Membrane Interactions of Cyclic Lipodepsipeptides

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Abstract

Cyclic lipodepsipeptides (CLPs) are a diverse group of secondary metabolites produced by various bacteria with important biological functions, but with yet unresolved molecular mechanisms. Our previous efforts have gone towards characterizing with NMR the conformation and self-assembling properties of a collection of CLPs known as the viscosin group. [1-4] CLPs increasingly attract attention because of their antifungal and antibiotic properties through membrane permeabilization. A full understanding of their membrane interactions is essential to elucidate the exact working mechanism of CLPs.

Comprehensive structural information in a membrane environment can be obtained by liquid-state NMR of model membrane systems, such as micelles and isotropic lipid bicelles. Like micelles, isotropic bicelles display favourable NMR relaxation properties, while possessing structural characteristics of lipid bilayers. [5] Specifically, the orientation and insertion depth of CLPs in a membrane environment is investigated using diffusion NMR and paramagnetic relaxation enhancement. The latter is achieved by introducing paramagnetic probes at various locations. By introducing a water-soluble paramagnetic complex to a bicelle sample, NMR signals from nuclei closer to the aqueous phase can be identified. Covalently linking paramagnetic radicals to the lipid molecules at different positions reveal the orientation and insertion depth of the peptides in the bilayer.

The NMR results are further supplemented with other experimental techniques, including fluorescence to study membrane permeability and fusion, circular dichroism and infrared spectroscopy. We have also performed all-atom molecular dynamics (MD) simulations of CLPs within lipid membranes, which can be confronted with the experimental NMR results.

[1] Sinnaeve, D., P. M. Hendrickx, et al., Chemistry - A European Journal 15(46): 12653-12662, 2009
[2] Sinnaeve, D., M.-A. Delsuc, et al., Chemical Science 3: 1284-1292, 2012
[3] De Vleeschouwer, M., D. Sinnaeve, et al., Chemistry - A European Journal 20(25): 7766-7775, 2014
[4] Geudens N., De Vleeschouwer M., et al., ChemBioChem 15: 2736-2746, 2014
[5] Durr, U. H., M. Gildenberg, et al., Chem Rev 112(11): 6054-6074: 2012