Experimental report

19/07/2024

Proposal: **8-02-1007**

Council: 4/2023

Title: A piece in the puzzle of deciphering the antimicrobial properties of Histatin 5: The effect of chain length

Research area: Chemistry

This proposal is a new proposal

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Samples: Histatin 5

Instrument	Requested days	Allocated days	From	To
D17	3	0		
FIGARO	4	2	30/08/2023	01/09/2023

Abstract:

A lack of stable tertiary structure characterizes IDPs under physiological conditions in vitro. 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 shown that IDPs are functional and that the lack of folded structure is related to function. The hypothesis is that IDPs adopt a structure upon adsorption to surfaces that give rise to a function. Hence, understanding how interactions with surfaces or membranes induce the secondary structure of IDPs will shed light on how IDPs perform their functions - that is a fundamental biology question. Here we aim to study the chain length that affects the peptide-membrane interactions by adding a tandem repeat of the Histatin 5 sequence and a peptide half of the chain (11-24). In both peptides, the percentage of histidines in the sequence is kept constant and the same as for Hst5 (29%).

Experimental report for 8-02-1007 and 8-03-1063 on FIGARO

Users: Marie Skepö (PI), Amanda Eriksson Skog, Yuri Gerelli
Local contact: Nicolo Paraccini
28-8-2023 to 1-9-2023

During the experiments 8-02-1007 and 8-03-1063 performed on FIGARO we measured the interaction of a salivary protein, Histatin 5 (Hst5), with model lipid membranes. In previous experiments (confirmed by data collected during some tests on the SuperAdam instrument in September 2019), we have observed that Hst5 penetrates the bilayer and cumulates in the proximity of the solid substrate (silica in this case) (see report 9-13-656). The aim of the current experiment was to investigate the effect of the sequence length of Hst5, as well as the order of the amino acids within the sequence for the ability of the peptide to penetrate the bilayer and form a cushion. Five variants of Hst5 in total were designed, one shorter, one longer, two with random sequence and one peptide consisting only of seven histidine residues. It was found that the length of the peptide was not of great importance at low salt concentration, however, at a high salt concentration Hst5 does not interact with the bilayer while both variants do. These results are published in *Molecular Pharmaceuticals* (doi: 10.1021/acs.molpharmaceut.4c00450).

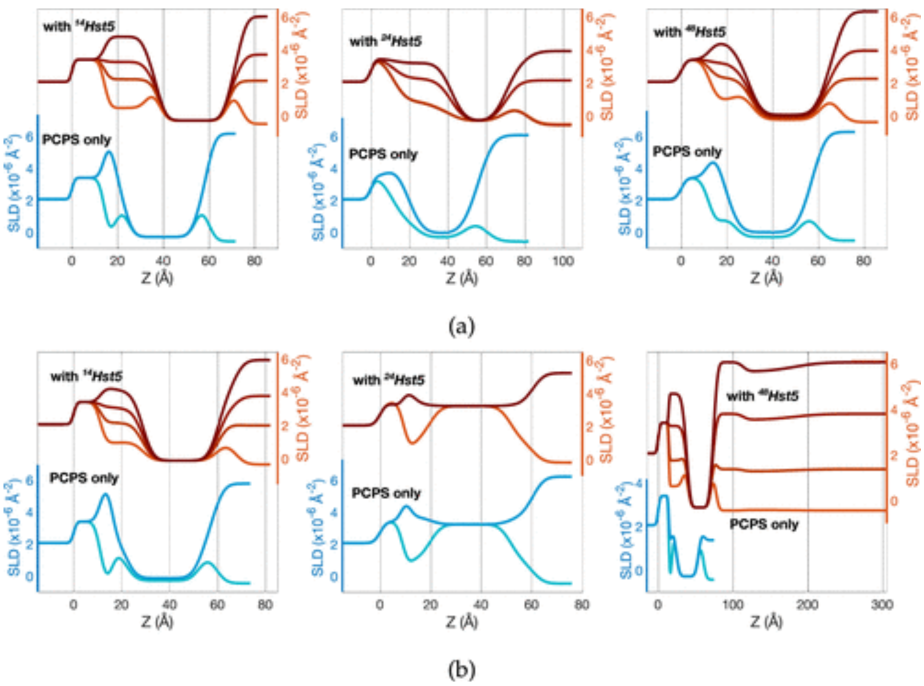

Figure 1. SLD curves for the length variants

It was also concluded that the order of the amino acids was of importance for the interaction where one of the variants with a similar distribution of all amino acids as Hst5, named $Hst5^{rand1}$ behaves similarly forming an equally thick cushion as Hst5. The other variant, $Hst5^{rand2}$ gives rise to a cushion of half the size compared to the other two peptides. These results correspond to 10mM NaCl conditions. These data will be published during autumn 2024.

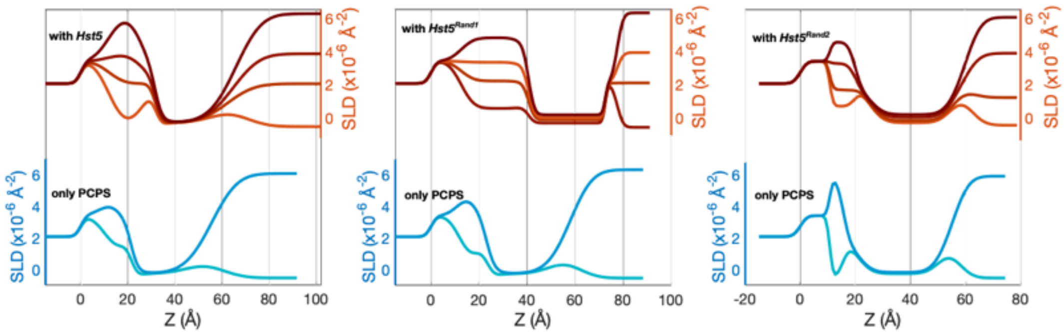

Figure 2. SLD curves for the random variants.

All the data reported here, thus both chain length effect and effect of the primary sequence, is part of Amanda Eriksson Skog's PhD-thesis, that will be defended in December 2024.