Experimental report

06/09/2022

Proposal: 8-03-1036 **Council:** 10/2020

Title: A piece in the puzzle of deciphering the antimicrobial properties of Histatin 5: The role of arginine-phosphoryl group interaction

Research area: Chemistry

This proposal is a new proposal

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Samples: Histatin 5
Variants of Histatin 5

Instrument	Requested days	Allocated days	From	To
D17	5	2	03/07/2021	05/07/2021
FIGARO	5	0		

Abstract:

IDPs are characterized by a lack of stable tertiary structure under physiological conditions in vitro and it has been shown that ~30% of all proteins in eukaryotic organisms belong to this group, and that IDPs are involved in many central biological processes and diseases. This discovery challenges the traditional protein structure paradigm, which states that a specific, well-defined structure is required for the correct function of a protein. Biochemical evidence has since shown that IDPs are functional, and that the lack of folded structure is related to function. The hypothesis is that IDPs adopts a structure upon adsorption to surfaces that gives rise to a function. Hence, understanding how interactions with surfaces or membranes induces secondary structure of IDPs will shed light on how IDPs perform their functions - that is a fundamental biology question. Here we aim to underpin the role of Arginine-phosphoryl group interactions by studying Histatin 5-membrane interactions, where the lipid contains a phosphorylated headgroup, and compare with our own designed variants of Histatin 5, where the number of Arginine as well as their location in the primary sequence of are altered.

Report for 8-03-1036

The experiment 8-03-1036 was performed on the reflectometer D17 from 03/07/2021 to 05/07/2021. The aim of the experiment was to elucidate the role of arginine groups present in the primary sequence of Histatin 5 variants. This experiment is inline with previous ones conducted on the role of charges in the antimicrobial activity of this salivary cationic peptide. This project is a part of a project "Structure of membrane proteins under solution conditions" supported by a grant for Neutron Research awarded by NordForsk, https://www.nordforsk.org/en to Prof. Marie Skepö.

During the experiment we performed the characterisation, in 2 contrasts, of the silica surfaces as well as of the POPC:POPS (9:1) target bilayers. Subsequently, the structure of these bilayers exposed to three different peptides was investigated by collecting NR data in 3 contrasts per sample as well as "before rinsing", i.e. in the final state of the incubation. The peptides used were:

- LL37 and LS37, two variants of Histatin 5 in which the number of arginine groups was changed.
- M17, a random sequence of Histatin 5 that maintains the number of arginine groups constant.

There is a clear effect of all peptides on the reflectivity curves as shown in Figures 1-3.

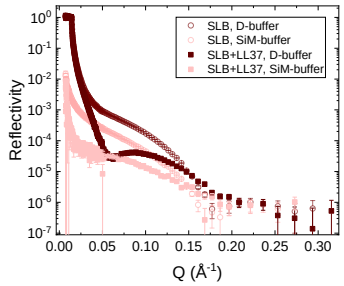

Figure 1. NR data collected for a POPC:POPS (9:1) SLB on silica before (open symbols) and after (filled symbols) the incubation of **LL37**. Data measured in H-buffer show changes, but they are not shown for improving the visibility of the other datasets.

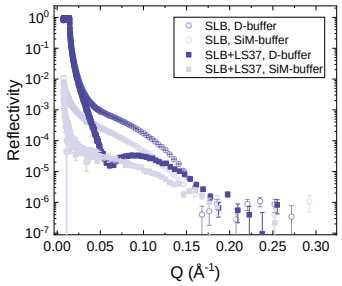

Figure 2. NR data collected for a POPC:POPS (9:1) SLB on silica before (open symbols) and after (filled symbols) the incubation of **LS37**. Data measured in H-buffer show changes, but they are not shown for improving the visibility of the other datasets.

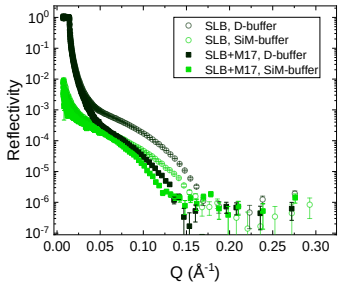

Figure 3. NR data collected for a POPC:POPS (9:1) SLB on silica before (open symbols) and after (filled symbols) the incubation of **M17**. Data measured in H-buffer show changes, but they are not shown for improving the visibility of the other datasets.

The changes in the curves induced by the LS37 and LL37 peptides are similar, suggesting a similar structural modification of the bilayer and a similar mechanism of action. On the contrary, changes induced by the M17 variant are completely different and they indicate that the overall structure of the SLB is maintained.

A full understanding of the structural changes induced need a proper modelling approach. At present, data analysis is still in progress.