Experimental report

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Proposal: 8-02-826 **Council:** 4/2018

Title: Impact of an antimicrobial peptidefrom Lactobacillus gasseri supernatant on the structure of lipid bilayer mimicking the bacterial inner me

Research area: Soft condensed matter

This proposal is a new proposal

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Samples: C39H76NO8P
EFVFVAHAVPVM peptide
C40H76O10PNa

Instrument	Requested days	Allocated days	From	To
D17	3	0		
FIGARO	3	3	16/10/2018	19/10/2018

Abstract:

Antimicrobial peptides (AMPs) are promising candidates as future therapeutics against the antibiotic resistance caused by pathogenic bacteria. Most known AMPs are small peptides, formed of 12 to 60 amino acids with molecular masses < 10 kDa. Recently, several AMPs have been identified in the marine environment, which act as the first line of defense against a broad spectrum of pathogens. A short peptide, composed of 12 amino acids (EFVFVAHAVPVM) with a molecular weight of 1333 Da, and presenting a strongly hydrophobic character, was recently isolated from Lactobacillus gasseri supernatant (named hereafter AMP12). This peptide has shown antimicrobial activity and may also bind the TLR4 receptor or lipopolysaccharides (LPS), which can result in blocking the activation of the inflammatory pathway. We are interested in the characterization of the interaction between AMP12 and lipid bilayers and NR can provide unique information on the action mechanism of AMP12. We propose to characterize lipid bilayers containing LPS from Escherichia Coli as mimics of the bacterial membrane and to investigate the location of AMP12 with respect to the bilayer.

Experimental Report – Proposal n. 8-02-826

Impact of an antimicrobial peptide from Lactobacillus gasseri supernatant on the structure of lipid bilayer mimicking the bacterial inner membrane.

1. Scientific Background

Antimicrobial peptides (AMPs) are promising candidates as future therapeutics against the antibiotic resistance caused by pathogenic bacteria. AMPs are an essential part of the innate immune response and their ubiquitous presence in nature attests their key role in building up the defense strategies of almost every organism. Most known AMPs are small peptides, formed of 12 to 60 amino acids with molecular masses < 10 kDa. Recently, several AMPs have been identified in the marine environment, which act as the first line of defense against a broad spectrum of pathogens [1]. A short peptide, composed of 12 amino acids (EFVFVAHAVPVM) with a molecular weight of 1333 Da, and presenting a strongly hydrophobic character, was recently isolated from Lactobacillus gasseri supernatant (named hereafter AMP12) [2]. This peptide has shown antimicrobial activity and may also bind the TLR4 receptor or lipopolysaccharides (LPS), which can result in blocking the activation of the inflammatory pathway [2]. Neutron Reflectometry (NR) measurements can provide unique information on the mechanism of action of AMP12. In particular we propose to characterize lipid bilayers containing LPS from *Escherichia Coli* as mimics of the bacterial membrane and to investigate the location of AMP12 with respect to the bilayer (i.e. the peptide exclusively interacts with the lipid headgroup or it can aslo penetrate the bilayer).

2. NR experiments

We tried to characterize lipid bilayers composed by 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (POPE), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-(1'-*rac*-glycerol) (POPG) and LPS and how their structure is affected by injection of AMP12 solution at a concentration of 0.1 mg/mL. NR measurements will be performed on FIGARO at 25 °C on lipid bilayers prepared by vesicles fusion deposition. Measurements will be performed at 25 °C in the q-range (0.01-0.3 $Å^{-1}$) with the buffer (phosphate buffer pH=7.4, 150mM NaCl) being prepared with D_2O and H_2O according to the desired contrast.

3. NR results

We favourably characterized POPE/POPG bilayers at different lipid weight ratios while we had some problems to form LPS-containing lipid bilayers due to difficulties in the vesicle rupture and subsequent deposition on the silicon support. On the other hand, we studied the effect of AMP12 addition on POPE/POPG which did not show a significant destabilizing effect, supporting the idea that a LPS-specific interaction could be decisive for the peptide interaction.

However, we did not collect enough data to write an article. Unfortunately, for national balance issues, there was no more beamtime in the next time to continue the study and acquire new data necessary for the paper.

References

[1] Jenssen, H. et al. *Clin. Microbiol. Rev.* **19** (2006) 491; [2] Zanfardino, A. et al. *Benef. Microbes*. **8** (2017) 133; [3] Vitiello, G. et al. *Soft Matter* **11** (2015) 3003.