Acta Crystallographica Section C
Crystal Structure Communications
ISSN 0108-2701

Cyclic lipoundecapeptide tensin from *Pseudomonas fluorescens* strain 96.578

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Received 24 August 1999
Accepted 19 October 1999

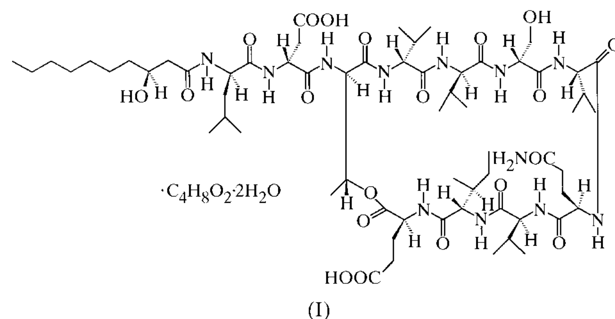
The crystal structure of the non-ribosomal lipoundecapeptide tensin from *Pseudomonas fluorescens* has been solved as an ethyl acetate/bis-water solvate (tensin ethyl acetate dihydrate, $C_{67}H_{115}N_{12}O_{20}\cdot C_4H_8O_2\cdot 2H_2O$) to a resolution of 0.8 Å. The primary structure of tensin is β-hydroxydecanoyl-D-Leu-D-Asp-D-*allo*-Thr-D-Leu-D-Leu-D-Ser-L-Leu-D-Gln-L-Leu-L-Ile-L-Glu. The peptide is a lactone linking the Thr3 O_γ atom to the C-terminal C atom. The stereochemistry of the β-hydroxy acid has been shown to be *S*. The peptide shows structural resemblance to the non-ribosomal cyclic lipopeptide fengycin from *Bacillus subtilis*. The structure of tensin is essentially helical (3_{10}-helix), with the cyclic peptide wrapping around a hydrogen-bonded water molecule. The lipopeptide is amphipathic in good agreement with its function as a biosurfactant.

Comment

Several bacterial genera characteristically produce a wide variety of extracellular low molecular weight compounds classified as siderophores, antibiotics and toxins. Some of these are peptides produced non-ribosomally by large multifunctional peptide synthethases (Marahiel, 1992). 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). It is now accepted that this non-ribosomal synthetic route utilizing L-, D- and modified amino acids is an alternative way of producing specialized peptides.

Bacillus subtilis has been a source of these bioactive peptides, especially the cyclic lipopeptides 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 and characterized from *Bacillus cereus* (Umezawa *et al.*, 1986). All of these compounds represent amphiphilic membrane active biosurfactants with specific antimicrobial activities, and fengycin and the plipstatins inhibit phospholipase A2 (Umezawa *et al.*, 1986).

Pseudomonas fluorescens strains produce a number of cyclic lipopeptides with biosurfactant and antifungal properties, *e.g.* viscosinamide (Nielsen, Christophersen *et al.*, 1999) and tensin (Nielsen, Thrane *et al.*, 1999). In this paper we present the structure of tensin, (I), which is related to surfactin, a cyclic lipopeptide with seven amino acids in the lactone ring and with the hydroxy-O atom of the β-hydroxy fatty acid involved in the lactone formation. The three-dimensional structure of surfactin has been established by NMR spectroscopy (Bonmatin *et al.*, 1994). Tensin is even more closely related to the cyclic lipopeptide fengycin with ten amino acids and to the white-line-inducing principle from *Pseudomonas reactans* with eight amino acids and known crystal structure (Han *et al.*, 1992). Many studies have been undertaken addressing the interaction of biosurfactants and membranes or lipid layer model systems. Surfactin has been used for many of these investigations (Sheppard *et al.*, 1991; Grau *et al.*, 1999). The general model of interaction between membranes and surfactin (and anionic biosurfactants in general) is pore formation by clustered peptides. In surfactin two closely situated carboxylic groups on the hydrophilic side also bind metal ions at high pH. The degree of surface penetration is still a subject of debate, but it appears as if the biosurfactants do not penetrate deep into the membranes. Direct microscopic observations of the antagonistic activity against the plant pathogenic micro fungus *Rhizoctonia solani* suggested that tensin may affect the fungi differently as compared with viscosinamide studied previously (Nielsen, Thrane *et al.*, 1999), which has Ca^{2+} channel forming properties (Thrane *et al.*, 1999).



(I)

The structure of tensin is shown in Fig. 1. The absolute configuration fixed by the side-chain stereochemistry of isoleucine and threonine was supported by a Marfey analysis (Marfey, 1984). The Flack refinement was inconclusive. The peptide shows a disordered lipid and two disordered side-chains. This degree of disorder is comparable to what is seen in high-resolution protein structures. The peptide is essentially

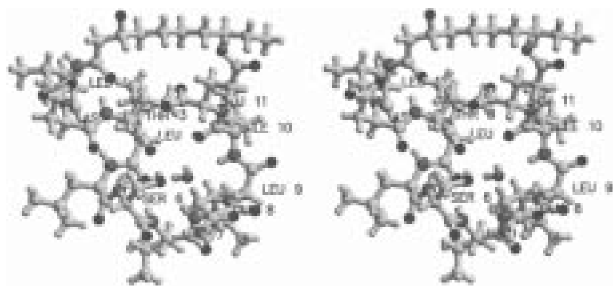


Figure 1
Stereo *VMD* (Humphrey *et al.*, 1996) drawing of the title compound. Only the most occupied sites of the lipid, Leu5 and Glu11 are drawn. The lipid is in the N-terminal end, while the C-terminal end forms a lactone with Thr3. The molecule is viewed from its polar side with the hydrogen-bonded water molecule included.

helical (3_{10}-helix from Leu1 to Leu7), with the rest of the cyclic peptide wrapping around a water molecule. The coordination of the water is close to tetrahedral and it donates two and accepts two peptide hydrogen bonds. Another water molecule and an ethyl acetate moiety are also found in the crystal structure. It is obvious that the molecule has two very different sides: a hydrophobic and a hydrophilic, in agreement with its function as a biosurfactant. The two residues that are negatively charged at high pH are situated on the hydrophilic side of the molecule, but are quite distant from each other. The closest distance between atoms of the two residues is 9.364 (7) Å, much longer than the difference observed in surfactin. This makes it unlikely that tensin can form 1:1 complexes with Ca^{2+} as does surfactin (Maget-Dana *et al.*, 1992), at least in the conformation observed in this crystal structure. It is however common that these non-ribosomal lipopeptides can adopt a number of distinct and different conformations. This is, for example, the case for surfactin, where the NMR studies revealed two very different conformations (Bonmatin *et al.*, 1994). The conformation of surfactin has also been shown to be highly dependent on the nature of the solvent (Itokawa *et al.*, 1994). Inspection of the structure seems to suggest that major degrees of freedom of the tensin molecule are associated with the φ and ψ angles of Asp2 and the φ value of Thr3. The conformation of the rest of the molecule appears to be fixed by the lactone-ring formation. Changes of one of these three angles will make it possible to position the lipid chain orthogonal to the plane of the tensin ring. This in turn would make tensin an analogue of a phospholipid with a very bulky head group. If the rings now associate (*e.g. via* complexation of metal ions by the carboxylate groups from adjacent tensins) this could be the structural basis of the pore formation in biological membranes.

The packing in the crystal does not suggest multimerization of the tensin molecules, and the hydrophobic tail of the lipid is seen to pack closely to the rest of the peptide. Hence, in order to obtain information on more biologically relevant conformations, structural and functional studies of the molecule when absorbed into living membranes should be undertaken.

Experimental

Tensin was isolated and purified as described elsewhere (Nielsen, Thrane *et al.*, 1999). The crystals were grown from a water-saturated ethyl acetate solution at 278 K. Crystals suitable for single-crystal X-ray diffraction appeared after one month.

Crystal data

$C_{67}H_{115}N_{12}O_{20}\cdot C_4H_8O_2\cdot 2H_2O$
$M_r = 1532.84$
Orthorhombic, $P2_12_12_1$
$a = 13.245$ (10) Å
$b = 21.984$ (10) Å
$c = 30.732$ (10) Å
$V = 8948$ (8) Å^3
$Z = 4$
$D_x = 1.138$ Mg m^{-3}
Cu $K\alpha$ radiation
Cell parameters from 20 reflections
$\theta = 40.29$–$43.62°$
$\mu = 0.708$ mm^{-1}
$T = 122.0$ (2) K
Prism, colourless
$0.32 \times 0.23 \times 0.15$ mm

Data collection

Enraf–Nonius CAD-4 diffractometer
ω–2θ scans
21 476 measured reflections
10 036 independent reflections plus 1822 Friedel-related reflections
8739 reflections with $I > 2\sigma(I)$
$R_{int} = 0.087$
$\theta_{max} = 74.96°$
$h = -16 \rightarrow 16$
$k = 0 \rightarrow 27$
$l = -37 \rightarrow 38$
5 standard reflections
frequency: 166.7 min
intensity decay: 5.6%

Refinement

Refinement on F^2
$R[F^2 > 2\sigma(F^2)] = 0.070$
$wR(F^2) = 0.205$
$S = 1.035$
11 858 reflections
1031 parameters
H atoms treated by a mixture of independent and constrained refinement
$w = 1/[\sigma^2(F_o^2) + (0.1131P)^2 + 1.4802P]$ where $P = (F_o^2 + 2F_c^2)/3$
$(\Delta/\sigma)_{max} = 0.025$
$\Delta\rho_{max} = 0.41$ e Å^{-3}
$\Delta\rho_{min} = -0.40$ e Å^{-3}
Absolute structure: Flack (1983)
Flack parameter = −0.3 (3)

All ordered and major conformation (~70% occupancy) non-H atoms were refined with anisotropic displacement parameters. All H atoms were located in difference Fourier maps and treated as riding on the appropriate heavier atoms. A number of the residues were found to have more than one conformation. The last seven C atoms of the lipid group were found to occupy two positions. Also, all atoms after C_α of L5 and all atoms after C_α of E12 showed two different conformations. This relatively high degree of disorder is probably the reason for the R factor and internal R factor being somewhat high compared with average small molecule data.

Data collection: *CAD*-4 *EXPRESS* (Enraf–Nonius, 1994); cell refinement: *CAD*-4 *EXPRESS*; data reduction: *XCAD*4 (Harms, 1996); 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); software used to prepare material for publication: *SHELXL*97.

We thank Mr Flemming Hansen for help with the crystallographic experiment. THN was supported by the Danish Ministry of Agriculture (contract No. 93S-2466-A95-00788) in cooperation with Danisco Seeds and NOVO Nordisk A/S.

Supplementary data for this paper are available from the IUCr electronic archives (Reference: GD1059). Services for accessing these data are described at the back of the journal.

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