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A right handed peptide helix containing a central double D-amino acid segment

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The crystal structure of the 13 residue peptide Boc-Leu-Aib-Val-Ala-Leu-Aib-Val-DAla-DLeu-Aib-Leu-Aib-Val-OMe reveals a continuous helical conformation providing an unambiguous characterization of contiguous D-residues in a right handed peptide helix.

The chirality of L-amino acids determines the right-handed twist of α-helical secondary structures in proteins. The effect of amino acid configuration on the handedness of polypeptide helices was noted in passing by Linus Pauling in his seminal work on α-helices.[1] The incorporation of D-residues into helices composed of L-amino acids is expected to be destabilizing, and indeed model studies with the helix coiled-coils have revealed a measurable destabilization of helical structures by substitution of L-Ala by D-Ala.[2] The incorporation of D-amino acid doublets into a helical peptide amplifies the helix destabilization effects.[3] Recent studies have focussed on double D-amino acid replacements in amphipathic peptides[4] and peptide antibiotics like magainin.[5] The all D analogs of helical all L-peptides are expected to yield left handed helices, a feature which has been used in generating novel peptide ligands,[6] in designing heterochiral coiled coils[7] and in constructing ambidextrous structures containing both right and left handed helical segments.[8] Inspection of the Ramachandran map for L-Ala reveals a small sterically allowed segment of ϕ,ψ-space corresponding to the left handed helical (α_L) conformations. Consequently, the enantiomeric D-residue has a sterically allowed region corresponding to the right handed helical (α_R) conformation. The superposition of L-Ala and D-Ala conformational maps is used to generate the sterically allowed region for the conformationally constrained, achiral residue, Aib (α-aminoisobutyric acid), which may be considered to possess the steric attributes of both L- and D-alanine.[9] Aib does indeed adopt helical conformations with no intrinsic preference for helix handedness, a feature that results in centrosymmetric crystals containing helical molecules of both senses of twist in crystals of homooligopeptides.[10] Interestingly for the chiral α,α-dialkylated residue, isovaline (–HN–C(CH_3)(C_2H_5)–CO–), *both* senses of helix twist were observed in crystals of protected tri-, tetra- and hexa-homooligopeptides.[11] In principle, therefore, D-amino acids should be accommodated into right handed helical structures, albeit with a minor energy penalty.[2] We describe in this report the crystal structure of a 13-residue peptide, with a central *double* D-amino acid segment, which adopts a completely *right handed helical* conformation.

The crystal structure of the decapeptide Boc-Leu-Aib-Val-Ala-Leu-Aib-Val-DAla-DLeu-Aib-OMe reveals an interesting helix terminating motif stabilized by a C–H···O hydrogen bond, DLeu (9) CO···HC$^\alpha$ Ala(4).[12] A similar motif is also observed when Ala(4) is replaced by Gly(4) (unpublished). Based on these structures, we decided to explore the consequences of lengthening the sequence at the C-terminus. The crystal structure of a 13-residue peptide Boc-Leu-Aib-Val-Ala-Leu-Aib-Val-DAla-DLeu-Aib-Leu-Aib-Val-OMe (peptide **1**) reported here reveals a continuous right handed helical conformation spanning residues 1 to 12.[13] This is the first example of the structural characterization of a double D-segment in a right handed helical peptide.

Fig. 1 shows a view of the molecular conformation of peptide **1**. The backbone and side chain torsion angles are summarized in Table 1, and the hydrogen bond parameters in Table 2. Residues 1 to 12 adopt backbone torsion angles (ϕ,ψ) lying in the *right handed* helical (α_R) region of conformational space. Both DAla (8) and DLeu (9) have unusual *negative* ϕ, ψ values, indicative of an α_R conformation. The intramolecular hydrogen bonding pattern reveals an almost continuous string of 5⟶1 (α-helical) hydrogen bonds, with only two 4⟶1 (3_{10}-helical) interactions at the N-terminus. A lone water molecule bridges helices which are arranged in columns running antiparallel to each other. An interesting feature of the observed structure is the rather large amide bond distortion observed at Leu(11) (ω = −166.9°) and Aib(12) (ω = −163.8°). Inspection of the crystal structure suggests that the deviations from the planarity are more likely to be a consequence of the hydrogen bonding interaction with the water molecule (O1w), rather than a result of unfavorable intrapeptide interactions. The DLeu(9) side chain adopts an unusual side chain conformation with χ^1 = 97.4° and χ^{21} = 11.5°, which appear to be a result of the intramolecular contacts to Val(13) C$^\delta$H$_3$ and Aib(12) C$^\beta$H$_3$ groups. Here also, the distortion in local conformation appears to be unrelated to the double D-segment in the right handed helical structure. The structure of peptide **1** clearly demonstrates that the double D-

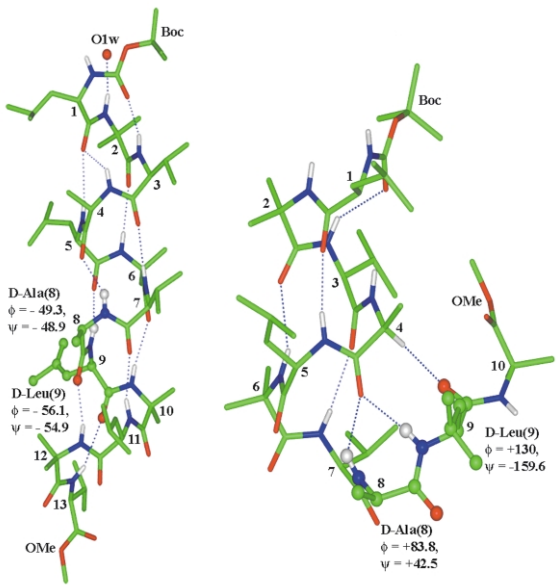

Fig. 1 (Left) Molecular conformation of peptide **1** in crystals. (Right) Molecular conformation of a decapeptide 'Boc-Leu-Aib-Val-Ala-Leu-Aib-Val-DAla-DLeu-Aib-OMe' containing a double D-amino acid segment at C-terminus (from ref: 12). Dotted lines indicate hydrogen bonds. The atoms of the double D-segment are shown in ball and stick representation for easy identification.

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segment can be comfortably accommodated into a conventional right handed α-helical peptide structure. Studies in solution reveal that the extent of destabilization of right handed helical structures by single D-amino acid substitution is about 4 kJ mol^{-1} for Ala in a model peptide. For an adjacent pair of double D-amino acids placed in the middle of a helical sequence, the values range from −0.3 to 5.7 kJ mol^{-1}, with a value of 4.38 kJ mol^{-1} for Ala and 2.77 kJ mol^{-1} for Leu.[3] Thus, the energetic penalties for incorporation of double D-segments may be offset by the greater number of cooperative hydrogen bonds formed in a continuous helix. In aqueous media, solvent competition for hydrogen bonding sites may be an additional factor determining the net stability of helical structures. In the present case of an apolar peptide helix, the number of intramolecular hydrogen bonds formed, together with the facility of packing helices into crystals[14] presumably promotes continuous helix formation. The use of larger segments of D-amino acids as guests in host L-peptide helices may be expected to provide greater insights into the role of amino acid chirality in determining helix sense and may also permit the design of novel structures with alternating senses of helix twist.

Table 1 Hydrogen bonds for peptide **1**[a]

Type	Donor	Acceptor	N···O/Å	H···O/Å	O···H–N/°
Intermolecular					
	N(1)	O(11)[b]	2.884	2.036	168.5
	O1w	O(10)[b]	2.808		
	O1w	O(13)[b]	2.873		
Solvent	N(2)	O1w	2.918	2.122	153.6
Intramolecular					
4 → 1	N(3)	O(0)	3.103	2.303	154.9
4 → 1	N(4)	O(1)	2.894	2.356	121.0
5 → 1	N(5)	O(1)	3.243	2.410	163.4
5 → 1	N(6)	O(2)	3.055	2.202	163.1
5 → 1	N(7)	O(3)	2.941	2.088	171.6
5 → 1	N(8)	O(4)	2.845	2.089	146.3
5 → 1	N(9)	O(5)	2.853	2.051	154.9
5 → 1	N(10)	O(6)	3.085	2.252	162.9
5 → 1	N(11)	O(7)	3.049	2.229	159.2
5 → 1	N(12)	O(8)	2.927	2.111	158.3
5 → 1	N(13)	O(9)	3.341	2.543	154.6

[a] The average estimated standard deviations are 0.01 Å and 1° for bond lengths and bond angles, respectively. [b] Symmetrically related by $x - 1, y, z - 1$.

Table 2 Torsion angles (°)[a] for peptide **1**

Residue	ϕ	ψ	ω	χ^1	χ^2
Leu(1)	−61.0[b]	−19.9	174.9	−158.1	66.3, −166.2
Aib(2)	−46.6	−41.6	−177.6		
Val(3)	−64.2	−40.1	177.7	−67.4, 170.4	
Ala(4)	−59.4	−42.7	178.6		
Leu(5)	−61.7	−50.7	−174.8	−105.9	−29.3, −170.4
Aib(6)	−59.6	−53.4	−176.5		
Val(7)	−71.1	−44.8	174.6	−61.2, 172.9	
DAla(8)	**−49.3**	**−48.9**	**−178.7**		
DLeu(9)	**−56.1**	**−54.9**	**−176.2**	**97.4**	**11.5, 171.6**
Aib(10)	−55.4	−42.5	−176.3		
Leu(11)	−75.5	−47.3	−166.9	−173.7	53.5, −177.5
Aib(12)	−68.2	−37.2	−163.8		
Val(13)	−65.1	+135.6[c]	177.8[d]	−67.1, 175.4	

[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, χ^2) as suggested by the IUPAC-IUB Commission on Biochemical Nomenclature. *Biochemistry*, 1970, **9**, 3471. [b] C′(0)–N(1)–Cα(1)– C′(1). [c] N(13)–Cα(13)–C′(13)–O(OMe). [d] Cα(13)–C′(13)–O(OMe)–C(OMe). The estimated standard deviation ≈ 1°.

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13 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 (HOBT). Purification of the peptide was achieved by reverse phase medium pressure liquid chromatography (C_{18}, 40-60 μm), using methanol–water gradients. Crystals of peptide **1** were grown by slow evaporation from methanol–water. X-Ray data were collected at room temperature from a crystal, mounted on a Bruker AXS SMART APEX CCD diffractometer, using Mo-Kα radiation (λ = 0.71073 Å). ω-Scan type was used, with 2θ = 53.64°, for a total of 16075 independent reflections. The space group is $P2_1$ with a = 17.179(5), b = 13.686(4), c = 19.053(6) Å, β = 108.86°, V = 4239(2) Å^3, Z = 2 for chemical formula $C_{67}H_{121}N_{13}O_{16}\cdot H_2O$, with one molecule per asymmetric unit; D_c = 1.082 g cm^{-3}, μ = 0.078 mm^{-1}, $F(000)$ = 1500. The structure was obtained by direct methods using SHELXS-97.[15a] The water molecule was located from a difference Fourier map, Refinement was carried out against F^2 with full matrix least squares methods using SHELXL-97.[15b] 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 0.0868 (wR_2 = 0.218) for observed reflections 8457 with $F_o \geq 4\sigma(|F_o|)$, GoF = 1.039. CCDC reference number 191531. See http://www.rsc.org/suppdata/cc/b2/b207960g/ for crystallographic data in CIF or other electronic format.

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