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COMMUNICATIONS

Published on Web 03/16/2007

Helix Bundle Quaternary Structure from α/β-Peptide Foldamers

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Extensive effort over the past decade has identified many families of non-natural oligomers ("foldamers") that display a variety of specific secondary structures.[1] There has been considerable interest in developing foldamers that display higher levels of organization, tertiary and quaternary structure, but progress toward this goal has been limited.[2−4] Here we describe helix bundle quaternary structures formed by oligomers containing a mixture of *β*- and α-amino acid residues ("α/*β*-peptides") in aqueous solution and in the crystalline state.

Previous design efforts toward foldamer quaternary structure can be characterized as "bottom-up" approaches in which a known foldamer helix is used in the de novo design of a primary sequence of non-natural building blocks anticipated to result in segregation of hydrophilic and lipophilic side chains in the helical conformation. Self-association of such globally amphiphilic helices in aqueous solution can be driven by burial of hydrophobic side chains, as occurs when natural proteins form helix bundle tertiary or quaternary structure.[5] We undertook an alternative "top-down" approach in which a previously characterized α-amino acid sequence of known secondary and quaternary structure is used directly in the design of hybrid α/*β*-peptides intended to form helix bundles.

We selected a natural α-amino acid sequence that has a high propensity for self-association in the α-helical conformation, the dimerization domain of yeast transcriptional regulator GCN4,[6] and replaced a subset of the α-residues with *β*-residues. Oligomers containing regular alternation of α- and *β*-residues have recently been shown to adopt helical secondary structures,[7] but there has been no prior report of higher order structure among α/*β*-peptides.[8] Our results show that selective α→*β* replacements in GCN4 lead to hybrid α/*β*-peptides that retain the ability to form a self-complementary recognition surface in the helical conformation, an essential behavior of the natural sequences. The *β*-residue substitutions are not without consequence, however; changes are observed in the solution behavior (stability and stoichiometry of the complex) and in the helix association geometry of the hybrid α/*β*-peptides relative to the parent α-peptide sequences.

GCN4-p1, a well-studied 33-residue segment of GCN4, adopts a dimeric parallel α-helical coiled-coil quaternary structure in solution and the crystalline state.[6] Formation of this dimer is driven by burial of hydrophobic surfaces that extend along the length of each α-helix.[9] It has been shown that modifications to the GCN4-p1 sequence can change the stoichiometry of self-association, leading to helix bundles with up to seven components.[10] We introduced *β*-residues systematically throughout the primary sequence of GCN4-derived peptides using the heptad repeat of the α-helix as the template for substitution (Figure 1). The original side chain was retained at each site of α→*β* modification through use of β^3-residues (Figure 1A). We hypothesized that the additional backbone methylene units introduced upon α→*β* substitution would not alter the heptad repeat of the side chains along the helical axis. Furthermore, we reasoned that placing the *β*-residue substitutions

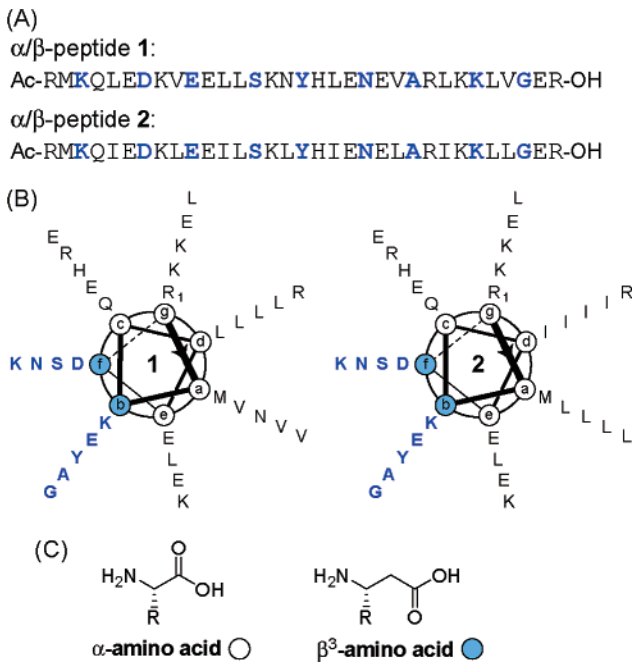

Figure 1. (A) Primary sequences and (B) helical wheel diagrams of α/*β*-peptides **1** and **2**. Blue letters in (A) and (B) indicate positions occupied by β^3-amino acid residues. (C) Structures of an α-amino acid and of a β^3-amino acid.

at positions distal to the hydrophobic surface, in the helical conformation, would minimize perturbation of the side chain array that forms the interhelical interface (Figure 1C). This design feature was intended to maximize the likelihood that the resulting α/*β*-peptide would retain, in some form, the folding and self-assembly tendencies of the original α-sequence.

α/*β*-Peptide **1** has the side chain sequence of GCN4-p1 but has β^3-residues incorporated at all *b* and *f* heptad positions (9 of the 33 residues are *β*). Circular dichroism (CD) analysis suggests that little helix formation occurs for 100 μM peptide **1** in aqueous solution (Figure S1), and analytical ultracentrifugation (AU) measurements indicate that the α/*β*-peptide is monomeric at 200 μM (Figure S2). Despite the solution behavior at low concentration, α/*β*-peptide **1** could be crystallized at higher concentrations. X-ray diffraction data were collected to 2.0 Å resolution, and the structure was solved by molecular replacement and geometrically restrained refinement (Figure 2, Table S1). α/*β*-Peptide **1** forms a parallel helix bundle in the crystal (Figure 2A), but each bundle contains three α/*β*-peptide molecules, whereas GCN4-p1 crystallizes as a dimer.[6b] Switching from dimeric to trimeric assembly is well-precedented among α-peptide GCN4-p1 variants: mutation of Asn_{16} to Val, Gln, Thr, or Ser induces formation of three-helix bundles.[10a,11] Overall, we can conclude that although the multiple α→*β* replace-

10.1021/ja070396f CCC: $37.00

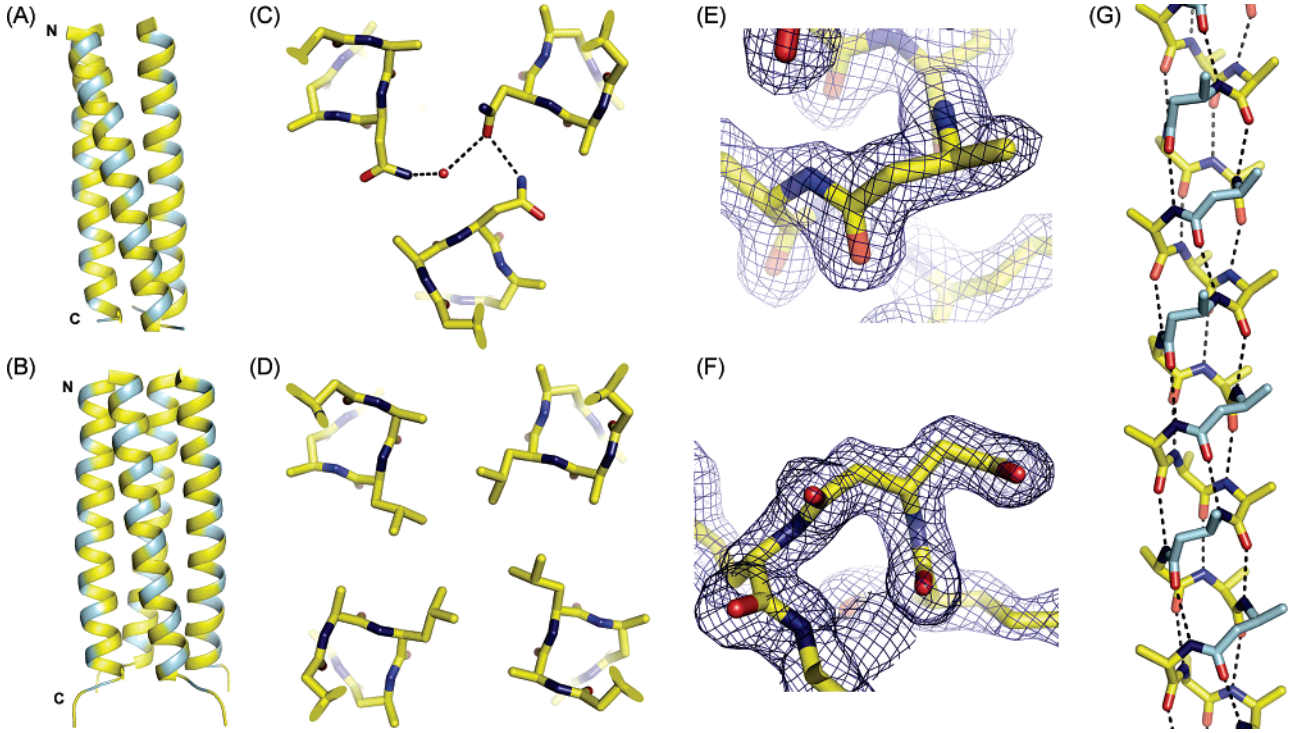

Figure 2. Crystal structures of α/*β*-peptides **1** (PDB: 2OXJ) and **2** (PDB: 2OXK). (A and B) Cartoon representations of the quaternary folds of (A) **1** and (B) **2** with *β*-residues colored cyan; (C and D) top-down views of core residues (C) Asn_{16} in the coiled-coil trimer of **1** and (D) Leu_{16} in the coiled-coil tetramer of **2**; (E and F) views of a σ_a weighted $2F_o-F_c$ electron density map contoured at 1.1σ around (E) β^3-$hAla_{24}$ in **2** and (F) β^3-$hAsn_{21}$ in chain A of **1**; (G) view of the backbone hydrogen bonding in **2** with *β*-residue carbons colored cyan. Some side chain atoms are omitted for clarity.

ments in **1** significantly diminish the drive for and alter the preferred stoichiometry of self-association relative to GCN4-p1, the α/*β*-peptide nevertheless retains the ability to form a discrete helix bundle quaternary structure.

Structural comparison of the GCN4-p1 dimer with the trimer formed by **1** suggests a rationale for the low self-association propensity displayed by **1** in solution. In both structures, Asn_{16} provides the only polar side chain that is situated at the hydrophobic interhelical interface. In GCN4-p1, a hydrogen bond is formed between the amide groups of the two Asn_{16} side chains.[6b] In **1**, three Asn_{16} side chains are juxtaposed, but only one hydrogen bond is formed (Figure 2C). The remaining Asn_{16} side chain projects away from the interface, creating a small hydrophilic cavity that is occupied by a water molecule (Figure S3), which is presumably unfavorable. We therefore prepared a second α/*β*-peptide that was expected to fold and assemble around a purely hydrophobic interface.

Elegant GCN4-p1 engineering efforts have revealed that placing Leu residues at most *a* heptad positions and Ile at most *d* heptad positions generates a sequence (GCN4-pLI) that forms a very stable four-helix bundle quaternary structure.[10a] We prepared α/*β*-peptide analogue **2**, which has a backbone substitution pattern identical to **1** (α→*β* modifications at every *b* and *f* position) and the primary side chain sequence of GCN4-pLI. CD analysis of 25 μM **2** revealed a very strong minimum at 207 nm, and little change in this minimum was observed when the sample was heated, suggesting that the folded form is very stable (Figure S1). Comparably high thermal stability has been reported for the four-helix bundle formed by GCN4-pLI.[10a] AU analysis indicated self-association of α/*β*-peptide **2** in aqueous buffer, and global fitting to data obtained for three concentrations (100, 200, and 300 μM) indicated a trimeric species in solution (Figure S4). In contrast to the association state indicated by AU, a crystal structure obtained for **2** at 2.0 Å resolution reveals a helix bundle quaternary structure comprised of four molecules, in parallel orientation (Figure 2B). The asymmetric unit consists of a single molecule, and the remaining three copies in the tetramer are related to the first by a crystallographic 4-fold symmetry axis. The folded structure appears to be stabilized by close packing of hydrophobic side chains in the core (Figure 2D) as well as by several favorable interactions among polar residues at the periphery of the assembly (Figure S5).

The electron density maps derived from the final refined structures of α/*β*-peptides **1** and **2** at 2.0 Å resolution clearly indicate the path of the *β*-residues along the helical backbone (Figure 2E,F). In both **1** and **2**, the *i*→*i*+4 backbone hydrogen bonding pattern is maintained (Figure 2G) regardless of the residue type that provides the carbonyl oxygen or amide hydrogen (i.e., α→α, α→*β*, *β*→α, and *β*→*β* C═O···H−N hydrogen bonds are observed). Qualitative and quantitative structural comparisons among the foldamers and analogous GCN4-based α-peptides (Figure 3, Table 1) proved informative with respect to the effects of *β*-residue backbone substitution on secondary and quaternary structure. Excellent overlap is observed at the level of individual helices (Figure 3A); despite the extra carbon atom in their backbone, the *β*-residues do not substantially alter the trace of the foldamer helix relative to that of an α-helix (Figure 3D,E). These qualitative similarities in secondary structure are borne out quantitatively by the calculated parameters defining the helical secondary structures (Table 1). Specifically, the helical pitch (described by residues per turn and rise per residue) of each α/*β*-peptide helix is almost identical to that of an α-helix, and the foldamer helical radius is only slightly larger (0.15 Å). In contrast to the similarities in secondary structure, significant differences between the α- and α/*β*-peptides are evident in the helix bundle quaternary structures (Figure 3B,C). Specifically, the packing of helices appears subtly altered, leading to a decrease in the interhelical crossing angle between helices in the foldamer

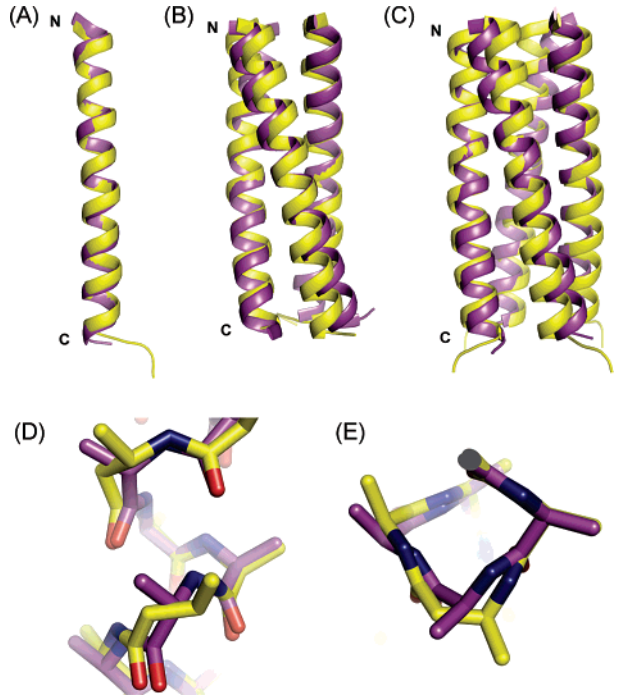

Figure 3. Comparison of secondary and quaternary structure among α/*β*-peptides **1** (PDB: 2OXJ) and **2** (PDB: 2OXK) and analogous α-peptides GCN4-p1-N_{16}→T (PDB: 1IJ2)[11] and GCN4-pLI (PDB: 1GCL).[10a] (A) Overlay of an individual helix from **2** with an α-helix from GCN4-pLI (rmsd = 0.62 Å for C_α atoms of α-residues); (B) overlay of the helix bundle quaternary structures of **1** with that of GCN4-p1-N_{16}→T and (C) **2** with that of GCN4-pLI; (D and E) close up views contrasting the backbone conformations adopted by *β*-residues in **2** with those of the α-residues they replace. The color scheme in all panels is yellow for hybrid α/*β*-peptides and magenta for α-peptides.

Table 1. Calculated Helical and Superhelical Parameters of α/*β*-Peptides **1** and **2** in the Crystalline State Compared to α-Helical Coiled-Coils of Analogous Side Chain Sequence[a]

	GCN4-p1-N_{16}→T[b]	**1**	GCN4-pLI[b]	**2**
Single Helix Parameters				
residues per turn	3.63	3.58	3.58	3.57
rise per residue (Å)	1.50	1.49	1.53	1.49
radius (Å)	2.30	2.44	2.25	2.42
Superhelix Parameters				
association state	trimer	trimer	tetramer	tetramer
supercoil radius (Å)	6.32	6.48	7.13	7.73
residues per supercoil turn	97	164	129	219
supercoil pitch (Å)	139	240	193	323
crossing angle (°)	32	19	26	17

[a] The definitions of the helical and superhelical parameters were described previously[12] and were calculated using the program TWISTER.[13] [b] Parameters for GCN4-p1-N_{16}→T (PDB: 1IJ2)[11] and GCN4-pLI (PDB: 1GCL)[10a] were calculated from published coordinates.

bundles relative to those of α-helix bundles of identical association state (Table 1).

In summary, we have introduced a new design strategy for generating foldamers that form discrete quaternary structure, and we have obtained some of the first high-resolution structural data for foldameric helix bundle assemblies.[14] Our selective and systematic α-residue to *β*-residue replacements in GCN4-p1 and GCN4-pLI caused significant changes in physical behavior relative to the original α-sequences, and so it is clear that altering the backbone while retaining the side chain sequence does not lead to completely faithful mimicry of the α-peptide prototype. Nevertheless, the resulting α/*β*-peptides display considerable structural homology to α-helices and an interesting property, formation of discrete quaternary structures, which represents a significant advance in foldamer science. We suggest that the top-down design approach described here will provide a general framework for development of diverse heterogeneous backbone foldamers with a wide array of activities that emerge from the adoption of specific high-order structure, by harnessing information embedded in natural α-amino acid sequences that display those structures and activities.

Acknowledgment. This work was supported by the NIH. W.S.H. was supported, in part, by an NIH postdoctoral fellowship (CA119875). We thank Dr. Jack Sadowsky for helpful discussions, Dr. Darrell McCaslin for assistance with AU experiments, Prof. Sergei Strelkov for providing the software used for superhelical parameter analysis, and PepTech for providing Fmoc-protected β^3-amino acids at a discounted price. Some experiments related to this work were carried out at the Advanced Photon Source, and we thank the staff of beamline 14-BMC (BioCARS) for their assistance.

Supporting Information Available: Figures S1−S5, Table S1, and experimental methods. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA070396F