The state-of-the-art on CLP structure

Cyclic lipodepsipeptides (CLPs) are secondary metabolites typically produced by Pseudomonas and Bacillus bacterial species via non ribosomal pathways [1]. These biomolecules are constituted of a fatty acid moiety linked to the N-terminus of a peptide chain which is cyclized by an ester (or depsipeptide) bond formation between its C-terminus and a hydroxyl group capped side chain of a Ser or Thr residue. Due to the nature of the biosynthesis, the familiar prevalence of L-amino acids is flawed by the significant presence of residues maintaining D-configuration. Additional peculiarity is the alternation of amino acids with polar and apolar side chain.

The tertiary structure of CLPs is yet poorly understood, however, some common features are already obvious from this respect. Their peptide backbone is composed of a α-helix proceeding into a loop which enables the depsipeptide bond connection. Moreover, the perfect separation of polar and apolar side chains established by the helix is maintained in the loop, which’s conformation seems adapted to preserve the amphipathic character [2].

Structure – function relationship not well understood!

Though it would be crucial en route to the development of novel pharmaceuticals...

Aim: to gain more detailed structural information ➔ Direct H-bond detection

Methods: bacterial growth in minimal salt medium

For direct (N-H–O=C)-type H-bond detection solution state NMR spectroscopy was used. The experiment in question (modified HNCO [6]) involves 1H, 13C and 15N nuclei. Unfortunately, the natural abundance of 13C and 15N is very low (1.1% and 0.4%, respectively). For the sake of sensitivity, the investigated peptide had to be enriched in 13C and 15N isotopes (a.k.a. isotopelabelling). This required to modify our routinely applied biosynthetic protocol, i.e. the bacteria had to be grown in minimal salt medium instead of the α-proteus routinely applied King’s B (KB).

➢ Tested bacterial strain: viscosinamide producer Pseudomonas sp. DRS5

• KB medium: salts + proteose peptone (N-, C-source) + glycerol (C-source)
• M9 medium: salts + NH4Cl (N-source) + glucose (C-source)

M9 not only worked, but provided more CLP and restricted the evolution of other metabolites compared to KB! Interpretation: bacterial swarming... [1]

Results: the H-bond network of viscosinamide

The isolated and purified 13C/15N-labelled viscosinamide was dissolved in Acn-d3 for NMR investigations. The HNCO experiment indicated the presence of three (N-H–O=C)-type H-bonds.

<table>
<thead>
<tr>
<th>Experimental</th>
<th>1H NMR of Acn-d3</th>
<th>15N NMR of Acn-d3</th>
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<tbody>
<tr>
<td>1H: Val4(N-H)-HDA (O=C)</td>
<td>I</td>
<td>15N: Leu5(N-H)-HDA (O=C)</td>
</tr>
<tr>
<td>1H: Ser8(N-H)-Thr9(O=C)</td>
<td>K</td>
<td>15N: Ser8(N-H)-Thr9(O=C)</td>
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</tbody>
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For peptide 'structure elucidation' in solution state, it is common to collect 1H–1H distance restraints by NOESY-NMR experiment which guides a series of simulated annealings [7] to find realistic conformations. Thanks to the restraints imposed on (NH) and αH positions this method leads to valid backbone conformation, but the C–O bond orientations can be doubtful (see below). We complemented this method with time-dependent MD simulation [8] as refinement step, and a static conformation was assessed by a self-tailored cluster analysis [9] protocol. The improved in silico result was in perfect agreement with the experimental observations.

In silico: simulated annealing + MD/cluster analysis

Conclusion and future prospects

We managed to give a precise picture about the H-bond pattern of a cyclic lipodepsipeptide using advanced NMR and MD methods. This work is pioneering in that context, since earlier neither the production and 13C/15N-labeling using minimal salt medium, nor direct detection of H-bonds had been performed for CLPs. In the future both intra- and intermolecular H-bonds will be identified in a similar fashion for CLPs to provoke coneggregates and self-assemblies. To further exploit the advantages of 13C/15N-labeling NMR-wise, isoipeptide-5H–5N NOESY is planned to be used for the structure calculation of CLP self-assemblies. That would allow the first ever investigation of such supramolecular construction at atomic details.