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Folding of an Ala-Ala-Ala Tripeptide into a β-Turn via Hydrophobic Encapsulation

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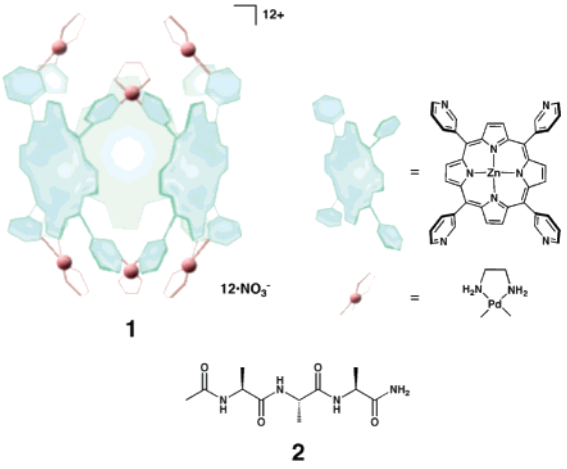
β-Turn conformation is one of the most important secondary structures of peptides. This fundamental motif is, like other peptide secondary structures, very unstable if a peptide exists as a short fragment.[1] The turn structure can be, in principle, constituted by four amino acid residues via hydrogen bonding between C*O*(*i*) and N*H*(*i*+3). In general, however, β-turn conformation is stable in water only if it is involved in β-hairpin structures consisting of more than nine amino acid residues.[2] Here, we observe that even a three-residue peptide fragment, Ac-Ala-Ala-Ala-NH_2, can be folded into a β-turn[3] (which is also regarded as a minimal 3_{10}-helix) through encapsulation by a porphyrin-assembled synthetic host. Despite the presence of only one intramolecular hydrogen bond, the turn conformation is shown to be very stable because of efficient host−guest interaction.

For the efficient accommodation of a short peptide fragment, we employed prism-like porphyrin cage **1**[4] that provides a large hydrophobic binding pocket. This cage can be prepared by the self-assembly of tetrakis(3-pyridyl)-substituted porphyrin and (en)Pd-$(NO_3)_2$. The target tripeptide, Ac-Ala-Ala-Ala-NH_2 (**2**), contains only Ala that is the simplest α-substituted amino acid residue.

The complex **1**·**2** was easily prepared by mixing the aqueous solutions of both components ([**1**] = [**2**] = 2 mM) at room temperature for a few minutes. Encapsulation of **2** within the cavity of **1** was confirmed by ^{1}H NMR analysis. Proton signals of complexed **2** were fully assigned from TOCSY and NOESY experiments. All the signals of **2** were considerably shifted upfield. In particular, the Hβ protons of Ala1, Ala2, and Ala3 were observed around −5.0 ppm (Figure 1). This result suggested that all residues of peptide **2** were deeply encapsulated by the cavity of **1**. The association constant was estimated to be 1×10^6 M^{-1} by ^{1}H NMR competition experiment.[5]



The turn conformation of **2** was elucidated from a NOESY experiment. NOE cross-peaks in NOESY were clearly observed between the N-terminal acetyl group and Ala3 protons (HN and Hβ), indicating the proximity of the N- and C-terminal of **2** (Figure 2). The turn structure of **2** was also supported by $^3J_{NHC\alpha}$ coupling constants. All $^3J_{NHC\alpha}$ values were below 6 Hz, characteristic of helical or turn rather than random-coil conformation.[6]

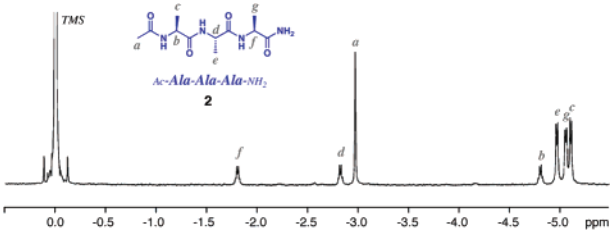


Figure 1. ^{1}H NMR spectrum of complex **1**·**2** in D_2O (500 MHz, 2 mM, 27 °C, TMS as external standard).

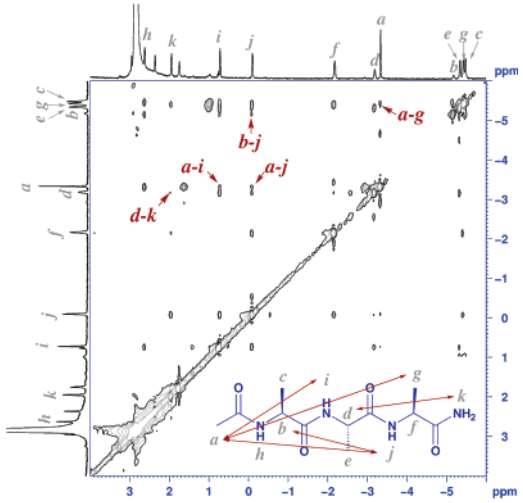


Figure 2. Selected NOESY spectrum (500 MHz, H_2O/D_2O = 9/1, 27 °C) of **1** ([**1**] = 2 mM) and **2** ([**2**] = 2 mM). Interresidue NOEs are denoted as letters in the spectrum and as arrows in the chemical structure of **2**.

Furthermore, the turn structure was strongly suggested by molecular dynamics (MD) simulation with the CNS program[7] that was run under restraints of NOE distances (11 intraresidue, 7 sequential, 5 medium range) and 3 backbone ϕ angles from $^3J_{NHC\alpha}$ values. Among randomly generated 50 initial structures, 48 of them were converged, with low backbone pairwise RMSD (0.09 Å), into almost identical lowest-energy structures that clearly showed the turn conformation (Figure 3). The MD calculation also suggested a hydrogen bond between the N-terminal acetyl group and NH of Ala3. This turn structure is a minimal 3_{10}-helix and is also categorized as a β-turn conformation.[8]

We confirmed that the MD-minimized structure fits the cavity of **1**. The crystal structure of **1**[4] and the MD-optimized structure of **2** were combined so that **2** was fully accommodated in the cavity

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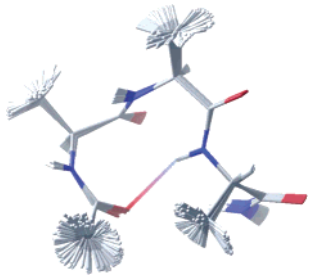

Figure 3. Superposition of the 48 lowest-energy structures by CNS[7] for bound peptide **2**.

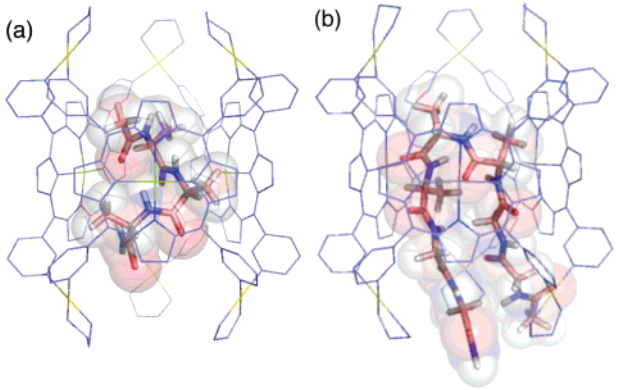

Figure 4. Refined structures of (a) complex **1·2** and (b) complex **1·9** obtained after combining the crystal structure of **1** and the MD-minimized structures of **2** and **9**, respectively.[9]

of **1**. When the combined structure was refined,[9] the conformation of **2** remained almost unchanged, suggesting that host **1** provides an ideal cavity for recognizing the most stable conformation of **2** (Figure 4a). For comparison, tripeptide **2** in an extended conformation was also refined in the cavity of **1**, but efficient host−guest interactions were hardly observed.

In the refined **1·2** structure, the methyl groups of Ala residues and the porphyrin ligands showed efficient CH−π contact, which presumably induces the folding of **2** into the β-turn conformation. In particular, the CH−π contact of Ala2 seems to be the most important because the association constant of **1** with a singly Gly-mutated tripeptide, Ac-Ala-*Gly*-Ala-NH_2 (**3**), was considerably reduced (9×10^3 M^{-1}). Other Gly-mutated tripeptides, Ac-*Gly*-Ala-Ala-NH_2 (**4**) and Ac-Ala-Ala-*Gly*-NH_2 (**5**), did not show significant decrease in association constants (8×10^5 and 1×10^6 M^{-1} for **4** and **5**, respectively).[5] Binding was no longer observed for Ac-Gly-Gly-Gly-NH_2 (**6**) because of the absence of the CH−π contact. Tripeptides, Ac-Val-Val-Val-NH_2 (**7**) and Ac-Leu-Leu-Leu-NH_2 (**8**), were also not bound at all because they are too bulky to be fit in the cavity of **1**.

Particularly interesting is that, through host−guest interaction with **1**, a stable β-hairpin-like structure is generated from a longer oligopeptide involving Ala-Ala-Ala sequence at the middle. When a heptapeptide, Ac-Gly-Gly-Ala-Ala-Ala-Gly-Gly-NH_2 (**9**), was treated with a molar equivalent of **1** in water, significant upfield shifts of proton signals were observed only around the Ala-Ala-Ala region ($\Delta\delta = -6.2$, -6.5, and -5.5 ppm for the methyl groups of Ala3, Ala4, and Ala5, respectively). The methylene groups of adjacent Gly2 and Gly6 were also shifted upfield ($\Delta\delta = -6.2$ and -6.9 ppm for Gly2; -4.2 and -5.1 ppm for Gly6), while terminal Ac-Gly1 and Gly7-NH_2 protons were hardly shielded. The association constant was 8×10^4 M^{-1}.[5] MD simulation under NOE distance restraints followed by force-field optimization[9] clearly indicated a β-hairpin-like structure with the turn conformation at the Ala-Ala-Ala region, which should be deeply accommodated in the cavity of **1** (Figure 4b).[10]

In summary, self-assembled porphyrin cage **1** showed remarkable ability to fold Ala-Ala-Ala sequence into β-turn via encapsulation. The result demonstrated that peptide recognition within the large cavity of self-assembled cages[11,12] is a powerful method to produce the secondary structures of peptides even if the peptide fragment is considerably short. Folding relatively long peptide fragments into protein partial structures showing a protein's native activities is the ultimate goal of the present study.

Supporting Information Available: Experimental procedures, physical properties, NMR assignments, MD calculations, and binding studies (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(8) Calculated structure by CNS was exactly categorized as β-turn type I.
(9) Structure refinements were carried out using MacroModel 8.0 and the OPLS-AA force field.
(10) Unfortunately, we could not assign NOEs between the terminal Gly residues due to fatal overlapping of their proton signals. Nevertheless, we considered that **9** must be folded into a hairpin-like structure because **9** cannot take other conformations in the cavity of **1** when optimized under restraints of NOE distances at the turn Ala3-Ala4-Ala5 region. Of course, the conformation of terminal Gly residues can be dynamic.
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