Influence of Antimicrobial Peptides of Lactoferrin Family on the Phase Behavior of Model Membranes

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Lactoferrin (LF), a mammalian iron-binding glycoprotein, is a multifunctional protein that has antibacterial, antiviral, antifungal and antiparasitic activities and is also implicated in protection against cancer. Within Lactoferrin, there is a highly cationic N1 terminal domain, where two antimicrobial peptides are found namely, lactoferricin (LFcin) and lactoferrampin (LFampin) [1, 2].

The present work was focused on three peptides from this family, namely LFcin17-30, LFampin265-284 and LFchimera, a hybrid peptide formed by the first two linked through the side chain of an additional lysine [3]. Their interaction with model membranes of different lipid compositions was assessed, using DMPC/DMPG (3:1), POPE and POPE/POPG (3:1). We prepared mixtures of the peptides and model membranes at different P:L ratios, in HEPES (100 mM NaCl) or in PBS buffer (150 mM NaCl).

Small angle X-Ray diffraction (SAXD) experiments were performed at beamline A2 in HASYLAB at DESY. The sample was equilibrated at each selected temperature for 5 min before exposure to radiation. Temperature scan was performed at a scan rate 1ºC/min and diffractograms were recorded every minute for 10s.

Due to the negatively charged DMPG, the mixture DMPC/DMPG (3:1) does not show a well organized lamellar phase (typical for DMPC), and in SAXD we observed a broad scattering with intensity at the level of background (Fig. 1), compatible with oligolamellar vesicles. Our DSC experiments shows that this system goes from gel lamellar phase (L_β) to fluid lamellar phase (L_α) with increasing temperature (T_M = 25 ºC). When the peptides were added to the liposome suspension, the phase behavior of DMPC/DMPG (3:1) membranes was affected differently depending on the peptide and ratio.

For LFcin17-30 at P:L 1:8 (mol/mol), a very well defined fluid lamellar phase and two gel lamellar phases are seen on cooling. These results suggest that the peptide induces lipid segregation (Fig. 1), which can be seen as a result of a preferential interaction of the cationic antimicrobial peptide with the negatively charged lipid, DMPG. Thus the presence of two lamellar gel phases when cooling from the fluid lamellar phase (Fig. 1) could reflect peptide rich DMPG and peptide poor domains.

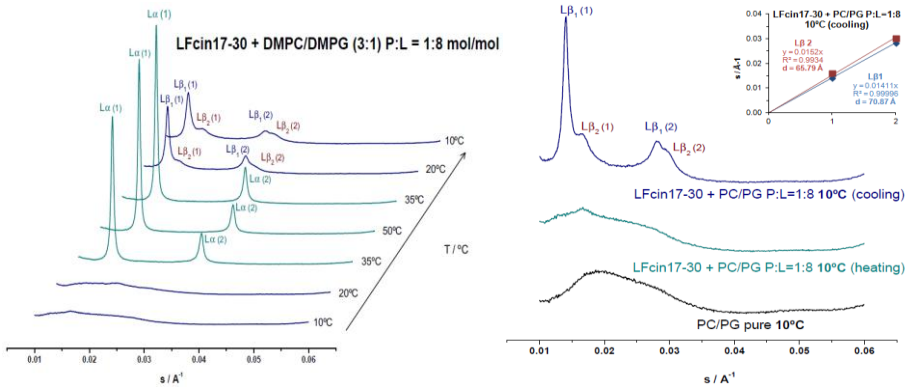

Fig. 1: Left: SAXD patterns for DMPC/DMPG (3:1) + LFcin17-30 P:L=1:8 at selected temperatures. Right: DMPC/DMPG (3:1) and DMPC/DMPG (3:1) + LFcin17-30 at 10ºC. Insert - s($Å^{-1}$) = $f(h^2+k^2+l^2)^{1/2}$.

In the case of LFampin 265-284 with the same lipid system, at P:L ratio 1:5 (mol/mol), the peptide disrupts the lipid bilayer, forming a cubic phase of space group Pm3n (< 28 ºC) and at high temperatures a lamellar phase I seen (Fig. 2). These results are published as "Lactoferrin-derived antimicrobial peptide induces a micellar cubic phase in a model membrane system", Bastos M, Silva T, Teixeira V, Nazmi K, Bolscher JG, Funari SS, Uhríková D *Biophys J*. 101(3):L20-2 (2011).

Finally for LFchimera we found a superposition of two cubic phases of different symmetry and lattice parameters: one of Pn3m space group with lattice parameter a=107.5Å, and another of Im3m space group with lattice parameter a=153.8Å (fig. 2). The ratio of the obtained unit cell parameters is aIm3m/aPn3m=1.4

The observed behavior confirms recent observations on the importance of cubic phases in the mechanism of action of antimicrobial peptides, and also shows that the peptide-lipid interaction strongly depends on the peptide. The present results are in line with microbiological results previously obtained with these peptides against the fungi *Candida albicans* (which is rich in PC and has also PG [4]), where by Freeze fracture microscopy different structures were obtained for these three peptides

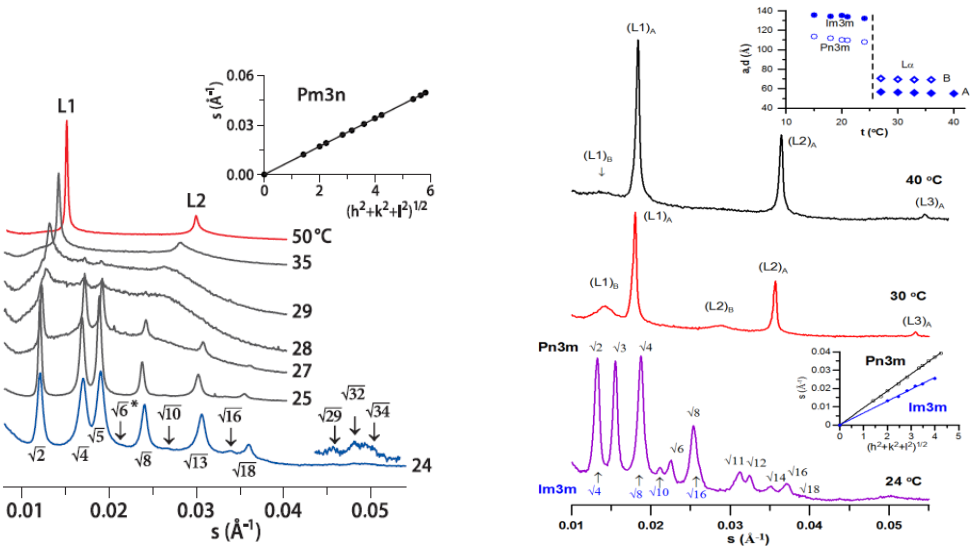

Fig. 2: Left: SAXD patterns of LFampin + DMPC/DMPG (3:1) at P:L=1:8 at selected temperatures. (Insert) A plot of $s=f(h^2+k^2\ l^2)^{1/2}$ for all observed reflections at 24 ºC. Right: SAXD patterns of LFchimera + DMPC/DMPG (3:1) at P:L=1:33 at selected temperatures. (Insert) A plot of lattice parameters vs. temperature (above) and plot of $s=f(h^2+k^2\ l^2)^{1/2}$ for all observed reflections at 24 ºC (below).

In the case of POPE, the main lipid of bacterial membranes, as the temperature increases there is a transition from gel lamellar phase (L_β) to fluid lamellar phase (L_α) at 26 °C, and from fluid lamellar (L_α) to hexagonal phase (H_{II}) at 71 °C (Fig. 3). When the peptides are added at P:L 1:8 for LFcin and LFampin and 1:18 for LFchimera, the lamellar and hexagonal phase parameters are not affected. However, when cooling from the hexagonal phase a new structure of cubic symmetry is observed. Fig. 3 shows a diffractogram from a temperature down scan of LFcin with POPE membranes at 50 °C. The peaks observed at the low s ($Å^{-1}$) range fit well with reflections that can be indexed to a superposition of two cubic phases of different symmetry, namelly Pn3m (a=128.2 Å) and Im3m (a=163.9 Å), that coexist with a fluid lamellar phase. The ratio of the obtained unit cell parameters, aIm3m/aPn3m=1.28, is close to the ideal Bonnet relation of 1.279 [5], supporting the chosen indexing.

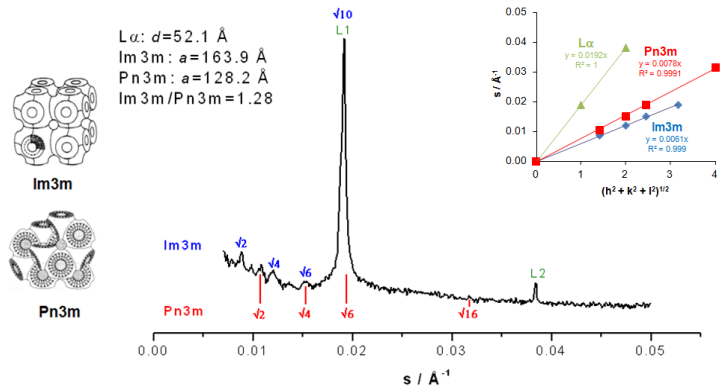

Fig. 3: SAXD pattern of LFcin17-30 + POPE at P:L=1:8 at 50ºC on cooling. (Inset) A plot of $s=f(h^2+k^2\ l^2)^{1/2}$.

Important conclusions can be derived from these results: a) antimicrobial peptide induce (or at best enhance) cubic phases in model membrane systems, when they are studied at physiological conditions (buffer at pH 7 and moderate to high ionic strength); b) the particular cubic phase retrieved depends on peptide and lipid system.

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