Beta-sheet-forming Antimicrobial Peptide Arenicin at the Air-Water Interface

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The recently discovered new antimicrobial peptide Arenicin [1], which was isolated from sandworm, is now widely under investigation. Especial interest represents the study of its properties at the air-water interface as a pre-study of its behaviour at model membrane surfaces. The non-cyclic form of Arenicin, which differs from the original sequence by substitution of two cysteine residues forming a disulfide bridge by two serine residues, is hydrophilic and can be therefore dissolved in aqueous phosphate buffer with additional sodium chloride. The peptide possesses surface activity and adsorbs to the air-buffer interface.

The peptide secondary structure in bulk was determined by CD measurements. The main contribution is from a beta-turn with some beta-sheet and random coil contents. Time-dependent surface pressure measurements of the buffer solution of this peptide showed that the adsorption to the air-buffer interface reaches an equilibrium value after 2 hours. The adsorption layer is very stable and can be investigated by specular X-Ray reflectivity (XR) and infrared reflection-absorption spectroscopy (IRRAS) experiments. XR measurements were performed at the liquid-surface diffractometer at BW1 in order to gain detailed information about the electron density profile. The depicted electron density profile (Figure 1) was obtained from the reflectivity curve using a linear combination of b-splines following the approach of Pedersen and Hamley [2, 3]. The profile (Figure 1) can be described by a 'one box model'. The box thickness is approximately 12 Å, and the roughness of the layer is only 3 Å showing that the peptide adsorption layer is tightly packed and very smooth.

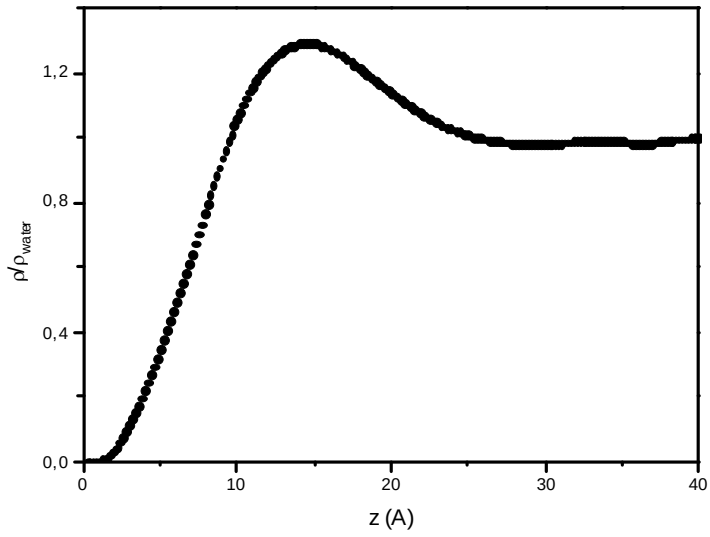

Figure 1: Electron density profile of the Arenicin adsorption layer at the air-buffer interface.

IRRAS has been applied to determine the secondary structure of the adsorbed peptides and their orientation. IRRA spectra show the presence of an anti-parallel beta-sheet at the air-water interface (amide I bands at 1627 cm^{-1} and 1691 cm^{-1}). The beta-sheet lies flat at the interface what agrees

well with the observed layer thickness of 12 Å. The question arose whether this adsorption layer is crystalline with well-ordered structures, which can be studied using Grazing Incidence X-Ray Diffraction (GIXD). Indeed, Bragg peaks have been observed. The Bragg peak at 1.317 $Å^{-1}$ corresponds to a repeat distance of 4.77 Å, which is the typical interstrand distance defined by the hydrogen-bond network in a beta-sheet conformation (Figure 2). The intensity and position of this peak does not vary during the compression of the film. The full-width at half-maximum (fwhm) of this peak shows a correlation length of 60 Å. A second Bragg peak can be seen at lower Q_{xy} values. This peak is associated with the peptide ordering along the peptidic backbone with a repeat distance of 36.3 Å, which nicely coincides with half the length of the peptide in a fully extended configuration. Both peaks do not change during compression. This means that the peptide after adsorption does not change its conformation and remains as a hairpin forming anti-parallel intramolecular beta-sheets (with a beta-turn). The correlation length in the longitudinal direction amounts to 370 Å.

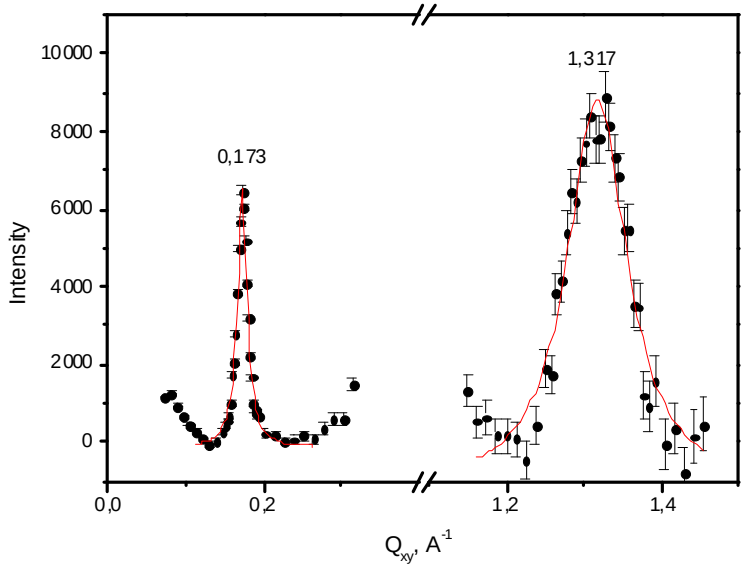

Figure 2: Bragg peaks (in-plane scattering vector component) of the Arenicin adsorption layer at the air-buffer interface.

The full-width at half-maximum (fwhm) of the longitudinal Bragg rod allows an estimation of the film thickness Lz : $Lz = 0.9 \cdot 2\pi/\text{fwhm}(Q_z) = 16.5$ Å. This value is larger than expected for a flat beta-sheet monolayer, and larger than determined by XR. A possible explanation could be that the peptide molecules at the air-water interface have not only the turn between two beta-strands, but they might be also twisted or bended in the middle of each beta-sheet part of the molecule.

References

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