The First Solution Stucture of a Single α-Helical Turn. A Pentapeptide α-Helix Stabilized by a Metal Clip

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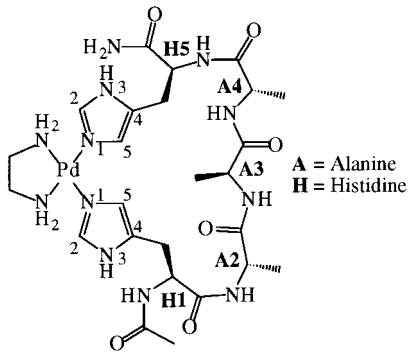
α-Helices on exposed surfaces of proteins are often critical motifs for recognition and binding by DNA, RNA, and proteins.[1] However, short peptides (<15 residues) excised from these bioactive surfaces are rarely helical in solution due to inherent thermodynamic instability.[2] If shorter α-helices could be stabilized, they might prove to be valuable biological probes and drug leads.[1] Helicity has previously been stabilized in peptides of 2 or more turns by incorporating unnatural amino acids,[3] noncovalent side chain constraints such as salt bridges and hydrophobic interactions,[4] covalent side chain linkers such as disulfide, lactam, and hydrazone bridges,[5] helix-nucleating or capping templates,[6] and metal ions.[7] We now report the first example of a stable single α-helical turn in solution, a native pentapeptide (Ac-His-Ala-Ala-Ala-His-NH_2) constrained by a metal clip to the shortest α-helix known (**1**).

Compound **1** was the major product formed in the reaction of the pentapeptide Ac-HAAAH-NH_2 with [Pd(en)$(ONO_2)_2$] in either water or DMF.[8] A combination of 2D TOCSY and ROESY spectra enabled unambiguous assignment of ^{1}H NMR resonances for both the free peptide and **1**. Resonances for each His were distinguished by the known downfield chemical shift of the imidazole C2 proton relative to the C5 signal[9] and by their strong correlations in the TOCSY spectrum.



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2D ROESY spectra for the free peptide in DMF-d_7 showed no structurally relevant cross-peaks suggesting a random coil conformation in solution. However, 2D ROESY spectra for **1** (Figure 1) showed numerous medium-range ROE's and three $^3J_{NHCH\alpha}$ coupling constants typical of an α-helical conformation (Figure 2).[10] Long-range cross-peaks between aromatic protons of His1 and His5 (Figure 1b) established that Pd was coordinated to both histidines bringing them close together. Chemical shifts for amide NH's of Ala4 and His5 were temperature independent ($\Delta\delta/T \leq 3$ ppb/K)[11] consistent with participation in hydrogen bonds as expected for an α-helix.

The three-dimensional structure of **1** was initially calculated from 44 ROE distance and 3 $^3J_{NHCH\alpha}$ ($\phi = -60 \pm 25°$) coupling constant restraints using a dynamic simulated annealing and energy minimization protocol in XPLOR (Version 3.851).[12] Resulting structures (calculated without Pd(en)$^{2+}$) showed reasonable convergence but a number of ROE violations remained. These disappeared when Ala4 and His5 amide NH's were constrained by 5→1 α-helical hydrogen bonds to the acetyl and His1 carbonyl oxygens, respectively. These two constraints dramatically improved structural convergence and markedly reduced the energies of calculated structures. Both were suggested by variable-temperature NMR data (Figure 2), and correspond to 2 of 3 H-bonds expected for an α-helical **1**.

The final calculated structure was a surprisingly well-defined α-helical turn, as demonstrated by the tight superimposition of the final 14 structures (Figure 3a) onto the backbone of an idealized α-helix (Figure 3b). In a perfect α-helix, a third hydrogen bond would be observed corresponding to the C-terminal amide-NH donating to the carbonyl oxygen of Ala2. The structure of **1**, however, shows two orientations of the C-terminal amide and VT-NMR experiments provided no evidence for this hydrogen bond. Additionally, Ramachandran analysis of the 14 lowest energy structures showed $\phi(-59°$ to $-71°)$ and $\psi(-31°$ to $-60°)$ angles typical of an α-helix for all residues except His5 $\phi(-75°$ to $-84°)$, $\psi(-34°$ to $-164°)$. These features suggest

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(8) Ac-HAAAH-NH_2 (12 mg, 22 μmol) and [Pd($^{15}NH_2(CH_2)_2{}^{15}NH_2$)-$(ONO_2)_2$] (6.4 mg, 22 μmol) were mixed in DMF-d_7 (1.0 mL) and analyzed by ^{15}N, ^{1}H, and ^{13}C NMR spectroscopy. Three linkage isomers of [Pd(en)-(peptide)]$^{2+}$ were identified in DMF (ratio 2:1:0.7) and in water. Each isomer is a 1:1 peptide:Pd(en) complex based on ^{1}H integration and electrospray MS and will be fully characterized elsewhere (Hoang et al., submitted for publication). Isomers differ in which His-N bound to Pd.

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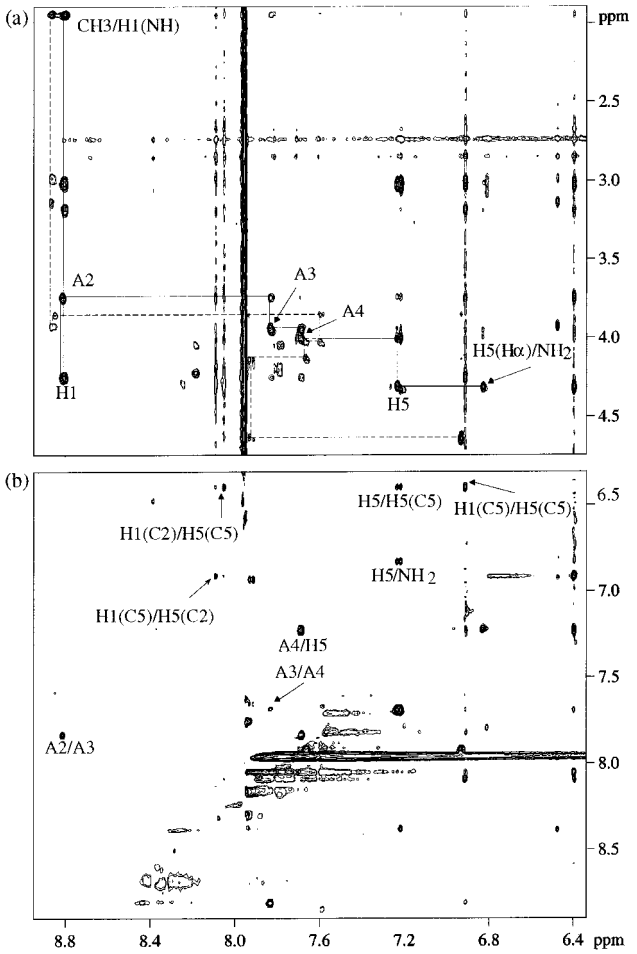

Figure 1. ROESY spectrum of **1** (DMF-d_7, 300 K, mixing time 250 ms, spin-lock 16 dB (3 kHz)). (a) Sequential connectivity[10] for **1** (solid line) and a linkage isomer (dashed line).[8] Intraresidue NH−CHα cross-peaks are labeled for His (H1, H5) and Ala (A2, A3, A4) residues of **1**. Sequential cross-peaks from N-terminal acetyl (CH_3) and C-terminal amide (NH_2) groups are also shown. (b) Amide NH−NH cross-peaks are labeled by their residue numbers in **1** and aromatic His cross-peaks are labeled by residue number and imidazole ring position (C2 or C5).

Figure 2. Summary of the sequential and medium range ROE information. ROE intensities were classified as strong (upper distance constraint 2.5 Å), medium (3.5 Å), weak (5.0 Å), and very weak (6.0 Å) and are proportional to bar thickness. $^3J_{NHCH\alpha}$ coupling constants less than 6 Hz are indicated by an ↓. Amide NH's for which chemical shifts changed by ≤3 ppb/K are indicated by a ●. The asterisk represents an ROE missing due to its proximity to the diagonal.

fraying at the C-terminal His5, a phenomenon often observed in short peptide helices.[13]

The NMR data precisely locate the positions of the imidazole rings of His1 and His5, which identifies the Pd-coordinating atoms (N1 of each histidine) and the position of Pd. Assuming a N1−Pd−N1 angle of 90° (usually 90 ± 5°)[14] for square-planar Pd(II), the average separation (2.69 Å) observed between coordinating His nitrogens in **1** implies a Pd−N bond length (1.90 Å) that is consistent with known Pd−His complexes.[14] Both N1 and N3 linkage isomers are known for His coordination to Pd(II)[9] but HXXXH has not previously been found chelated via both His residues as in **1**.[15]

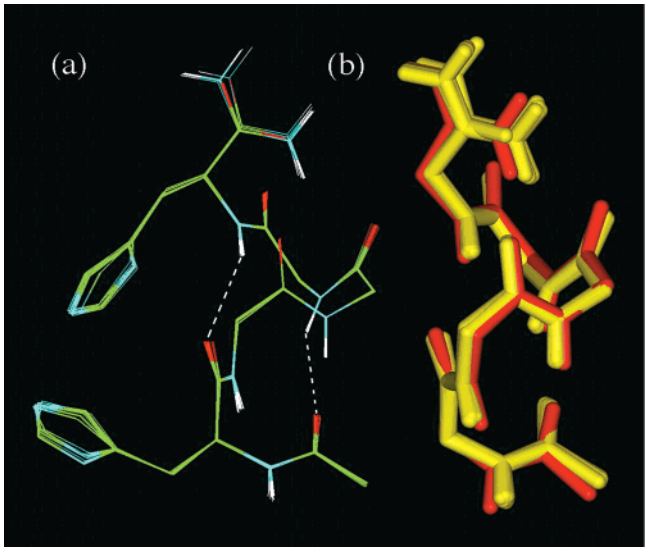

Figure 3. (a) Backbone superimposition of the 14 lowest energy refined structures of **1** (average backbone pairwise rmsd 0.0657 Å) with Ala-methyls omitted for clarity. White dashed lines represent hydrogen bonds identified from VT-NMR experiments. (b) Backbone superimposition of a theoretical α-helix (red: ϕ and ψ angles of −65° and −40°, respectively) on the 14 superimposed structures of **1** (yellow) showing almost perfect α-helicity for **1**.

On the basis of CD spectral changes, metal ions have previously been reported to stabilize native and synthetically modified peptides in an α-helical conformation through presumed coordination of two histidines or cysteines at i and $i + 4$ positions in the sequence.[7] The peptides studied were invariably >15 residues long, often with salt bridges, and spectra were usually recorded in the presence of TFE or alcohol or at 4 °C, all features which predispose peptides toward helicity. Only metal ions with a tendency for octahedral coordination geometry were previously thought to promote helicity, while square-planar Pt(II) and Pd(II) reportedly destabilize helices.[7a,9b] We now provide compelling structural evidence that a metal ion clip (Pd(II)) can chelate 2 histidines (via N1 nitrogens) arranged at i and $i + 4$ positions, inducing α-helicity even in a pentapeptide. This is the first structurally characterized example of a stable, single α-helical turn in solution.

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Supporting Information Available: Table 1 containing NOE, H-bond, and ϕ angle restraints used in the structure calculation for **1** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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