



# DOPC-DOPE composition dependent $L\alpha$ - $H_{II}$ thermotropic phase transition: SAXD study



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## ABSTRACT

The structural polymorphism and parameters of lyotropic phases formed in the mixed dioleoylphosphatidylcholine-dioleoylphosphatidylethanolamine (DOPC-DOPE) system upon heating and varying DOPC:DOPE composition were studied by means of small-angle X-ray diffraction (SAXD). In the temperature range 5–80 °C a sequence of fluid lamellar  $L\alpha$  – inverse hexagonal  $H_{II}$  – inverse cubic  $Q_{II}$  phases was detected at DOPE mole fractions  $X_{DOPE} \geq 0.65$ . A superposition of two bicontinuous cubic  $Q_{II}$  phases of  $Pn3m$  and  $Ia3d$  space groups was identified. The  $L\alpha$  to  $H_{II}$  phase transition temperature, the onset of the  $Q_{II}$  phase formation, as well as the lattice spacings of the  $L\alpha$  and  $H_{II}$  phases were found to decrease with rising DOPE content. Moreover, evidence of structural rearrangement during the  $L\alpha$  to  $H_{II}$  phase transition is given and change of transition mechanism with varying  $X_{DOPE}$  is suggested.

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## 1. Introduction

The thermotropic phase behavior of hydrated amphiphilic phospholipids is very diverse, ranging from fluid lamellar  $L\alpha$  phase, where equidistantly stacked bilayers are separated by aqueous layers, to more complex mesophases with increasing negative curvature, such as inverse hexagonal  $H_{II}$  or cubic phase (Seddon and Templer, 1995). Non-lamellar lipid structures perform a number of key roles in biological processes. They are indispensable in modification of membrane flexibility underlying the proper function of integral membrane proteins (Lee, 2004; van den Brink-van der Laan et al., 2004) and in local formation of nonbilayer structures, e.g. during the biological processes of endo- and exocytosis and cell division (Hague et al., 2013). However, lamellar to non-lamellar phase transition plays an important role not only in cell life but also for application in food science and pharmacology. For instance, lipidic nanoscale systems for drug delivery, including liposomal formulations and inverted-type fluid phases and their aqueous dispersions (cubosomes and hexosomes), have attracted much attention in recent years ((Rappolt, 2013) and references therein). Therefore, understanding the formation and stability of curved model membrane systems is essential.

The structure of the self-organized system is primarily determined by the chemical character and the geometrical parameters of lipid molecules. This is the basis of the so-called shape-structure concept (Israelachvili et al., 1980) which provides a framework for explaining the polymorphic phase preferences of lipid dispersions. Factors responsible for controlling the stability of formed phase are of two types, which are interrelated: (i) transversal interactions (van der Waals, hydration, fluctuation, electrostatic) between adjacent lipid layers and (ii) lateral interactions within a lipid bilayer (Seddon and Templer, 1995). In lamellar systems the lateral repulsive chain and head-group pressure are counterbalanced by the interfacial pressure, consequently each monolayer exhibits zero spontaneous curvature. When the distribution of lateral forces across the bilayer, i.e. the lateral pressure profile, changes so that these forces are not balanced any more, the monolayers tend to curve. Using this approach the “curvature concept” was formulated by Gruner (1985). If the curvature frustration is high, monolayers curve round into long cylinders which pack into hexagonal lattice,  $H_{II}$  phase is formed (Seddon, 1990). Another possibility is the formation of membranes with a saddle-like shape, as in the bicontinuous cubic phases, in which curvature frustration may be released. The cubic phase has peculiar characteristics that are intermediate between  $L\alpha$  and  $H_{II}$  phases (Gruner, 1989).

The amount of stored curvature elastic energy may be changed i.a. by altering the bilayer lipid composition. In this work we intend to follow the structural polymorphism of the

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dioleoylphosphatidylcholine (DOPC) and dioleoylphosphatidylethanolamine (DOPE) mixture, i.e. the mixture of a typically bilayer-forming and a nonbilayer lipid, respectively. For this system different non-lamellar structures, such as rhombohedral, distorted hexagonal (Yang et al., 2003) and cubic phases (Templer et al., 1998a; Fenske and Cullis, 1992), were observed upon hydration and/or heating, aside of  $H_{II}$  phase. Raising the temperature increases conformational disorder in the hydrocarbon chains, what tends to expand the interfacial area per molecule. Conversely, the rising the water content increases the lateral repulsions between the headgroups and concomitantly the interfacial area per molecule. Although both of these situations raise the energy of the system, other phases can have a smaller amount of frustration than  $L\alpha$  and  $H_{II}$  phases, and can thus be expected to occur in this system.

## 2. Materials and methods

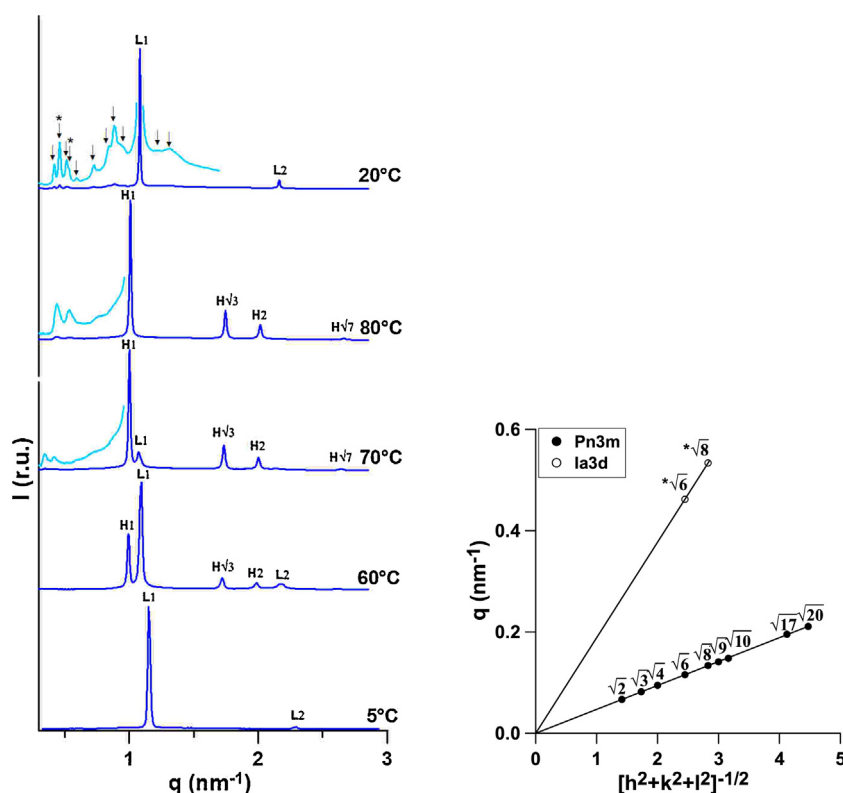
Synthetic DOPE and DOPC were purchased from Avanti Polar Lipids (Alabaster, USA) and used without further purification. The organic solvents of spectral purity were obtained from Slavus (Bratislava, Slovakia). Stock solutions of DOPE and DOPC were prepared in methanol+chloroform mixture. Samples were prepared at required DOPE mole fractions by pipetting appropriate amounts of stock solutions. The solvent was evaporated under a stream of gaseous nitrogen and its traces removed by an oil vacuum pump. Finally, deionized MilliQ water (18.2 M $\Omega$  cm, Millipore, Molsheim, France) was added at H<sub>2</sub>O:(DOPE + DOPC) = 105:1 mol/mol. Samples were homogenized at ~5 °C by forth-and-back centrifugation in a flame-sealed glass tube. Prior to measurement, samples were transferred into thin-walled capillaries (Hilgenberg, Germany) with a diameter of 1 mm. After centrifugation, to remove air bubbles, the capillaries were sealed with a two-component synthetic resin.

SAXD measurements were performed using a modified Kratky Compact camera (Anton Paar, Graz, Austria) equipped with a position-sensitive detector (M. Braun, Garching, Germany). The detector was calibrated using silver stearate. The Ni-filtered CuK $\alpha$  radiation ( $\lambda = 0.1542$  nm) was provided by Seifert X-ray tube with 1000W power. Samples were stored in metal capillary holders that were placed into an aluminium block positioned directly into the beamline and used as a thermal incubator. After 15 min incubation at respective temperature, diffractograms were recorded for 600 s. As the primary beam was line-focused, the intensity curves were corrected with respect to the geometry of the beam profile (Glatter, 1981). Before evaluation, normalization on water and background subtraction was done. Corrected curves were fitted using Gaussian function and positions of maxima were estimated with a non-linear least-squares program.

## 3. Results and discussion

### 3.1. Phase identification

Fig. 1 shows typical diffractograms of the DOPC-DOPE system at  $X_{DOPE} = 75\%$  mole fraction obtained on heating in the temperature range 5–80 °C and after rapid cooling back to 20 °C. In the monitored  $q$ -interval at the low temperature range two sharp reflections corresponding to the lamellar  $L\alpha$  phase were detected. On heating, a coexistence of lamellar and inverse hexagonal  $H_{II}$  phases occurred. With a further increase of temperature, three reflections, with the ratio of reciprocal spacings 1: $\sqrt{3}$ :2, characteristic for pure  $H_{II}$  phase, were observed. Few degrees before completion of the  $L\alpha$  to  $H_{II}$  phase transition further low-intensity reflections appeared. These reflections became more pronounced in coexistence with  $L\alpha$  phase, after cooling the sample back to 20 °C.



**Fig. 1.** Left: Typical SAXD diffractograms of DOPC-DOPE system at  $X_{DOPE} = 75\%$  at designated temperatures. Insets: 15 times enlarged region of  $Q_{II}$  phase reflections. Arrows mark the positions of fitted reflections. Right: Space group identification of the cubic phases at 20 °C after heat/cool cycle. Lines represent the best linear fits of experimental points ( $r^2 = 0.99999$ ).

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