Modulation of Nanochannels Hydration in Lipid Cubic Phases Studied by SANS and SAXS

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ABSTRACT

The hydration of a bicontinuous lipid cubic phase, respectively the sizes of the aqueous nanochannels formed in such a supramolecular structure of hydrated monoolein, was modulated by incorporation of small nonionic guest molecule (octyl glucoside). Small-angle neutron (SANS) and X-ray scattering (SAXS) were employed to monitor the structural evolutions of the cubic lattice nanochannel networks induced by the incorporations of octyl glucoside as well as via temperature scans. The obtained results allowed to precisely determine the topology and the dimensions of the aqueous nanochannels, which are critical for the encapsulation of biomolecules of therapeutic or cosmetic interest. Different levels of hierarchical organization of the soft-matter nanocompartment formations were established in the cubic phase supramolecular assembly. Among them an impressive growth of oriented domains with nanocubosome features was experimentally revealed. Such hierarchically organized nanoporous building blocks may potentially serve also as protein drug delivery vehicles, nanostructured enzymatic bioreactors, and protein-encapsulating fluid nanomaterials.

Keywords: nanostructured fluid, structure analysis by small-angle scattering, soft-matter nanostructure

1 INTRODUCTION

Bicontinuous cubic phases (BCP) exist either in condensed solid [1], polymeric [2], or in liquid crystalline [3] forms. Their formation and stability is one of the central topics in modern natural sciences. BCPs from lipids and amphiphiles are attracting a significant research interest as nanostructured drug delivery carriers of therapeutic molecules and templates for membrane protein crystallization. The typical structure of a lipid/water BCP consists of two intertwined water channel networks that are separated by a lipid bilayer with headgroups oriented toward the aqueous phase [4]. An idealized model of a particle from BCP [5,6] with normal size of the water channels is presented in Fig.1. Such particles have been registered under the name cubosomes®. One of the two networks of water channels is open and molecules from the aqueous environment can enter inside, while the other network of water channels is closed without contact with the medium.

BCPs appear as stable long living intermediates between lamellar and inverted hexagonal phases in the phase diagram of lipid/water systems. The detailed mechanism of the lamellar-nonlamellar transition from a flat to a curved bilayer is not yet known, although many intermediates have been found [7]. Recent studies [8-10] revealed that a highly hydrated form of a BCP can appear during the transition from a lamellar to a cubic phase. The highly hydrated BCP involves large water channels that may allow new applications in protein formulation or in the context of membrane fusion and membrane protein crystallization [11].

The present work focuses on investigation of the nanochannels hydration in a BCP formed by the model lipid monoolein. By incorporation of the nonionic hydrating agent octyl glucoside, the hydration is enhanced, which results in increase of the water channel diameters. The structural parameters are monitored by SANS and SAXS. SANS appeared to be a very suitable method to establish structural features of systems that reach thermodynamic equilibrium relatively slowly, while for fast processes SAXS was employed.

2 MATERIALS AND METHODS

2.1 Chemicals and Samples Preparation

Samples were prepared by the method described by Angelov et all [12]. A powder of 1-monooleoyl-rac-glycerol [C18:1, cis-9] (MO) (MW 356.5) (purity >99.5% from Sigma Co.) was hydrated and dispersed in excess aqueous phase containing n-octyl beta-D-glucopyranoside (OG) (MW 292.4) (purity>99.5% from Sigma Co.) dissolved in phosphate buffer (NaH_2PO_4/Na_2HPO_4 (1:1 mol/mol) pH 7.0, p.a. grade from Merck), yielding a lipid-

to-detergent molar ratio of 95/5, 90/10 and 85/15. Full hydration of the lipid MO was achieved under excess aqueous phase conditions (20 wt% dry lipid). The buffer was prepared with heavy water (D_2O) for SANS measurements. Hydration of the lipid powder, to yield dispersion of lipid in the OG solution, was performed at temperature 24 °C. For every sample, eight cycles of vortexing (for 1 min) and incubation (for 5 min) at room temperature were applied. After homogenization, the hydrated samples were stored at 0 ^{o}C before SANS measurements and at 4 ^{o}C before SAXS measurements.

2.2 Small Angle X-ray Diffraction

The structure of the lipid/detergent/water mixtures was investigated by means of Synchrotron X-ray diffraction performed at the A2 beam line at HASYLAB, DESY, Hamburg, Germany. The principle of the experimental setup was analogous to that described in refs [12,13]. Two detectors covering the small-angle (SAXD) and the wide-angle (WAXD) diffraction regions were used. The temperature scans from 1 to 101 ^{o}C and the scan rate of 2 ^{o}C/min were programmed and controlled automatically. The recorded one-dimensional X-ray diffraction data were presented as intensities versus wave vector (q). The latter was defined as $q = (4\pi/\lambda) Sin(\theta) = 2\pi/d$, where 2θ is the scattering angle, λ=1.5 Å is the X-ray wavelength, and d is the repeat spacing. For determination of the q values, the rat-tail tendon (RTT) was employed as a calibration sample.

2.3 Small Angle Neutron Scattering

Small-angle neutron scattering experiments were performed with the SANS1 instrument at the FRG1 research reactor at the GKSS Research Center, Geesthacht, Germany [14]. The neutron wavelength was 8.1 Å with a wavelength resolution of 10% (fwhm). A range of scattering vectors $0.005 < q < 0.24$ Å^{-1} was obtained using four sample-to-detector distances (0.7-9.7 m). Samples were measured at temperatures from 15 ^{o}C to 65 ^{o}C with a step of 5 ^{o}C. The lipid/OG dispersions were filled in quartz cells. Raw spectra were corrected for backgrounds from solvent, sample cell, and other sources by conventional procedures. The time for measurement of one sample at a given temperature was about 3h. The two-dimensional scattering spectra were azimuthally averaged, converted to an absolute scale, and corrected for detector efficiency via dividing by the incoherent scattering spectrum of pure water. On Figs. 4-6, the right column q range is 0.05-0.24, while for left column q range is 0.025-0.1 (Å^{-1})

3 RESULTS AND DISCUSSION

Small-angle X-ray diffraction scans as a function of temperature (Fig.2) indicated that the functionalization of the monoolein BCP by incorporation of OG, as a hydration-enhancer guest molecule, causes enlargement of the water channels. A structurally distinct D_{Large} cubic phase forms at temperatures between 1 and 45 ^{o}C.

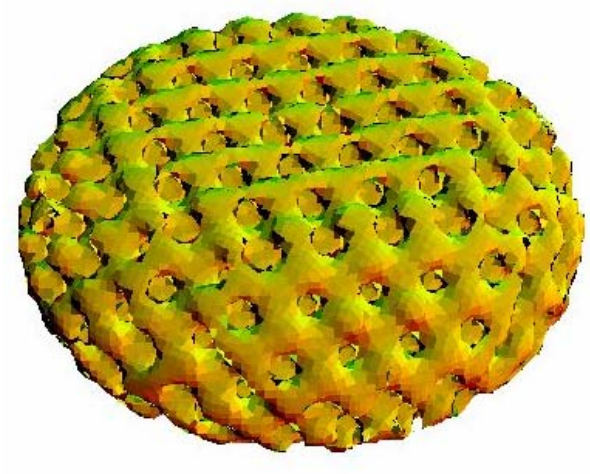

Figure 1: Nanosponge particle from a diamond type bicontinuous lipid cubic phase. The nanoparticle has open water channels on its surface.

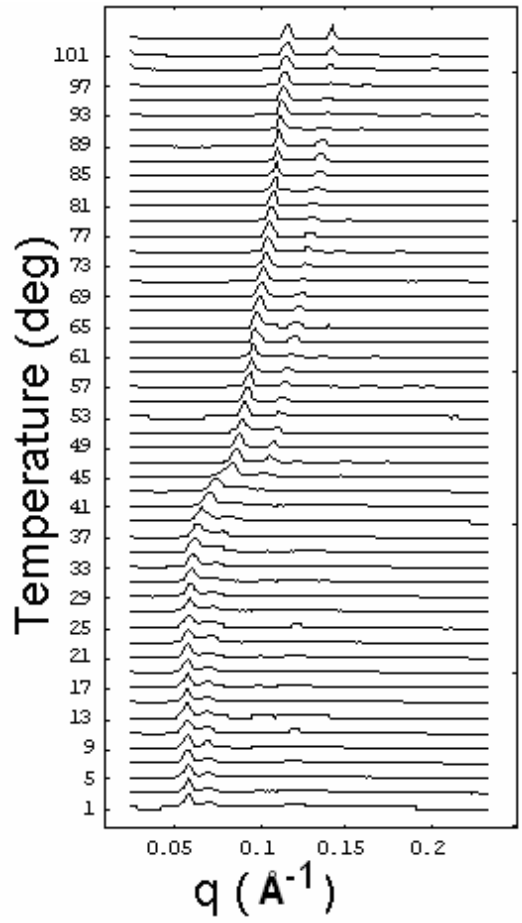

Figure 2: Small angle Xray diffraction of a MO/OG system at 90/10 molar ratio *versus* temperature. The large water channels are present at temperatures up to 45 ^{o}C.

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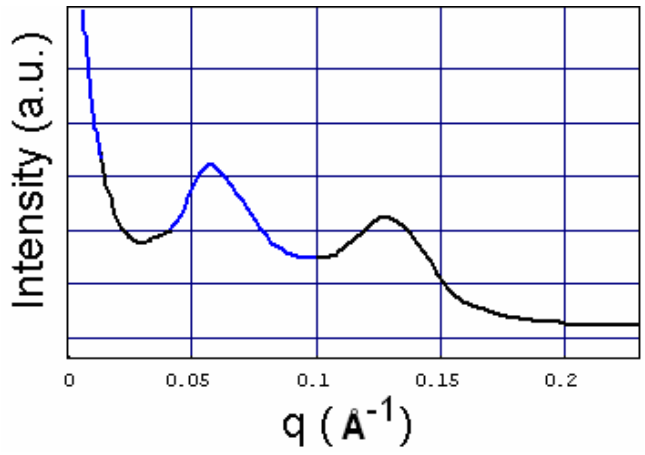

Figure 3: SANS of MO/OG at 90/10 (mol/mol) and 25 °C.

The water channels diameter, estimated from the SAXS patterns, are correspondingly *d = 31 Å* (for D_{Normal}) and *71 Å* (for D_{Large}), indicating a dramatic increase in the hydration. Table 1 summarizes the temperature dependence of the structural parameters, which were calculated using a modification of the Garstecki and Holyst model [15].

Sample Molar Ratio	**Temperature [°C]**	**Pn3m Lattice [*Å*]**	**Water Channel Diameter [*Å*]**
MO/OG 95/5	25	125.5	55
MO/OG 90/10	25	150.5	71
	65	89.7	31
MO/OG 85/15	25	Lamellar	n.a.

Table 1: Structural parameters of the investigated systems.

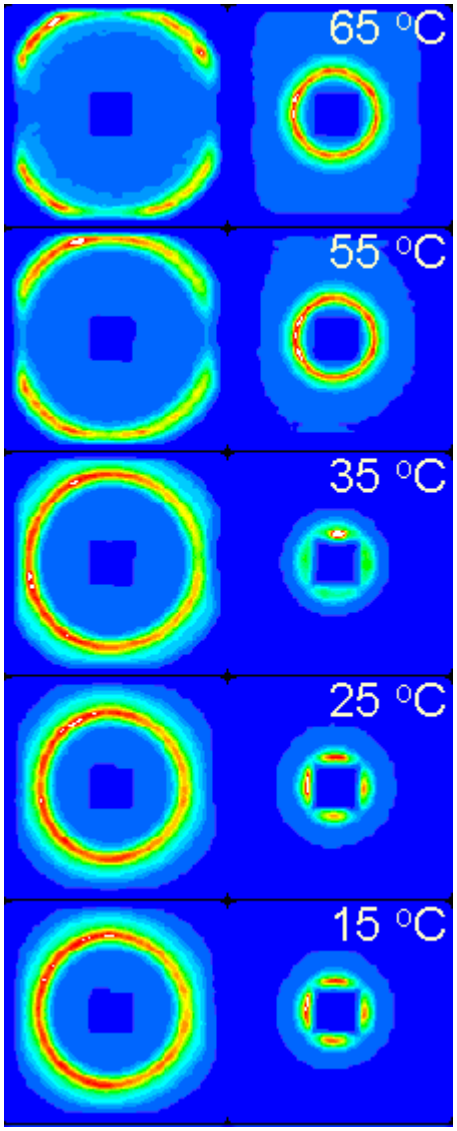

Figure 4: SANS of MO/OG at 95/5 molar ratio.

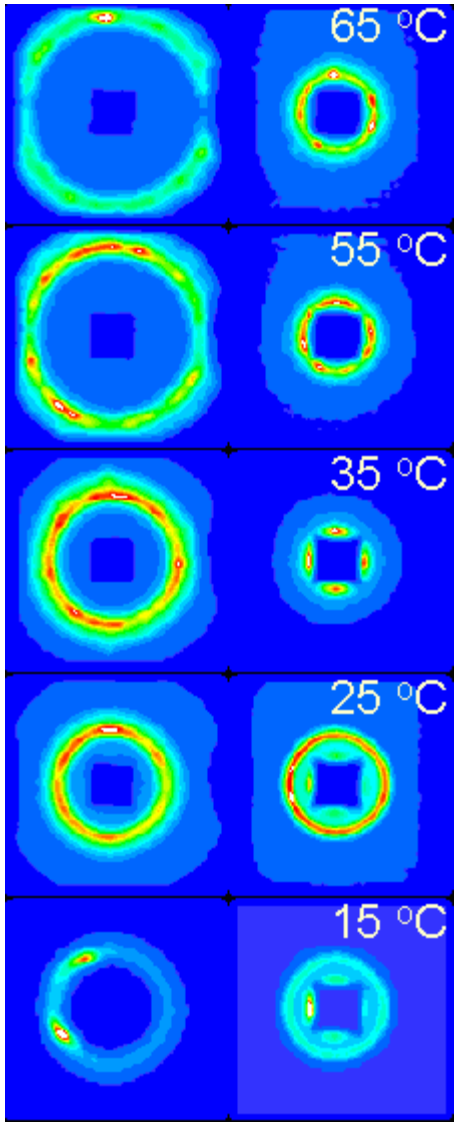

Figure 5: SANS of MO/OG at 90/10 molar ratio.

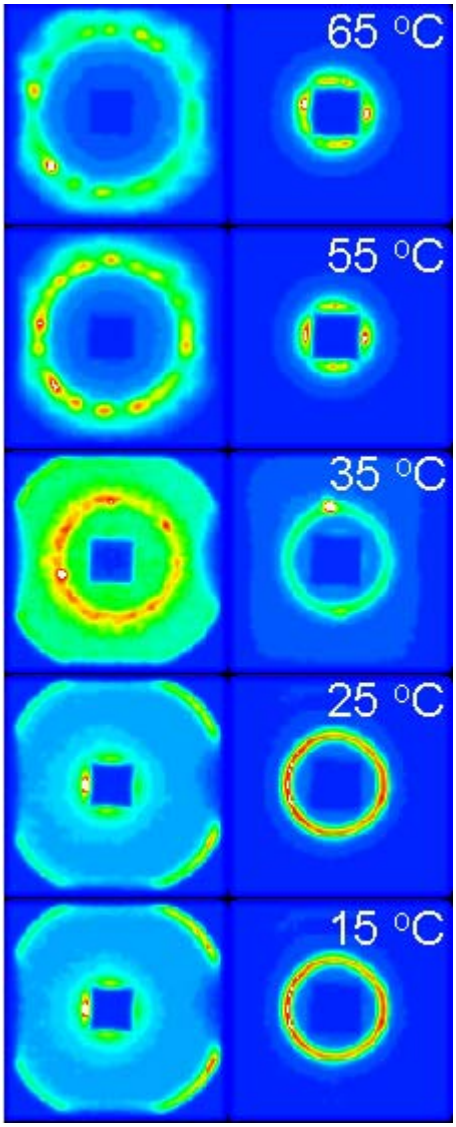

Figure 6: SANS of MO/OG at 85/15 molar ratio.

The SANS pattern in Fig. 3 of the D_{Large} BCP is dominated by a main peak associated to the first allowed (110) reflection of the cubic structure. The 2nd peak is due to formation of a small amount of a lamellar phase. The SANS behavior of the MO/OG 95/5 (mol/mol) system (Fig.4) is analogous to that of the pure-MO BCP. Fig. 5 presents the temperature effect on the SANS patterns of the MO/OG 90/10 (mol/mol) cubic phase. The 2D diffraction images show that the scattering patterns are no longer radially homogeneous. This reveals new structural features of the supramolecular organization of the D_{Large} cubic phase, which could not be resolved via one-dimensional SAXS or SANS curves. The asymmetry could be due to spontaneous orientation of the initial domains upon the application of the hydration stimuli. Increasing the OG concentration, to yield a total molar ratio MO/OG 85/15 (mol/mol), causes a shift in the transition temperature to a D_{Large} cubic phase to higher temperatures (T~35 °C). The SANS study of the MO/OG 85/15 system established that a lamellar phase is dominant for this molar ratio at temperatures below 35 °C (Fig. 6). Work is in progress to encapsulate proteins in such hierarchically organized soft nanostructured carriers.

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