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An α/β -Peptide Helix Bundle with a Pure β^3 -Amino Acid Core and a Distinctive Quaternary Structure

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The connection between function and folding among proteins has inspired a growing number of efforts to identify unnatural oligomers that adopt discrete tertiary and/or quaternary structures.¹ Recently we have shown that modification of a self-assembling α -amino acid sequence by systematic replacement of some α -residues with analogous β^3 -amino acid residues (identical side chains) can generate α/β -peptide “foldamers” that display protein-like helix-bundle quaternary structure. The designs reported thus far have placed the β^3 -residues mostly or entirely on the quaternary structure periphery.² Here we describe a new α/β -peptide that forms a helix bundle with a hydrophobic core comprised exclusively of β^3 -amino acid residues; this unique quaternary structure displays unprecedented features.

Much of our previous work on α/β -peptide helix bundles has focused on the dimerization domain of yeast transcription factor GCN4 and an engineered variant designated GCN4-pLI.³ The wild type sequence encodes a parallel coiled-coil dimer, while GCN4-pLI forms a parallel helix-bundle tetramer. The sequence of GCN4-pLI features a typical *abcdefg* heptad repeat pattern, with hydrophobic residues at the *a* and *d* positions (Leu and Ile respectively; Figure 1a,c). The *a* and *d* side chains align upon folding, resulting in an amphipathic α -helix.⁴ Burial of hydrophobic side chains provides the driving force for assembly. In the new α/β -peptide **β ad**, residues at all *a* and *d* positions of GCN4-pLI have been replaced with the homologous β^3 residues (e.g., Leu $\rightarrow\beta^3$ -hLeu, Ile $\rightarrow\beta^3$ -hIle; Figure 1).

Circular dichroism (CD) data for 5, 10, 25, and 100 μ M **β ad** in aqueous buffer show a strong minimum at 207 nm, which is consistent with extensive α/β -peptide helicity.⁵ Little change in CD intensity occurs upon heating to 98°C or dilution from 100 μ M to 5 μ M, suggesting a very stable assembly. Analytical ultracentrifugation (AU) data for 200 μ M **β ad** at 25°C are consistent with a tetrameric species. We crystallized **β ad** and solved the structure to gain insight on the tetramer assembly.

β ad forms a four-helix bundle in the crystalline state; the hydrophobic core is comprised entirely of β^3 -residues (Figure 2). The conformation of each **β ad** molecule closely mimics an α -helix, as illustrated by the overlay with GCN4-pLI (Figure 3a,b); **β ad** retains the $i\rightarrow i+4$ C=O--H-N hydrogen bonding pattern that is characteristic of the α -helix. Despite the similarities between **β ad** and α -peptide GCN4-pLI in terms of stoichiometry and helical secondary structure, the quaternary structures are quite dissimilar. Neighboring helices are antiparallel in the **β ad** tetramer, while all α -helices are parallel in the GCN4-pLI tetramer. Previously, only parallel orientations have been observed in α/β -peptide helix bundles.^{2a,c,3} Furthermore, the hydrophobic packing arrangement within the core of the **β ad** tetramer has no precedent among known proteins.

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Supporting Information Available: Coordinates and structure factors for **β ad** were deposited in the PDB with ID codes 3F86 and 3F87. Experimental protocols, biophysical data, and crystallographic statistics are available free of charge online at <http://pubs.acs.org>

Two of the antiparallel helix pairings within the **βad** tetramer involve very close backbone contacts (Figure 3c), which results in an unusual rectangular arrangement of the four subunits about the helix bundle axis. In contrast, a more symmetrical (square) arrangement about the helix bundle axis is typical of both parallel and antiparallel coiled-coils (Figure 3c,d).⁶ The closely interacting **βad** helices have an 8.1 Å separation (center-to-center); the other interhelical separation is 13.2 Å. In contrast, a typical α -helix tetramer displays uniform 10-11 Å interhelical separation.^{5,6} The short interhelical distance in **βad** is a result of a “stripe” of backbone methylene groups that is created by alignment of β^3 -hLeu residues (*a* positions) along the helical axis. The backbone-backbone interactions between close-packed helix pairs cause their *a* and *d* side chains to generate a relatively flat hydrophobic surface. Packing of two of these flat surfaces against one another leads to tetramer formation. Thus, the core side chain arrangement in the **βad** tetramer is quite different from the “knobs-into-holes” packing that is characteristic α -helical coiled-coil quaternary structures (Figure 3c,d)^{7a,b} and previously reported α/β -peptide helix bundles (which have mostly or entirely α -residues in their cores).

The backbone methylene stripes displayed by helical **βad** molecules represent patches of non-polar surface, and it is possible that burial of these patches stabilizes the **βad** tetramer via a hydrophobic effect. Another structural role is possible as well: formation of multiple C_{α} -H--O=C hydrogen bonds between the close-packed helices. C_{α} -H--O=C hydrogen bonds have been proposed to play a role in the folding and association of integral membrane proteins, specifically, the dimerization of helical domains that contain a GxxxG motif.⁸ Whether or not these interhelical C_{α} -H--O=C interactions contribute to dimer stability, however, remains a subject of debate.⁹ For each close antiparallel pairing within the **βad** four-helix bundle there are 10 C_{α} -H--O=C interactions (i.e., 20 such interactions per tetramer). The interatomic distances and angles for these interactions are within the parameters proposed based upon membrane protein structural data.^{8b} An extended C_{α} -H--O=C interaction array of the type seen in the **βad** crystal structure would appear to be impossible for α -peptides because of the large crossing angle dictated by GxxxG-mediated α -helix association.⁵

In order to determine whether the unique quaternary structure observed for **βad** requires β^3 -residues at both the *a* and *d* positions of the heptad repeat we examined two isomers, α/β -peptides **βae** and **βdg** (Figure 1a,b). These isomers have the same $\alpha\alpha\beta\alpha\alpha\beta$ backbone pattern and side chain sequence of **βad**, but the locations of the β^3 -residues differ. For **βae**, CD data indicate extensive helix formation at room temperature, but helicity is disrupted at higher temperatures and at concentrations below 25 mM.⁵ AU data suggest that **βae** forms a trimer. Isomer **βdg** is relatively unstructured according to CD data, and AU data suggest indiscrete aggregation.⁵ For a previously reported GCN4-based, α/β -peptide crystal structure^{2b}, we observed that β^3 -substitution significantly altered the orientation of *a* or *d* side chain projection from the helix. Extension of this analysis to *e* and *g* position β^3 -residues reveals comparable effects and may explain why global *a-e* β^3 -substitution of GCN4-pLI allows assembly, while global *d-g* β^3 -substitution nearly abolishes assembly.⁵ We conclude that the placement of β^3 -residues at all the hydrophobic core positions of the GCN4-pLI sequence is necessary for formation of the unique quaternary structure observed for **βad**.

The asymmetry of interaction within the **βad** helix bundle and the face-to-face side-chain packing motif in the **βad** tetramer core are, to our knowledge, unprecedented among naturally occurring or designed α -helical assemblies or among β - or α/β -peptide helix bundles.^{4a,5,10a,b} Although we do not have high-resolution structural information for **βad** in solution, AU and CD data are consistent with the hypothesis that the tetrameric assembly observed in the crystalline state forms in aqueous solution as well, and that this assembly is quite stable. The occurrence of antiparallel helix orientations within the **βad** tetramer raises the exciting prospect that foldamer tertiary structures could be generated by linking helix-forming α/β -peptide segments.¹¹

Supplementary Material

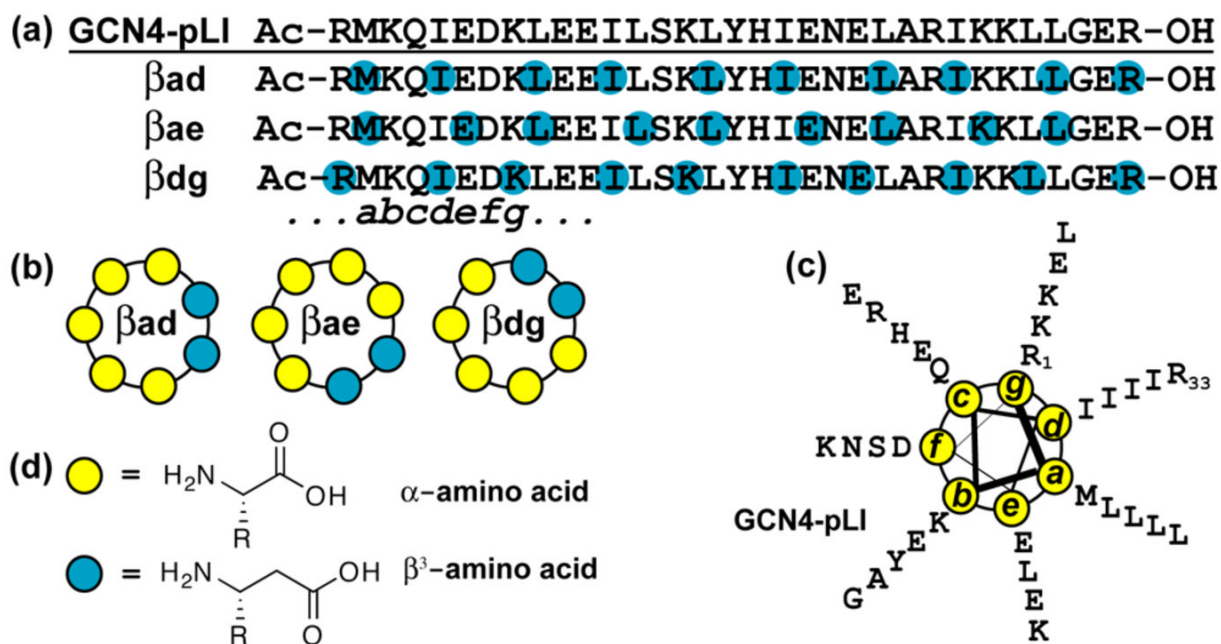
Refer to Web version on PubMed Central for supplementary material.

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**Figure 1.**

(a) Sequences of GCN4-derived α/β -peptides. Heptad positions are shown in italics. (b) Helical wheel diagrams of α/β -peptides β_{ad} , β_{ae} , and β_{dg} . Letters refer to substituted heptad positions. (c) Helical wheel diagram of the GCN4-pLI sequence. (d) Structures of an α -amino acid and a β^3 -amino acid.

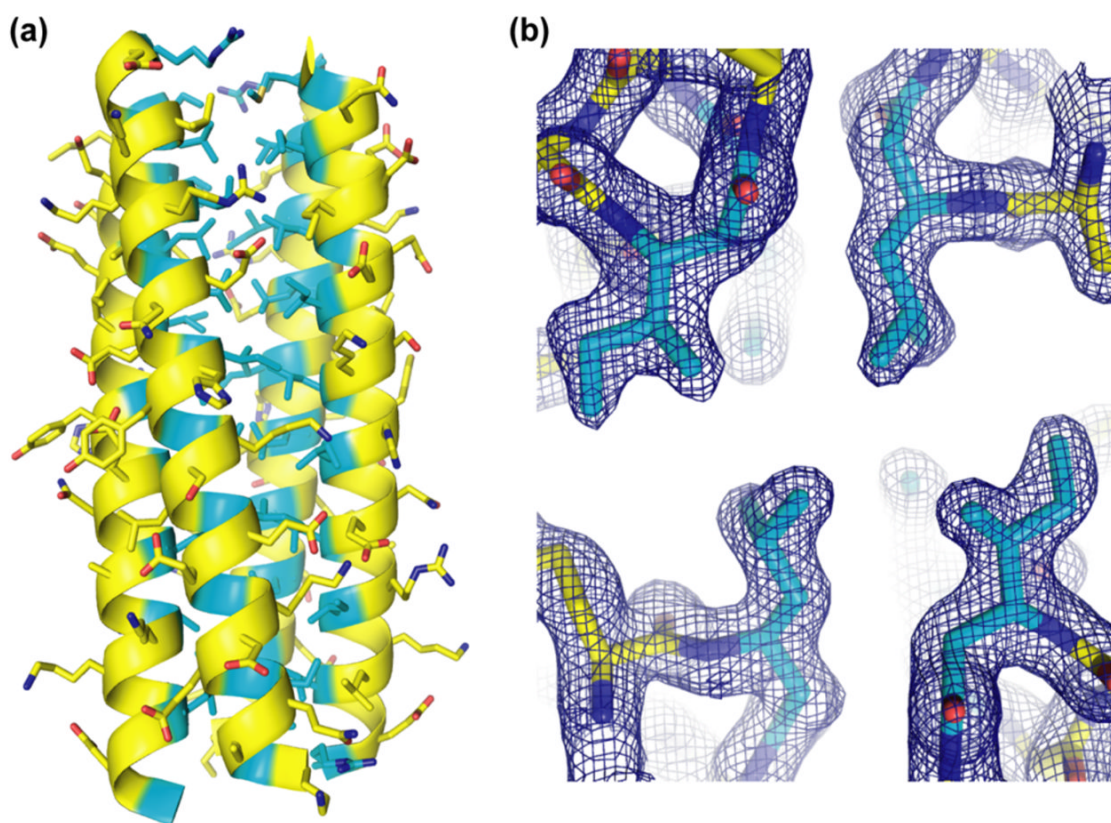


Figure 2.
(a) 2.0 Å x-ray crystal structure of α/β -peptide β ad shown as cartoon helices displaying amino acid side chains (PDB code: 3F86). β^3 -amino acids are shown in cyan, α -amino acids are shown in yellow. (b) Single layer of β ad hydrophobic core residues fit into $2F_o - F_c$ electron density at a map level of 1.2σ .

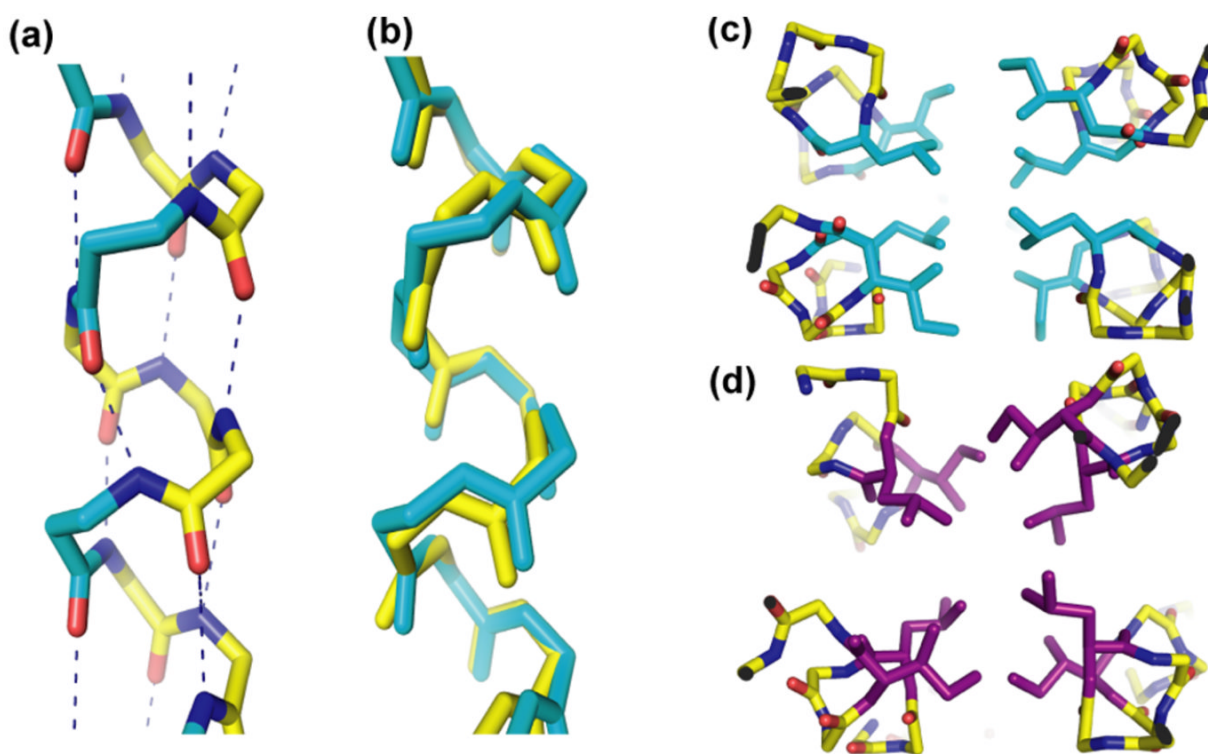
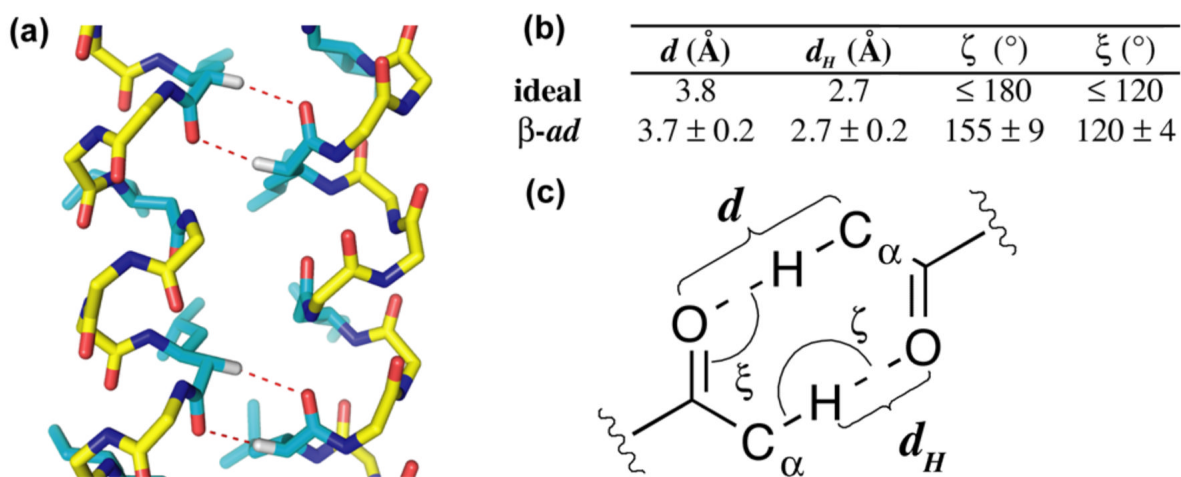


Figure 3.

(a) i to $i+4$ hydrogen bonding in β ad. β^3 -amino acids are shown in cyan. (b) Overlay of β ad (cyan) with GCN4-pLI (yellow). C_{α} RMSD for residues 4-30 = 0.55 Å. (c) and (d) Hydrophobic cores of β ad and an antiparallel GCN4-pLI derivative (PDB: 2CCF),⁵ respectively. β^3 -amino acids are shown in cyan, α -residues are shown in purple.

**Figure 4.**

(a) Side view of close-packed helices with potential C_α -H-O hydrogen bonds shown as red dashes. (b) Comparison of idealized C_α -H-O hydrogen bond parameters with average values calculated from the crystal structure of β ad. (c) Diagram defining the geometric parameters of the C_α -H-O hydrogen bond.⁹