Experimental report

05/03/2020

Proposal: 8-02-856 **Council:** 10/2018

Title: Antimicrobial action and synergistic interaction of two cationic linear peptides on oriented lipid bilayers - focus on T = 260 - 320 K

Research area: Biology

This proposal is a continuation of 8-02-817

Main proposer: **Burkhard BECHINGER**

Experimental team: Judith PETERS
Arnaud MARQUETTE
Aline CISSE

Local contacts: Judith PETERS

Samples: POPE/POPG (3:1) + magainin 2 (2%)
POPE/POPG (3:1) lipid membranes only
POPE/POPG (3:1) + PGLa (2%)
POPE/POPG +magainin 2 (1%) +PGLa (1%)

Instrument	Requested days	Allocated days	From	To
IN13	5	7	08/07/2019	15/07/2019

Abstract:

We want to complement our previous measurements performed at ILL in June 2018 with data in the range of temperature T = 260 - 320 K. This will slightly extend the range of measurements but more importantly strongly increase the statistics on the data previously recorded. This will permit without any ambiguity the determination of the dynamic and thermodynamic parameters (Enthalpy and Entropy changes) in this region of particular interest where the peptides are biologically active and where we have performed solid-state NMR measurements. Four samples will be made of lipid bilayers (POPE/POPG 3:1), alone or supplemented with peptides (PGLa and/or magainin 2). Elastic incoherent neutron scattering (EINS) measurements will be performed on IN13 - fortunately before it's renewal - to ensure the same experimental conditions.

Antimicrobial action and synergistic interaction of two cationic linear peptides on oriented lipid bilayers – focus on the temperature range T = 260 - 320 K

Experiments 'TEST 3079' (10/01-17/01/2020), '8-02-856' (8/07-15/07/2019) and 'CRG 2604' du (15/07-16/07/2019) on the IN13 spectrometer.

Arnaud Marquette, Burkhard Bechinger, Aline Cisse and Judith Peters

To gain further insight into the antimicrobial activities of cationic linear peptides, we previously investigated the topology the two peptides PGLa and magainin 2 by solid-state NMR, in oriented phospholipid bilayers in the presence and absence of the other peptide and as a function of the membrane lipid composition [1]. Whereas magainin 2 always exhibits stable in-plane alignments, PGLa adopts several different membrane topologies with considerable variations in tilt angle. In equimolar mixtures of PGLa and magainin 2, the former is transmembrane in dimyristoyl-, but not in 1-palmitoyl-2-oleoyl- phospholipid bilayers, whereas magainin 2 remains associated parallel to the surface in all cases [1, 2]. Recent investigations suggest that the two peptides have a strong disordering effect on the fatty acyl chains of the lipids, and that peptide induced membrane disorder could be a major driving force for PGLa re-alignment. These results have important consequences for the mechanistic models explaining synergistic activities of the peptide mixtures. The ensemble of data suggests that the lipophobic effect, and to a lesser extent membrane curvature and the thinning of the dimyristoyl membranes, caused by magainin 2 tips the topological equilibrium of PGLa toward a membrane-inserted configuration.

This is within this context, where the mode of action of the two peptide systems on lipids remains at the best unclear, that we proposed to investigate by elastic incoherent neutron scattering how the dynamics/thermodynamics parameters describing the phospholipid interactions could be modulated by the presence of PGLa and/or magainin 2. We focused here on high temperatures where the peptides are biologically active and where we performed solid-state NMR measurements [4].

After facing problems of controlling the hydration level on some of our samples in summer 2019 (experiment '8-02-856') we finally succeeded to solve this issue during the later runs in 2020 (experiment 'TEST 3079'). The samples were made of oriented phospholipid membranes composed with a mixture of POPE/POPG (3:1) which mimic the bacterial membrane. Apart from pure lipid systems, membranes including 2 mol% of magainin, 2 mol% of PGLa, and a mixture of 1 mol% of magainin plus 1 mol% of PGLa were deposited on Si wafers. The experiments were performed on IN13 as function of temperature between 260 and 316 K and under two different angles with respect to the incoming beam (135 and 45°), e.g. in the in-plane and out-of-plane directions with respect to the membrane plane in order to extract two orthogonal dynamical contributions. The treatment of the data was done at ILL through a standard procedure using the LAMP program [5].

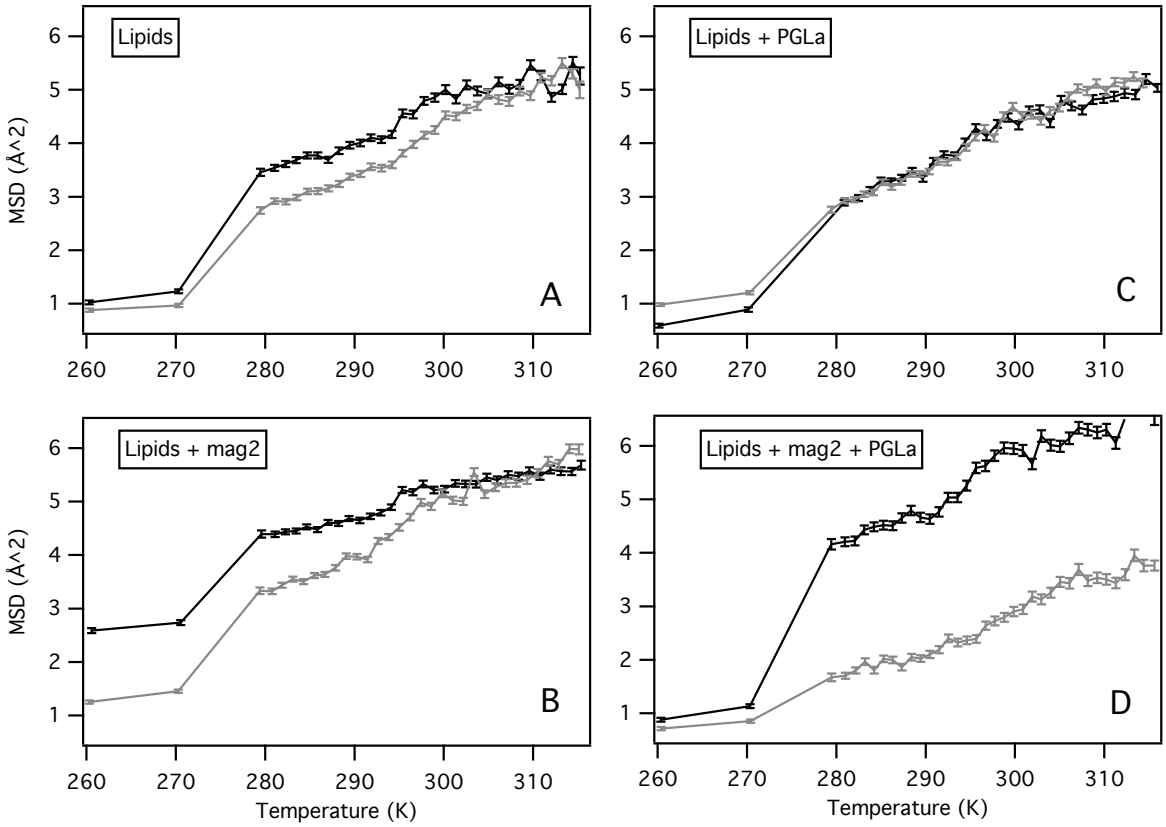

Figure 1. Total mean square displacements (MSD) for the lipid sample alone (A) and for the three different lipid-peptide mixtures (B, C and D) as function of the temperature. The black and grey curves correspond to motions out- and in-plane relative to the membrane surface, respectively.

The total mean square displacements (MSD) as a function of temperature are displayed on figure 1, for the four samples and for the in-plane and out-of-plane contributions. More data points were registered above 280 K where the phospholipids undergo 'gel-fluid' phase transitions and where the two peptides have synergistic effects on living systems. Important increases of the MSD between T = 270 and 280 K are due to 'solid-liquid' phase transition of water while at higher temperature near $T \approx 293$ K the 'gel-fluid' transition of the lipids can be clearly identified on most of the samples. A striking effect of the two peptides added in cocktail is visible by considering the important differences between the in-plane and out-of-plane curves (fig. 1D) *vs* the differences observed for the magainin 2 and PGLa peptide systems added separately to the lipid bilayers (fig. 1B and 1C, respectively). A fine analysis of the data is under process and will lead to the quantification of the thermodynamic parameters describing the lipid phase transition for the four different samples.

References

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[5] D. Richard, M. Ferrand, G. J. Kearley, G. J., J. Neutron Res. 1996, 4, 33-39.