

## Phase behaviour, molecular conformation and compressibility of the monoolein-water system

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In this allocated time we have investigated the effects of hydrostatic pressure on the structure and phase behaviour of some lyotropic lipid mesophases. In addition, the analysis of the curvature elastic parameters to prove theoretical models for the energetics of these lipid structures has been made possible. Structural investigations of different lipid systems have been carried out at room temperature and at different pressures from 1 bar to 3 kbar.

The systems that we have studied are the monoolein in water, both in the presence and in the

absence of cytochrome C and the dodecyl-trimethyl ammonium chloride (DTAC) in water. X-ray diffraction measurements were performed at 25°C for different pressures, from 1 bar to 3 kbar, with steps of about 100 bar. To avoid radiation damage, the exposure time was 2-5 s/frame and a lead shutter was used to protect the sample from excess radiation within periods where no data were recorded. Particular attention has been devoted to check for equilibrium conditions and for radiation damage: in several cases, measurements were repeated several times (up to 200) at the same constant pressure to account for stability in position and intensity of the Bragg peaks. Accordingly, a gently compression of the sample, at a rate of 0.5-2 bar/s, was observed to ensure the establish of equilibrium conditions, also in the regions of phase coexistence. In all cases, once stabilised the pressure (in a few minutes), the X-ray diffraction measurements were repeated at least 2 times, with an interval of at least 5 minutes. In each experiment, a number of sharp low angle reflections were observed and their spacings measured following the usual procedure. In all the cases discussed in this paper, a diffuse band in the 3-5 Å region of the scattering curves indicates the disordered nature of the lipid short-range conformation. Low angle diffraction profiles were indexed using equations which define the spacing of reflections for the different symmetry systems usually observed in lipid phases (lamellar, hexagonal, or 3-dimensional cubic

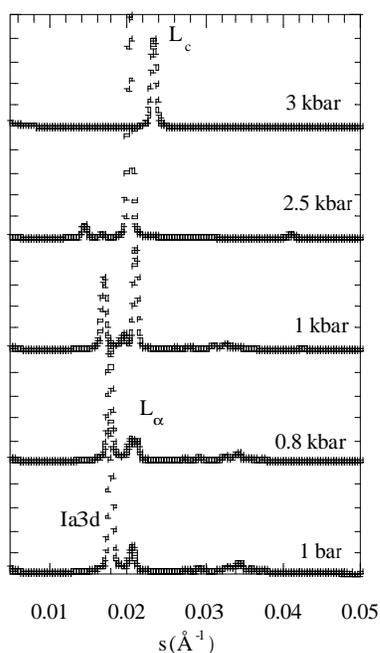
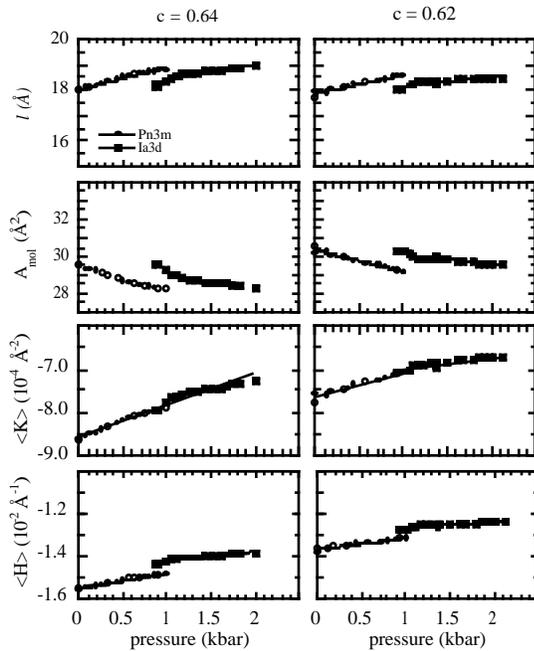


Fig.1: Azimuthally averaged diffraction patterns at different pressures for a monoolein sample at  $c = 0.7$  w/w lipid.

lattices): the indexing problem was easy to solve, because in no case extra peaks, which can be ascribed to the presence of unknown phases or to crystalline structures, were observed. Once the symmetry of the lipid phase was found, the dimension of the unit cells were calculated. Considering the sample composition, the structural parameters can be derived and analysed as a function of pressure.

The results can be summarized as follow:

- in the **monoolein/water** system the pressure-dependent phase diagram has been confirmed, and a clear elucidation of the effects of pressure on this system has been obtained. The pressure induces in the more hydrated samples a transition from the  $Pn3m$  cubic phase to a lamellar  $L\alpha$  or to a  $Ia3d$  cubic phase, according to the sample composition, while in the dryer samples compression determine a transition from the  $Ia3d$  cubic to the  $L\alpha$  lamellar phase. The underlying mechanism for



the phase transitions appears related to a continuous change in shape of monoolein molecules induced by pressure. In the cubic phases, these changes imply the stretching of the hydrocarbon chains and the consequent reduction of its cross-sectional area, resulting in the decrease of the mean curvature at the polar/apolar interface. These deformations are clearly involved in the associated curvature elastic energy contributions to the cubic phase free energy which explains the observed phase behaviour. The figure 1 shows that the pressure induces a transition from the *Ia3d* cubic to the lamellar  $L_\alpha$  phase and then to the lamellar crystalline phase. In Figure 2, some of the derived structural parameters are reported as a function of pressure.

Fig 2: Pressure dependence of the monolayer thickness ( $l$ ), area-per-molecule at the polar apolar interface ( $A_{mol}$ ), gaussian ( $\langle K \rangle$ ) and mean curvature ( $\langle H \rangle$ ) for the *Pn3m* and *Ia3d* cubic phases in monoolein samples

- for the **monoolein/cytochrom c/water** system, we have examined samples of full hydrated monoolein in cytochrome c solution of 100 mg/ml. This protein induces the formation of a bicontinuous inverted cubic phase with space group *Im3m*. As a function of pressure, this cubic phase has been detected to transform in the *Pn3m* phase, which forms at about 1 kbar and room temperature. At higher pressures (2 kbar), the lamellar liquid-crystalline phase finally appears. As detected in the absence of the protein, the lattice periodicities strongly depend on pressure, ranging from about 120 Å to 150 Å for *Im3m* and from about 105 Å to 113 Å for *Pn3m*. In the lamellar phase, the unit cell is of the order of 50 Å and appears rather independent on compression. These preliminary results give new information about lipid-protein-water phase diagram as a function of pressure.

- in order to compare the pressure induced phase behaviour, we extend our analysis to the **DTAC/water** system, which present as a function of lipid concentration a cubic micellar *Pm3n* phase, a hexagonal *H* phase and a cubic bicontinuous *Ia3d* phase, all of type I. Due to the limited measurement time, we analysed only a few samples, then exploring a very limited region of the phase diagram: the preliminary results show that the lattice periodicities don't strongly depend on pressure, but several phase transitions appear: the unit cell variations were only from 82 Å to 84 Å for the *Pm3n* and from 33.5 Å to 34 Å for the *H* phase ( $c = 0.55$  w/w). In the range between room pressure and 500 bar, the *H* phase was the only phase observed, while a re-entrant *Pm3n* phase was detected at low hydration. These preliminary data indicate an unusual phase behaviour, that merits be fully investigated..