Host–Guest Systems

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Folding a De Novo Designed Peptide into an α-Helix through Hydrophobic Binding by a Bowl-Shaped Host**

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Spontaneous folding of polypeptides into their latent secondary structures such as the α helix and β sheet is both a chemically and biologically important process. It is anticipated that studies on peptide folding will lead to the construction of functional architectures with peptide backbones and help the understanding of protein folding in nature.[1] Extensive studies have thus been made on the formation of peptide secondary structures from de novo designed oligopeptides.[2,3] However, secondary structures from synthetic peptides are normally ephemeral unless fixed by covalent links or metal coordination between the residues.[4–6] Fragmental units remain stable in proteins as they are sustained by protein scaffolds through weak interactions (hydrophobic, electrostatic, etc.). We anticipated that the large hydrophobic cavity of a synthetic host could replace the protein scaffolds and stabilize the secondary structures of oligopeptides.

Bowl-shaped host **1**, assembled from $[Pt(en)(NO_3)_2]$ (en = ethylene-1,2-diamine) and a triazine-cored tridentate ligand, has a large hydrophobic pocket,[7] and is potentially capable of accommodating two or more amino acid residues.[8] De novo designed oligopeptide **2** consists of only nine residues with a Trp group at the two terminals.[9] Assuming that **2** has an α-helical conformation, the hydrophobic indole rings of the i and $i+8$ Trp residues are oriented on the same face of the helix,[10] whereas the negative charges of the i and $i+4$ Glu

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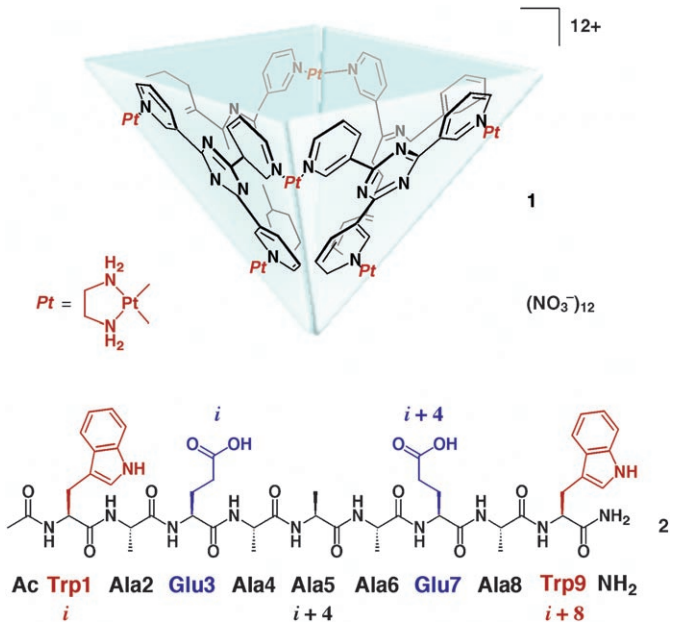

residues are on the opposite face. Accordingly, the bowl complex **1** is expected to bind **2** in an α-helical conformation through two types of host–guest interactions: primarily by hydrophobic interactions between the Trp residues and the pocket of **1** and, secondarily through electrostatic interactions between the Glu residues and the high positive charge of **1** (12+).[11] The remaining Ala residues have latent propensity for an α-helical conformation.[2c]

Compounds **1** and **2** were mixed in an aqueous solution in the expectation of an induction of the α helix. Disappointingly, a somewhat complex NMR spectrum was obtained when **1** (3 equiv) was added to a solution of **2** (2.5 mM) in phosphate buffer (100 mM) and the conformation of **2** could not be determined. However, the addition of a small amount of chloroform (1 v/v%) resulted in the signals of **2** becoming simpler (Figure 1) and all nine residues of **2** could be assigned by TOCSY and NOESY measurements. Most signals of **2** were shifted upfield, thus indicating the encapsulation of **2** within the hydrophobic pocket of **1**. Indeed, some NOE interactions between **1** and **2** were observed. The Job plot supported the formation of a 1:1 complex between **1** and **2**. The association constant was estimated from NMR titration experiments to be about $1 \times 10^3\,\text{M}^{-1}$.

The α-helical conformation of **2** in the cavity of **1** was elucidated by careful NMR spectroscopic analysis. We noted that the upfield shifts of the Hβ signals of the Trp1, Trp9, and Ala5 residues ($\Delta\delta$ = ca. −1.2 ppm) were somewhat larger than those of the others, thus suggesting that these residues were co-enclathrated in the cavity of **1**, namely, Trp1, Ala5, and Trp9 in the *i*-, *i*+4-, and *i*+8-positions were oriented on the same face and in good agreement with the α-helical conformation of peptide **2**.

The α-helical conformation was more reliably confirmed by analyzing NOE cross-peaks of peptide **2**. Several medium-range NOE interactions, for example, $d_{\alpha\beta}(i,i+3)$, $d_{\alpha N}(i,i+3)$, and $d_{\alpha N}(i,i+4)$ were observed in the NOESY spectrum and are characteristic of a typical α-helical conformation (Figure 2). Some sequential $d_{NN}(i, i+1)$ NOESY cross-peaks also supported the presence of helical structure.

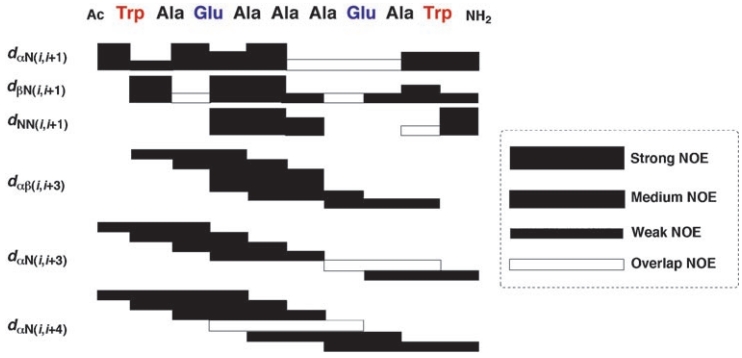

Figure 2. NOE correlations for bound peptide **2** in H_2O/D_2O (9:1; 100 mM phosphate buffer, pH 6.8). The black bars indicate the intensity of each NOE signal. The white bars indicate ambiguous NOE signals arising because of overlapping of the cross-peaks.

The conformation of **2** was also studied by a molecular dynamics (MD) simulation with the CNS program[12] that contains NOE distance restraints (39 intra-residue + sequential constraints, 25 medium-range constraints).[13] The 15 lowest energy structures for **2** all predicted an α-helical conformation (Figure 3). These ensembles showed low back-

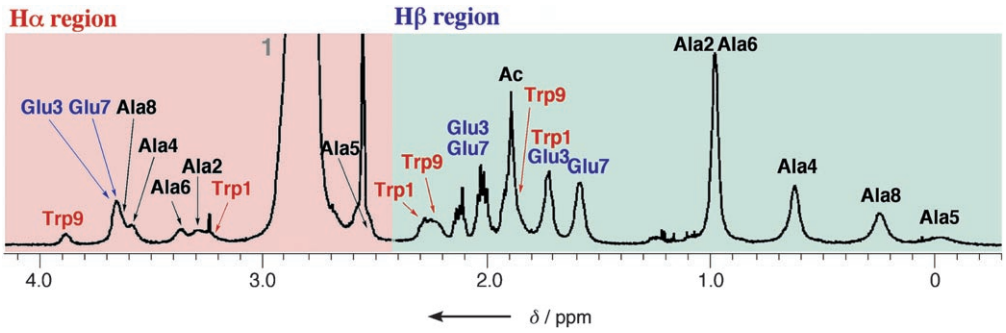

Figure 1. ^{1}H NMR spectrum (600 MHz) of **1**·**2** in H_2O/D_2O (9:1; 100 mM phosphate buffer, pH 6.8). The pale red and green backgrounds represent the regions of the Hα and Hβ signals of bound peptide **2**.

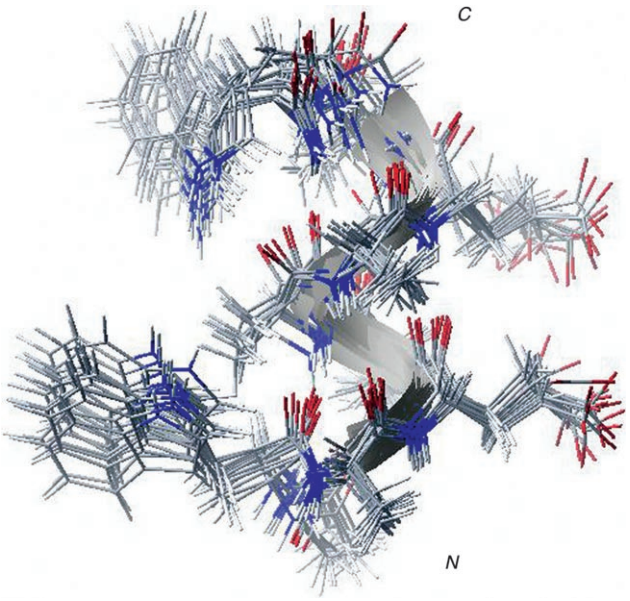

Figure 3. Superposition of the 15 lowest energy structures by CNS[12] for bound peptide **2**.

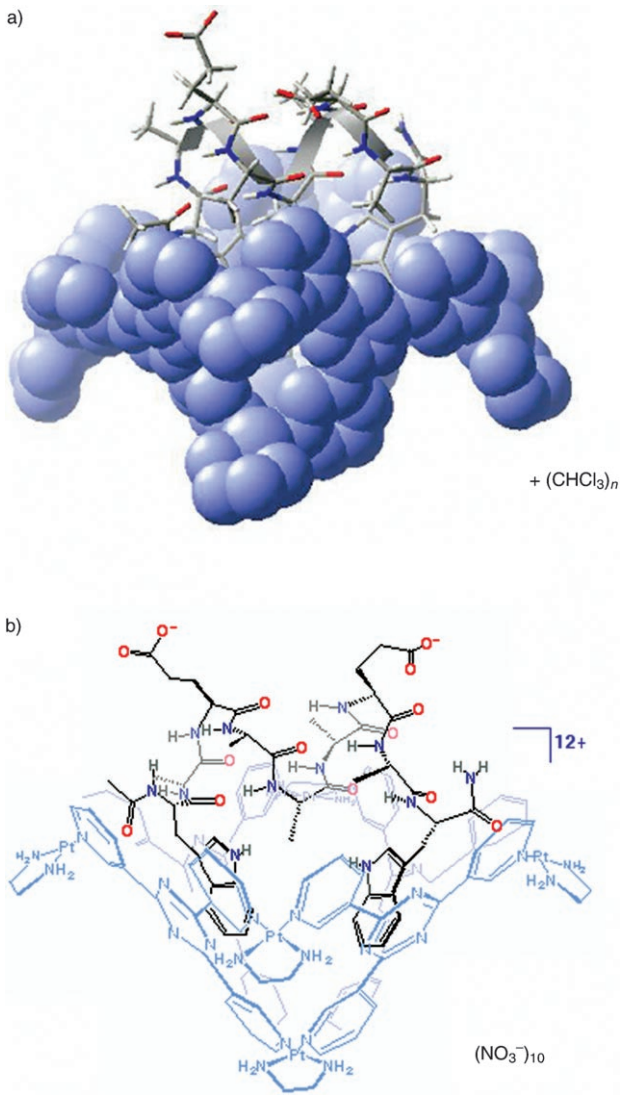

Figure 4. The proposed structure of complex **1**·**2**. a) The bowl-shaped cavity of **1** and peptide **2** are represented by space-filling and cylindrical models, respectively. b) Schematic representation of complex **1**·**2**.

bone pairwise root-mean-square deviation (0.68 Å) and no violation of the ideal bond lengths and angles.[14] The observation of some NOE interactions between the Ala 5 methyl group and the indole rings of both Trp residues illustrate the two indole rings were on the same face.

From the NMR studies, Job's plot, and the MD simulation, we conclude that oligopeptide **2** adopts an α-helical conformation and the Trp 1, Ala 5, and Trp 9 residues are on the same face of the helix and deeply accommodated in the cavity of **1** to form the stable **1**·**2** complex (Figure 4).[10] Chloroform, which is essential to induction of the α helix, is probably co-enclathrated in a small void surrounded by Trp 1, Ala 5, and Trp 9 in the **1**·**2** complex, although we could not specify the number and position of the co-enclathrated chloroform molecules by NMR spectroscopic analysis.[15]

In summary, we have demonstrated that bowl **1** has the ability to induce and stabilize the α-helix conformation of a de novo designed peptide. It is noteworthy that the stabilization of secondary structures by the hydrophobic environment is what nature does. Our bio-inspired method has potential applications for stabilizing not only other secondary structures but, ultimately, tertiary structures of proteins.

Experimental Section

Peptide **2** was synthesized with an automated peptide synthesizer (ABI 433A, Applied Biosystems) by using standard 9-fluorenylmethoxycarbonyl (Fmoc) based FastMoc coupling chemistry (0.1 mmol scale). Peptides were cleaved from the resin with trifluoroacetic acid (TFA, 10 mL) containing 5% (v/v) water and 5% (v/v) 1,2-ethanedithiol as a scavenger at room temperature for 3 h. Free peptides were washed from the resin with TFA (3 mL) followed by dichloromethane (5 mL), and then evaporated. A large volume of Et_2O was added to the residue and the precipitate was collected by filtration. Crude peptides were purified by reversed-phase HPLC on an Inertsil Peptides C18 (GL Sciences Inc.) semipreparative column (20 mm × 250 mm) using 10 mM ammonium hydrogencarbonate solution with a 0.05% TFA (pH 6.0) and acetonitrile gradient. The peptide was obtained as a white powder by lyophilization. Characterization of the peptide was carried out by ^{1}H NMR spectroscopy and MALDI-TOF mass spectrometry (Voyager-DE STR, Applied Biosystems).

1: 2,4,6-Tris(3-pyridyl)-1,3,5-triazine (0.20 mmol), $[Pt(en)(NO_3)_2]$ (0.30 mmol), and 2-naphthoic acid (0.25 mmol) as a template were combined in H_2O (10 mL) and stirred for 6 days at 90 °C. After filtration of the mixture, a small amount of nitric acid was added to the solution and the slightly acidic solution was washed five times with chloroform and then the solution was concentrated. A large volume of acetone was added to the concentrated solution to give a white precipitate, which was collected and then washed with acetone. After drying the white powder, it was dissolved in water, and then this solution was lyophilized (for complete evaporation of residual acetone). Yield 90%. ^{1}H NMR (500 MHz, D_2O, 300 K, tetramethylsilane (TMS) as external standard): $\delta = 10.63$ (s, 8H), 9.90 (s, 4H), 9.25 (d, $J = 5.0$ Hz, 8H), 9.19–9.14 (m, 16H), 7.87–7.81 (m, 12H), 3.02–2.86 ppm (m, 24H); ^{13}C NMR (125 MHz, D_2O, 300 K, TMS as

external standard): $\delta = 169.2$ (Cq), 169.1 (Cq), 155.6 (CH), 155.2 (CH), 153.5 (CH), 153.0 (CH), 140.6 (CH), 140.3 (CH), 134.3 (Cq), 134.2 (Cq), 127.7 (CH), 127.4 (CH), 47.7 (CH_2), 47.2 ppm (CH_2); IR (KBr, cm^{-1}): 3066, 1608, 1587, 1533, 1384, 1320, 1192, 1175, 1053; m.p. $>250\,^{\circ}C$ (decomp); elemental analysis calcd for $C_{84}H_{96}N_{48}O_{36}Pt_6 \cdot 16\,H_2O$: C 26.46, H 3.38, N 17.63; found: C 26.72, H 3.44, N 17.37. The structure of **1** was the same as that of the Pd-bowl[7a] as determined by ^1H NMR spectroscopy.

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[9] Circular dichroism spectra showed that free peptide **2** took an almost unordered structure. The conformation of complexed **2** could not be determined from CD measurements because strong absorption of **1** around 200–250 nm interfered with the CD spectrum of **2**. The unordered structure of free **2** is also elucidated by comparison of the ^{13}C chemical shifts of the Cα carbon atoms; See the Supporting Information.

[10] Ideally, the Trp resides should be located at the *i*- and *i*+7-positions. We originally designed peptide **2** so that it would be encapsulated within the dimeric capsule of **1**, which we have previously reported.[7b] In this study, we unexpectedly observed the binding of **2** by monomeric **1**. Despite the subtle mismatch in the location of the Trp residues, the α-helical conformation of **2** was sufficiently stabilized because of flexible orientation of the terminal Trp residues.

[11] The importance of two interactions (hydrophobic and electrostatic) for peptide binding has been discussed in one of our previous papers.[8] Namely, a related Pd^{II}-linked host compound recognized the Trp-Trp-Ala sequence strongly, whereas the non-aromatic peptide Gly-Gly-Ala and cationic peptide Trp-His-Ala were much less strongly bound by the host.

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[13] We could not estimate the $^3J_{NHC\alpha}$ coupling constants for the CNS restraint because of broadening of the signals of the bound peptide.

[14] From the observed 68 NOE correlations, four unreasonable correlations presumably arising from very minor conformational isomers were eliminated for the CNS calculation.

[15] Titration experiments were difficult since chloroform shows very low solubility in water. Stabilization of the α-helical conformation of **2** was observed when more than approximately 10 equivalents of chloroform were added to the solution.

