Experimental report

30/08/2022

Proposal: 9-13-1014 **Council:** 4/2021

Title: Structural organization of HoLA-containing bilayers: the effect of the hopanoid moiety

Research area: Soft condensed matter

This proposal is a new proposal

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Samples: 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-(1'-rac-glycerol) (POPG)
BTAi1 strain LPS
1-palmitoyl-2-oleoyl-glycero-3-phosphocholine (POPC)

Instrument	Requested days	Allocated days	From	To
D17	4	2	01/09/2021	03/09/2021
FIGARO	4	0		

Abstract:

Hopanoids are membrane lipids with similar structure and function to the steroids commonly found in the eukaryotic cell membrane, i.e. cholesterol. The peculiar characteristic of Bradyrhizobium Hopanoid Lipid A (HoLA) molecule is that the hopanoid is covalently bound to the lipid A instead of being dispersed in the membrane as for most of the Gram-Negative bacteria. Vesicles were successfully prepared with POPC, POPG, HoLA or its mutant (BTAi1Dshc) without hopanoid moiety as mimics of the bacterial outer membrane. The structure and dynamic of the vesicles was preliminarily investigated, which confirmed the presence of HoLA in the vesicles. Here we propose to complete this study by performing Neutron Reflectometry (NR) measurements on asymmetric supported POPC/POPG bilayer containing HoLA and/or its mutant BTAi1Dshc alternatively in the outer bilayer leaflet. Asymmetric lipid bilayers could be prepared by Langmuir-Blodgett method, in collaboration with PSCM laboratories. NR can provide unique information on the structure of HoLA lipid bilayer.

Experimental Report – Proposal n. 9-13-1014

Structural organization of HoLA-containing bilayers: the effect of the hopanoid moiety.

1. Scientific Background

Gram-Negative Bacteria are characterized by two different membranes (the inner and outer membrane) which primary act as protective barriers for the cells. The inner membrane is mainly composed by phospholipids, among which 1-palmitoyl-2-oleoyl-glycero-3-phosphocholine (POPC) and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-(1'-*rac*-glycerol) (POPG) are the most abundant, and cardiolipin (CL), while the outer membrane is also characterized by the presence of lipopolysaccharides (LPSs) in the outer leaflet. LPSs are composed by a hydrophilic portion, which includes the distal polysaccharide and the proximal oligosaccharide. The LPS hydrophilic portion is bound to a glycolipid known as lipid A. Some of these molecules, such as the lipid A extracted from *Bradyrhizobium* BTAi1 strain (HoLA), is characterized by the presence of a hopanoid unit bound to one of the acyl chains [1]. Hopanoids are membrane lipids with similar structure and function to the steroids commonly found in the eukaryotic cell membrane, i.e. cholesterol. The definition of the structural properties of lipid bilayers composed by phospholipid/cardiolipin/LPS is crucial to understand the biological mechanism as well as to define new antimicrobial peptides (AMPs) as future therapeutic candidates against the antibiotic resistance caused by pathogenic bacteria. Recently, 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].

2. NR experiments

We characterized lipid bilayers composed by a mixture of PC and PG phospholipids, in the absence and presence of cardiolipin (CL) and LPS, also focusing on how their structure is affected by injection of AMP12 solution. NR measurements on lipid bilayers prepared by vesicles fusion deposition were performed on D17 at 25 °C in the q-range (0.01-0.3 $Å^{-1}$). Four different D_2O/H_2O mixtures were used as contrast matching solvents. Then, a phosphate buffer pH=7.4, 150mM NaCl was also used to create biomimetic conditions for membranes both before and after the peptide addition.

3. NR analysis

We favourably characterized PC/PG bilayers at different lipid weight ratios, obtaining a great coverage of the silicon supports. A similar situation was obtained in the case of PC/PG/CL bilayers, while we had some problems to form LPS-containing lipid bilayers due to difficulties in the vesicle rupture and subsequent complete deposition on the silicon support (Fig. 1). Then, we studied the effect of AMP12 addition on PC/PG and PC/PG/CL bilayers, observing a significant destabilizing effect induced by a partial insertion of the peptide within the bio-membrane.

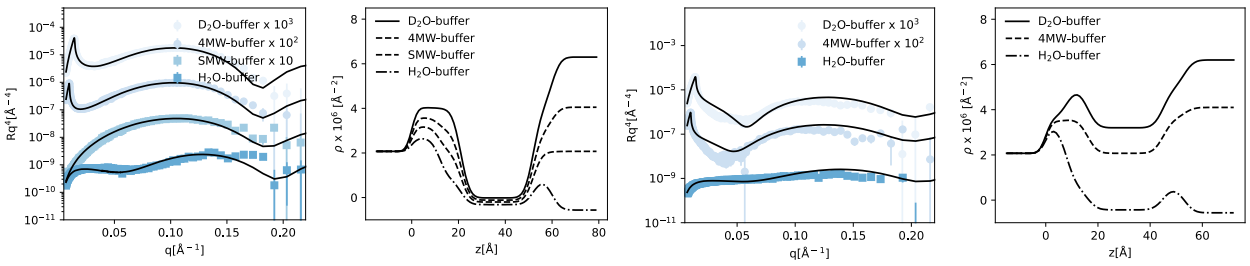

Fig. 1 – Experimental NR curves for PC/PG and PC/PG/CL bilayers in different contrast matching solvents before the AMP12 addition.

References

[1] Silipo, A., *Nature Communications* (2014); [2] Zanfardino, A. et al. *Benef. Microbes*. **8** (2017) 133; [3] Vitiello, G. et al. *Soft Matter* **11** (2015) 3003.