Parallel β-sheet assemblage in a model dipeptide: an X-ray diffraction study

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The dipeptide Boc-L-Ala-*m*-ABA-OMe has been synthesised in order to understand the structure of β-sheet polypeptides. Its crystal structure shows that it forms an infinite, distorted, parallel β-sheet assemblage by intermolecular hydrogen-bonding and π–π interactions between the benzene rings.

Introduction

It is well established that the occurrence of β-sheet aggregation of polypeptides causes several fatal diseases, such as neurodegenerative prion protein disease,[1,2] Alzheimer's disease,[3,4] *etc*. In order to understand the molecular basis of such diseases, the design of β-sheet structures through self-assembly of small peptides is very important. There are a limited number of reports of β-sheet formation in crystals as well as in solution, stabilized by purely intermolecular interactions. Generally, in most cases, we observe the stabilization of the β-sheet by intramolecular hydrogen bonding in β-hairpins.[5] Schrader and Kirsten[6] were the first to show that small peptides based on a rigid template like 3-aminopyrazole can promote β-sheet formation through purely intermolecular interactions. The formation of amyloid-like parallel β-sheet assemblages in solution has been reported for a series of designed peptides by Yamada and co-workers.[7] Recently Kelly and co-workers[8] have shown the importance of β-sheet-mediated self-assembly in the formation of highly ordered amyloid-like fibril structures in some model peptides based on 2,8-disubstituted dibenzofuran. A crystallographic study of the amyloid-like fibril-forming β-sheet assemblage in a tripeptide of non-coded amino acids has been reported recently.[9] In the present paper, the crystallographic study is reported of a model dipeptide, Boc-L-Ala-*m*-ABA-OMe, which can potentially form an infinite parallel β-sheet assemblage by both intermolecular hydrogen-bonding and π–π interactions between the benzene rings. It has been shown that the self-assembly of aromatic-bridged macrocyclic peptides through π–π interactions is useful in the creation of peptide nanotubes.[10,11] In the present design we demonstrate the importance of π–π stacking along with hydrogen bonding in the formation of a distorted, parallel β-sheet structure. The peptide was synthesised following conventional solution-phase peptide synthesis.

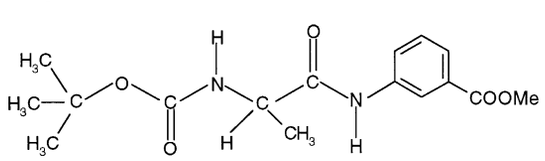

Table 1 Selected bond lengths (Å)

C(1)–O(2)	1.443(3)	C(12)–C(14)	1.531(2)
O(2)–C(3)	1.326(3)	C(14)–N(16)	1.454(2)
C(3)–O(4)	1.205(3)	C(14)–C(15)	1.519(3)
C(3)–C(5)	1.485(3)	N(16)–C(17)	1.336(2)
N(11)–C(12)	1.349(2)	C(17)–O(18)	1.214(2)
C(12)–O(13)	1.222(2)	O(19)–C(20)	1.482(2)

Table 2 Selected bond angles (°)

C(3)–O(2)–C(1)	117.7(2)	N(16)–C(14)–C(12)	108.5(1)
O(4)–C(3)–O(2)	123.0(2)	C(15)–C(14)–C(12)	111.0(2)
O(13)–C(12)–N(11)	123.7(2)	C(17)–N(16)–C(14)	122.1(2)
O(13)–C(12)–C(14)	121.2(2)	O(18)–C(17)–N(16)	124.2(2)
N(11)–C(12)–C(14)	115.1(2)	O(18)–C(17)–O(19)	125.3(2)
N(16)–C(14)–C(15)	110.4(2)	N(16)–C(17)–O(19)	110.5(1)

Results and discussion

Selected bond lengths and angles of the crystal structure are presented in Tables 1 and 2, respectively. Study of the crystal structure of the peptide shows it to adopt a distorted, parallel β-sheet structure, with a sharp turn at the C(14) atom (Fig. 1), the torsion angle N(16)–C(14)–C(12)–N(11) (φ_1) being 96.3(2)°. The backbone torsion angles that characterize the structure include O(19)–C(17)–N(16)–C(14) (ω_0) = 176.7(2)°, C(17)–N(16)–C(14)–C(12) (ϕ_1) = −101.6(2)°, C(14)–C(12)–N(11)–C(9) (ω_1) = −175.9(2)°. The backbone atoms on the two sides of the C(14) atom show an extended conformation.

There are two intermolecular hydrogen bonds between N(11) and O(13) ($x-1, y, z$) and between N(16) and O(18) ($x+1, y, z$) with donor–acceptor distances of 2.94(2) and 2.96(2) Å, respectively. These hydrogen bonds result in the formation of a twelve-membered pseudo-ring that connects neighbouring molecules. A parallel β-pleated sheet along the direction of the *a* axis is thus formed with strands running in the same direction (Fig. 2). The molecules are packed by π–π interactions between the rings at a distance of 5.09 Å between the phenyl rings. This arrangement is further stabilized by the segregation of alternate hydrophobic and hydrophilic zones.

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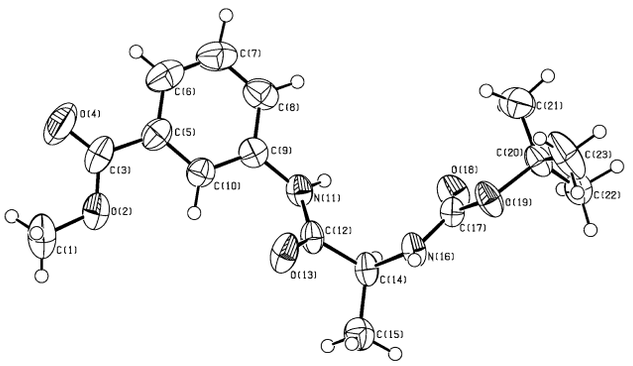

Fig. 1 Crystal structure of the model dipeptide Boc-L-Ala-*m*-ABA-OMe.

Conclusion

The dipeptide shows remarkable properties of self-assembly to form a distorted, parallel β-sheet structure through intermolecular hydrogen-bonding and π–π interactions. The present model may increase our understanding of β-sheet polypeptides. Importantly, the system may provide insights about the structure and function of peptides involved in the pathogenesis and therapeutics of conformational diseases.

Experimental

Synthesis

Methyl *m*-aminobenzoate. A mixture of absolute methanol (20 ml) and *m*-aminobenzoic acid (10 mmol, 1.37 g) was cooled in an ice–salt bath for 30 minutes. Then thionyl chloride (about 15 ml) was added dropwise with stirring. The reaction mixture was slowly allowed to come to room temperature. After 15 h of stirring of the clear reaction mixture, the excesses of methanol and thionyl chloride were removed and the residue was treated with ether. The solid methyl ester hydrochloride was then filtered and washed with ether. This hydrochloride salt (1.03 g) was dissolved in water (25 ml) and neutralized with Na_2CO_3. Then methyl *m*-aminobenzoate was extracted with ethyl acetate (2 × 25 ml) and isolated by removal of solvent. This liquid ester (3 ml) was used for the synthesis of the dipeptide without further purification. IR (KBr): 1711 (CO) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 3.73 (2H, s, -NH_2), 3.78 (3H, s, -OCH_3), 6.79, 7.14, 7.32, 7.37 (4 Ar-H).

Boc-L-Ala-*m*-ABA-OMe. The isolated methyl *m*-aminobenzoate (3 ml) was added to an ice-cooled solution of Boc-L-Ala-OH (0.51 g, 2.7 mmol) in 15 ml of DMF (*N*,*N*-dimethylformamide). Then DCC (0.56 g, 2.7 mmol; 1,3-dicyclohexylcarbodiimide) was added to the cooled mixture, which was stirred for 2 days at room temperature. The residue was then taken into ethyl acetate (50 ml) and the DCU (*N*,*N*′-dicyclohexylurea) was filtered off. The organic layer was washed with 2 M HCl (3 × 50 ml), 1 M sodium carbonate (3 × 50 ml) and brine (2 × 50 ml), dried over anhydrous sodium sulfate and evaporated *in vacuo* to yield a solid material. The crude peptide was purified by silica-gel column chromatography using CH_3OH–$CHCl_3$ as eluent (yield 65%). The pure peptide was crystallized from CH_3OH–H_2O. R_f = 0.47 ($CHCl_3$–CH_3OH = 9.5 ml : 0.5 ml) in TLC. $[\alpha]_{578}^{26} = -44.96°$ (c = 0.10 g per 100 ml; CH_3OH). IR (KBr): 3327 (N–H), 1722 (ester CO), 1675 (amide CO) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 1.43 (3H, d, Ala-C^{β}-H), 1.46 (9H, s, -$C(CH_3)_3$), 3.90 (3H, s, -OCH_3), 4.37 (1H, m, Ala-C^{α}-H), 5.19 (1H, d, Ala-NH), 7.35, 7.74, 7.80, 8.08 (4 Ar-H), 8.82 (1H, s, *m*-ABA-NH).

Crystal data †

$C_{16}H_{22}N_2O_5$, M = 322.36, monoclinic, a = 5.092(2), b = 28.928(9), c = 5.702(2) Å, β = 90.02(2)°, V = 839.97(5) Å^3, T = 294 K, space group $P2_1$, Z = 2, μ = 0.10 mm^{-1}, 3553 reflections measured, 1818 unique reflections, R_{int} = 0.024.

The experimental data were collected at room temperature using a Nonius-Kappa CCD diffractometer with Mo-Kα radiation (λ = 0.71069 Å) and the ϕ-ω scan technique [$-6 \leqslant h \leqslant 6$, $-35 \leqslant k \leqslant 37$, $-6 \leqslant l \leqslant 7$]. The structure was solved by direct methods (SHELXS-97).[12] The non-hydrogen atoms were refined anisotropically using full-matrix least squares on F^2 (SHELXL-97).[12] The final R-value was 0.0335, $wR2$ = 0.0884, S = 1.011 using 263 parameters and 1 restraint with a Flack parameter of 0.8(11). The weighting scheme, $w = 1/[\sigma^2(F_o^2) + (0.0531\ P)^2 + 0.06\ P]$ where P = max (F_o^2, 0) + $2F_c^2$)/3 gave satisfactory analysis of the variance. The absolute configuration of the system could not be determined by X-ray methods. Data collection: COLLECT;[13] cell refinement: DENZO-SMN;[14] data reduction: DENZO-SMN; molecular graphics: PLATON.[15]

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† CCDC reference number 183160. See http://www.rsc.org/suppdata/p2/b2/b203164g/ for crystallographic files in .cif or other electronic format.

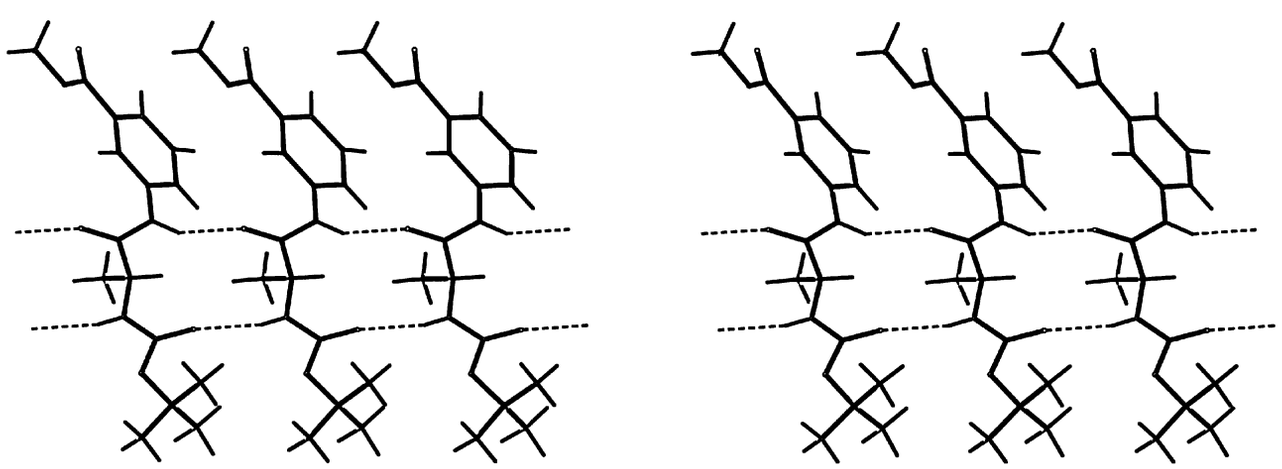

Fig. 2 A stereoview of the hydrogen-bonding pattern in the system.

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