoside (purity >99.5%) in 0.1 M NaCl (phosphate buffer pH 7.0), yielding a lipid-to-detergent molar ratio of 90/10. This molar ratio between MO and OG was chosen to be close to the critical value for the induction of a nonlamellar-to-lamellar phase transition in MO, which was estimated in our previous work.[8] The structure of the lipid/detergent/water mixture was studied by means of synchrotron X-ray diffraction. Figure 1 shows time-resolved X-ray diffraction patterns recorded in the SAXS region in the interval from 1 to 100 °C. The sample was scanned at a rate 2 °C/min at beamline D24 of LURE (Orsay, France). The principle of the experimental setup is analogous to that described in refs 8 and 14. We present one-dimensional X-ray diffractograms as intensities vs reciprocal spacings (s) for selected temperatures. The patterns define periodic 3D structures with diamond *Pn*3*m* cubic lattices (Figure 1). The characteristic X-ray diffraction peaks with reciprocal spacings spaced in the ratio $\sqrt{2}:\sqrt{3}:\sqrt{4}:\sqrt{6}:\sqrt{8}:\sqrt{9}:\sqrt{10}:\sqrt{12}:\sqrt{14}$ ... were used for the determination of the *Pn*3*m* cubic lattice parameter a.

A detailed analysis of the time-resolved X-ray diffraction patterns was performed at every 2° of the scan from 1 to 100 °C. The lipid bilayer thickness, L, and the dimensionless bilayer thickness ratio, L/a, were determined explicitly from the experimental X-ray data

[†] Bulgarian Academy of Sciences.
[‡] IMEC.
[§] CNRS UMR 8612.
[⊥] LURE.

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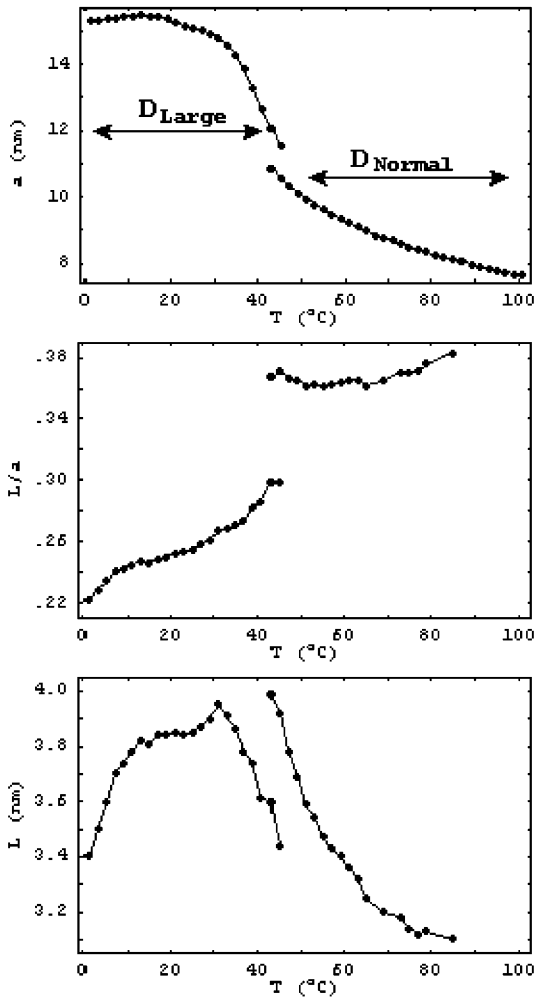

Figure 2. Temperature dependences of the unit cell constant *a*, the dimensionless bilayer thickness *L*/*a*, and the bilayer thickness *L* in MO/OG (90/10, mol/mol) cubic phase. The *Pn*3*m* spacegroup keeps throughout. The transition $D_{large} \rightarrow D_{normal}$ occurs at 44 °C.

using the GH model[10] and our procedure for intensity curve fitting (see the Supporting Information). The intensities of the diffraction patterns were calculated by formula (7) from ref 10b. The simultaneously fitted parameters were the following: (1) the dimensionless bilayers thickness *L*/*a*; (2) the *s* value of the first peak that best optimizes the *s* values of the first six peaks of the *Pn*3*m* structure; (3) the peak width (the *Pn*3*m* peaks were supposed to be Gaussian peaks of equal width); (4) the intensity of the first peak that best optimizes the intensities of the first six *Pn*3*m* peaks; (5) another four parameters were added to model a coexistence of *Im*3*m* and *Pn*3*m* phases or of two *Pn*3*m* phases during the phase transitions; (6) the background was modeled as a Gaussian peak with three parameters. The transition $D_{large} \rightarrow D_{normal}$ was thus modeled by 11 parameters. To better fit the small-intensity peaks from the third to sixth reflection, we worked with logarithmic intensities. The obtained temperature dependencies of the cubic lattice parameter, the dimensionless bilayer thickness, and the thickness of the lipid bilayer are presented in Figure 2.

We verified that the MO/OG/water system displays a *Pn*3*m* cubic symmetry over the entire interval from 1 to 100 °C. Around 44 °C, a cubic−cubic transition from *Pn*3*m* (D_{large}) to *Pn*3*m* (D_{normal}) is established (Figure 2). At temperature below 5 °C, superimposed reflections of *Im*3*m* and *Pn*3*m* cubic phases were resolved, the intensity of the strongest *Im*3*m* peak being nearly 10 times smaller than that of the strongest *Pn*3*m* peak. A third isotropic phase, modeled as a single broad Gaussian peak, was well resolved for every temperature at *s*-maximum in the interval $s \sim 0.20 \div 0.25$ nm^{-1}. Such background scattering was interpreted as a disordered bicontinuous phase.[10b] If some amount of OG separates from MO to form micelles, a typical maximum for OG micelles at $s \sim 0.34$ nm^{-1} would be observed. The micellar maximum could shift to $s \sim 0.27 \div 0.37$ nm^{-1} if mixed lipid/OG micelles[15] were formed. Considering that the isotropic ("disordered") phase in the present system displays a peak maximum at $s < 0.25$ nm^{-1}, we conclude that OG micelles are not formed. Hence, the obtained results suggest that the detergent OG homogeneously mixes with MO at a molar ratio of 90/10. The shape of the X-ray patterns changes notably on heating at around 44 °C during the transition $D_{large} \rightarrow D_{normal}$ (Figure 1). The intensity of the isotropic disordered phase decreased by about 50% for the normal *Pn*3*m* structure (D_{normal}) as compared to that for the cubic phase with large water channels (D_{large}).

The variation of the bilayer thickness and the remarkable features of the $D_{large} \rightarrow D_{normal}$ cubic−cubic structural transition are reported here for the first time (Figure 2). The induction of D_{large} is associated with a disorder in the bicontinuous structure resulting from the dilatation of the water channels caused by OG. The downward jump of the lattice parameter *a* around 44 °C could be due to changes in the OG solubility upon increasing temperature (OG cmc ~ 25 mM at 25 °C). This reduces the hydration of D_{large}, and thus a portion of the phase transforms into an ordered D_{normal} structure. The presented structural information is important for the entrapment of proteins in lipid nanochannel networks as well as for the studies on stability of cubic phases.[6,7] The results suggest that addition of 10 mol % of OG to MO considerably increases the hydration of the lipid cubic phase without causing a phase transformation into a lamellar phase.

Supporting Information Available: Details on X-ray data analysis for experimental results at selected temperatures. This material is available free of charge at http://www.pubs.acs.org.

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