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Inducing α-Helices in Short Oligopeptides through Binding by an Artificial Hydrophobic Cavity

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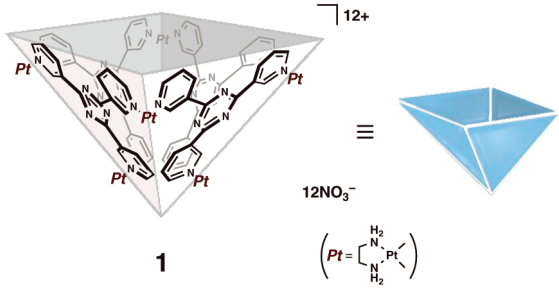
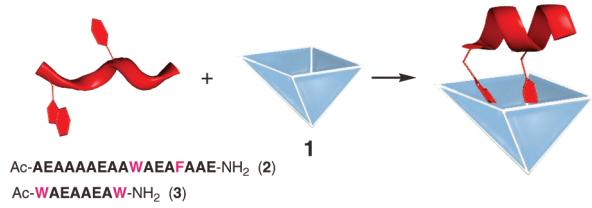
The de novo design of oligopeptides often introduces extra intramolecular interactions between the *i* and *i* + 4 peptide residues to stabilize latent α-helical structures.[1] Weak interactions such as salt bridges,[2] hydrogen bonds,[3] aromatic−aromatic interactions,[4] cation−π interactions,[5] and hydrophobic interactions[6] have been employed to strengthen (*i*)−(*i* + 4) interactions. Metal chelation,[7] S−S bonds,[8] or even olefin metathesis[9,10] have also been used to effectively link the *i* and *i* + 4 residues and enforce helical conformations. Without these extra interactions, short polypeptides generally do not adopt α-helical structures in solution, as the *i* + 4 → *i* hydrogen bonds cannot compensate for the entropic cost associated with the folding of the polypeptide chain. Within the hydrophobic pockets of proteins, however, the α-helical secondary structures of even small peptide fragments can be stabilized through local inter-residue interactions.

Inspired by the use of hydrophobic pockets in biological systems, we envisioned that short peptides containing hydrophobic residues at the *i* and *i* + 4 positions would assume α-helical conformations in the presence of the suitably sized artificial hydrophobic host **1**.[11−13] In a preliminary study, the hydrophobic cavity of host **1** induced the formation of secondary structure in a specific nine-residue peptide with the aid of a solvent molecule, but the folding was not general.[14] We now report that oligopeptides with hydrophobic *i* and *i* + 4 residues adopt α-helical conformations within the large hydrophobic cavity of bowl-shaped host **1**.



In the helical conformation, residues at the *i*, *i* + 4, and *i* + 7 positions occupy the same face of the α-helix.[15] Peptides **2** and **3**, with aromatic residues at the *i*, *i* + 4 and *i*, *i* + 7 positions, respectively, were thus chosen to examine the ability of **1** to recognize and induce a helical conformation in peptides with increasingly spaced hydrophobic residues (Scheme 1). Titrations of aqueous solutions of **2** and **3** at 5 °C with bowl-shaped host **1** were followed using CD spectroscopy.

Scheme 1. Schematic Representation of Short Peptide Folding via Enclathration



In the absence of bowl **1**, the CD spectrum of an aqueous solution of *i*, *i* + 4 peptide **2** displayed negative CD absorptions at 208 and 222 nm, characteristic of an α-helix conformation (Figure 1a).[16] Upon addition of **1**, the intensities of the two bands increased, reaching a maximum at a 1:1 ratio of host **1** and peptide **2**.[17] The hydrophobic cavity of **1** recognized and further stabilized the latent α-helix structure of peptide **2**. The new optically active absorption bands at 245, 270, and 285 nm are mostly likely due to a new chiral conformation of achiral bowl **1** induced by binding of the chiral peptide.[18,19]

The shorter peptide **3** decorated with hydrophobic residues at positions *i* and *i* + 7 exhibited a CD spectrum characteristic of a random-coiled structure, with a strong negative absorption at 196 nm and a weak positive absorption at 222 nm (Figure 1b). The peptide is too short to stabilize an α-helix in solution. Upon addition of bowl **1**, the two negative CD bands at 208 and 222 nm that are indicative of an α-helix conformation appeared. Presumably,

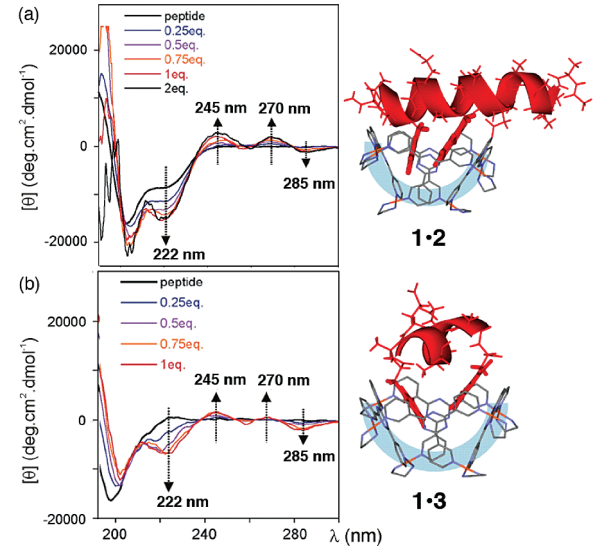


Figure 1. CD spectra showing the titration of peptides (a) **2** and (b) **3** in the presence of increasing amounts of bowl **1**. Molecular models of the complexes **1**•**2** and **1**•**3** are shown.

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Table 1. Association Constants of Bowl·Peptide Complexes[a]

entry	peptides	K_a (M^{-1})
1	Ac-AEAAAAEAA**W**AEA**F**AAE-NH_2 (**2**)	3.5×10^5
2	Ac-**W**AEAAEA**W**-NH_2 (**3**)	2.4×10^5
3	Ac-**W**AEA**G**A-NH_2 (**4**)	1.0×10^4
4	Ac-**W**AEA**A**A-NH_2 (**5**)	1.0×10^4
5	Ac-**W**AEA**L**A-NH_2 (**6**)	3.1×10^4
6	Ac-**W**AEA**F**A-NH_2 (**7**)	1.6×10^5
7	Ac-**W**AEA**W**A-NH_2 (**8**)	3.1×10^5
8	Ac-**W**AEA**W**EAA-NH_2 (**9**)	5.5×10^5

[a] Obtained from UV−vis titrations by monitoring the charge-transfer absorption maxima of the bowl·peptide complexes (350−550 nm).

hydrophobic and aromatic interactions of the two tryptophan residues with the cavity of bowl **1** led to the induction and stabilization of the α-helix conformation of peptide **3**. Again, the new absorption bands at 245, 270, and 285 nm are due to the induced chirality of bowl **1**.[20,21]

Systematic variation of the residue X at the $i + 4$ position in the hexapeptides Ac-**W**AEA**X**A-NH_2 (X = G, A, L, F, W) shed light on the role of the second $i + 4$ hydrophobic residue and the necessity of both aromatic residues. Association constants (K_a) between host **1** and the peptides were evaluated by monitoring the charge-transfer absorption maxima of the bowl·peptide complexes at 350−550 nm during the titrations of hexapeptides **4**−**8** in phosphate buffer solutions (pH 7) (Table 1).[22] Overall, the association constants for hexapeptides **4**−**8** stressed the importance of aromatic−aromatic interactions in the recognition of peptides by host **1** (Scheme 1 and Figure 1a). Hexapeptides **4**−**6** all possess a single tryptophan residue and displayed similar association constants on the order of 10^4 M^{-1} with only slight variations as the size of the aliphatic hydrophobic residue at the $i + 4$ position increased (Table 1, entries 3−5). Addition of a second aromatic residue at the $i + 4$ position resulted in a significant increase in the binding constant (entries 6 and 7). Aromatic−aromatic interactions with the electron-deficient triazine panels of **1** are known to be major driving forces for encapsulation,[23] and accordingly, increasing the electron density of the second aromatic residue from phenylalanine (F) to tryptophan (W) resulted in stronger binding.

Although the helical conformations of both **2** and **3** (each with two aromatic residues) were stabilized by bowl **1** (Figure 1), tighter association was obtained when the aromatic residues were separated by single helix turn. Octapeptides **3** and **9** have tryptophans at the i, $i + 4$ and i, $i + 7$ positions, respectively, and direct comparison of the obtained association constants revealed a 2-fold improvement for the i, $i + 4$ binding motif (Table 1, entries 2 and 8). Molecular modeling supported this interpretation and indicated that aromatic residues separated by a single turn of the α-helix (i, $i + 4$) are better arrayed for efficient packing within the cavity of bowl **1** (Figure 1).

The preorganization of the aromatic residues also plays a large role in the recognition process. The 17-residue peptide **2** contains the same aromatic residue recognition motif as hexapeptide **7** but was bound twice as strongly (Table 1, entries 1 and 6). The hexapeptide is too short to stabilize an α-helix conformation in solution and is random-coiled. The longer peptide **2**, however, adopts an α-helix conformation in solution (Figure 1), and this preorganized structure presumably oriented both aromatic residues onto the same face and facilitated the enclathration and strong binding by host **1**.

In conclusion, we have demonstrated that even short peptides can be induced into α-helix conformations in aqueous solution through binding to an artificial hydrophobic pocket. Systematic variation of hydrophobic residues in short oligopeptides evidenced aromatic−aromatic interactions as a key driving force for enclathration. Peptides with two aromatic residues showed the highest affinity for host **1**, but the spacing and preorganization of the residues were also important. We anticipate that artificial cavities could be used to alter and control biological processes occurring at the protein surface, such as protein−protein recognition and interaction.

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Supporting Information Available: Experimental details and spectroscopic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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(15) The position of aromatic residues on the α-helix was examined by molecular modeling. See the Supporting Information.
(16) Maximum intensities of this peptide **2** were reached at 60% (v/v) 2,2,2-trifluoroethanol and defined as 100% helicity. Peptide **2** had 33% helicity in solution before enclathration with **1**. See the Supporting Information for details.
(17) This 1:1 complex structure of **1**·**2** was also supported by DOSY NMR experiments. See the Supporting Information.
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(21) Helical folding of peptide **3** was not observed at all by simple addition of Pt^{II} ions. See the Supporting Information.
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