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

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Introduction

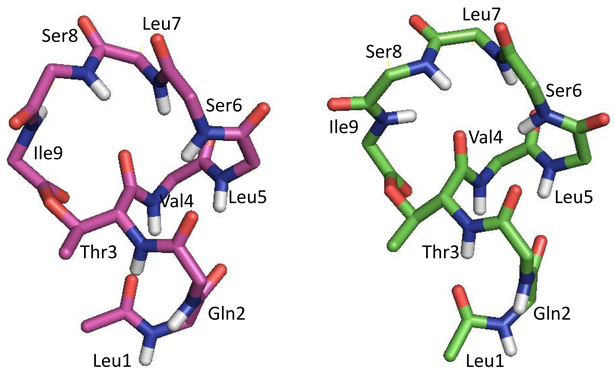
Cyclic lipodepsipeptides (CLPs) are non-ribosomal peptides of bacterial origin, which exhibit antagonistic activity for several bacterial and fungal species. The CLPs produced by *Pseudomonas* species can be classified in several groups, whereby we are specifically interested in the viscosin group.

Previously, we extensively investigated the structure and conformation of **pseudodesmin 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.

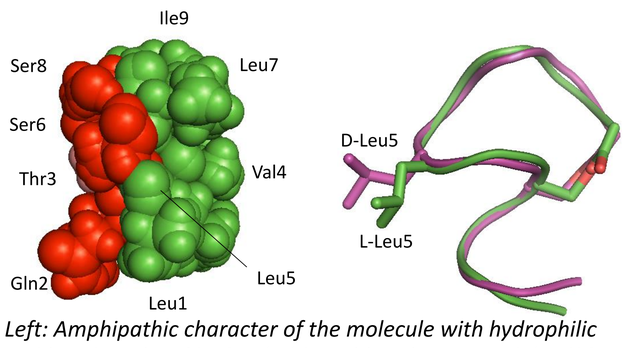
Viscosin D-subgroep										
		1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
Pseudodesmin A	***β-HDA***	***L-Leu***	***D-Gln***	***D-aThr***	***D-Val***	***D-Leu***	***D-Ser***	***L-Leu***	***D-Ser***	***L-Ile***
Pseudodesmin B	*β -HDA*	*L-Leu*	*D-Gln*	*D-aThr*	*D-Val*	*D-Leu*	*D-Ser*	*L-Leu*	*D-Ser*	*L-Val*
WLIP	*β -HDA*	*L-Leu*	*D-Glu*	*D-aThr*	*D-Val*	*D-Leu*	*D-Ser*	*L-Leu*	*D-Ser*	*L-Ile*
Viscosin L-Subgroep										
Viscosin	*β -HDA*	*L-Leu*	*D-Glu*	*D-aThr*	*D-Val*	*L-Leu*	*D-Ser*	*L-Leu*	*D-Ser*	*L-Ile*
Viscosinamide	***β -HDA***	***L-Leu***	***D-Gln***	***D-aThr***	***D-Val***	***L-Leu***	***D-Ser***	***L-Leu***	***D-Ser***	***L-Ile***

Amino acid sequences of CLPs in the viscosin group. This group can be divided into two subgroup according the stereochemistry of the fifth residue. Underlined residues are part of the cyclised part of the molecules.

Structure calculation



Left: Backbone conformation of pseudodesmin A (left, purple) and viscosinamide (right, green).



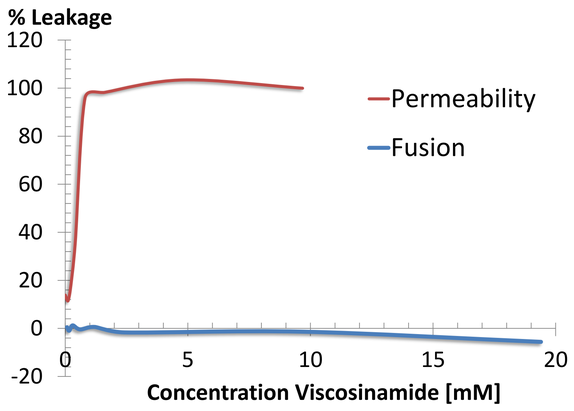
Left: Amphipathic character of the molecule with hydrophilic residues in red and hydrophobic residues in green. Right: Side chain orientation of Leu5 in viscosinamide (green) and pseudodesmin (purple)

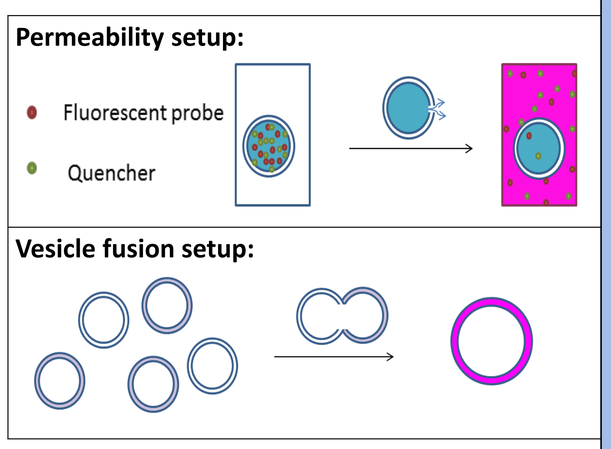
Structure calculations are performed for both viscosinamide (L-Leu5) and pseudodesmin A (D-Leu5) using NMR-derived distance restraints and homo- and heteronuclear coupling constants. Simulated annealing molecular dynamics provides an ensemble of structures, from which the lowest energy structure are 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 pseudodesmin 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 permeablise model membranes or cause membrane fusion of the model vesicles.

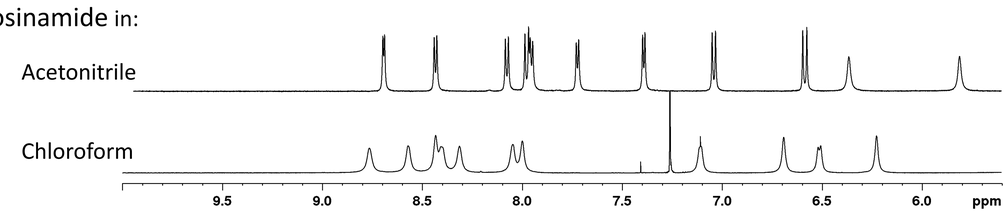




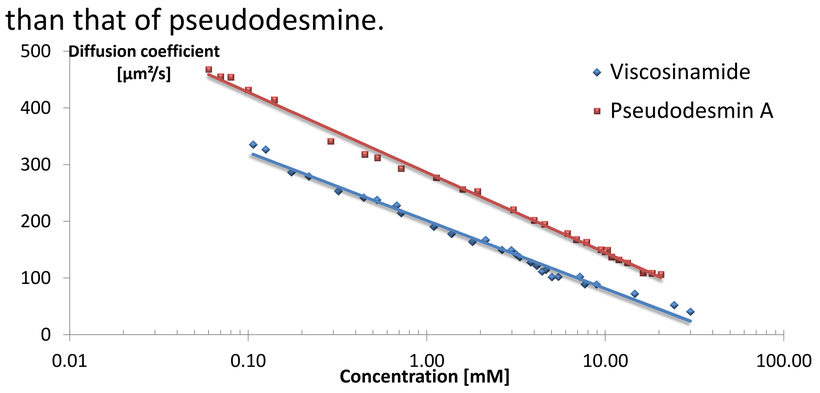
The results (left) clearly show that viscosinamide causes permeability once a certain critical concentration is reached. No vesicle fusion is observed at comparable concentrations.

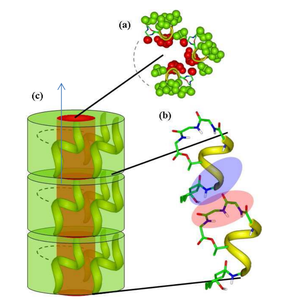
Self-assembly

Viscosinamide in:



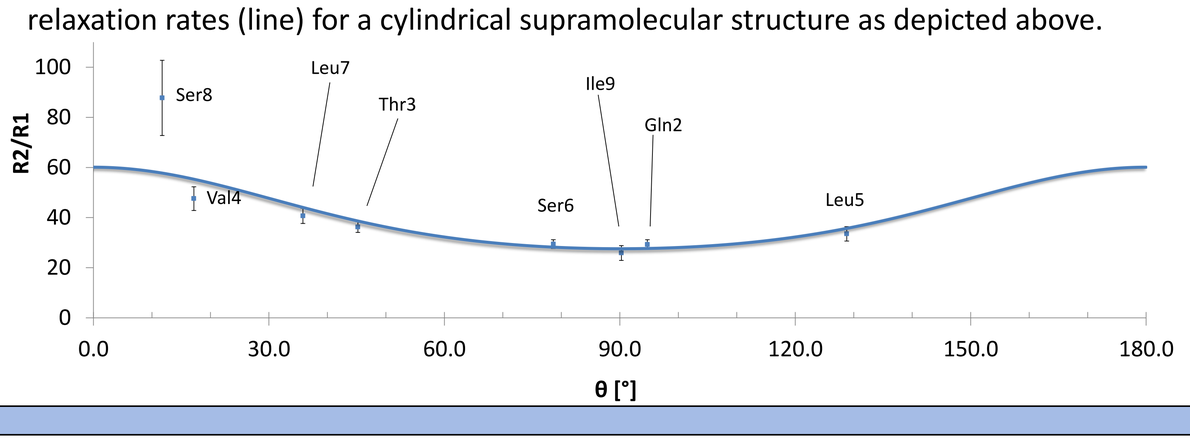
The self-assembly of the CLPs in an apolar solvent such as chloroform is already apparent in the ^{1}H-NMR spectra due to the **extensive and uniform line broadening** which is not present in polar solvents (e.g. acetonitrile). Moreover, the self-assembly is **concentration dependent** as can be seen 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 pseudodesmine.





Hypothetical supramolecular model

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 pseudodesmin A, a significant variation is observed in both R_1 and R_2 values over the different $^{13}C^{\alpha}$ nuclei. When correlating R_1 and R_2 values with the $^{13}C^{\alpha}$-^{1}H 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, R_2/R_1 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 pseudodesmine A was found to have a no impact on the backbone conformation;
- Self-assembly of viscosinamide appears stronger than that of pseudodesmin A;
- CLP's can permeabilise model membrane, but do not cause vesicle fusion.

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