Structural investigations of bacterial lipopeptides: challenges and (some) solutions

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Pseudomonas are ubiquitous bacteria and outstanding producers of bioactive secondary metabolites in support their eclectic lifestyle (e.g., iron scavenging, swarming motility, biofilm formation, pathogenicity, cooperation or antagonism)[1]. Of these metabolites, cyclic lipopeptides – CLIPs in short – have enjoyed the attention of numerous researchers because of their antimicrobial activity profile and anti-proliferative properties, which holds some potential for biomedical applications[2]. Their biosynthesis through non-ribosomal peptide synthetases creates a large structural diversity, introducing opportunities to learn how Nature uses the same molecular blueprint to generate a swiss-army like diversity of effects.[2]

As always, understanding CLiP structure and linking it to biological activity is considered essential to uncover the molecular mode of action and ultimately design analogues with improved potency while mitigating undesirable properties. Although NMR based approaches to determine the structure and conformation of peptides is well-established and considered rather routine, we found the NMR road to be marred by potholes in the case of CLiPs, requiring non-standard and partly novel approaches. First, the incorporation of non-proteinogenic amino acids with a majority of residues displaying D-configuration through the (currently) unpredictable action of epimerization domains in the non-ribosomal assembly line, magnifies the chemical structure elucidation challenge [3]. This is addressed using an NMR based spectral fingerprinting approach, which can also be used for dereplication approaches [4]. Second, it is generally accepted that CLiPs act through perturbation and/or permeation of the cellular membrane [2]. Thus, the conformation needs to be investigated under membrane mimicking circumstances rather than merely aqueous solution. The determination of the conformation of various CLiPs in such conditions as well as the investigation of location and orientation of the CLiP across the water/lipid interface using NMR and modelling, and insight derived therefrom will be discussed [5]. In particular, the added value generated by the possibility to biosynthetically introduce ^{13}C and ^{15}N isotope labelling using the Pseudomonas own NRPS will be highlighted. Their insertion allows the application of NMR methodologies allowing to extract conformations sensitive scalar couplings while directly identifying and monitor hydrogen bonds under a variety of conditions. Using these, conformational changes upon insertion in DPC or SDS micelles can be investigated. A final challenge, relating to understanding the molecular basis for the generation of well-defined supramolecular structures by certain CLiPs under low polarity solvent conditions may be touched upon, time permitting.

References:

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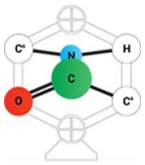

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