Experimental report

26/07/2018

Proposal: 8-02-817 **Council:** 4/2018

Title: The antimicrobial action and the synergistic interaction of two cationic linear peptides on oriented lipid bilayers

Research area: Biology

This proposal is a new proposal

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Samples: DMPE/DMPG (3:1) lipid membranes
DMPE/DMPG (3:1) lipid membrane + 2 mol% magainin
DMPE/DMPG (3:1) lipid membrane + 2 mol% PGLa
DMPE/DMPG (3:1) lipid membrane + 2 mol% magainin + 2 mol% PGLa

Instrument	Requested days	Allocated days	From	To
IN13	9	8	07/06/2018	12/06/2018
			12/06/2018	15/06/2018

Abstract:

We propose to investigate the mechanism of antimicrobial action of two different cationic linear peptides, magainin 2 and PGLa, embedded in oriented lipid bilayers formed by DMPE/DMPG phospholipids. The peptides strongly influence each other in the membrane by communicating through the lipid matrix. Thus their antimicrobial and membrane activities are an order of magnitude enhanced when added in combination. In a recent study, we were able to show that a model proposed by Bicout and Zaccai (D. Bicout and G. Zaccai, Biophys. J. 80, 2001, 1115 - 1123) permitted to fit the mean square displacements extracted from elastic incoherent neutron scattering data and to obtain thermodynamic parameters such as enthalpy and entropy. We expect that the internal dynamics and thermodynamics of the lipid bilayer are strongly influenced by the presence of the peptides inside the membrane as recently presented by Barrett et al. (M.A. Barrett et al., Soft Matter 12, 2016, 1444 - 1451) and thus we would like to correlate the corresponding quantities with the functional mechanisms of the membrane-embedded peptides and their synergistic activity.

The antimicrobial action and the synergistic interaction of two cationic linear peptides on oriented lipid bilayers

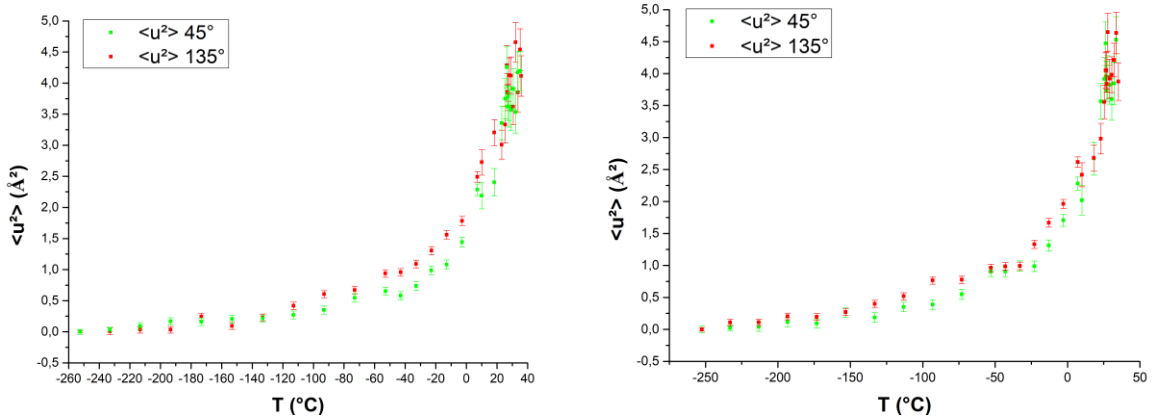
Experiment 8-02-817 on the instrument IN13: 06 – 15/06/2018

Experimental team: Burkhard Bechinger, Arnaud Marquette, Aline Cisse, Judith Peters

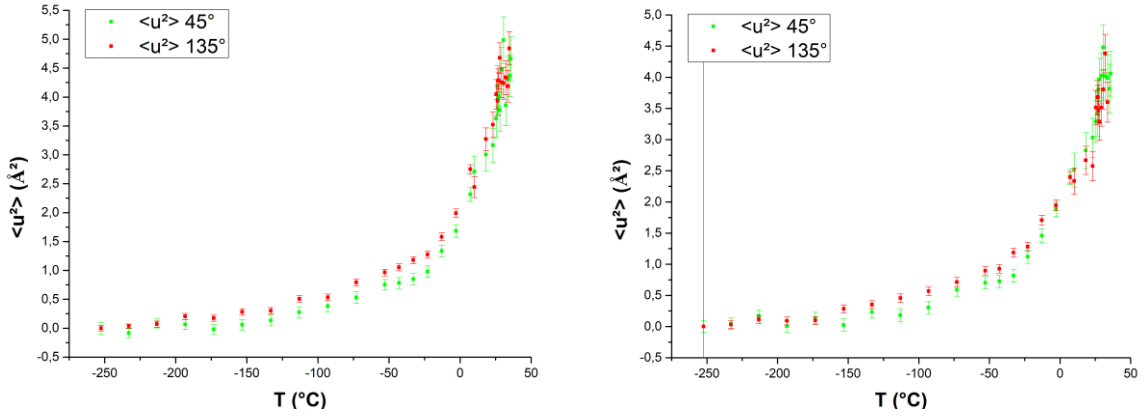
To gain further insight into the antimicrobial activities of cationic linear peptides, we previously investigated the topology the two peptides PGLa and magainin 2 by solid-state NMR, in oriented phospholipid bilayers in the presence and absence of the other peptide and as a function of the membrane lipid composition [1]. Whereas magainin 2 always exhibits stable in-plane alignments, PGLa adopts a number of 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.

The measurements were performed at ILL on the IN13 spectrometer as function of temperature in the range -250 – +40 °C. 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 made. The samples were measured at the angles of 135 and 45° with respect to the incoming beam, e.g. in the in-plane and out-of-plane directions with respect to the membrane plane (see figure 1), in order to extract two orthogonal dynamical contributions.



(a) POPE/POPG (b) POPE/POPG + PGLa



(c) POPE/POPG + Mag2 (d) POPE/POPG + PGLa + Mag2

Figure 1. Mean square displacements as function of temperature of the four different lipid-peptide mixtures. 45 and 135° refer to motions out- and in-plane of the bilayer, respectively.

We observe in Fig. 1 the typical increase of the mean square displacements (MSD) as a function of temperature. More data points were registered above 20 °C, where the phospholipids are undergoing phase transitions and where the two peptides exhibit synergistic effects on living systems. Unfortunately, this region of interest suffers of a low signal to noise ratio due to the limited resolution of the spectrometer. In general, the samples show more flexibility in the in-plane than in the out-of-plane direction.

Neutron scattering measurements were complemented by DSC investigations in order to help the interpretation of our data (see fig. 2). Clear differences in the heat capacities between the four different samples were observed.

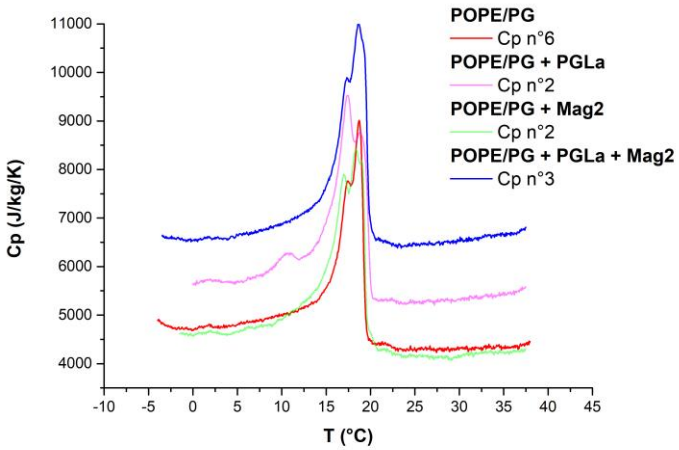


Figure 2. Heat capacity extracted from DSC measurements on SUV made of POPE/POPG, POPE/POPG/PGLa, POPE/POPG/magainin-2 and POPE/POPG/PGLa/magainin-2 in D20.

A detailed data analysis is at this time under progress. We aim to study in more in detail the high temperature region where the phase transition occurs. Thermodynamic parameters will be extracted from the MSD by applying the model proposed by Bicout and Zaccai [3].

References

[1] E.S. Salnikov and B. Bechinger, Biophys. J. 100, 2011, 1473 – 1480.
[2] E. Strandberg, E., et al., Biophys. J. 104, 2013, L09-L11.
[3] D. Bicout and G. Zaccai, Biophys. J. 80, 2001, 1115 – 1123.