ESRF	**Experiment title: Interaction of the antimicrobial frog peptide, PGLa, with lipid monolayers studied by grazing incidence diffraction**	**Experiment number**: SC-517
Beamline: ID10B	**Date of experiment**: from: 16 September 1998 to: 22 september 1998	**Date of report**: 10/08/99
Shifts: 18	**Local contact(s)**: Detlef SMILGIES	*Received at ESRF:*

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Report:

Surface pressure-area (π-A)-isotherms measured previously in laboratory by one of us (K.Lohner) indicated that frog peptide PGLa interacts differently with monolayers of DPPG and DPPC formed at the air/water interface. These result suggest that the peptides do not mix at a molecular level with DPPC but with DPPG. So, the aim of this experiment was to understand, using X-ray grazing incidence diffraction (GID), the structural changes taking place in monolayers of DPPG and DPPC at presence of PGLa. An understanding of how these peptides distinguish between e.g. bacterial (mimicked by DPPG monolayer) and eucaryotic (mimicked by DPPC monolayer) cell membranes and perturb the barrier function of cell membranes of the first would allow to design novel peptide antibiotics which can selectively kill bacteria.

To reach the posed aim we applied GID technique to measure in plane Bragg peaks from monolayer of pure substances and their mixtures. The monochromatic incident beam (λ=1.55 Å) was sent onto the air/water interface at grazing angle 2.14 mrad ($0.8\,\theta_c$). We analysed the diffractted intensity with Sollers collimator and vertically oriented linear PSD. The Langmuir trough was flowed with He to reduce background and film damage due to ozone that is created by X-ray beam if trough is filled with the air. All measuremets was done at room temperature (20^oC). Position and shape of the Bragg rods for all systems was measured at surface pressure (π) 15, 20, 25, 30, 35 and 40 mN/m. This range of π is defined by collaps of phospholipids above 40 mN/m and collaps of peptide monolayer at 25 mN/m. This set of π gave possibility to compare molecular ordering of phospholipids without and with presence of PGLa both before and after peptide collaps. Intensity of the Brag rods at π < 15 mN/m is expected to be very weak.

We start measurements from monolayers of pure substances to have later a reference for the mixtures. The result is presented on the Table. Bragg rods in vertical direction was recorded only for $Q_z < 0.35$ Å^{-1}. At this condition we were able to see for lipids layers only inplane Bragg peak <11>. Brag peaks intensity from DPPC and DPPG monolayers was weak and we decided to take longer molecules: DSPC and DSPG. Phospholipids with longer chains gave stable results and more intense Bragg peaks. We would like to stress that the **results obtained for monolayers of DPPG and DSPG are novel**, there is no a publication about structure of this molecules in monolayers at the air/water interface. Analysis of the Bragg peaks positions for lipidic monolayers shows that lipds with PG at the head of aliphatic chan have larger compressibility than with PC. We explane this by size of the PG group which is smaller than PC group and the chains cross section. That is why molecules packing defines manly by chains odering in case of PG lipids and heads in

case of PC lipids. The Bragg peaks position for all measured lipids has linear dependence on the applied surface pressure (see Table and Figure).

The GID data obtained for the first time for the monolayer of pure peptide overcame all our expectations. First we were not sure at the beginning of experiment that PGLa film will give any Bragg peaks. But we were able to measure up to 3 order of Bragg peaks, that is not indexed yet and we assign to them just ordinal numbers (see Table). Second, width and position of this Bragg peaks in Q_{xy} and Q_z directions do not depend on surface pressure variation (see Figure). Only the peak intensity grows with increase of π, that we explane with increase of number of molecules per square unit. It seems that observed Bragg peaks come from inner structure of peptide molecule. In this case Bragg peak width ΔQ_{xy} is much smaller than the value expected for one molecule.

Measurements of the PGLa and DSPC mixtures clearly show (see Figure) that they did not mix on a molecular level. The measured spectrum looks like the sum of the spectrum from pure substances with the same behaviour versus surface pressure change. But the spectrum obtained in case of the PGLa and DSPG mixtures are completely new. Ordering of DSPG molecules are destroied by PGLa but new order of molecules packing did not apper as it was expected for the homogeneous mixture. Observed Bragg peaks are the same as in case of film from pure PGLa. This spectra consistent with the idea that this peaks originate from inner structure of peptide molecule.

The main ideas of the experiment are reached. Structural transformation of DSPG layer in presens of peptide are found. New questions is posed by the measurement: 1) what is the detailed inplane structure of PGLa layer and petide molecules in the bulk and in the layer at water interface; 2) where PGLa molecules located in DSPC and DSPG layers that differ only by size and charge of the heads.

Reply on the second question we are going to get using X-ray reflectivity technique with our next proposal.

Table. In plane Bragg peaks position for the monolayers of pure substances.

π, mN/m	$Q_{xy<11>}$, Å^{-1}			
	DPPC	DPPG	DSPC	DGPG
15	1.459	1.464	1.453	1.454
20	1.466	1.470	1.454	1.459
25	1.467	1.474	1.459	1.463
30	1.4674	1.479	1.461	1.468
35	1.470	1.483	1.463	1.473
40	1.472	1.488	1.466	1.479

π, mN/m	PGLa		
	Q_{xy} (1)	Q_{xy} (2)	Q_{xy} (3)
15	1,510	1,678	2,812
22	1,511	1,679	2,814
27	1,510	1,678	2,814

Figure. In plane Bragg peaks spectrum measured for some of the systems.