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Discrete Heterogeneous Quaternary Structure Formed by α/β-Peptide Foldamers and α-Peptides

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The development of unnatural oligomers that adopt predictable secondary structures has led to interest in expanding the conformational repertoire of foldamers to include discrete, cooperatively folded tertiary and quaternary structures.[1] Such advances could lead ultimately to protein-like activities. Reported efforts have focused on inter- or intramolecular association of helical foldamers, with the aim of generating helix bundle architectures.[2−5] In the examples described to date, all helical elements in the assembly have had the same type of backbone, as is the case with helix bundles found in proteins;[6] we refer to such systems as homogeneous helix bundles.[7] Here we report the first examples of heterogeneous quaternary structure, tetrameric helix bundles that contain both α-peptide and α/β-peptide segments.

α/β-Peptide foldamers with 1:1 alternation of α- and β-amino acid residues have been studied recently by several groups.[8] We have shown that five-membered ring-constrained β-residues, such as ACPC and APC (Figure 1), favor a helical conformation with *i*, *i*+4 C═O···H−N backbone H-bonds ("14/15-helix") when the α/β-peptide contains at least 15 residues.[8c,j] Our structural data suggested that a 14/15-helical α/β-peptide could mimic the side chain display found among α-helical α-peptides that form natural helix bundles. Such α-peptides display a heptad sequence repeat, with hydrophobic side chains at the first and fourth position (positions *a* and *d* in an *abcdefg* heptad).[6] We hypothesized that an appropriately designed α/β-peptide sequence would display an analogous set of side chains and therefore allow formation of heterogeneous (α/β-peptide + α-peptide) quaternary structure.

The Acid-pLL/Base-pLL α-peptides of Kim et al.[9] (**1** and **2**; Figure 1), which form a 2:2 tetrameric helix bundle, served as the basis for our test of this hypothesis. Association in water is driven by interactions among Leu side chains at *a* and *d* positions. Electrostatic interactions guide the assembly: self-association of only **1** or only **2** is less favorable than association of **1**+**2**. α/β-Peptide **3** was generated by replacing every other α-residue of **2** with a β-residue. β^2-Homoleucine (β^2-hLeu) in **3** replaces Leu in **2** at *a* and *d* positions, and all other replacements are cyclic β-residues, which should promote 14/15-helicity.[8c] Simple helix-net overlay analysis suggested that β^2-hLeu would be preferable to β^3-hLeu for mimicry of α-helical **2**.

Comparisons among the circular dichroism (CD) spectra for 100 μM **1**, 100 μM **3**, and 50 μM **1** + 50 μM **3** in phosphate-buffered saline (PBS) provide strong evidence for intermolecular association between **1** and **3** with concomitant increase in helicity (Figure 2A). The CD spectrum of **1** is characteristic of a partially folded α-helical peptide,[9] and the minimum near 206 nm for **3** is consistent with α/β-peptide helicity.[8j] The CD spectrum for **1**+**3** is much more intense than the average of **1** alone plus **3** alone, which suggests that **1** and **3** associate in a way that promotes helical folding. A Job plot indicates 1:1 stoichiometry for the association between **1** and **3** (Figure 2A, inset).

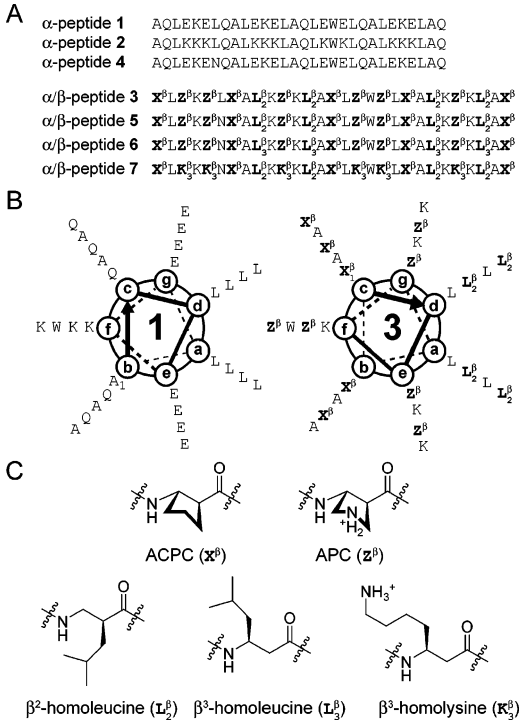

Figure 1. (A) Sequences of α- and α/β-peptides. Bold letters represent β-amino acids, using the following abbreviations: $\mathbf{X}^\beta$ = ACPC, $\mathbf{Z}^\beta$ = APC, $\mathbf{L}_2^\beta$ = β^2-homoleucine, $\mathbf{L}_3^\beta$ = β^3-homoleucine, $\mathbf{K}_3^\beta$ = β^3-homolysine. Non-bold letters represent α-amino acids according to the standard one-letter code. (B) Helical wheel representations of α-peptide **1** and α/β-peptide **3** viewed from the N-terminus of each peptide. (C) Structures of β-amino acids.

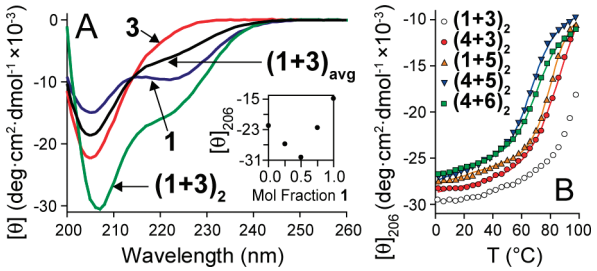

Figure 2. (A) CD spectra of 100 μM **1**, 100 μM **3**, and 50 μM **1** + 50 μM **3** [**(1**+**3)$_2$**] in PBS at 25 °C. **(1** + **3)$_{avg}$** is the average of the CD spectra of **1** alone plus **3** alone. Inset shows a Job plot of $[\theta]_{206}$ for 100 μM solutions differing in relative amounts of **1** and **3**. (B) Variable temperature CD at 100 μM total peptide for tetramers **(1** + **3)$_2$**, **(4** + **3)$_2$**, **(1** + **5)$_2$**, **(4** + **5)$_2$**, and **(4** + **6)$_2$**. Solid lines are fits of the data from which T_m values were obtained.

Analytical ultracentrifugation (AU) of 50 μM **1** + 50 μM **3** indicates a single species with the molecular weight expected for a 2:2 heterotetramer.[10] In contrast, AU results for 100 μM solutions of **1** alone or **3** alone are consistent with monomer−dimer equilibria,

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Table 1. CD Intensities and Melting Temperatures for Tetrameric Helix Bundles Formed by α/β-Peptide Foldamers and α-Peptides

	$[\theta]_{206}$/(deg·cm²·dmol⁻¹)[a]	T_m[b]/° C	
tetramer	100 μM[c]	100 μM[c]	25 μM[c]
(1+3)₂	−30200	>95	>91
(4+3)₂	−29000	>87	69
(1+5)₂	−26900	80	66
(4+5)₂	−26100	67	50
(4+6)₂	−26500	70	58

[a] Data obtained at 25 °C in PBS. [b] T_m for each mixture is the temperature at the midpoint of the unfolding transition shown in Figure 1. [c] Indicated values represent total peptide concentration.

with the monomers predominant in both cases.[10] The heterotetramer **(1+3)₂** is extremely stable; for 50 μM **1** + 50 μM **3**, we observe only the beginning of an unfolding transition at 95 °C (Figure 2B). This transition occurs at lower temperatures when peptide concentration is diminished (Table 1). In contrast, for 100 μM **1**, a partial unfolding transition is observed at low temperature, and 100 μM **3** displays no transition.[10] These results indicate that α-peptide **1** and α/β-peptide **3** combine to form a stable, cooperatively folded helix bundle quaternary structure that is comparable to the heterotetrameric α-helix bundle formed by α-peptides **1** and **2**.[9] It is noteworthy that heterotetramer **(1+3)₂** is fully associated at micromolar concentrations.[2,3,5]

We probed the predicted hydrophobic interface of **(1 + 3)₂** by replacing a single *a* position Leu in α-peptide **1** and in α/β-peptide **3** with Asn to generate α-peptide **4** and α/β-peptide **5**, respectively. Combinations **1**+**5**, **4**+**3**, and **4**+**5** were examined by CD and AU. Each pair forms a cooperatively folded 2:2 heterotetramer[10] that is less stable than **(1+3)₂** (Figure 2B, Table 1). Variable temperature CD data indicate that heterotetramer **(4+5)₂**, which has four Asn substitutions, is less stable than **(4+3)₂** or **(1+5)₂**, which each have two Asn substitutions. This progressive loss of stability upon Leu→Asn mutation is consistent with the energetic penalty expected for burial of the polar Asn side chain at the hydrophobic interface of the heterotetramer.[11] The Leu→Asn effects could arise also, at least in part, from diminished helical propensity of Asn relative to Leu.[12] Addition of NaCl to the buffer leads to an increase in the stability of **(4+5)₂**,[10] which supports the hypothesis that interhelical association is driven primarily by hydrophobic rather than electrostatic interactions. Whether the helices in the tetrameric assembly have a preferred relative orientation is currently under investigation.

Our next test of the design hypothesis involved α/β-peptide **6**, the isomer of **5** in which the four β^2-hLeu residues are replaced by β^3-hLeu. CD and AU data indicate that heterotetramer **(4 + 6)₂** is moderately more stable than **(4 + 5)₂** (Figure 2B, Table 1). This enhancement may arise from more favorable packing of β^3-hLeu relative to that of β^2-hLeu at the helix bundle interface, from a difference in helical propensities, or from a combination of these effects.

The contribution of the cyclically constrained β-amino acid residues to the stability of the heterotetrameric assembly was evaluated with α/β-peptide **7**, in which the six cationic APC residues of **5** are replaced with acyclic cationic β^3-homolysine residues. CD data for the 1:1 mixture of **4** and **7** indicate substantially diminished helicity relative to all other α-peptide + α/β-peptide pairs.[10] AU analysis of 50 μM **4** + 50 μM **7** suggests multiple species, with monomers predominant.[10] These results imply that backbone preorganization due to cyclic β-residues is critical for stability of the heterogeneous quaternary assemblies.

We have provided the first evidence that helices formed by different types of oligomeric backbones can associate to form discrete heterogeneous assemblies.[13] Our results suggest that the intimate packing of secondary structural elements that underlies tertiary and quaternary structure in proteins does not require that the subunits all have the same backbone. These observations highlight the prospect that many combinations of foldamer and α-peptide subunits, or of different types of foldamers, could lead to folding and/or assembly behavior reminiscent of that necessary for complex function among proteins. Such heterogeneous assemblies could combine functional mimicry of a natural protein, conferred by α-peptide components, with the high conformational and metabolic stability provided by foldameric components.

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Supporting Information Available: Supplementary CD and AU data, and experimental methods. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Gellman, S. H. *Acc. Chem. Res.* **1998**, *31*, 173−180. (b) Kirshenbaum, K.; Zuckermann, R. N.; Dill, K. A. *Curr. Opin. Struct. Biol.* **1999**, *9*, 530−535. (c) Hill, D. J.; Mio, M. J.; Prince, R. B.; Hughes, T. S.; Moore, J. S. *Chem. Rev.* **2001**, *101*, 3893−4011.

(2) β-Peptide helix bundles: (a) Raguse, T. L.; Lai, J. R.; LePlae, P. R.; Gellman, S. H. *Org. Lett.* **2001**, *3*, 3963−3966. (b) Cheng, R. P.; DeGrado, W. F. *J. Am. Chem. Soc.* **2002**, *124*, 11564−11565. (c) Qiu, J. X.; Petersson, E. J.; Matthews, E. E.; Schepartz, A. *J. Am. Chem. Soc.* **2006**, *128*, 11338−11339. (d) Daniels, D. S.; Petersson, E. J.; Qiu, J. X.; Schepartz, A. *J. Am. Chem. Soc.* **2007**, *129*, 1532−1533.

(3) Peptoid helix bundles: (a) Burkoth, T. S.; Beausoleil, E.; Kaur, S.; Tang, D.; Cohen, F. E.; Zuckermann, R. N. *Chem. Biol.* **2002**, *9*, 647−654. (b) Lee, B.-C.; Zuckermann, R. N.; Dill, K. A. *J. Am. Chem. Soc.* **2005**, *127*, 10999−11009.

(4) Delsuc, N.; Leger, J.-M.; Massip, S.; Huc, I. *Angew. Chem., Int. Ed.* **2007**, *46*, 214−217.

(5) Horne, W. S.; Price, J. L.; Keck, J. L.; Gellman, S. H. *J. Am. Chem. Soc.* **2007**, *129*, 4178−4180.

(6) For leading references, see: (a) Woolfson, D. N. *Adv. Protein Chem.* **2005**, *70*, 79−112. (b) Lupas, A. N.; Gruber, M. *Adv. Protein Chem.* **2005**, *70*, 37−78.

(7) For a stereochemically heterogeneous helix bundle formed from α-helical α-peptides, see: Sia, S. K.; Kim, P. S. *Biochemistry* **2001**, *40*, 8981−8989.

(8) (a) De Pol, S.; Zorn, C.; Klein, C. D.; Zerbe, O.; Reiser, O. *Angew. Chem., Int. Ed.* **2004**, *43*, 511−514. (b) Hayen, A.; Schmitt, M. A.; Ngassa, F. N.; Thomasson, K. A.; Gellman, S. H. *Angew. Chem., Int. Ed.* **2004**, *43*, 505−510. (c) Schmitt, M. A.; Weisblum, B.; Gellman, S. H. *J. Am. Chem. Soc.* **2004**, *126*, 6848−6849. (d) Sharma, G. V. M.; Nagendar, P.; Jayaprakash, P.; Krishna, P. R.; Ramakrishna, K. V. S.; Kunwar, A. C. *Angew. Chem., Int. Ed.* **2005**, *44*, 5878−5882. (e) Schmitt, M. A.; Choi, S. H.; Guzei, I. A.; Gellman, S. H. *J. Am. Chem. Soc.* **2005**, *127*, 13130−13131. (f) Baldauf, C.; Gunther, R.; Hofmann, H. J. *Biopolymers* **2006**, *84*, 408−413. (g) Srinivasulu, G.; Kumar, S. K.; Sharma, G. V. M.; Kunwar, A. C. *J. Org. Chem.* **2006**, *71*, 8395−8400. (h) Seebach, D.; Jaun, B.; Sebesta, R.; Mathad, R. I.; Flögel, O.; Limbach, M.; Sellner, H.; Cottens, S. *Helv. Chim. Acta* **2006**, *89*, 1801−1825. (i) Jagadeesh, B.; Prabhakar, A.; Sarma, G. D.; Chandrasekhar, S.; Chandrashekar, G.; Reddy, M. S.; Jagannadh, B. *Chem. Commun.* **2007**, 371−373. (j) Schmitt, M. A.; Weisblum, B.; Gellman, S. H. *J. Am. Chem. Soc.* **2007**, *129*, 417−428.

(9) (a) O'Shea, E. K.; Lumb, K. J.; Kim, P. S. *Curr. Biol.* **1993**, *3*, 658−667. (b) Lumb, K. J.; Kim, P. S. *Biochemistry* **1995**, *34*, 8642−8648.

(10) Relevant data may be found in the Supporting Information.

(11) (a) Harbury, P. B.; Zhang, T.; Kim, P. S.; Alber, T. *Science* **1993**, *262*, 1401−1407. (b) Akey, D. L.; Malashkevich, V. N.; Kim, P. S. *Biochemistry* **2001**, *40*, 6352−6360.

(12) Munoz, V.; Serrano, L. *Proteins: Struct., Funct., Genet.* **1994**, *20*, 301−311.

(13) A reviewer suggested that complexes between foldamer ligands and proteins represent a type of heterogeneous quaternary structure. For examples, see: (a) Werder, M.; Hauser, H.; Abele, S.; Seebach, D. *Helv. Chim. Acta* **1999**, *82*, 1774−1783. (b) Kritzer, J. A.; Lear, J. D.; Hodsdon, M. E.; Schepartz, A. *J. Am. Chem. Soc.* **2004**, *126*, 9468−9469. (c) Sadowsky, J. D.; Schmitt, M. A.; Lee, H.-S.; Umezawa, N.; Wang, S.; Tomita, Y.; Gellman, S. H. *J. Am. Chem. Soc.* **2005**, *127*, 11966−11968.

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