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Cyclic lipoundecapeptide amphisin from *Pseudomonas* sp. strain DSS73

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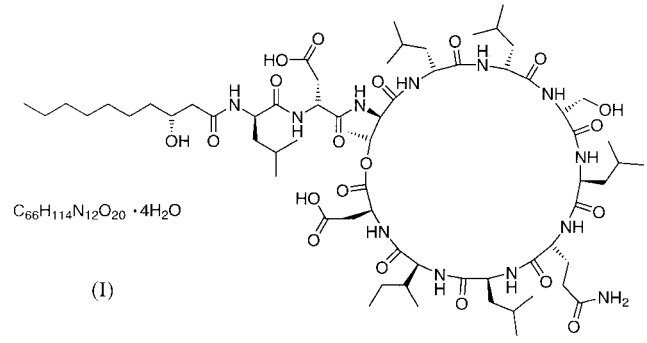
The crystal structure of the lipoundecapeptide amphisin, presented here as the tetrahydrate, $C_{66}H_{114}N_{12}O_{20}\cdot 4H_2O$, originating from non-ribosomal biosynthesis by *Pseudomonas* sp. strain DSS73, has been solved to a resolution of 0.65 Å. The primary structure of amphisin is β-hydroxydecanoyl-D-Leu-D-Asp-D-*allo*-Thr-D-Leu-D-Leu-D-Ser-L-Leu-D-Gln-L-Leu-L-Ile-L-Asp (Leu is leucine, Asp is aspartic acid, Thr is threonine, Ser is serine, Gln is glutamine and Ile is isoleucine). The peptide is a lactone, linking Thr4 O_γ to the C-terminal. The stereochemistry of the β-hydroxy acid is *R*. The peptide is a close analogue of the cyclic lipopeptides tensin and pholipeptin produced by *Pseudomonas fluorescens*. The structure of amphisin is mainly helical (3_{10}-helix), with the cyclic peptide wrapping around a hydrogen-bonded water molecule. This lipopeptide is amphiphilic and has biosurfactant and antifungal properties.

Comment

A rich variety of bioactive extracellular low-molecular-weight compounds are produced by bacteria. They may be classified as siderophores, antibiotics or toxins according to their biological activities. From a biosynthetic viewpoint, some of these are peptides produced non-ribosomally by large multifunctional peptide synthethases (Marahiel *et al.*, 1997). The component amino acids and lipids are activated in the form of adenylate, acylphosphorylate or coenzyme A derivatives before enzymatic condensation (Stachelhaus *et al.*, 1998). This non-ribosomal synthetic route utilizing L-, D- and modified amino acids is able to produce a broad diversity of specialized peptides (Konz & Marahiel, 1999). Genetic engineering of the peptide synthethases has been successfully applied to produce modified peptides (Schneider *et al.*, 1998).

Bacillus subtilis has traditionally been a source of bioactive peptides, especially the cyclic lipopeptide surfactin (Arima *et al.*, 1968), with many isoforms, fengycin (Vanittanakom *et al.*, 1986) and the members of the iturin family: iturin, mycosubtilin and bacillomycin (Peypoux *et al.*, 1980). The plipstatins, which are very similar to fengycin, have been isolated from *Bacillus cereus* and characterized (Umezawa *et al.*, 1986). All of these compounds are amphiphilic membrane-active biosurfactants with specific antimicrobial activities, and fengycin and the plipstatins inhibit phospholipase A2 (Umezawa *et al.*, 1986). *Pseudomonas flourescens* also produces a number of cyclic lipopeptides with biosurfactant and antifungal properties, *e.g.* viscosinamide (Nielsen *et al.*, 1999) and tensin (Nielsen *et al.*, 2000). *Pseudomonas fluorescens* was the source of the phospholipase C inhibitor pholipeptin (Ui *et al.*, 1997), and another *Pseudomonas* sp. yielded the antimycobacterial massetolides (Gerard *et al.*, 1997). The present study is part of our research program to discover and describe novel cyclic lipopeptides from various soil-associated *Pseudomonas* strains with a particular emphasis on their biosurfactant and antifungal properties.

The few crystal structures of cyclic lipopeptides comprise tensin (Henriksen *et al.*, 2000) and the white-line-inducing principle (WLIP) from *Pseudomonas reactans* (Han *et al.*, 1992). In this paper, we present the structure of amphisin tetrahydrate, (I), a cyclic lipopeptide with 11 amino acids, a β-hydroxydecanoyl N-terminal and a lactone formation between the side chain of Thr4 and the C-terminal. Close analogy exists between amphisin and the structurally related tensin, which differs only by incorporating Glu12 in place of Asp12.



The structure of amphisin is shown in Fig. 1. The absolute configuration was substantiated by chiral gas chromatographic analysis of the constituents in the hydrolyzed peptide. Asp3 has a disordered side chain and minor disorder was observed around residues Leu10 and Leu8 (not included in the model), but unlike the structure of tensin, the lipid shows no sign of disorder. The peptide incorporates a helical motif (3_{10}-helix from Leu2 to Leu8). The cyclic part of the peptide forms a 'cobra-head'-like backbone around a water molecule. The coordination of the water is almost planar, accepting one and donating two peptide hydrogen bonds. Three additional water molecules are found in the crystal structure.

The molecule has both a hydrophobic and a hydrophilic side, in agreement with its function as a biosurfactant. With the exception of Leu6, the hydrophobic side chains in the ring

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are aligned in a parallel formation. The alternating stereoconfigurations of the amino acids are required for this organization. The two acidic residues, charged at high pH, are situated on the hydrophilic side of the molecule, quite far apart and facing in opposite directions. The peptide backbones of amphisin and tensin are similar and not affected by the amino acid substitution. Interestingly, WLIP shares some of the 'cobra-head' features of amphisin and tensin even though WLIP contains two amino acids less than the latter. It is common, however, that these cyclic lipopeptides can adopt a number of distinct and different conformations. The conformation of WLIP in the crystal structure is thus significantly different from the one deduced by NMR in dimethyl sulfoxide solution (Mortishire-Smith *et al.*, 1991). Another example is surfactin, where NMR studies revealed two very different conformations (Bonmatin *et al.*, 1994). The conformation of surfactin has furthermore been shown to be highly dependent on the nature of the solvent (Itokawa *et al.*, 1994). The amphiphilic property combined with conformational flexibility could be of crucial importance in the interaction with biological membranes or receptor sites.

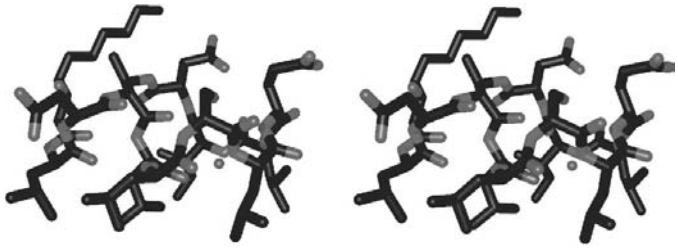

Figure 1
Stereo-*VMD* (Humphrey *et al.*, 1996) drawing of amphisin. Only the most occupied sites of Asp3 are shown. The lipid is attached to the N-terminal, while the C-terminal forms a lactone with the Thr4 side chain. The molecule is viewed from the side, showing the amphiphilic arrangement of side chains and the central hydrogen-bonded water molecule.

Experimental

Amphisin (10 mg) was isolated from the EtOAc extract of 50 Petri dishes with potato dextrose agar (PDA) medium incubated with *Pseudomonas* strain DSS73 at 298 K for 4 d, and purified by various types of Sephadex LH-20, Si-60 and RP-18-based chromatography. Final crystallization occurred overnight from a solution of CH_3CN/H_2O (70:30) with 0.5% trifluoroacetic acid at 278 K.

Crystal data

$C_{66}H_{114}N_{12}O_{20}\cdot 4H_2O$
M_r = 1467.76
Orthorhombic, $P2_12_12_1$
a = 12.329 (10) Å
b = 20.245 (10) Å
c = 31.691 (10) Å
V = 7910 (8) Å^3
Z = 4
D_x = 1.232 Mg m^{-3}
Mo $K\alpha$ radiation
Cell parameters from 7276 reflections
θ = 0.0–27.4°
μ = 0.09 mm^{-1}
T = 293 (2) K
Prism, colourless
0.3 × 0.2 × 0.1 mm

Data collection

Nonius KappaCCD diffractometer
ω scans
71 388 measured reflections
9869 independent reflections
8726 reflections with $I > 2\sigma(I)$
R_{int} = 0.037
θ_{max} = 27.4°
$h = -15 \rightarrow 15$
$k = -26 \rightarrow 26$
$l = -41 \rightarrow 41$

Refinement

Refinement on F^2
$R[F^2 > 2\sigma(F^2)]$ = 0.044
$wR(F^2)$ = 0.157
S = 0.86
9869 reflections
941 parameters
H-atom parameters constrained
$w = 1/[\sigma^2(F_o^2) + (0.1550P)^2 + 1.0565P]$ where $P = (F_o^2 + 2F_c^2)/3$
$(\Delta/\sigma)_{max}$ = 0.053
$\Delta\rho_{max}$ = 0.56 e Å^{-3}
$\Delta\rho_{min}$ = −0.52 e Å^{-3}

All ordered and major conformation (occupancy ∼0.7) non-H atoms were refined with anisotropic displacement parameters. All H atoms were located in difference Fourier maps and treated as riding on the appropiate heavy atoms (C—H = 1.05 Å). The Asp3 residue was found to have more than one conformation.

Data collection: *XPRESS* (MacScience, 1989); cell refinement: *DENZO* (Otwinowski & Minor, 1997); data reduction: *DENZO*; program(s) used to solve structure: *SHELXS*97 (Sheldrick, 1990); program(s) used to refine structure: *SHELXL*97 (Sheldrick, 1997); molecular graphics: *VMD* (Humphrey *et al.*, 1996).

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Supplementary data for this paper are available from the IUCr electronic archives (Reference: DA1195). Services for accessing these data are described at the back of the journal.

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