J|A|C|S COMMUNICATIONS

Published on Web 07/06/2009

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

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Received December 19, 2008; E-mail: gellman@chem.wisc.edu

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 showed 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 to date have placed the β^3-residues mostly or entirely on the periphery of the quaternary structure.[2] Here we describe a new α/β-peptide that forms a helix bundle with a hydrophobic core composed 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 as 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 of the *a* and *d* positions in GCN4-pLI have been replaced with the homologous β^3 residues (i.e., Leu → β^3-hLeu, Ile → β^3-hIle; Figure 1).

Circular dichroism (CD) data for 5, 10, 25, and 100 μM ***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 the CD intensity occurs upon heating to 98 °C or dilution from 100 to 5 μM, suggesting a very stable assembly. Analytical ultracentrifugation (AU) data for 200 μM ***β*ad** at 25 °C are consistent with a tetrameric species.[5] We crystallized ***β*ad** and solved the structure to gain insight into the tetramer assembly.

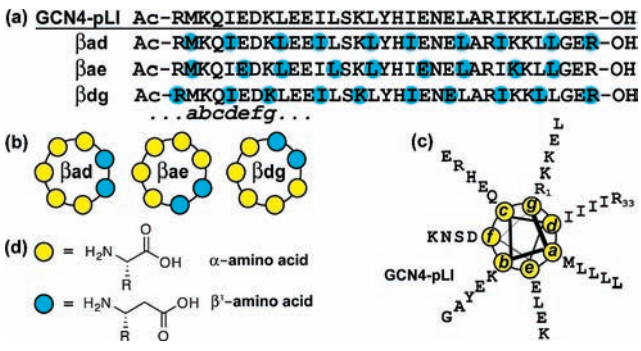

Figure 1. (a) Sequences of GCN4-derived α/β-peptides. Heptad positions are shown in italics. (b) Helical wheel diagrams of α/β-peptides ***β*ad**, ***β*ae**, and ***β*dg**. Letters refer to substituted heptad positions (orientation as in (c)). (c) Helical wheel diagram of the GCN4-pLI sequence. (d) Structures of an α-amino acid and a β^3-amino acid.

***β*ad** forms a four-helix bundle in the crystalline state; the hydrophobic core is composed 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, whereas all of the 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.

Two of the antiparallel helix pairings within the ***β*ad** tetramer involve very close backbone contacts (Figure 3c), resulting 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 ***β*ad** helices have a center-to-center separation of 8.1 Å; the other interhelical separation is 13.2 Å. In contrast, a typical α-helix tetramer displays a uniform 10−11 Å interhelical separation.[5,6] The short interhelical distance in ***β*ad** 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 ***β*ad**

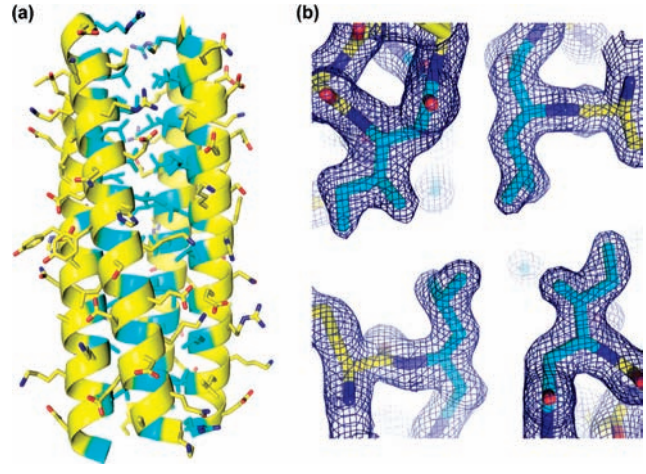

Figure 2. (a) X-ray crystal structure (2.0 Å) of α/β-peptide ***β*ad** (PDB code 3F86) shown as cartoon helices displaying amino acid side chains. β^3-Amino acids are shown in cyan and α-amino acids in yellow. (b) Single layer of ***β*ad** hydrophobic core residues fit into the $2F_O - F_C$ electron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

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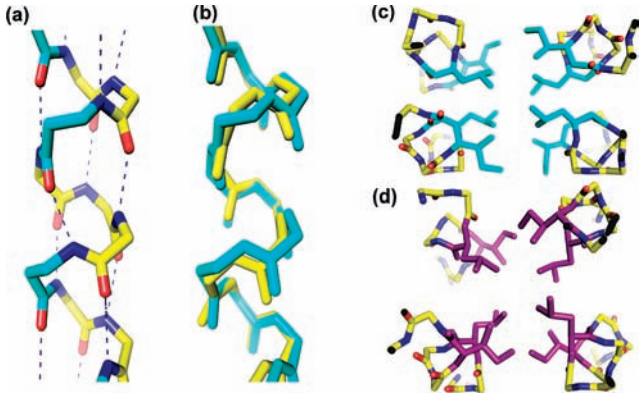

Figure 3. (a) Illustration of the $i \rightarrow i + 4$ hydrogen bonding in ***β*ad**. β^3-Amino acids are shown in cyan. (b) Overlay of ***β*ad** (cyan) with GCN4-pLI (yellow). The C_α rmsd for residues 4−30 (including β^3-residues) is 0.55 Å. (c, d) Hydrophobic cores of ***β*ad** and an antiparallel GCN4-pLI derivative (PDB code 2CCF),[5] respectively. β^3-Amino acids are shown in cyan and core α-residues are shown in purple.

tetramer is quite different from the "knobs-into-holes" packing characteristic of α-helical coiled-coil quaternary structures (Figure 3c,d)[7a,b] and previously reported α/β-peptide helix bundles (whose cores consist mostly or entirely of α-residues).

The backbone methylene stripes displayed by helical ***β*ad** molecules represent patches of nonpolar surface, and it is possible that burial of these patches stabilizes the ***β*ad** 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 (Figure 4). 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 ***β*ad** four-helix bundle, there are 10 C_α−H···O═C interactions (i.e., there are 20 such interactions per tetramer). The interatomic distances and angles for these interactions (Figure 4b) are within the range of parameters proposed on the basis of membrane protein structural data.[8b] An extended C_α−H···O═C interaction array of the type seen in the ***β*ad** 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 ***β*ad** requires β^3-residues at both the *a* and *d* positions of the heptad repeat, we examined two isomers, α/β- peptides ***β*ae** and ***β*dg** (Figure 1a,b). These isomers have the same ααβααααβ backbone pattern and side-chain sequence of ***β*ad**, but the locations of the β^3-residues differ. CD data for ***β*ae** indicate extensive helix

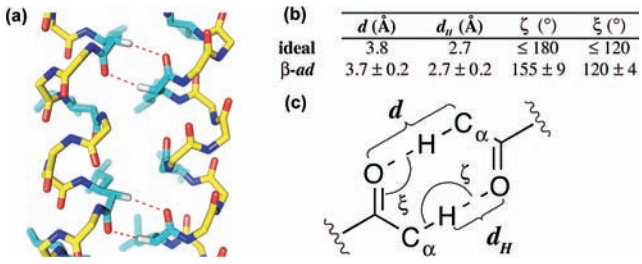

Figure 4. (a) Side view of close-packed helices with potential C_α−H···O hydrogen bonds, which are shown as red dashes. (b) Comparison of idealized C_α−H···O hydrogen-bond parameters with average values calculated from the crystal structure of ***β*ad**. (c) Diagram defining the geometric parameters of the C_α−H···O hydrogen bond.[9]

formation at room temperature. However, folding is disrupted at higher temperatures and at concentrations below 25 μM.[5] The AU data suggest that ***β*ae** forms a trimer. Isomer ***β*dg** is relatively unstructured according to the CD data, and the AU data suggest indiscrete aggregation.[5] For a previously reported GCN4-based α/β-peptide crystal structure,[2b] we observed that β^3-subsitution 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 of the hydrophobic core positions of the GCN4-pLI sequence is necessary for formation of the unique quaternary structure observed for ***β*ad**.

The asymmetry of interaction within the ***β*ad** helix bundle and the "face-to-face" side-chain packing motif in the ***β*ad** 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 ***β*ad** in solution, the 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 ***β*ad** tetramer raises the exciting prospect that foldamer tertiary structures could be generated by linking helix-forming α/β-peptide segments.[11]

Acknowledgment. This research was supported by NIH Grant GM61238. M.W.G. was supported in part by the UW-Madison NSEC (NSF DMR-0425880), and W.S.H. was supported in part by an NIH fellowship (CA119875). We thank Professors J. Keck and K. Forest for the use of X-ray crystallography facilities, Dr. D. McCaslin for assistance with the AU experiments, and Peptech for providing discounted Fmoc-β^3-amino acids.

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

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JA8099294