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An Unusual C–H · · · O Hydrogen Bond Mediated Reversal of Polypeptide Chain Direction in a Synthetic Peptide Helix

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An unusual C-terminal conformation has been detected in a synthetic decapeptide designed to analyze the stereochemistry of helix termination in polypeptides. The crystal structure of the decapeptide Boc-Leu-Aib-Val-Ala-Leu-Aib-Val-^{D}Ala-^{D}Leu-Aib-OMe reveals a helical segment spanning residues 1–7 and helix termination by formation of a Schellman motif, generated by ^{D}Ala(8) adopting the left-handed helical (α_L) conformation. The extended conformation at ^{D}Leu(9) results in a compact folded structure, stabilized by a potentially strong C-H · · · O hydrogen bond between Ala(4) C$^{\alpha}$H and ^{D}Leu(9) CO. The parameters for C-H · · · O interaction are Ala(4) C$^{\alpha}$H · · O=C ^{D}Leu(9) distance 3.27 Å, C$^{\alpha}$-H · · O angle 176°, and O · · H$^{\alpha}$ distance 2.29 Å. This structure suggests that insertion of contiguous D-residues may provide a handle for the generation of designed structures containing more than one helical segment folded in a compact manner. © 2000 Academic Press

Key Words: **C-H · · · O hydrogen bond; conformational analysis; helix termination; peptide design; Schellman motif; X-ray structure.**

The design of helical peptide modules for incorporation into synthetic protein mimics, based on a Meccano (or Lego) set approach, requires the nucleation and specific termination of secondary structures (1). Stable helical conformations in peptides can be readily generated by incorporation of α,α-dialkylated residues, most notably α-aminoisobutyric acid (Aib) (2, 3). In proteins, helix termination is accomplished by placement of the terminating residue (T) in the non-helical regions of conformational space (4). In particular, the Schellman motif where residue T adopts the left-handed helical (α_L) conformation ($\phi \approx +60°$, $\psi \approx +30°$) is a commonly observed terminating signal for right-handed helices (α_R) (5, 6). In synthetic peptides, the Schellman motif can be generated by placing achiral residues (Gly, Aib or α,β-dehydrophenylalanine, ΔPhe) near the C-terminus end, most often at the penultimate position (7–10). During the course of investigations designed to rationally terminate a helical segment, followed by further extension of the polypeptide backbone, we have examined the molecular conformation of the synthetic decapeptide Boc-Leu-Aib-Val-Ala-Leu-Aib-Val-^{D}Ala-^{D}Leu-Aib-OMe (**1**). In this sequence a previously characterized heptapeptide helix (Boc-Leu-Aib-Val-Ala-Leu-Aib-Val-OMe) (8) is followed by the segment ^{D}Ala-^{D}Leu-Aib. Helix sense reversal is anticipated to occur at ^{D}Ala(8). The positioning of two contiguous D-residues was designed to promote formation of a Type I′ β-turn with ^{D}Ala-^{D}Leu at positions i + 1/i + 2. In such a structure, the NH groups of ^{D}Leu(9) and Aib(10) should both be internally hydrogen bonded. Indeed, in an earlier structural investigation of the 14-residue peptide, (Boc-D(Val-Ala-Leu-Aib-Val-Ala-Leu) − L(Val-Ala-Leu-Aib-Val-Ala-Leu)-OMe (11), containing fused, continuous helical segments of opposite chirality, we obtained such a conformational motif at the junction between the segments. The crystal structure determination of peptide **1** reported here, reveals an unanticipated conformation at the C-terminus, which appears to be stabilized by a significant C-H · · O interaction. The structure assumes particular relevance in the context of the growing body of recent literature, which addresses the issue of whether C-H · · O hydrogen bonds constitute an important determinant of molecular conformation and crystal packing (12–14).

MATERIALS AND METHODS

Peptide **1** was synthesized by conventional solution phase procedures using a fragment condensation strategy. The t-butyloxycarbonyl (Boc) group was used for N-terminal protection and the C-terminus was protected as a methyl ester. Deprotections were performed using 98% formic acid and saponification for N- and C-terminus, respectively. Couplings were mediated by dicyclohexylcarbodiimide/1-hydroxybenzotriazole (DCC/HOBt). The final coupling of the 10-residue peptide was achieved by the fragment

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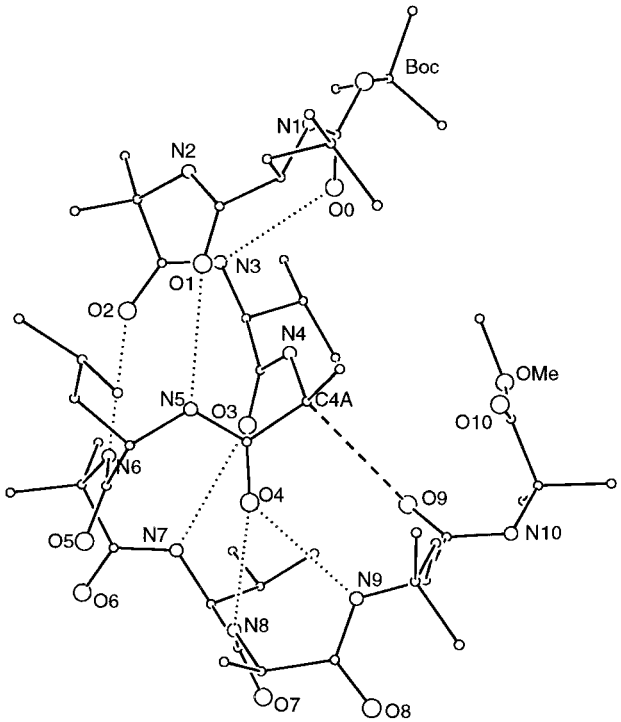

FIG. 1. Molecular conformation of peptide **1** in crystals. Backbone NH · · O hydrogen bonds are shown by dotted lines and the C-H · · O interaction between Ala(4) $C^{\alpha}H \cdots {}^{D}$Leu(9) CO is highlighted by broken lines.

condensation of Boc-Leu-Aib-Val-Ala-Leu-Aib-Val-OH and H-^{D}Ala-^{D}Leu-Aib-OMe, using DCC/HOBt as coupling agents. Purification of the peptide was achieved by reverse phase medium pressure liquid chromatography (C_{18}, 40–60 μ), using methanol-water gradients. The purified peptide was analyzed by electrospray mass spectrometry (MNa^{+}_{obs} = 1089.7, M_{cal} = 1066.7) on a Hewlett Packard 1100 LCMSD mass spectrometer. Crystals of peptide **1** were grown by slow evaporation from methanol.

For the structure analysis of peptide **1,** $\omega - 2\theta$ scan type was used with a variable scan rate, and $2\theta_{max}$ = 136°, for a total of 6322 independent reflections using CuK_{α} (λ = 1.5418 Å). The space group is $P2_1$ with a = 11.818(3) Å, b = 22.109(2) Å, c = 14.242(3) Å, β = 114.24(1)°, V = 3393.24(4) Å^3, Z = 2 for chemical formula C_{52} H_{94} N_{10} O_{13} (M_r = 1067.4) with one molecule per asymmetric unit. ρ_{calcd} = 1.045 gcm^{-3}, μ = 6.14 cm^{-1}, F(000) = 1160. The structure was obtained by direct methods using SHELXS-97 (15). 4862 reflections [$|F_o| \geq 4\sigma(|F_o|)$] reflections were used for structure solution. Refinement was carried out against F^2 with full matrix least squares methods using SHELXL-97 (16). 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 0.055 (wR_2 = 0.13) for observed reflection, with $F_o \geq 4\sigma(|F_o|)$. S = 1.09. The coordinates have been deposited at Cambridge Data Centre (ID code CCDC − 138695).

RESULTS AND DISCUSSION

Figure 1 shows a view of the conformation of peptide **1** determined in crystals. The molecule adopts a right-handed helical conformation over the segment residues 1–7. The backbone torsion angles (ϕ, ψ) (17) which describe the peptide fold are, Leu(1) (−67°, −18°), Aib(2) (−51°, −45°), Val(3) (−69°, −44°), Ala(4) (−55°, −45°), Leu(5) (−68°, −44°), Aib(6) (−59°, −45°), Val(7) (−107°, −7°), D-Ala(8) (+84°, +42°), D-Leu(9) (+130°, −160°), Aib(10) (+51°, −149°). Leu(1) ϕ is defined as the rotation about the bond C′(0)-N(1)-C^{α}(1)-C′(1) and Aib(10) ψ is defined as the rotation about the bond N(10)-C^{α}(10)-C′(10)-O(Me). ^{D}Ala(8) adopts positive ϕ, ψ values corresponding to a left-handed helical (α_L) conformation resulting in helix termination by formation of the classical Schellman motif (5, 6). Table 1 lists all the intermolecular and intramolecular hydrogen bond parameters in peptide **1.** A strong 6 → 1 hydrogen bond between ^{D}Leu(9) NH and Ala(4) CO is observed, a commonly observed characteristic of the Schellman

TABLE 1

Hydrogen Bonds in Peptide **1**

Type	Donor	Acceptor	N · · O (Å)	H · · O (Å)	C=O · · H (deg)	C=O · · N (deg)	O · · HN (deg)
				Intermolecular			
	N (1)	O (8)[a]	2.839	2.002			158.81
	N (2)	O (7)[a]	3.110	2.293			164.16
	N (10)	O (6)[b]	2.897	2.069			161.38
				Intramolecular			
4 → 1	N (3)	O (0)	3.052	2.352	122.88	129.06	138.86
5 → 1	N (5)	O (1)	2.948	2.102	163.47	164.02	167.95
5 → 1	N (6)	O (2)	3.132	2.342	140.07	147.09	152.88
5 → 1	N (7)	O (3)	3.085	2.268	160.13	162.22	158.71
5 → 1	N (8)	O (4)	3.040	2.399	137.39	145.86	131.71
6 → 1	N (9)	O (4)	2.940	2.146	130.15	134.06	153.09

[a] Related by the symmetry equivalent (x, y, z − 1).
[b] Related by the symmetry equivalent (−x + 2, y + 1/2, −z + 1).

TABLE 2

Parameters for Potential C-H · · O Interactions at the C-Terminus in Helical Peptides[a]

Sequence	T ϕ, ψ (deg)	T + 1 ϕ, ψ (deg)	$C^{\alpha} \cdot \cdot O$ (Å)	$H^{\alpha} \cdot \cdot O$ (Å)	$C^{\alpha} - H^{\alpha} \cdot \cdot O$ (deg)	$H^{\alpha} \cdot \cdot O{=}C$ (deg)
Peptide **1**	84, 43	130, −160	3.271	2.293	176.44	101.47
Peptide **2**	60, 42	−113, 166	4.599	4.778	73.68	87.72
Peptide **3**	65, 35	−126, 167	4.069	3.267	140.20	153.50
Peptide **4**	67, 36	−115, 144	4.602	3.830	137.60	135.80
Peptide **5**	84, 43	−114, 82	3.329	2.629	128.57	100.46

[a] T represents the helix terminating residue that is the site of chiral reversal. The C-H · · O parameters are listed for the interaction of the T+1 CO group with $C^{\alpha}H$ of the T-4 or T-3 residue. In the case of peptides **1** and **2** the hydrogen donor is the T-4 residue. In all other examples the donor is the T-3 residue. Note that in peptide **5** the hydrogen acceptor is the carboxylic acid. The examples chosen contain a chiral reversal at position T and an extended conformation at position T+1. Peptide **1** Boc-Leu-Aib-Val-Ala-Leu-Aib-Val-^{D}**Ala**-^{D}Leu-Aib-OMe (this study). Peptide **2** Boc-Pro-Aib-Gly-Leu-**Aib**-Leu-OMe.[5] Peptide **3** Boc-Leu-Aib-Val-Gly-Leu-**Aib**-Val-OMe.[6] Peptide **4** Boc-Leu-Aib-Val-Ala-Leu-**Aib**-Val-OMe.[7] Peptide **5** Boc-Val-ΔPhe-Phe-Ala-Leu-Ala-Δ**Phe**-Leu-OH.[8]

motif (5, 6). The peptide helix is stabilized by four successive 5 → 1 hydrogen bonds (α-helix) with a sole 4 → 1 interaction (3_{10}-helix) in the N-terminal turn. Interestingly, ^{D}Leu(9) adopts a largely extended conformation with $\phi = +129.9°$, $\psi = -159.7°$. This conformation places the carbonyl group of ^{D}Leu(9) and Aib(10) in proximity to the $C^{\alpha}H$ group of Ala(4) (Fig. 1). The parameters for a potential C^{α}-H · · O interaction in peptide **1** and related Schellman motifs reported in the literature are listed in Table 2. Notably, in peptide **1** the Ala(4) $C^{\alpha}H$ · · O=C ^{D}Leu(9) distance is extremely short, 3.27 Å and the C^{α}-H · · O angle is ≈176°, corresponding to an almost perfectly linear arrangement. These parameters, together with the short O · · H^{α} distance of 2.29 Å, suggest that this interaction may be a genuinely stabilizing force in determining the C-terminal fold of peptide **1.** It is important to note that short C^{α}-H · · O distances in peptides should be considered as a stabilizing interaction only in the absence of any strong proximal C=O · · H-N interactions. For example, surveys of protein structures, which highlight C^{α}-H · · O distances in β-sheets (18, 19) ignore the fact the strong N-H · · O interactions determine the β-sheet structure, precluding analysis of whether the short C-H · · O distances are a *consequence* or a *cause* of the observed peptide conformation. This caveat is also equally applicable to studies which identify short $C^{\delta}H$ · · O=C distances involving Pro residues in protein and peptide helices (20). In the structure of peptide **1,** the originally anticipated conformation would have placed ^{D}Leu(9) in the left-handed helical region ($\phi \approx 60°$, $\psi \approx 30°$) resulting in a hydrogen bond between Aib(10) NH and Val(7) CO groups. Despite this stereochemically and energetically favorable possibility, the molecule has revealed an alternative conformation in crystals. We therefore believe that the Ala(4) $C^{\alpha}H$ · · · ^{D}Leu(9) CO "hydrogen bond" is indeed stabilizing. Examination of intermolecular hydrogen bond contacts do not reveal any obvious packing determinant of this conformation. Interestingly, Aib(10) CO is also oriented rather close to Ala(4) $C^{\alpha}H$, albeit with poor parameters for a potentially stabilizing interaction (C^{α} · · O = 3.95Å, H^{α} · · O = 3.36 Å, ∠ C^{α}-H^{α} · · O 120.7°).

A summary of parameters for potential C-H · · O interactions in four relevant peptides terminating with the Schellman motif is also given in Table 2. A significant C-H · · O interaction is identifiable in peptide **5,** involving the CO group of the C-terminus carboxylic acid. In the case of peptide **1** (this study), the C-H · · O interaction involves the $C^{\alpha}H$ of residue T-4 and CO of residue T+1, where T is the helix-terminating site of chiral reversal [^{D}Ala(8)]. In peptides **3** and **4** the T-4 residue is Aib, which lacks a C^{α} hydrogen. Small changes in backbone dihedral angles result as a consequence of the short distance between the T+1 CO group and $C^{\beta}H_3$ of T-4. As a consequence, the closest candidate for a potential C-H · · O interaction is the $C^{\alpha}H$ of residue T-3. In the case of peptide **2,** the potential candidate T-4 residue is Pro. The most favorable arrangement for the C-H · · O interaction is achieved when residue T+1 adopts an extended conformation. Recent theoretical studies of C-H · · O hydrogen bonds suggest a stabilizing interaction of ≈1.5 kcal/mole (12). Inspection of the molecular conformation of peptide **1** reveals that chain reversal has been effectively achieved by the conformation adopted at the residues 8 and 9, resulting in a compact polypeptide fold. This observation augurs well for the rational design of compact structural motifs mimicking those found in protein structures. The use of limited segments of D-residues as guests in all L-residue polypeptide sequences merits further investigation (21, 22).

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