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-Ala-Leu-Leu-Ala-Ala-Leu-Ala-Ala-OMe reveals a helical segment spanning residues 1–7 and helix termination by formation of a Schellman motif, generated by the Ala(8) adopting the left-handed helical (αL) conformation. The extended conformation at Leu(9) results in a compact folded structure, stabilized by a potentially strong C–H⋯O hydrogen bond between Ala(4) C-H and Leu(9) CO. The parameters for C–H⋯O interaction are Ala(4) C-H⋯O=C 3.27 Å, C–H⋯O angle 176°, and O⋯H 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 (αL) conformation (θ ~ +60°, ψ ~ +30°) is a commonly observed terminating signal for right-handed helices (αR) (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, following by further extension of the polypeptide backbone, we have examined the molecular conformation of the synthetic decapeptide Boc-Leu-Ala-Leu-Leu-Leu-Ala-Ala-Ala-OMe (1). In this sequence a previously characterized heptapeptide helix (Boc-Leu-Ala-Leu-Leu-Ala-Ala-Ala-OMe) (8) is followed by the segment Leu-Ala-Ala-Leu-Ala-Ala-Ala-Ala-Ala-OMe. Helix sense reversal is anticipated to occur at Ala(8). The positioning of two contiguous D-residues was designed to promote formation of a Type I β-turn with Ala-Ala-Leu at positions i + 1/i + 2. In such a structure, the NH groups of 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-Ala-Ala-Leu) – L(Val-Ala-Leu-Ala-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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For the structure analysis of peptide 1, \(\omega - 2\theta\) scan type was used with a variable scan rate, and \(2\theta_{\text{max}} = 136^\circ\), for a total of 6322 independent reflections using CuK\(\lambda\) (\(\lambda = 1.5418\) Å). The space group is \(P2_1\), with \(a = 11.818(3)\) Å, \(b = 22.109(2)\) Å, \(c = 14.242(3)\) Å, \(\beta = 114.24(1)^\circ\), \(V = 3393.24(4)\) Å\(^3\), \(Z = 2\) for chemical formula \(C_{52}H_{94}N_{10}O_{11}\) \((M_r = 1067.4)\) with one molecule per asymmetric unit. \(R_{\text{ref}} = 0.054\) \(g/cm^3\), \(\mu = 6.14\) \(cm^{-1}\), \(F(000) = 1180\). 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 \(F_o = 4\sigma(F_o)\), \(S = 1.09\). The coordinates have been deposited at Cambridge Data Centre (ID code CCDC - 138665).

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 \((\phi, \psi)\) (17) which describe the peptide fold are, \(\text{Leu}(1) (-67^\circ, -18^\circ), \text{Aib}(2) (-51^\circ, -45^\circ), \text{Val}(3) (-69^\circ, -44^\circ), \text{Ala}(4) (-55^\circ, -45^\circ), \text{Leu}(5) (-68^\circ, -44^\circ), \text{Aib}(6) (-59^\circ, -45^\circ), \text{Val}(7) (-107^\circ, -7^\circ), \text{D-Ala}(8) (+84^\circ, +42^\circ), \text{D-Leu}(9) (+130^\circ, -160^\circ), \text{Aib}(10) (+51^\circ, -149^\circ).\) \(\text{Leu}(1)\) \(\phi\) is defined as the rotation about the bond C\(^\text{N}(10)-\text{N}(1)-\text{C}^\text{N}(1)-\text{C}^\text{C}(1)-\text{C}(1)\) and \(\text{Aib}(10)\) \(\psi\) is defined as the rotation about the bond N\(^\text{N}(10)-\text{C}^\text{N}(10)-\text{C}^\text{C}(10)-\text{O}(\text{Me})\). \(^5\)\text{Ala}(8) adopts positive \(\phi, \psi\) values corresponding to a left-handed helical (\(\alpha_L\)) 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 \(\rightarrow\) 1 hydrogen bond between \(^5\text{Leu}(9)\) NH and \(^5\text{Ala}(4)\) CO is observed, a commonly observed characteristic of the Schellman motif.

### Table 1

<table>
<thead>
<tr>
<th>Type</th>
<th>Donor</th>
<th>Acceptor</th>
<th>N \cdot \cdot \cdot O (Å)</th>
<th>H \cdot \cdot \cdot O (Å)</th>
<th>C \cdot \cdot \cdot O \cdot \cdot H (deg)</th>
<th>C \cdot \cdot \cdot O \cdot \cdot N (deg)</th>
<th>O \cdot \cdot \cdot HN (deg)</th>
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<tbody>
<tr>
<td>Intermolecular</td>
<td>N (1)</td>
<td>O (8)(^a)</td>
<td>2.839</td>
<td>2.002</td>
<td>122.88</td>
<td>129.06</td>
<td>138.86</td>
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<tr>
<td></td>
<td>N (2)</td>
<td>O (7)(^a)</td>
<td>3.110</td>
<td>2.293</td>
<td>163.47</td>
<td>164.02</td>
<td>167.95</td>
</tr>
<tr>
<td></td>
<td>N (10)</td>
<td>O (6)(^a)</td>
<td>2.897</td>
<td>2.069</td>
<td>161.38</td>
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<td></td>
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<tr>
<td>Intramolecular</td>
<td>4 (\rightarrow) 1</td>
<td>N (3)</td>
<td>O (0)</td>
<td>3.052</td>
<td>2.352</td>
<td>122.88</td>
<td>129.06</td>
</tr>
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<td>N (5)</td>
<td>O (1)</td>
<td>2.948</td>
<td>2.102</td>
<td>163.47</td>
<td>164.02</td>
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<tr>
<td></td>
<td>5 (\rightarrow) 1</td>
<td>N (6)</td>
<td>O (2)</td>
<td>3.132</td>
<td>2.342</td>
<td>140.07</td>
<td>147.09</td>
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<td>N (7)</td>
<td>O (3)</td>
<td>3.085</td>
<td>2.268</td>
<td>160.13</td>
<td>162.22</td>
</tr>
<tr>
<td></td>
<td>5 (\rightarrow) 1</td>
<td>N (8)</td>
<td>O (4)</td>
<td>3.040</td>
<td>2.399</td>
<td>137.39</td>
<td>145.86</td>
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<tr>
<td></td>
<td>6 (\rightarrow) 1</td>
<td>N (9)</td>
<td>O (4)</td>
<td>2.940</td>
<td>2.146</td>
<td>130.15</td>
<td>134.06</td>
</tr>
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</table>

\(^a\) Related by the symmetry equivalent \((x, y, z - 1)\).

\(^b\) Related by the symmetry equivalent \((-x + 2, y + 1/2, -z + 1)\).
motif (5, 6). The peptide helix is stabilized by four successive \( \rightarrow 1 \) hydrogen bonds (\( \alpha \)-helix) with a sole 4 \( \rightarrow 1 \) interaction (3_{\text{hel}})-helix in the N-terminus tail. Interestingly, 5-Leu(9) adopts a largely extended conformation with \( \phi = +129.9^\circ \), \( \psi = -159.7^\circ \). This conformation places the carbonyl group of 5-Leu(9) and Aib(10) in proximity to the C-H group of Ala(4) (Fig. 1). The parameters for a potential C-H \( \cdots O \) interaction in peptide 1 and related Schellman motifs reported in the literature are listed in Table 2. A summary of parameters for potential C-H \( \cdots O \) interactions in four relevant peptides terminating with the Schellman motif is also given in Table 2. A significant C-H \( \cdots O \) 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 \( \cdots O \) interaction involves the C-H of residue T-4 and CO of residue T+1, where T is the helix-terminating site of chiral reversal.}

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**Parameters for Potential C-H \( \cdots O \) Interactions at the C-Terminus in Helical Peptides**

<table>
<thead>
<tr>
<th>Sequence</th>
<th>T(Å)</th>
<th>T+1(Å)</th>
<th>C-H ( \cdots O ) (Å)</th>
<th>H ( \cdots O ) (Å)</th>
<th>C-H ( \cdots O ) (Å)</th>
<th>H ( \cdots O ) (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptide 1</td>
<td>3.27</td>
<td>2.293</td>
<td>176.44</td>
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<tr>
<td>Peptide 2</td>
<td>4.599</td>
<td>4.778</td>
<td>73.68</td>
<td>140.20</td>
<td>153.50</td>
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<tr>
<td>Peptide 3</td>
<td>4.062</td>
<td>3.830</td>
<td>137.60</td>
<td>135.80</td>
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<tr>
<td>Peptide 4</td>
<td>3.329</td>
<td>2.629</td>
<td>128.57</td>
<td>100.46</td>
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<td></td>
</tr>
<tr>
<td>Peptide 5</td>
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</tr>
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</table>

\( ^a \) represents the helix terminating residue that is the site of chiral reversal. The C-H \( \cdots O \) parameters are listed for the interaction of the T+1 CO group with C-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. Noteworthy 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-Val-Aib-Val-Aib-Leu-Ome (this study). Peptide 2 Boc-Pro-Aib-Gly-Leu-Aib-Leu-Ome. Peptide 3 Boc-Leu-Aib-Val-Gly-Leu-Aib-Val-Ome. Peptide 4 Boc-C-Leu-Aib-Val-Ala-Leu-Aib-Leu-Ome. Peptide 5 Boc-Val-\( \Delta \)Phe-\( \Delta \)Leu-\( \Delta \)Leu-\( \Delta \)Phe-\( \Delta \)Leu-OH.
REFERENCES


