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Studies of phospholipid monolayer at liquid/liquid interface in presence of an antimicrobial peptide

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Abstract

An application of the antimicrobial peptides (AP) as antibiotics of new generation requires comprehensive studies of the interaction of these peptides with a cell membrane (CM). Despite the big progress in characterization of the AP-CM system, its elastic properties remain unclear. In the present work we present studies of the structure and the bending rigidity of phospholipid monolayers formed at hexadecane/water interface in presence or absence of antimicrobial peptides. It is shown that due to the interactions between the monolayer and the peptide, the bending rigidity decreases after the injection of the peptide.

Keywords: Bending rigidity; Langmuir–Blodgett films; X-ray scattering; Liquid–liquid interface

1. Introduction

An application of the antimicrobial peptides (AP) as antibiotics of new generation requires comprehensive studies of the interaction of these peptides with a cell membrane (CM). Despite the big progress in characterization of the AP–CM system, its elastic properties remain unclear.

Antimicrobial peptides activity is related to their interactions with the lipid bilayer itself, rather than specific protein receptor (s) within the cell membrane [1]. In this work, we study the structure and the bending rigidity of phospholipid monolayers in presence and absence of antimicrobial peptides. DiPalmitoyl-PhosphatidylGlycerol (DPPG) was chosen to mimic bacterial cell membrane because antimicrobial peptides were shown to discriminate between mammalian and bacterial cell membranes by their lipids composition [2] and DPPG is the main component of bacterial cell membranes.

Experiments were performed at liquid/liquid interface because, as it will be explained below, the X-ray measurements in Grazing Incidence geometry are sensitive to surface tension (γ) and bending rigidity (κ) through the height–height correlation function. On the other hand, at liquid/liquid interface, the surface tension can be reduced to the minimum; consequently the diffuse scattering signal will be due mainly to the elastic properties of membranes. So bending rigidity can be studied more accurately [3].

2. Experimental section

2.1. Materials

Phospholipid DiPalmitoyl-PhosphatidylGlycerol (DPPG, MW = 740 g/mol) was purchased from Sigma (France) (purity >99%) and used without further purification.

Antimicrobial peptide Peptidyl-Glycyl-Leucine-carboxylamide (PGLa, MW = 1969 g/mol) consisting of 21 amino acids (GMASKAGAIAGKIAKVALKAL-carboxylamide [4]) was purchased from Multiple Peptide System (San Diego, CA).

Chloroform was purchased from Sigma (France) and was of HPLC grade.

Pure water was obtained with an ELGA LABWATER system. Hexadecane was purchased from Sigma (France) (purity: 99%) and purified before use with two different techniques.

Aluminum oxide (Activated, Basic) (Al_2O_3), for hexadecane purification, was purchased from Sigma (France).

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2.2. Isotherms measurements

Surface pressure–Area (π–A) isotherms were recorded at Air/Water (A/W) and Hexadecane/Water (H/W) interfaces, using a home-made Langmuir trough specially designed for X-ray experiments at the liquid/liquid interface [5]. Liquid hexadecane used for the isotherm measurements was purified by five cycles of filtering through a basic Al_2O_3 column. Stock solution of DPPG lipids was prepared in chloroform at a concentration of 0.25 mg/ml. Water solution of peptide at a concentration of 0.17 mg/ml was prepared in 10 mM Na-Phosphate buffer, pH = 7.4.

The monolayers were prepared by spreading, with a Hamilton syringe, 120 μL and 150 μL of phospholipids solution on the interfaces A/W and H/W respectively. After deposition, the films were left for at least 20 min before compression in order to ensure complete evaporation of chloroform. The monolayers were compressed at the interface by symmetric move of two barriers with a velocity of 15 cm^2/min. The surface pressure (π) was recorded using a Wilhelmy balance (NIMA tensiometer PS4) with 10 mm wide filter paper, connected to a home made electronic that measures with an accuracy of ±0.2 mN/m. All π–A measurements of lipid monolayers were done at room temperature (20 °C).

2.3. X-ray experiments

X-ray Reflectivity (XRR) for structural characterization and Grazing Incidence diffuse X-ray Scattering in the plane of incidence (GIS) for obtaining bending rigidity of monolayers at liquid/liquid interface were performed.

For these experiments, the hexadecane purification was done differently than previously. Pure water was added to hexadecane in the bottle and the mixture was shaken to increase the exchange surface between the two immiscible components. After phase separation we took hexadecane above the polluted interface and used it for experiments. As we measure at the H/W interface this technique allows removing impurity surfactants from the bulk of hexadecane. When the purified hexadecane was put in the trough, the bare H/W interface was compressed and sucked a bit to remove last impurities.

Monolayer preparation for the X-ray measurements was the same as for the measurements of the π–A isotherm at H/W interface.

After spreading of the phospholipids onto the cleaned H/W interface, the vessel containing the trough was closed with a cap to minimize evaporation of hexadecane during the X-ray measurements.

The DPPG-peptide system was prepared from the previously formed lipid monolayer by injection in the water subphase of 50 μL of PGLa solution. We waited for 10 min before taking X-ray measurements to ensure complete interaction between PGLa and a monolayer.

X-ray measurements were carried out at the ID10B (Troika II) beamline of the European Synchrotron Facility (ESRF). The energy of the monochromatic beam was set to 22.5 keV (wavelength $\lambda = 0.055$ nm) to allow the X-ray beam to pass through 70 mm of the oil (Fig. 1). The grazing angle (α) was set to 0.384 mrad for GIS. The X-ray reflectivity measurements were done in the angular interval from 0.384 mrad to 16.93 mrad. At each scattering vector rocking curve measurements were performed in order to subtract background from the specularly reflected beam. The incident beam size was 0.017 mm × 1.0 mm ($V \times H$) defined using conventional slits. All X-ray measurements were performed at room temperature of 23 °C, to be well above of melting point of the hexadecane and to avoid its freezing on the filter paper, and at two surface pressures. One pressure is above ("High": 20 mN/m corresponding to 30 mN/m at A/W interface) and below ("Low": 5 mN/m corresponding to 15 mN/m at A/W interface) the collapse pressure ($\pi = 25$ mN/m at A/W interface) of PGLa monolayer [6].

Obtained XRR data were fitted using Parratt algorithm (Fig. 3) for calculation of the reflectivity curves [7]. Electron density profile was modeled analytically with a function

$$\rho(z) = \begin{cases} \rho_{ch2} - \Delta\rho_m \exp\left(-\frac{1}{2}(z/\sigma_m)^2\right) + \Delta\rho_h \exp\left(-\frac{1}{2}((z-Lz)/\sigma_h)^2\right), & 0 \le z \le Lz \\ \rho_{h2o} + (\Delta\rho_h + \rho_{ch2} - \rho_{h2o}) \exp\left(-\frac{1}{2}((z-Lz)/\sigma_h)^2\right), & z > Lz \\ \rho_{oil} - (\Delta\rho_m - \rho_{ch2} + \rho_{oil}) \exp\left(-\frac{1}{2}(z/\sigma_m)^2\right), & 0 < z \end{cases}.$$

This function describes the mean electron densities of the oil (ρ_{oil}), film (ρ_{ch2}) and water (ρ_{h2o}) as well as separated with distance Lz Gaussian like positive (at the film/water interface ($\Delta\rho_h$, σ_h)) and negative (at the oil/film interface ($\Delta\rho_m$, σ_m)) contributions to the electron density ρ_{ch2} (Fig. 4). Electron density profile obtained through the fit of XRR data was used for fitting the GIS data.

The GIS data were analyzed following to the approach described by S. Mora and co-workers [8]. Main contribution to the GIS signal measured on the gas/liquid or liquid/liquid interface comes from the scattering on the interfacial roughness that result from the thermally excited capillary waves. Presence

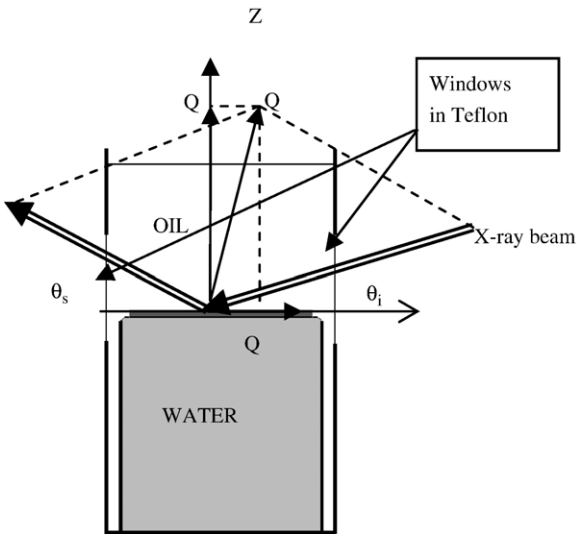

Fig. 1. Experimental geometry.