Structure analysis of antimicrobial peptides in solid supported lipid bilayers using method of reflectivity

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The structure of membrane-active peptides and their interaction with lipid bilayers can be studied in oriented lipid membranes deposited on solid substrates. We have investigated the structure of solid-supported, multilamellar membranes by x-ray reflectivity. Compared to isotropic scattering (SAXS), reflectivity offers the advantage of probing the density profile on an absolute scale without free scaling parameters. The preparation of well-defined, homogeneous and structurally intact membrane systems on solid support is an important problem[1], involving fundamental physical questions, for example related to wetting behaviour [2], thermal stability [3, 4], or defects typical for smectic liquid crystalline (LC) films. A particularly simple and low-cost approach to preparing oriented lipid membranes is to spread a solution of co-dissolved lipids and peptides onto solid surfaces like silicon, glass, quartz or other flat surfaces [5].

As a prerequisite to the application of x-ray for structural analysis, lipid films of sufficiently low mosaicity (in most cases below instrumental resolution) have been prepared by spreading or spin coating from organic solvent [5,6]. Samples composed of the following phospholipids have been prepared as highly oriented multilamellar or oligo film: DMPC, POPC as well as the mixture(3:1 mol ratio) of DMPC+DMPG and POPC+POPS (lipids all bought from Avanti Lipids, Al.).

The peptides magainin2 amide (GIGKFLHSACKFGEIMNS) and alamethicin (XXPXAXAQXV XGLXPV XXSEQ) were bought from Sigma.

For the data presented here, DMPC was solved in 1:1 Chloroform/TFE (2-2-2-trifluoroethanol) mixtures. The resulting solutions were spread on specially cleaned Si(100) wafers by spin coater
and subsequently put into vacuum overnight. For the measurements, the samples were kept with a water reservoir in a humidity and temperature controlled sample chamber, and were kept at $T \approx 45^\circ C$ and relative humidity (RH) close to 100%. The temperature was measured close to the sample holder by a Pt-100 sensor, the RH was measured by a humidity sensor ((HIH2610-003, Honeywell, Freeport IL). The parameters were chosen to keep the samples in the physiologically relevant state of the fluid $L_\alpha$ phase [7]. The measurements have been performed at 20 keV to reduce radiation damage. Fig.1 shows the reflectivity and the fitted curve of pure DMPC.

The electron density profiles are obtained by fitting the full q-range (preliminary fit only). The model uses the fourier coefficients of the bilayer density profile as fitting parameters[6]. The effect of hydration and the substrate boundary condition are taken into account, in view of the well known Landau-Peierls effect and its implications for structure determination. After the successful development of this fitting program the next step will be the interpretation and modeling of the profiles with respect to molecular conformation.

Fig. 2 shows the electron density profiles of representative curves at varied peptide to lipid ratio P/L on an absolute scale. Note that the curve fitting is still in progress and that these results are still preliminary.

![Figure 2: Electron density profiles of DMPC/magainin2 with different peptid concentration.](image-url)

References