Experimental Report

Proposal: **8-02-681** **Council:** 10/2012

Title: Elucidating the interaction mechanism of novel antimicrobial dendrimer on reconstituted bacterial cell membranes vs. mamma-lian cell mimics

This proposal is a new proposal

Researh Area: Soft condensed matter

Main proposer: **WACKLIN Hanna**

Experimental Team: LIND Tania
WACKLIN Hanna
DARRE Leonardo

Local Contact: BARKER Robert

Samples: hE. coli lipids, purified at home laboratory
Single crystal silicon
Dendrimer BAIN: branched analogue of indolicidin
dE. coli lipids, obtained via biodeuteration lab ILL and purified
dPOPC and hPOPC, obtained from Avanti

Instrument	Req. Days	All. Days	From	To
D17	3	3	30/07/2013	02/08/2013

Abstract:

Antibiotic multiresistance is an ever-increasing problem, which has to be addressed with novel approaches, if this already serious public health issue is to be contained. The prospect of exploiting dendritic structures as scaffolds for antimicrobial peptide (AMP) based agents with increased activity have led to synthesis of novel AMP dendrimers. We have already extensively studied a dendrimer of this type and these studies revealed a significant interaction with fluid membranes. They showed that the dendrimers were able to penetrate the lipid bilayer and form lipid-dendrimer micellar-like complexes, which resulted in solubilisation of the mammalian model membrane. The dendrimer (BAIN) selected for the present studies has shown high activity towards bacteria of different genera including even resistant laboratory strains. Furthermore and of paramount importance, this compound is non-toxic to human cells. We wish to study the interaction between dendrimer BAIN and supported lipid membranes of mammalian and bacterial lipids in order to clarify this selectivity. The outcome of this project will be of major relevance in the on-going optimization of the design of effective antibiotics.

Elucidating the interaction mechanism of novel antimicrobial dendrimer on reconstituted bacterial cell membranes vs. Mammalian cell mimics

Tania K. Lind[1], Anna Åkesson[1], Zofia Lipkowska[2], Hanna Wacklin[3], Marité Cárdenas[1]
[1]Nanoscience center and department of chemistry, Copenhagen University, Denmark.
[2]Institute of organic chemistry, Polish academy of Science, Poland.
[3]European Spallation Source ESS AB.

Naturally occurring antimicrobial peptides (AMPs), which are part of the innate immune response in many organisms, are likely to become one of the answers to antimicrobial resistance. They are believed to act on membranes in a non-specific manner via electrostatic and hydrophobic interactions and thus decrease the risk of antibiotic resistance.[1, 2] The prospect of exploiting the dendritic structures as scaffolds for AMP-based antibiotic agents with increased activity have led to the synthesis of novel antimicrobial peptide dendrimers. We have extensively studied a dendrimer of similar structure (a branched analogue of lysine; BALY) and found that its interaction with lipid membranes was dependent on the fluidity of the membrane.[3] In the present study, we investigated the interaction mechanism of a novel dendrimer. This compound has 5 tryptophan residues and is a branched analogue of indolicidin (BAIN). Indolicidin is a natural, linear 12-peptide with 5 tryptophans in the sequence. This dendrimer is of special interest since it has better properties than BALY in terms of high antimicrobial activity while being non-toxic (below 5 % up to 200 micromols).

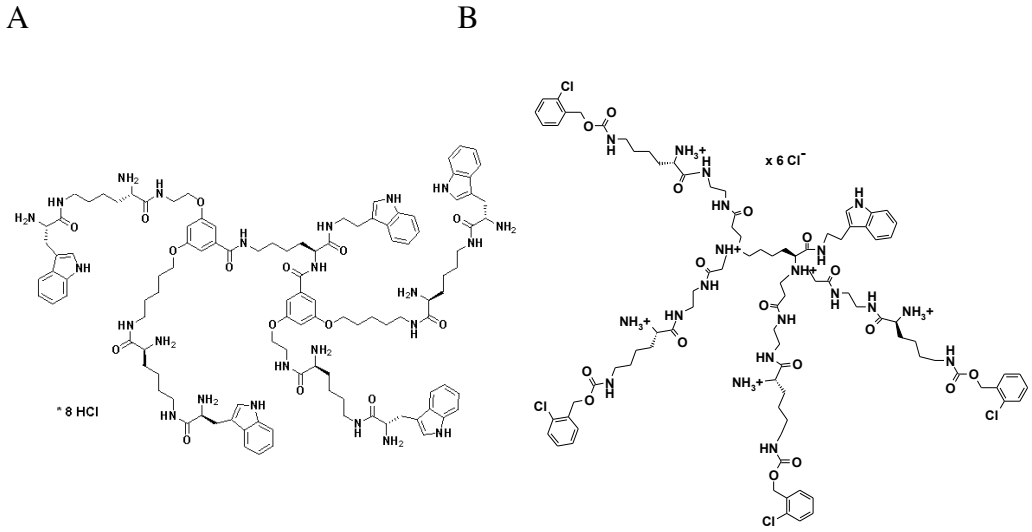

Figure 1. A: the novel dendrimer, which is a branched analogue of indolicidin (BAIN) . B: dendrimer BALY (branched analogue of lysine), which has been extensively studied by our group.

By the use of methanol/chloroform extraction we achieved a pure deuterated *E. coli* total lipid mixture (Biomass provided by the deuteration lab, ILL). The extracted lipids were stored in chloroform at -18°C until use. POPC, DPPC and POPG lipids were purchased from Avanti Lipids Inc. Under a stream of flowing nitrogen an appropriate volume of the lipids was dried onto the walls of a glass vial. The lipid films

were then resuspended in PBS (10 mM PBS, 100 mM NaCl, pH 7.4) to a concentration of 500 μg/ml. Small unilamellar vesicles (SUVs) were prepared by sonication of the suspension until clarity at 50 °C for *E. coli* and DPPC samples and at 25 °C for POPC and POPG containing samples. The vesicles were diluted to 200μg/ml in PBS. In the case of dEcoli and the POPG:POPC 1:3 mixture the vesicles were diluted in a Ca^{2+} containing PBS buffer (10 mM PBS, 100 mM NaCl and 2 mM $CaCl_2$).

After characterization of the bare silica surface in D_2O and H_2O, supported lipid bilayers were deposited at 25 °C (POPC and POPC:POPG 1:3) or at 50 °C (DPPC and dEcoli) onto silica surfaces *in situ* by vesicle fusion at the D17 beamline. After rinsing the flow cell with excess PBS, the bilayers were characterized in H_2O, D_2O and a 1:1 mixture of these.

After successful bilayer formation was confirmed, antimicrobial peptide dendrimers in a concentration of 6µM were introduced to the membranes in order to follow the interaction behaviour.

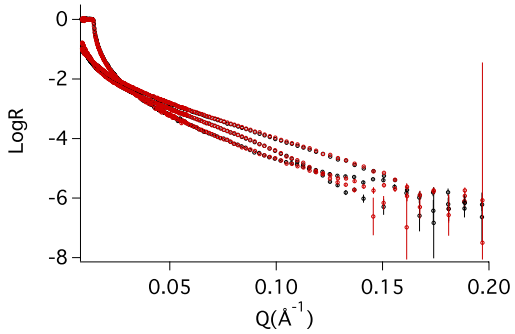

Figure 2: NR profiles measured in H_2O, 50v/v% D_2O and pure D_2O for 6µM BAIN interacting with dEcoli. The black data points correspond to the membrane prior to interaction and the red points that fall on top of them were measured after addition of BAIN.

The membrane/dendrimer interaction was measured in three different contrast environments: H_2O, D_2O and 1:1. In contrast to BALY, NR data on 6 µM BAIN showed no significant changes in reflectivity for interaction with POPC, DPPC, POPG:POPC 1:3 or dEcoli. An example of BAIN interaction with dEcoli is shown in figure 1. It was speculated that solubility issues could prevent dendrimer adsorption and integration because of phase separation or aggregation in solution, which would lead to a lower apparent monomer concentration. NR measurements were thus carried out with a tenfold decrease in dendrimer concentration (0.6 µM). No changes in reflectivity were seen at this concentration either. It is evident that BAIN interacts in a different way than BALY, probably by increasing the permeability of the membrane without major layer rearrangements.

1. Malmsten, M. Antimicrobial Peptides. *Ups. J. Med. Sci.* 2014, 119, 199-204.
2. Stromstedt, A. A.; Ringstad, L.; Schmidtchen, A.; Malmsten, M. Interaction between Amphiphilic Peptides and Phospholipid Membranes. *Curr. Opin. Colloid Interface Sci* 2010, 15, 467-478.
3. Lind, T. K.; Darré, L.; Domene, C.; Urbanczyk-Lipkowska, Z.; Cárdenas, M.; Wacklin, H. P. Antimicrobial Peptide Dendrimer Interacts with Phosphocholine Membranes in a Fluidity Dependent Manner: A Neutron Reflection Study Combined with Molecular Dynamics Simulations. *Biochimica et Biophysica Acta (BBA) - Biomembranes*.