Exploration of the structure-activity relation of natural, self-assembling cyclic lipodepsipeptides

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Introduction
Cyclic lipodepsipeptides (CLPs) are non-ribosomal peptides of bacterial origin, which exhibit antagonistic activity for several bacterial and fungal species. The CLPs produced by Pseudomonas species can be classified in several groups, whereby we are specifically interested in the viscosinamide group. Previously, we extensively investigated the structure and conformation of pseudosedmin A using X-ray analysis and elaborate NMR relaxation measurements (1-3). Currently, the conformation of its epimer viscosinamide is being investigated. The analysis of the conformation of this molecule is an essential first step for understanding the self-assembling behavior of this molecule.

Structure calculation
Structure calculations are performed for both viscosinamide (L-Leu5) and pseudosedmin A (D-Leu5) using NMR-derived distance restraints and homo- and heteronuclear coupling constants. Simulated annealing molecular dynamics provides an ensemble of structures, from which the lowest energy structure are selected. The backbone structures of both peptides are found to be very similar. This similarity is also found in the side chain orientations, except for Leu5, where the D/L-stereo inversion occurs. The stereo inversion does not have an impact on the amphipaticity of the molecules. Further insight into the supramolecular structure is needed to clarify the increased self-assembly of viscosinamide compared to pseudosedmin A.

Membrane interaction studies
To determine the mode of action of these CLP’s two different fluorescence spectroscopic experimental setups are performed. In this way, it can be determined whether CLP’s either permeable model membranes or cause membrane fusion of the model vesicles.

Permeability setup: Fluorescent probe Quencher

Vesicle fusion setup:

The results (left) clearly show that viscosinamide causes permeability once a certain critical concentration is reached. No vesicle fusion is observed at comparable concentrations.

Structure of Viscosinamide: Chain orientation of Leu5 in viscosinamide (green) and residues in red and hydrophobic residues in green. Right: Side view of the viscosinamide (right, green).

Self-assembly

Viscosinamide in: Acetonitrile Chloroform

The self-assembly of the CLPs in an apolar solvent such as chloroform is already apparent in the ¹H-NMR spectra due to the extensive and uniform line broadening which is not present in polar solvents (e.g. acetonitrile). Moreover, the self-assembly is concentration dependent as can be seen from the diffusion coefficient (measured by NMR) is plotted as a function of the concentration. Moreover, at similar concentrations, the supramolecular structure of viscosinamide appear to be larger than that of pseudosedmin.

Within our group, a methodology was developed to gain insight into the supramolecular structure using heteronuclear relaxation measurements (Poster 42 by D. Sinnaeve). In short, the relaxation rates of an NMR nucleus can be linked to the rotational diffusion properties of the molecule, assuming no contribution from internal motion or exchange processes. For viscosinamide and pseudosedmin A, a significant variation is observed in both R₂ and R₁ values over the different ¹H nuclei. When correlating R₁ and R₂ values with the ¹³C-¹H binding vector of the molecules, structural information on the shape (anisotropy), organisation and size of the supramolecular structure can be determined. In the figure below, R₁/R₂ relaxation rates of the individual residues are plotted as a function of theta, the angle of the C-H binding vector in respect to the rotation diffusion tensor. It is clear that the experimental relaxation rates (points) agree well with the theoretically calculated relaxation rates (line) for a cylindrical supramolecular structure as depicted above.

Conclusion
- Important measurements performed to elucidate the properties and working mechanism of cyclic lipodepsipeptides.
- The impact of the D/L stereo inversion of Leu5 between viscosinamide and pseudosedmin A was found to have no impact on the backbone conformation;
- Self-assembly of viscosinamide appears stronger than that of pseudosedmin A;
- CLP’s can permeable model membrane, but do not cause vesicle fusion.

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