Experimental report

20/11/2017

Proposal: 9-13-656 **Council:** 4/2016

Title: To understand the antimicrobial activity of the salivary protein Histatin 5.

Research area: Chemistry

This proposal is a resubmission of 8-02-744

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Samples: Histatin 5

Instrument	Requested days	Allocated days	From	To
FIGARO	3	0		
D17	3	2	28/11/2016	30/11/2016

Abstract:

The goal of this project is to understand the antimicrobial activity of the intrinsically disordered salivary protein Histatin 5, which acts in defence against oral candidiasis caused by Candida albicans. From the literature it is still unknown how the peptide transports itself through the membrane, but it is suggested to not be an ordinary pore-opening transport, instead it has been attributed to its metal binding abilities. Studies have established that various transitional metals, such as Zn, Ni, Cu, and Fe, are intrinsically present in the saliva. In this project, we aim to investigate not only how Histatin 5 interacts with the SiO2 surface but also how Histatin 5 interacts with membranes, to be able to detect structural changes of the membrane, as well as determine how Histatin 5 inserts into the membrane. For this purpose, a combined experimental and theoretical approach will be used, where quartz-crystal microbalance with dissipation, ellipsometry, and neutron reflectivity will be utilized together with computer simulations. Thereby we will obtain a molecular understanding of system that is both of medical and academic relevance.

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Experimental Report for Beamtime 9-13-656

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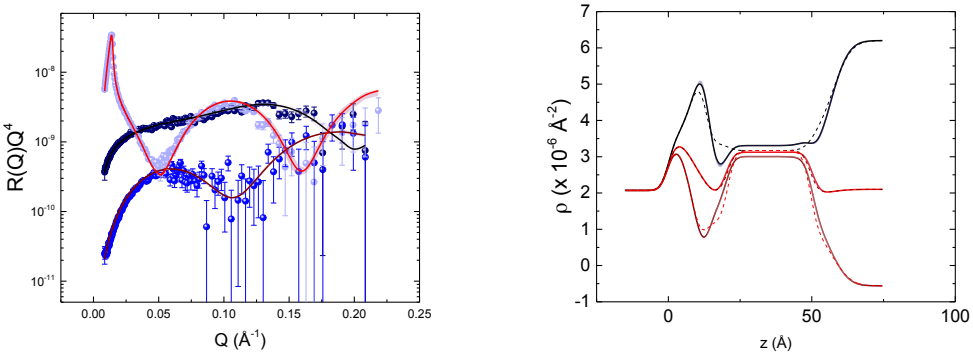
Intrinsically disordered proteins (IDPs)[1] are characterized by lack of stable tertiary structure under physiological conditions in vitro. More recently, it has been shown that ~30% of all proteins in eukaryotic organisms belong to this group and that IDPs are involved in a large number of central biological processes and diseases. Histatin 5, which is a cationic short saliva peptide, is an example of such an IDP. This peptide acts in defence against oral candidiasis caused by *Candida albicans,* and the antimicrobial activity has been ascribed to the high content of basic amino acids. Histatin 5 participate in the formation of a protective layer on smooth tooth surfaces,[2] and thereby prevent microbial colonization and stabilize mineral-solute interactions. Histatin 5 metal binding abilities have been suggested to be important for candidacidal mechanism, and studies have established that various transitional metals, such as Zn, Ni, Cu, and Fe, are intrinsically present in the saliva. The aim of this project is to understand the antimicrobial properties of Histatin 5. From the literature it is still unknown how the peptide transports itself through the membrane, but it is suggested to not be an ordinary pore-opening transport, instead it has been attributed to its metal binding abilities.[1]

Results from exp. 9-13-656

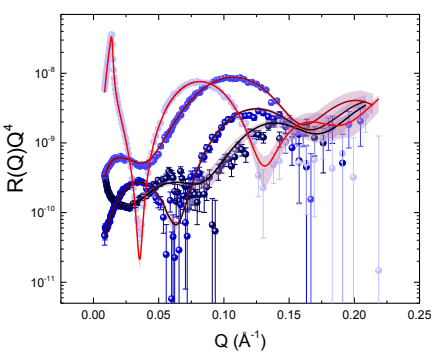
PCPS, effect of Hist5 in 20mM Tris and 80mM NaCl

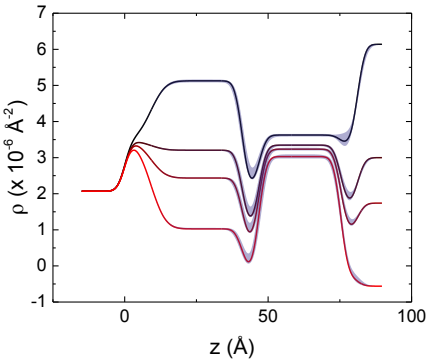
First we investigated the interaction of the Hist5 peptide with a partially charged bilayer composed by POPC and POPS (PCPS) in buffer at 80mM NaCl. The analyses of the data indicate that the peptide promotes water penetration in the bilayer (from the fit parameters approximately 4.5% by volume) interpreted as caused by a probable formation of holes in the bilayer. In this contrast scheme (phospholipids were partially deuterated) it is not possible to quantify the amount of inserted protein, if any.



Plots in RQ4 scale to highlight the high-Q region. SLD profiles are the result of data modeling. For reference the original profiles for the bilayer BEFORE addition of HIST5 are shown as dashed lines.

PCPS, effect of Hist5 in 20mM Tris and 10mM NaCl

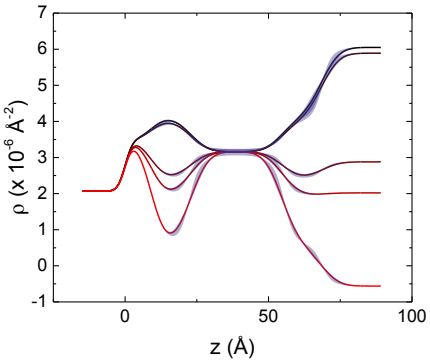




The same system was investigated at a lower ionic strength (10mM NaCl). In this case major changes in reflectivity, compared to the pristine bilayer, are visible. To model the data, after trying several models, we had to make the hypothesis of the presence of a large gap (~3 nm) between the bilayer and the SiOx surface. This gap was mostly occupied by water and by material having an SLD ~ 3 x 10^{-6} $Å^{-2}$. The reflectivity curve collected in a water having SLD=3 has indeed very poor statistic indicating that the overall contrast of the layer, in that particular case, was diminished a lot. This supports the model obtained by the fit.

100% d31POPC, effect of Hist5 in 20mM Tris and 10mM NaCl

For comparison purposes the measurements were repeated in presence of a pure zwitterionic bilayer systems (100% POPC). In this case it was not possible to observe any type of interaction; the structure of the bilayer was unperturbed and this confirms the electrostatic origin of the interaction.



Conclusions

The measurements performed on D17 during the experiment 9-13-656 indicate that at low ionic strength an excluded volume is created between the surface and the lipid bilayer that is deposited on the solid substrate. Because of the low protein concentration and the contrast scheme used it is not possible to evaluate if proteins are located within the bilayer structure or if they inserted in the water gap as indicated by be schematic figure below. Anyhow, this result was completely unexpected and further NR measurements and complimentary studies will be necessary to understand the underlying mechanisms and physics.

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References

1. S. Puri and M. Edgerton, Eukaryotic Cell **13** (8), 958-964 (2014).
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4. K. Hyltegren, T. Nylander, M. Lund and M. Skepö, JCIS **467**, 280-290 (2016).
5. K. Hyltegren and M. Skepö, JCIS **494**, 266-273 (2017).