Peptide Folding

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Conformational Preferences of Short Peptide Fragments**

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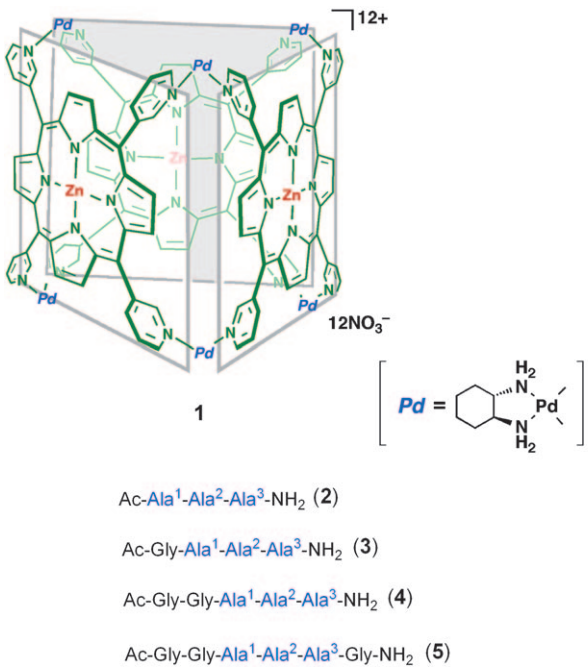
In contrast to the numerous studies on peptide folding in protein structures,[1] the folding of short peptide fragments is seldom discussed as they normally adopt random-coil conformations in water. When biologically active small peptides are bound to proteins, specific folded conformations are expected. Therefore, examining the latent folding propensity of short peptides[2] is important for understanding how they interact with proteins.[3] When peptide fragments are protected from water in aprotic environments, hydrogen bonding between amide groups is effectively induced and equilibrium should favor a folded structure. To study the latent propensity of short peptides to adopt folded conformations, alanine-rich tri- to hexapeptides **2**–**5** were placed in the cavity of self-assembled host **1** (Scheme 1).[4] We found that these peptide fragments adopted specific helical conformations within the protected cavity. In all cases, hybrid β-turn (3_{10})[5]/α-helix (4_{13}) conformations were found instead of pure α-helix conformations.[6,7] Thus, we propose that in the absence of solvent interference, short peptide fragments—effective protein termini mimics—adopt mixed $3_{10}/4_{13}$ conformations.

The porphyrin-prism host **1** self-assembles from zinc(II) tetrakis(3-pyridyl)porphyrin and [Pd(chxn)(NO_3)$_2$] (chxn = (*S*,*S*)-1,2-diaminocyclohexane) in aqueous solvents.[8] Host **1** has a large hydrophobic cavity suitable for accommodating short peptide fragments in water. The enclathration of peptides **2**–**5** was accomplished by simply mixing **1** (2.0 μmol) and the desired peptide (2.0–4.0 μmol) in D_2O (2.0 mL) at 70 °C for 3 h. NMR spectroscopic analysis clearly showed the formation of inclusion complexes **1**·G (G = **2**–**5**) in 70–80 % yields.

In all cases, single crystals suitable for crystallographic analysis were obtained by slow evaporation. The chiral Pd^{II} end cap ((*S*,*S*)-1,2-diaminocyclohexane) forces the inclusion complex to crystallize in a chiral space group, thus avoiding false symmetry issues and simplifying the crystallographic analysis of the entrapped chiral guests.[9] Short flexible peptides **2**–**5** should be conformationally free within the cavity of **1**, but significant guest disorder was not observed in the crystalline state. All of the atoms of the guest molecules were directly modeled from the electron-density maps and successfully refined, this possibility is indicative of static peptide conformations within the host crystal. Unlike standard X-ray crystallography of proteins, cage **1** ensured high data quality and facilitated a detailed discussion of the conformations of the peptide fragments.



Scheme 1.

Previously, a related inclusion complex of **2** with a host in which the end-capping group on the Pd^{II} center was ethylenediamine (**2**⊂**1′**) was examined. In this case, the 3_{10}-helix conformation of **2** in the cavity of **1′** was elucidated by NMR-constrained molecular-dynamics simulation.[4d] The new crystal structure of **2**⊂**1** also displayed the same 3_{10}-helix conformation and was consistent with the previous NMR-determined structure of **2**⊂**1′**. In both structures, the carbonyl group of the N-terminal acetyl moiety (*i*) and the amide nitrogen atom of alanine (*i* + 3) are hydrogen bonded and form the same 10-membered pseudocyclic 3_{10}-helix (or β-turn) structure (Figure 1b). The hydrogen-bonding distances determined from the crystal and NMR structure analysis are quite similar (3.1 and 3.4 Å respectively), and thus we believe that the solid and solution-state structures of peptides **3**–**5** in the cavity should also be similar.[10]

Tetrapeptide **3** is extended by one additional Gly unit at the N terminus. However, despite the small steric demand of

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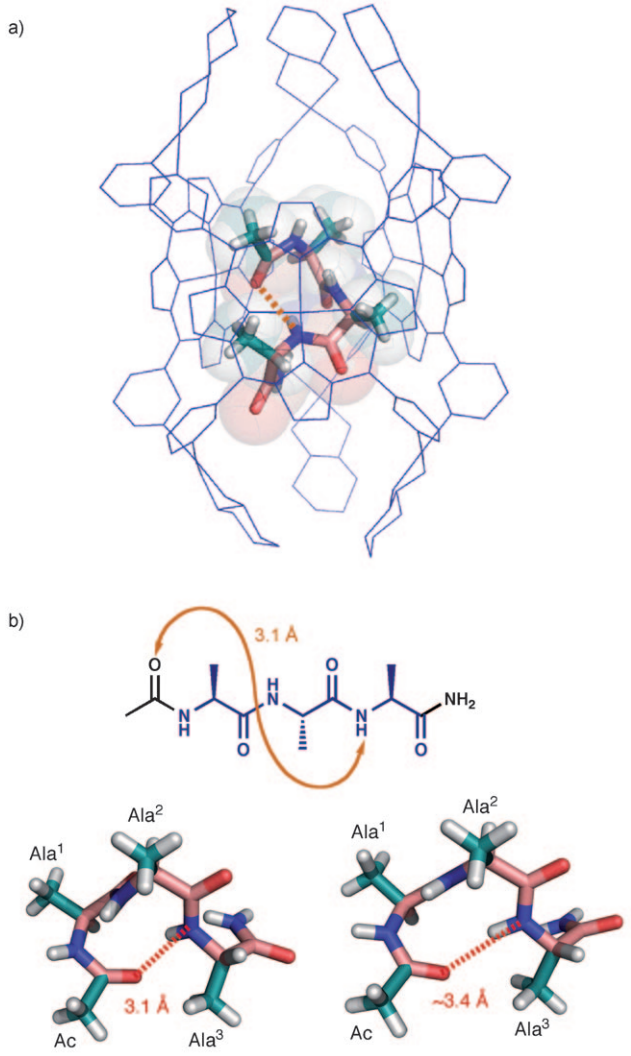

Figure 1. Structure of **2** in the cavity of **1**. a) Crystal structure of **2**⊂**1**. b) The 3_{10}-helix conformation of encapsulated **2** in the X-ray (left) and the NMR-determined (right) structures.

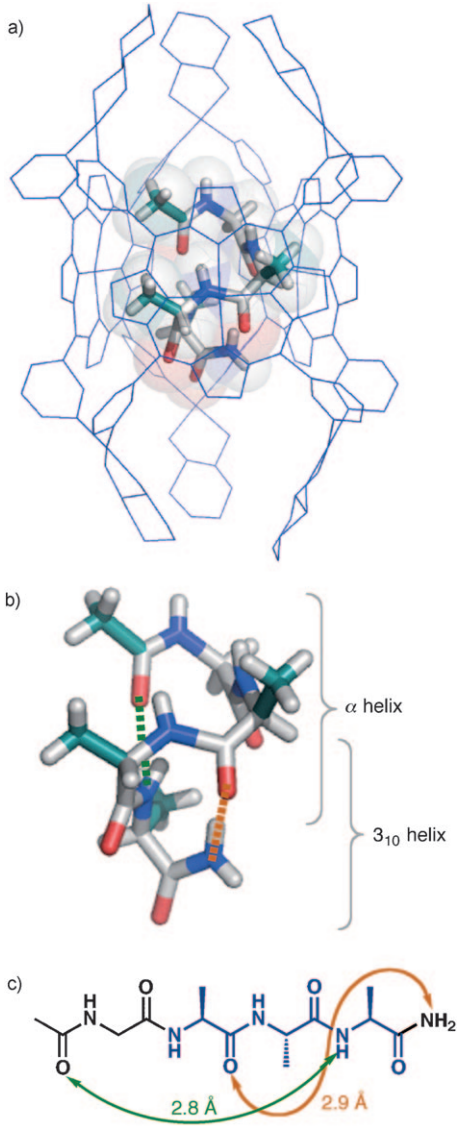

Figure 2. Folded structures of tetrapeptide **3** within **1** in the crystalline state. a) Crystal structure of **3**⊂**1**. b) Structure of **3** in the **3**⊂**1** complex. c) The hydrogen-bonding interactions within **3** in the cavity of **1**.

the Gly unit, the 3_{10}-helix conformation was no longer exclusive and an α-helix conformation appeared. In the crystal structure of inclusion complex **3**⊂**1**, the carbonyl group of the N-terminal acetyl moiety (*i*) is hydrogen bonded (2.8 Å) to the amide nitrogen atom of alanine ($i+4$) and forms a 13-membered pseudocyclic 4_{13}-helix (α-helix) structure (Figure 2). Interestingly, the C-terminal amide is also hydrogen bonded (2.9 Å) to the carbonyl group of Ala^1, rather than Gly, and a secondary 3_{10}-helix structure is evident. Thus, the conformation of tetrapeptide **3** is best described as a hybrid of 3_{10} and 4_{13} helices.

A similar mixed 3_{10}-/4_{13}-helix conformation was also observed for pentapeptide **4** in the crystal structure of **4**⊂**1**. The diffraction data was of high quality and only a single helical peptide conformation without disorder was clearly observed within the cavity. Within cage **1**, peptide **4** adopts a hybrid conformation composed of two 3_{10} and two 4_{13} helices with four hydrogen bonds (Figure 3).

A hybrid 3_{10}-/4_{13}-helix conformation was also formed by hexapeptide **5** (Figure 3).[11] The crystal structure revealed six hydrogen bonds forming three 3_{10} and three 4_{13} helices along the backbone of **4**. The central alanine sequences formed α helices in both **4**⊂**1** and **5**⊂**1**, and 3_{10}-helix domains were found at the flexible termini. These crystallographic observations strongly suggest that rigid α-helix conformations are not favored at peptide termini and engenders the hypothesis that 3_{10}- and α-helix conformations exist in equilibrium in the peripheral regions of protein structures.[12]

The helical conformations presented here arise from the innate conformational preferences of peptide fragments. Molecular dynamics simulation (MD) of peptide fragments **3**–**5** also produced similar helical conformations with minimal hydrogen-bond distance restraints (see the Supporting Information). Thus, crystallographic analysis of the series of complexes demonstrates the innate conformational prefer-

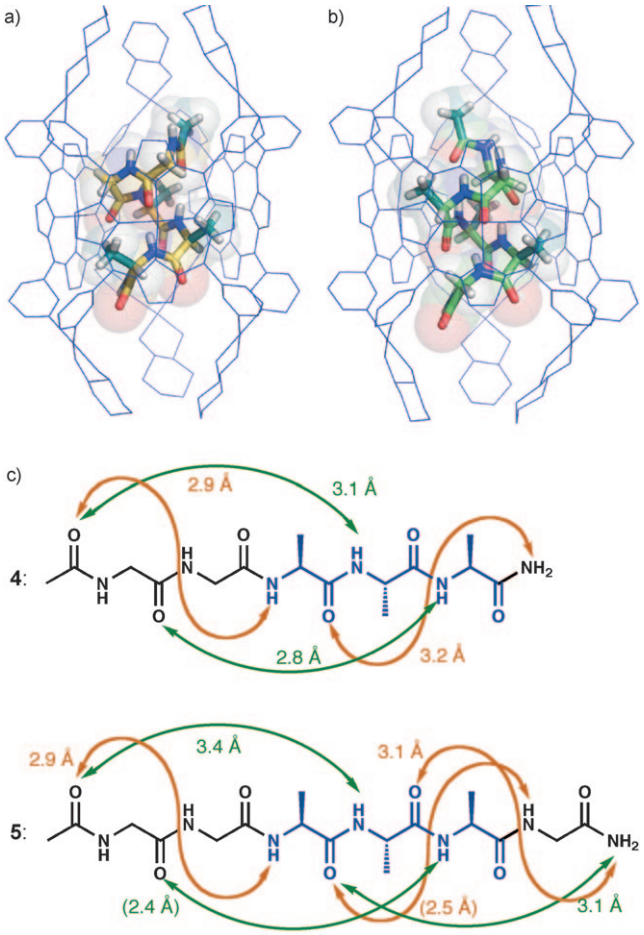

Figure 3. The crystal structures of a) **4**⊂**1** and b) **5**⊂**1**. c) The hydrogen-bonding interactions within **4** and **5** in the cavity of **1**.

ences of short peptides rather than a restricted conformation enforced by the confines of host **1**.

In summary, we have successfully obtained the crystal structures of short peptide fragments by confining them in the cavity of a self-assembled host. Short peptide fragments **3**–**5** adopted hybrid 3_{10}-/4_{13}-helix conformations rather than a pure α-helix conformation.[13] A good agreement between the solid- (X-ray) and solution-state (NMR) structures of **2**⊂**1** and **2**⊂**1′** illustrated that the synthetic cavity provides a local hydrophobic environment in water. The present study thus imparts useful insights into the conformational behavior of small biomolecules relevant to the folding processes of oligopeptides in hydrophobic protein pockets.

Experimental Section

Crystallization of peptide inclusion complexes: Single crystals were obtained after aqueous solutions of the encapsulation complexes (1.0 mM) were slowly condensed at ambient temperature for two weeks.

Crystallographic data: Data for **2**⊂**1** were collected on a RIGAKU Jupiter210 CCD area detector using synchrotron radiation ($\lambda = 0.7000$ Å) at SPring-8. Data for **3**⊂**1** and **4**⊂**1** were measured on a Bruker APEX-II/CCD diffractometer equipped with a focusing mirror ($Mo_{K\alpha}$ radiation $\lambda = 0.71073$ Å) with a cryostat system equipped with a N_2 generator. Data for **5**⊂**1** were collected on a RIGAKU MSC Mercury CCD X-ray diffractometer using synchrotron radiation ($\lambda = 0.6889$ Å) at KEK AR-NW2. The structures were solved by direct methods (SHELXS-97), and refined by full-matrix least-squares calculations on F^2 (SHELXL-97) by using the SHELX-TL program package. Hydrogen atoms were fixed at calculated positions and refined by using a riding model. The thermal temperature factors of all structures without Pd and Zn ions were isotropically refined as a consequence of the single crystals diffracting only weakly because of their poor crystallinity. All the helical structures of the peptides were refined on the basis of the chemical geometry of peptide bonds.

2⊂**1**: $C_{167}H_{213.50}N_{44.50}O_{36.25}Pd_6Zn_3$, $M_r = 4258.83$, space group $P222$, $a = 22.145(4)$, $b = 25.674(5)$, $c = 44.795(9)$ Å, $V = 25468(9)$ Å^3, $T = 100$ K, $Z = 4$, $\rho_{calcd} = 1.111$ Mg m^{-3}, 13334 unique reflections out of 84316 with $I > 2\sigma(I)$, $1.19 > \theta > 17.67°$, 1175 parameters, final R factors $R_1 = 0.1262$ ($I > 2\sigma(I)$) and $wR_2 = 0.3148$.

3⊂**1**: $C_{338}H_{445}N_{95}O_{92.50}Pd_{12}Zn_6$, $M_r = 8987.91$, space group $C222$, $a = 44.795(6)$, $b = 51.005(7)$, $c = 44.277(6)$ Å, $V = 101162(23)$ Å^3, $T = 85$ K, $Z = 8$, $\rho_{calcd} = 1.180$ Mg m^{-3}, 39521 unique reflections out of 309154 with $I > 2\sigma(I)$, $1.10 > \theta > 18.87°$, 2359 parameters, final R factors $R_1 = 0.1173$ ($I > 2\sigma(I)$) and $wR_2 = 0.3148$.

4⊂**1**: $C_{171}H_{225}N_{48}O_{45.50}Pd_6Zn_3$, $M_r = 4515.50$, space group $P4_22_12$, $a = 34.796(4)$, $c = 44.488(10)$ Å, $V = 53863(15)$ Å^3, $T = 85$ K, $Z = 8$, $\rho_{calcd} = 1.114$ Mg m^{-3}, 16243 unique reflections out of 137994 with $I > 2\sigma(I)$, $1.49 > \theta > 17.23°$, 1168 parameters, final R factors $R_1 = 0.1226$ ($I > 2\sigma(I)$) and $wR_2 = 0.3127$.

5⊂**1**: $C_{173}H_{221}N_{46.50}O_{35.50}Pd_6Zn_3$, $M_r = 4354.47$, space group $P222$, $a = 22.075(4)$, $b = 25.265(5)$, $c = 44.740(9)$ Å, $V = 24953(9)$ Å^3, $T = 85$ K, $Z = 4$, $\rho_{calcd} = 1.159$ Mg m^{-3}, 26186 unique reflections out of 140288 with $I > 2\sigma(I)$, $1.26 > \theta > 20.28°$, 1154 parameters, final R factors $R_1 = 0.1198$ ($I > 2\sigma(I)$) and $wR_2 = 0.3165$.

CCDC 736639 (**2**⊂**1**), 736640 (**3**⊂**1**), 736641 (**4**⊂**1**), and 736642 (**5**⊂**1**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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[10] The crystal structure of **2**⊂**1** also revealed a coordination bond between a Zn^{II} center and an acetyl carbonyl group, which was not, however, elucidated in the NMR study (see the Supporting Information).

[11] Since some of the hydrogen-bonding distances in the crystal structure of **5**⊂**1** are shorter than common values, disorder may be involved to some extent in the structure of guest **5**.

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[13] Since the terminal carbonyl group in both **4** and **5** is bifurcated to two different amide nitrogen atoms, the peptide conformation can also be expressed as in between 3_{10} and α helices.

