A Crystalline β-Hairpin Peptide Nucleated by a Type I′ Aib-D-Ala β-Turn: Evidence for Cross-Strand Aromatic Interactions**

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Recent progress in the design of β-hairpin peptides[1] and β-sheet models has been based on the ability to nucleate reverse turns of the appropriate stereochemistry. D-Pro-Gly[2] and to a lesser extent Asn-Gly[3] segments have been shown to facilitate formation of type I′ and II′ β-turns, which are most often found at the site of sharp polypeptide chain reversal, that is, β-hairpins in proteins.[4,5] The prime turns, I′ and II′, can exert differing influences on the relative twist of the antiparallel strands. The I′ turn has the sense of twist that matches the twisting of adjacent β-strands in proteins. In contrast, the II′ turn results in a more "planar arrangement" with the hairpin flattening to a considerable degree.[4a,e] The D-Pro-Xxx segment can in principle adopt both II′ and I′ turn conformations as ψ (D-Pro) values of $+30°$ and $-120°$ are energetically favorable.[1a,5]

To develop a definitive approach towards the design of I′ turn nucleated β-hairpins, we have been investigating Aib-D-Xxx nucleating segments. The achiral α-aminoisobutyric (Aib) residue is overwhelmingly constrained to adopt either right-handed αR ($\phi = -60°$, $\psi = -30°$) or left-handed αL ($\phi = +60°$, $\psi = +30°$) helical conformations.[1a,6,7] The incorporation of a D-residue is intended to favor formation of a type I′ β-turn, with the D-residue adopting a preferred αL conformation. Alternative β-turn conformations such as types I, II, and II′ are expected to be energetically less favorable as one of the two prospective turn residues will have to adopt disfavored conformations.[5] We report here the crystallographic characterization of a type I′ turn nucleated β-hairpin in the designed octapeptide Boc-Leu-Phe-Val-Aib-D-Ala-Leu-Phe-Val-OMe (**1**; Boc = *tert*-butoxycarbonyl).

Figure 1 shows a view of the molecular conformation of peptide **1** in the crystal, while Table 1 summarizes the observed backbone torsion angles. Three strong cross-strand intramolecular hydrogen bonds are observed between Val(3)-CO···HN Leu(6) (N···O 2.958 Å, ∢N-H···O 159.95°), Val(3) NH···OC Leu(6) (N···O 2.853 Å, ∢N-H···O 162.23°), and Leu(1) CO···HN Val(8) (N···O 2.913 Å, ∢N-H···O 167.84°). Peptide **1** adopts a β-hairpin structure with a type I′ β-turn formed at the Aib-D-Ala segment. Of the four anticipated cross-strand hydrogen bonds in an idealized β-hairpin, the terminal interaction Leu(1) NH···OC Val(8) is disrupted by a large reorientation about the C^{α}–CO bond of Val(8) ($\psi = -57.3°$). Such fraying at hairpin termini is not uncommon.[8c] Residues Leu(1), Phe(2), Val(3), and Leu(6) adopt ϕ and ψ values characteristic of β-sheets, while some distortion is observed at Phe(7), which takes up a "polyproline-like" conformation ($\phi = -69.9°$, $\psi = +140.5°$). The molecules are held together in the crystal by a network of water-mediated hydrogen bonds. Four water molecules bridge CO and NH groups in symmetry-related molecules that do not participate in intramolecular hydrogen bonds (N4···O2w 2.909, N7···O3w 2.955, O1w···O7 2.867, O4w···O5 2.842, and N5···O1w 3.048 Å (symmetry-related by $x+1, y, z$); O1w···O2 3.193 Å (symmetry-related by $x-1, y, z$); O2w···O4 2.763 Å (symmetry-related by $-x+1, y+1/2, -z+1/2$)). A single interpeptide hydrogen bond N(2)···O(8) of 2.957 Å (symmetry-related by $x+1, y, z$) is also observed. The retention of the β-hairpin conformation of peptide **1** in solution has been demonstrated by ^{1}H NMR spectroscopic studies in methanol (data not shown). The characteristic distribution of C^{α} and NH chemical shifts and interstrand NOEs as observed in

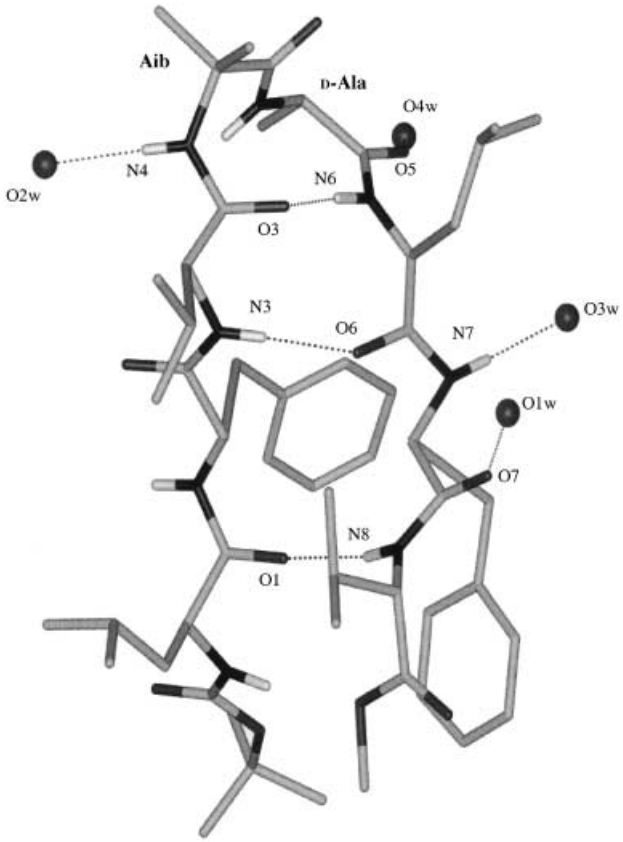

Figure 1. Molecular conformation of peptide **1** in the crystal. All the intramolecular hydrogen bonds and hydrogen bonds to the solvent molecules are shown as dotted lines.

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[**] This work was supported by a program grant in the area of Drug and Molecular Design by the Department of Biotechnology and a grant from the Council of Scientific and Industrial Research, India. We also thank the Department of Science and Technology, India, for data collection on the CCD facility under the IRHPA-DST program.

  0044-8249/02/4120-3863 $ 20.00+.50/0

Table 1. Torsion angles[a] of peptide **1**.

Residue	ϕ [deg]	ψ [deg]	ω [deg]	χ^1 [deg]	χ^2 [deg]
Leu(1)	−124.0[b]	133.4	−178.3	−173.9	72.3, −166.6
Phe(2)	−125.3	137.4	177.3	−67.3	73.8, −106.8
Val(3)	−128.2	125.9	179.8	−61.9, −179.6	
Aib(4)	56.4	34.13	173.2		
D-Ala(5)	83.2	4.3	−174.9		
Leu(6)	−115.8	139.6	178.6	−65.8	−59.9, 170.8
Phe(7)	−69.9	140.5	174.4	−174.1	73.0, −104.1
Val(8)	−117.7	−57.3[c]	−179.5[d]	−57.8, 178.9	

[a] The torsion angles for rotation about bonds of the peptide backbone (ϕ, ψ, and ω) and about bonds of the amino acid side chains (χ^1 and χ^2) as suggested by the IUPAC-IUB Commission on Biochemical Nomenclature.[16] Estimated standard deviation ≈ 1°. [b] C′0-N1-C$^\alpha$1-C′1. [c] N8-C$^\alpha$8-C′8-O(OMe). [d] C$^\alpha$8-C′8-O(OMe)-C(OMe).

related peptide β-hairpins[8] confirmed that the conformation determined in the crystal is also robust in solution.

Figure 2 shows the far-UV CD spectrum of peptide **1** in methanol. It is clear that the observed CD is anomalous and possibly the consequence of interaction between the two aromatic Phe chromophores, which occur at facing positions across the antiparallel strands. Indeed, anomalous CD bands have been demonstrated for the related peptide Boc-Leu-Phe-Val-D-Pro-Gly-Leu-Phe-Val-OMe in a study which demonstrated the utility of vibrational CD as compared to electronic CD.[9] The CD spectrum of the related peptide Boc-Leu-Val-Val-Aib-D-Ala-Leu-Val-Val-OMe (**2**) is also shown in Figure 2 for comparison. Notably, the CD spectrum of peptide **2** closely resembles that observed for well-characterized β-hairpins, with a strong negative CD band at approximately 220 nm. In contrast, the CD spectra of peptide **1** reveals positive bands at 223 and 203 nm, negative bands at 238 and 212 nm, and an intense negative band below 195 nm. This abnormal pattern is likely to be a consequence of the interaction of the two aromatic chromophores. This interpretation is supported by the absence of unusual CD bands in the spectra of model peptide hairpins which contain a single Phe residue[9] or of hairpins which have two Phe residues in nonfacing positions (unpublished results).

The inset in Figure 2 shows the approximately orthogonal disposition of the two phenyl rings, with the closest interatomic distance of 3.91 Å for Phe(7) CD1···CZ Phe(2) and a centroid-to-centroid distance of 5.52 Å. The positive and negative bands observed at 223 and 212 nm may be tentatively assigned to the exciton split π–π* transition centered at 217 nm. Such orthogonal arrangements of aromatic rings have been observed in proteins and are suggested to be a source of stabilization of tertiary structures. In proteins, the centroid-to-centroid distance for interacting phenyl pairs shows a peak in the distribution at approximately 5.5 Å.[10]

The ability to generate peptide hairpin structures nucleated by I′ and II′ β-turns permits a comparison of the effects of turn conformation on antiparallel strand orientation. Figure 3 shows a superposition of two octapeptide β-hairpin segments—namely, peptide **1** and Leu-Phe-Val-D-Pro-Gly-Leu-Phe-Val-OMe[11]—which contain the same strand residues but differ in the constitution and conformation of the β-turn units. For the superposition, only the C$^\alpha$ carbon atoms of the backbone have been used. The flattened nature of the type II′ turn is evident. More pronounced twisting and divergence of the strands in the I′ turn nucleated hairpin is observed, as

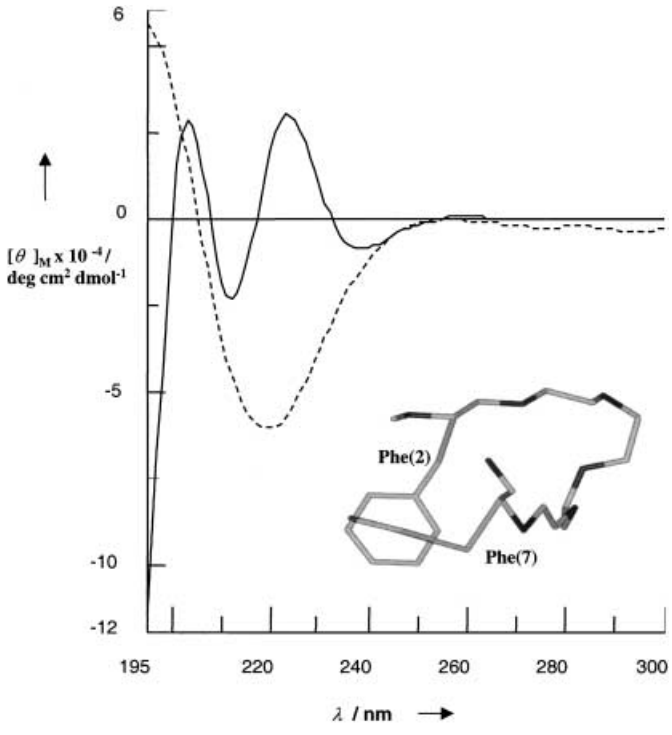

Figure 2. Electronic CD spectra of peptides **1** (——) and **2** (– – –) in the far-UV region in methanol. $[\theta]_M$ is the molar ellipticity. The inset shows the proximity of the aromatic rings of Phe(2) and Phe(7), which are approximately perpendicular to each other.

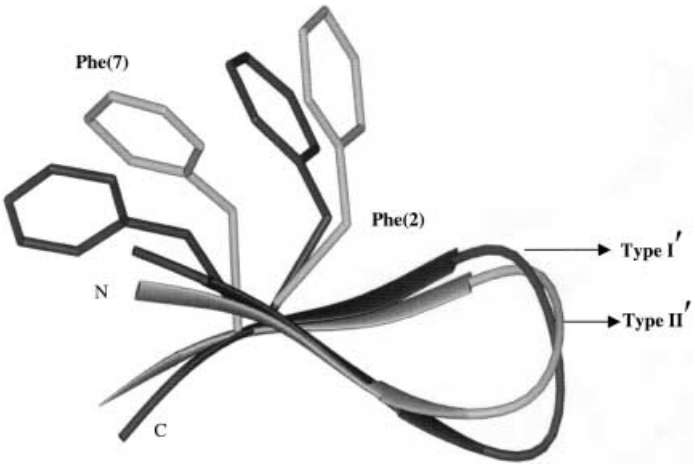

Figure 3. Superposition of peptide **1** (black) and the octapeptide segment Leu-Phe-Val-D-Pro-Gly-Leu-Phe-Val-OMe (gray) from the crystal structure of a 17-residue helix–hairpin peptide.[11] Only the side chains of the Phe residues are shown. This representation was generated using the program MolMol.[15] The virtual torsion angle[4d] (θ = C$^\alpha$3-C$^\alpha$4-C$^\alpha$5-C$^\alpha$6) is −56.2° for peptide **1** and −20.7° for the octapeptide segment.

  0044-8249/02/4120-3864 $ 20.00+.50/0 *Angew. Chem. Int. Ed.* **2002**, *41*, No. 20

compared to the II′ counterpart. It must be noted that the use of only C^{α} atoms avoids the distortion at the C-terminus observed in peptide **1**.

This study establishes the utility of an Aib-D-Xxx sequence in generating a type I′ β-turn, which in turn facilitates nucleation of β-hairpin structures incorporating an appreciable degree of strand twisting. Enhancing the degree of the strand twist in synthetic hairpins may be critical in the design of twisted multistranded structures in de novo approaches to β-barrels.[12] The conformationally constrained Aib residue has been extensively employed for the nucleation of helical structures in designed peptides.[1a,6,7] An Aib-L-Ala type II′ β-turn has been characterized in the crystal structure of the disulfide-bridged hairpin peptide Boc-Cys-Val-Aib-Ala-Leu-Cys-NHMe.[13] However, in the absence of the covalent S–S bond, the S-protected precursor favors a helical conformation in apolar solvents.[13c] The present study demonstrates that in conjunction with adjacent D-residues, Aib, and by extension related α,α-dialkyl residues, can be used to generate β-hairpin structures in de novo design strategies.

Experimental Section

Peptide **1** was synthesized by conventional solution-phase procedures using a fragment condensation strategy. Boc and methyl ester groups are used as N- and C-terminal protecting groups, respectively. Peptide couplings were mediated by *N*,*N*′-dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole.[8b] The peptide was purified by reverse-phase, medium-pressure liquid chromatography (C18, 40–60 μ) using methanol/water gradients. The peptide was characterized by 500 MHz ^{1}H NMR spectroscopy and MALDI mass spectrometry ($M+Na^+_{obs} = 1030.6$; $M_{calcd} = 1007.22$).

Crystal structure analysis: Crystals of peptide **1** were grown by slow evaporation from a solution in isopropanol/water. A single crystal ($0.5 \times 0.3 \times 0.2$ mm) was mounted on a capillary with a small amount of the mother liquor. The X-ray data were collected at room temperature on a Bruker AXS SMART APEX CCD diffractometer using MoK_{α} radiation ($\lambda = 0.71073$ Å), ω-scans ($2\theta = 53.8°$), for a total of 13913 independent reflections. Space group $P212121$, $a = 10.004(4)$, $b = 13.724(5)$, $c = 51.214(19)$ Å, $V = 7023(4)$ Å^3, $Z = 4$ for chemical formula $C_{53}H_{82}N_8O_{11}\cdot 4\text{-}H_2O$, with one molecule per asymmetric unit; $\rho_{calcd} = 1.012$ g cm^{-3}, $\mu = 0.074$ mm^{-1}, $F(000) = 2304$, $R_{int} = 0.14$. The structure was obtained by direct methods using SHELXS-97.[14a] The four water molecules were located from a difference Fourier map, Refinement was carried out against F^2 with full-matrix least-squares methods using SHELXL-97.[14b] All non-hydrogen atoms were refined isotropically. The R value at the end of isotropic refinement was 0.178, and dropped to 0.12 after anisotropic refinement. The hydrogen atoms were fixed geometrically in the idealized position and refined in the final cycle of refinement as riding over the atoms to which they are bonded. The final R value was 0.0958 ($wR2 = 0.246$) for 5055 observed reflections ($F_0 \geq 4\sigma(|F_0|)$) and 686 variables, where the data to parameter ratio is 7.4:1.0 and $S = 0.857$. The largest difference peak and hole were 0.47 and -0.23 e Å^3, respectively. The standard deviations in bond lengths and bond angles are approximately 0.01 Å and 1°. CCDC-184575 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk).

Received: May 7, 2002
Revised: June 25, 2002 [Z19250]

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