Parallel Sheet Secondary Structure in γ-Peptides

Matthew G. Woll,[†] Jonathan R. Lai,[‡] Ilia A. Guzei,[†] Stephen J. C. Taylor,[§] Mark E. B. Smith,*[,§] and Samuel H. Gellman*[,†,‡]

Department of Chemistry and Graduate Program in Biophysics University of Wisconsin, Madison, Wisconsin 53706 Chirotech Technology Limited, Unit 321, Cambridge Science Park, Milton Road, Cambridge CB4 0WG, UK

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Interest in unnatural oligomers ("foldamers") with discrete conformational propensities akin to those of proteins has led to numerous recent explorations of peptides constructed from β-, γ-, or δ-amino acids.[1] γ-Peptides containing residues bearing γ-substitution or α,γ-disubstitution or α,β,γ-trisubstitution have been shown by Hanessian et al.[2] and by Seebach et al.[3] to adopt a helical conformation defined by 14-membered ring C═O(*i*) → NH(*i*+3) hydrogen bonds. Hanessian et al. have reported reverse turn formation by a γ-peptide built from α,γ-disubstituted residues with a stereochemistry different from that leading to helical folding.[4] We now demonstrate formation of sheet secondary structure by cyclically constrained γ-amino acid residues that are derived from *trans*-3-aminocyclopentanecarboxylic acid (*trans*-3-ACPC).[5,6]

The hairpin architecture, two strands connected by a short loop, is essential for creating small increments of β-sheet secondary structure among conventional peptides.[7] Formation of sheet secondary structure requires noncovalent attraction between the strand segments and an appropriate conformational propensity in the loop segment; subtle variations in covalent structure can prevent sheet formation.[8] The loop segment need not be constructed from the same components as the strands. Several groups, for example, have shown that non-peptide loops can allow antiparallel β-sheet interactions between appended α-amino acid strands;[9] parallel β-sheet hairpins require a non-peptide loop, since the strands must be linked C-terminus-to-C-terminus or N-terminus-to-N-terminus.[10] Antiparallel sheet secondary structure has been documented among β-peptides with both non-β-peptide and β-peptide linkers.[8b,11]

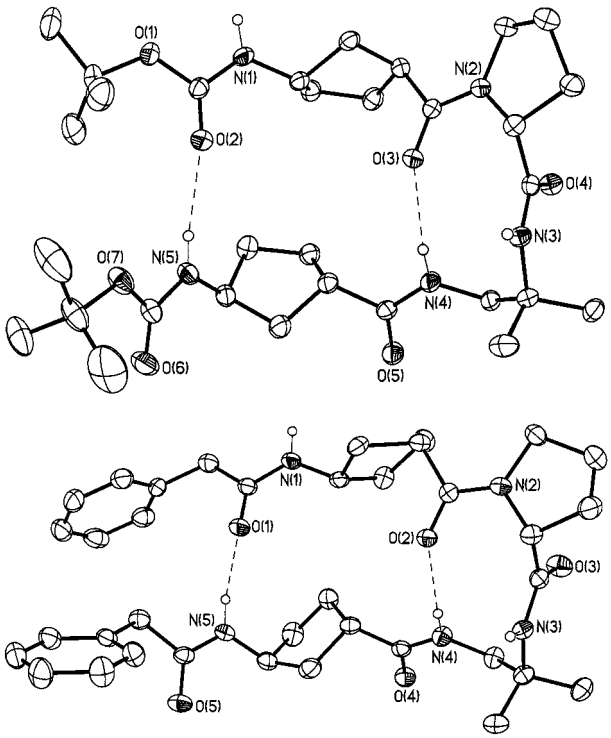

Figure 1. Solid-state structures: molecule **1** (top) and molecule **2** (bottom). All hydrogen atoms, except those attached to nitrogen, have been omitted for clarity.

CPK modeling suggested to us that *trans*-3-ACPC residues would have a high propensity for γ-peptide parallel sheet secondary structure, in contrast to the helical propensity previously documented for acyclic γ-amino acid residues. We tested this hypothesis by preparing molecules **1** and **2** in which two (*1S,3S*)-*trans*-3-ACPC residues are linked via a D-prolyl-(1,1-dimethyl)-1,2-diaminoethyl unit. This diamine linker has previously been shown to allow parallel β-sheet formation between attached α-amino acid residue strand segments.[8d,9h] Crystal structures of **1** and **2** show that both molecules adopt the desired hairpin conformation in the solid state (Figure 1). These results, particularly the similarity between two independent structures, show that

* To whom correspondence should be addressed: (e-mail) marksmith@chirotech.com, gellman@chem.wisc.edu.
† Department of Chemistry, University of Wisconsin.
‡ Graduate Program in Biophysics, University of Wisconsin.
§ Chirotech Technology Limited.

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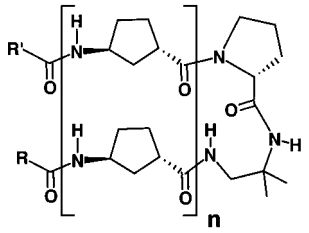
the non-γ-peptide linker allows a parallel sheet hydrogen bonding pattern between attached γ-peptide strands.



1, n = 1, R = R' = $OC(CH_3)_3$
2, n = 1, R = R' = CH_2Ph
3, n = 2, R = CH_2Ph, R' = $C(CH_3)_3$

Molecule **2** was examined by two-dimensional NMR methods in CD_2Cl_2 (3.6 and 5.7 mM, 25 °C), conditions that allow little or no aggregation,[12] in order to evaluate the propensity for parallel γ-peptide sheet formation under dynamic conditions. Previous work with small oligoamides, including hairpin molecules that contain α- and/or β-amino acid residues, has shown that intramolecular hydrogen bonding provides a modest drive for folding in nonpolar solvents, but that sheet-type hydrogen bonding will not occur unless both the strand and the turn segments have suitable conformational propensities.[8,13] A combination of COSY,[14] TOCSY,[15] and ROESY[16] data allowed us to assign nearly all proton resonances from **2**, which, in turn, enabled us to use amide proton chemical shift data to gain preliminary insight on folding. In nonpolar solvents C═O- -H−N hydrogen bond formation causes an increase (up to 2−3 ppm) in the chemical shift of an amide proton (δNH).[13] Equilibria between hydrogen bonded and non-hydrogen bonded states are usually rapid on the NMR time scale, and observed δNH values are therefore weighted averages of the contributing hydrogen bonded and non-hydrogen bonded states. For **2** in CD_2Cl_2 the pattern of δNH values suggests significant population of the conformation observed in the solid state. δNH-1 (5.52 ppm) and δNH-2 (5.63 ppm) are consistent with little or no hydrogen bonding at these amide protons, while δNH-4 (7.04 ppm) and δNH-5 (7.24 ppm) imply substantial hydrogen bond donation by these groups (numbering as in Figure 1).

More detailed structural insight was obtained from ROESY data for **2**. Most informative among the short-range NOEs was one between C_δH of proline and C_αH of the *trans*-3-ACPC residue attached to proline, which showed that the tertiary amide linkage has the *Z* configuration in solution, as observed in both crystal structures. In addition, six NOEs between the two γ-amino acid residues or immediately adjacent atoms could be assigned unambiguously (Figure 2A). Five of these NOEs are consistent with the conformation observed for **2** in the solid state or modest distortions from this conformation (NOEs listed proline-linked *trans*-3-ACPC → amino-linked *trans*-3-ACPC; the distances given after NOE intensities were measured in the crystal structure of **2**): $C_\gamma H \rightarrow C_\alpha H$ (strong; 2.29 Å), $C_\gamma H \rightarrow$ linker NH (weak; 3.64 Å), $C_\gamma H \rightarrow C_\epsilon H$ (medium; 2.41 Å), $C_\gamma H \rightarrow$ NH (weak; 3.72 Å), and phenacyl $CH_2 \rightarrow$ NH (weak; 3.95 Å). The sixth nonadjacent NOE, $C_\beta H \rightarrow$ phenacyl CH_2 (weak), suggests that an alternative mode of interstrand interaction occurs to at least a small extent for **2** in CD_2Cl_2, because the shortest distance between protons on these two methylene groups is 5.91 Å in the crystal structure of **2**.

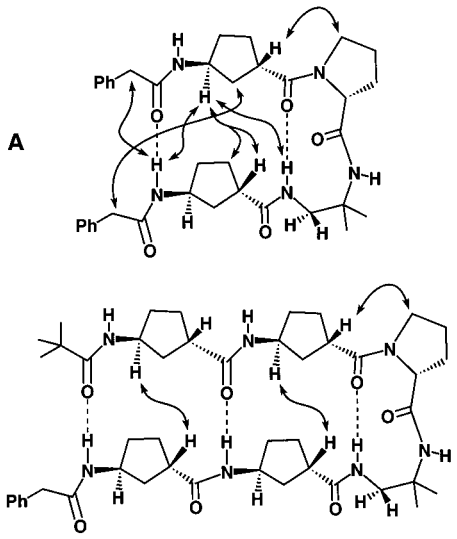


Figure 2. Graphical summary of selected NOEs: (A) molecule **2** (3.6 mM) in CD_2Cl_2, 25 °C, and (B) molecule **3** (2 mM) in pyridine-d_5, 25 °C.

Molecule **3**, with two-residue γ-peptide strands on either side of the loop, was examined to determine whether parallel sheet secondary structure could propagate out from the loop. Two-dimensional NMR analysis was carried out in pyridine-d_5 (2 mM, 25 °C) because **3** is nearly insoluble in CD_2Cl_2. Extensive overlap among proton resonances prevented definitive assignment of most ring protons. Nevertheless, several key NOEs were unambiguously identified (Figure 2B). The tertiary amide involving the proline nitrogen was shown to have the *Z* configuration by observation of a strong NOE between proline C_δH and C_αH of the adjacent *trans*-3-ACPC residue. Strong $C_\gamma H \rightarrow C_\alpha H$ NOEs were observed between the inner pair of *trans*-3-ACPC residues and between the outer pair of *trans*-3-ACPC residues. These two NOEs suggest significant population of a hairpin conformation in which the parallel γ-peptide sheet involves all four *trans*-3-ACPC residues.

Three types of secondary structure, helix, reverse turn, and sheet, are observed among conventional peptides (α-amino acid residues)[17] and among β-peptides.[1] Dill and co-workers have suggested that the two secondary structure classes with long-range order, helix and sheet, will be universal among polymers that fold to compact conformations.[18] The results reported here complete the secondary structure set for γ-peptides. It will be interesting to see whether *trans*-3-ACPC residues can be induced to form antiparallel sheet secondary structure, when appropriately linked, and whether the pyrrolidine analogue of *trans*-3-ACPC available from *trans*-4-hydroxyproline[19] allows γ-peptide sheet secondary structure to form in water.[20]

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(12) The amide proton chemical shifts of **2** displayed minimal variation over the concentration range 0.3 to 10 mM in CD_2Cl_2 (maximum change = 0.06 ppm), indicating that there is little or no self-association of **2** under these conditions.

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