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COMMUNICATIONS

Published on Web 03/22/2006

New Helical Foldamers: Heterogeneous Backbones with 1:2 and 2:1 α:*β*-Amino Acid Residue Patterns

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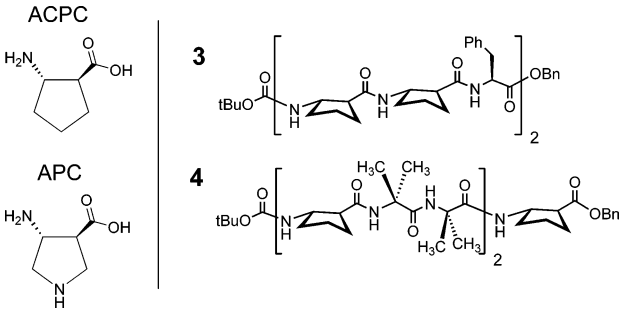
Specific folding of protein backbones creates specific side-chain arrangements that lead to complex molecular activities. The relationship between biopolymer function and conformation has inspired many chemists to seek unnatural oligomers with strong folding propensities ("foldamers"), which provide a new basis for creating useful molecules.[1] Foldamer design strategies that depart from the specific architectural features of proteins could be particularly valuable. One such strategy is the use of heterogeneous backbones, i.e., backbones that contain subunits of different types.[2] Several groups have recently shown that short oligomers with a 1:1 alternation of α- and *β*-amino acid residues ("α/*β*-peptides") can adopt helical conformations.[3] Cyclic constraints within the *β*-amino acid residues are essential for conformational stability in polar solvents, and the size of the constraining ring determines the type of helix formed.

Here we report an expansion of the heterogeneous α/*β*-peptide family to include backbones with 2:1 and 1:2 α:*β*-amino acid residue repeat patterns. We provide NMR and crystallographic evidence for two new types of foldameric helices, and NMR evidence for two additional helices. These findings demonstrate that a large set of discrete molecular scaffolds can be created by combining monomers from just two classes, which should encourage a broader exploration of heterogeneous backbones. Each new foldamer scaffold offers a unique way to arrange sets of side chains in space; therefore, expanding the scaffold set should expand the range of accessible functions. We have recently illustrated this point by demonstrating the superiority of a 1:1 α/*β*-peptide helix scaffold relative to *β*-peptide helix scaffolds for mimicry of a protein interaction domain.[4]

We adopted a systematic approach to searching for foldamers with 2:1 or 1:2 α:*β*-amino acid residue patterns, based on previous experience in our laboratory.[3b−d] (1) We focused on combinations of α-amino acid residues and cyclically rigidified *β*-amino acid residues. The former provide readily accessible side-chain diversity, and the latter are intended to confer strong conformational propensities. (2) We examined short oligomers (≤10 residues) because secondary structures with the level of stability we seek will be manifested at this length. (3) Our conformational evaluation was conducted in methanol, which is sufficiently polar that conformations of short oligomers stabilized primarily by hydrogen bonds will not be appreciably populated. (4) We used 2D NMR as the primary probe for folding; NOEs between protons on residues that are not adjacent in sequence provide very strong evidence for folding.

For both 1:2 and 2:1 α/*β*-peptide backbones, we explored four specific variations, containing *β*-residues constrained by either a five-membered ring ((*1S*,*2S*)-ACPC or heterocyclic analogue APC) or a six-membered ring and either L- or D-α-residues. Among these eight backbone variants, five showed suggestive NOEs. Thus, the 1:2 and 2:1 α/*β*-peptide families seem to offer richer sources of new foldamers than did the analogous 1:1 α/*β*-peptides, in which



only one of the four variants folded.[3b] To date we have focused on two of the backbone variants with promising behavior, the 1:2 and 2:1 α/*β*-peptides containing (*1S*,*2S*)-ACPC and L-α-residues, since this combination supports folding among 1:1 α/*β*-peptides.[3b]

Large networks of *i*,*i*+2 and *i*,*i*+3 NOEs were observed for 1:2 α/*β*-peptide hexamer **1** and for 2:1 α/*β*-peptide heptamer **2** in methanol (Figure 1). In both cases, NOEs were observed even at the termini, which are commonly frayed among α-helical α-peptides,[5] suggesting substantial folded populations among these new α/*β*-peptides. The crystal structures of **3** and **4**, nonpolar analogues of **1** and **2**, display helical conformations that contain *i*,*i*+3 C═O- -H−N H-bonds (Figure 2). Following previous conventions,[1b,3b] we propose the names 11/11/12-helix and 10/11/11-helix, based on H-bond ring sizes.

Six of the 11 nonsequential NOEs observed for 1:2 hexamer **1** in methanol can be mapped onto the crystal state conformation of hexamer **3** (the other five involve terminal groups in **1** that are absent in **3**). The six "mappable" NOEs from **1** fall into four patterns: (i) *β*-residue $C_\beta H(i)$ - - α-residue NH(*i*+2), (ii) *β*-residue $C_\alpha H(i)$ - - *β*-residue NH(*i*+2), (iii) *β*-residue $C_\beta H(i)$ - - *β*-residue NH(*i*+3), and (iv) *β*-residue $C_\beta H(i)$ - - *β*-residue $C_\alpha H$ (*i*+3). For

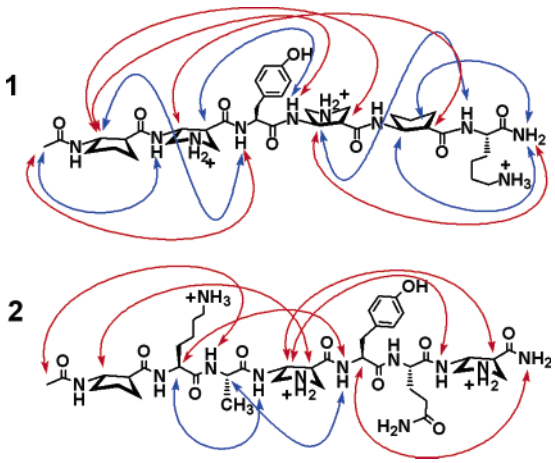


Figure 1. NOEs for oligomers **1** and **2**. Blue lines indicate *i*,*i*+2 NOEs. Red lines indicate *i*,*i*+3 NOEs.

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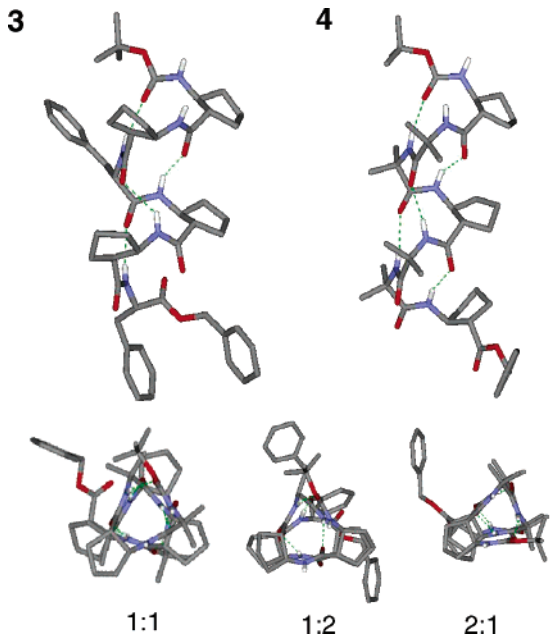

Figure 2. (Top) Crystal state conformations of **3** and **4**. (Bottom) End-on comparisons of a 1:1 α/*β*-octamer,[3d] 1:2 α/*β*-hexamer **3**, 2:1 α/*β*-heptamer **4**. All H atoms except those on the N atoms are omitted.

NOE patterns (i−iii), the corresponding H- -H distances in the crystal structure of **3** are <4 Å; therefore, these three NOE patterns observed for **1** in methanol are consistent with the 11/11/12-helical conformation observed for **3** in the crystal state. NOE pattern (iv), however, corresponds to relatively large H- -H distances in the crystal state, 4.60(2) Å between ACPC-1 and ACPC-4, and 5.13(2) Å between ACPC-2 and ACPC-5. Two additional 1:2 α/*β*-peptide hexamers have been characterized in the crystal state, and the average ACPC $C_\beta H(i)$ - - ACPC $C_\alpha H$ (i+3) distance among these structures is ca. 5.2(3) Å. H- -H distances >5 Å do not give rise to NOEs,[6] and we conclude that NOE pattern (iv) is not consistent with the 11/11/12-helix. Modeling suggests that NOE pattern (iv) could arise if the helix-defining H-bonds expanded from i,i+3 to i,i+4 (i.e., a 15-helix). Populations of both i,i+3 and i,i+4 H-bonded helices have previously been demonstrated for short α-peptides[7] (3_{10}-helix and α-helix) and for short 1:1 α/*β*-peptides (11-helix and 14/15-helix).[3b−d] Our structural data and the precedents lead us to conclude that 1:2 α/*β*-peptide hexamer **1** adopts two different helical conformations in solution, with rapid interconversion between these helices on the NMR time scale.

Three nonsequential NOE patterns are observed among backbone protons for 2:1 α/*β*-peptide **2** in methanol: (v) *β*-residue $C_\beta H(i)$ - - *β*-residue NH(i+3), (vi) *β*-residue $C_\beta H(i)$ - - *β*-residue $C_\alpha H$ (i+3), and (vii) α-residue $C_\alpha H(i)$ - - α-residue NH(i+3). H- -H distances corresponding to patterns (v) and (vi) can be evaluated in **4**, but pattern (vii) cannot because no α-residue in **4** bears a proton on the α-carbon. (All α-residues in **4** are Aib, which is well-known to promote helical folding among α-peptides.[8]) The crystal lattice formed by **4** and solvent molecules contains seven independent molecules, all with very similar conformations. The average H- -H distance corresponding to NOE pattern (v) is 3.53(14) Å, while the average H- -H distance corresponding to (vi) is 5.3(2) Å. Thus, NOE pattern (v) can be explained by invoking the helical conformation seen in the crystal structure of **4**, but (vi) is not consistent with this helix. NOE pattern (vi) is, however, consistent with a 14-helix, defined by i,i+4 H-bonds. We conclude that the i,i+3 and i,i+4 H-bonded helices equilibrate rapidly on the NMR time scale for 2:1 α/*β*-peptide **2**, which parallels behavior among short α-peptides[7] and 1:1 and 1:2 α/*β*-peptides.

The existence of two new foldamer secondary structures, the i,i+3 H-bonded helices of 2:1 and 1:2 α/*β*-peptides, has been demonstrated here by a combination of NMR and crystallographic data, and strong NMR evidence has been provided for two additional secondary structures formed by these backbones, the i,i+4 H-bonded helices. That these secondary structures offer unique scaffolds for display of side chains is illustrated by Figure 2, which juxtaposes axial views of the i,i+3 H-bonded helices adopted by 1:1, 1:2 and 2:1 α/*β*-peptides. All three helices have ca. three residues per turn; therefore, three linear residue arrays run along the sides of each helix. For the 1:2 and 2:1 α/*β*-peptide helices, these linear arrays comprise purely α- or purely *β*-amino acid residues, but for the 1:1 α/*β*-peptide these arrays are α−*β*−α−*β*. A distinction between the 1:2 and 2:1 α/*β*-peptide helices is apparent in the molecular surfaces formed by pairs of these linear residue arrays. Only the 1:2 α/*β*-peptide offers an all-*β*/all-*β* helical face, and only the 2:1 α/*β*-peptide offers an all-α/all-α helical face. These new helices should be valuable additions to the set of foldamer scaffolds that can be used to create specifically functionalized surfaces by rational or combinatorial methods.

Acknowledgment. This research was supported by NSF Grant CHE-0551920. M.A.S. was supported in part by a Biophysics Training Grant from NIGMS. S.H.C. was supported in part by The Samsung Lee Kun Hee Scholarship Foundation. NMR equipment purchase was supported in part by grants from NIH and NSF, and X-ray equipment by NSF. We thank Prof. G. Sheldrick for advice and Dr. M. Pink for obtaining diffraction data at ChemMatCARS Sector 15, which is principally supported by NSF/DOE (CHE0087817) and by the Illinois Board of Higher Education. The Advanced Photon Source is supported by DOE (Contract No. W-31-109-Eng-38).

Supporting Information Available: Chemical shift assignments and crystallographic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA060281W