A Parallel β-Sheet Model System that Folds in Water

John D. Fisk† and Samuel H. Gellman*,†,‡

Graduate Program in Biophysics and Department of Chemistry, University of Wisconsin Madison, Wisconsin 53706

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Proteins display a vast array of tertiary structures, but only a few regular motifs are common at the level of secondary structure. Establishing sequence-stability relationships for the common secondary structures should enhance our understanding of protein-folding preferences. Many groups have used model systems to examine α-helix[1] and, more recently, antiparallel β-sheet[2] conformational stability in aqueous solution. In both cases, autonomously folding secondary structures can be created with short peptides because a single α-helix or antiparallel β-sheet can readily form from a continuous peptide segment. In contrast, small units of *parallel* β-sheet are not readily available from short peptides, because N- and C-termini of adjacent parallel strands do not lie near one another. A variety of unnatural segments have been used to link peptide strands, N-terminus to N-terminus or C-terminus to C-terminus, in a manner that promotes parallel sheet formation;[3−5] these systems have been studied largely in organic solvents.[6] Water is widely regarded as the most important solvent for peptide model studies, and it is well-established that aqueous solution is less conducive to secondary structure formation than are organic or mixed aqueous/organic solvents.[7] Here we describe a parallel β-sheet model system that is shown unambiguously to fold in water.

Our model system, **1**, is illustrated in Figure 1. This molecule is designed to form a two-stranded parallel β-sheet ("parallel hairpin"), as drawn. Two six-residue strands, Ac-Ser-Lys-Phe-Ile-Gln-Val and Ac-Lys-Val-Leu-Tyr-Thr-Arg, are linked via their C-termini by a diamine derived from D-Pro and 1,2-diamino-1,1-dimethylethane (DADME). The D-Pro-DADME linker was identified in an earlier study, which focused on small model systems (single-residue strands) in organic solvents.[5] The strands of **1** were designed based on four considerations. First, we positioned hydrophobic residues (e.g., Phe/Leu and Ile/Tyr) so as to allow interstrand side chain−side chain interactions, which are expected to stabilize β-sheet secondary structure. Second, we included three basic residues to generate a net positive charge that would discourage aggregation at neutral or mildly acidic pH. Third, we maximized sequence diversity and incorporated aromatic side chains to enhance dispersion of ^{1}H NMR resonances. Fourth, we selected residues with high intrinsic propensities for β-sheet.[8]

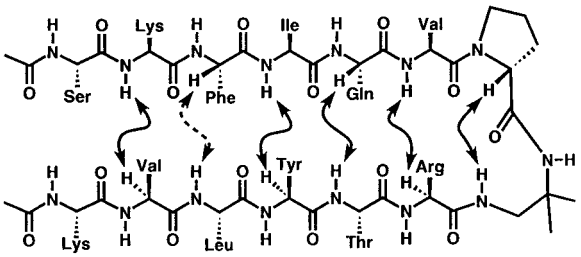

Figure 1. Backbone−backbone NOEs between nonadjacent residues observed in ROESY analysis for 2.5 mM **1** in 100 mM aqueous sodium deuterioacetate buffer, pH 3.8 (uncorrected), 4 °C. Resonance assignments were deduced from a combination of TOCSY and COSY data and sequential NOEs from ROESY data, obtained at 600 and 750 MHz.

Parallel hairpin **1** was prepared by a combination of solution- and solid-phase synthesis methods. The segment Alloc-Glu-Val-D-Pro-DADME-Fmoc was prepared in solution and then attached to Rink amide resin via the Glu side chain carboxyl. Standard Fmoc-based procedures were used to complete the "lower" strand. The Alloc group was then removed, and Fmoc-based procedures were used to complete the "upper" strand. Cleavage from the solid support and removal of side chain protecting groups were accomplished in a single step, followed by HPLC purification and structure confirmation by MALDI mass spectrometry. Analytical ultracentrifugation indicated that **1** does not self-associate at 0.5 mM in 100 mM sodium acetate buffer, pH 3.8. Two-dimensional NMR analysis[9] was conducted in a similar solvent system (9:1 H_2O:D_2O, 100 mM sodium acetate buffer, pH 3.8), with 2.5 mM peptide samples. NMR chemical shifts were identical for 0.5 and 2.5 mM samples, and we conclude that there is no change in aggregation state over this concentration range.

NOEs between residues that are not adjacent in sequence have proven to be the most incisive criteria for β-sheet formation in previously reported antiparallel model systems.[2,10] A total of 22 NOEs between nonadjacent residues were observed for **1**, all of which are consistent with the parallel hairpin folding pattern. Figure 1 shows a subset of these NOEs, including five backbone−backbone C_αH- -NH NOEs, which are characteristic of parallel β-sheet.[10] The identity of these backbone NOEs [e.g., C_αH(Gln)- -NH(Thr) but not C_αH(Thr)- -NH(Gln)] indicates that there is a unique hydrogen-bonded registry between the two strands. The fact that these NOEs are observed out to the penultimate residue in each strand suggests that the hairpin is well formed over almost the entire length of the molecule. An NOE within the linker, between C_αH of D-Pro and 2-NH of DADME, is consistent with

* To whom correspondence should be addressed. E-mail: gellman@chem.wisc.edu.
† Graduate Program in Biophysics.
‡ Department of Chemistry.

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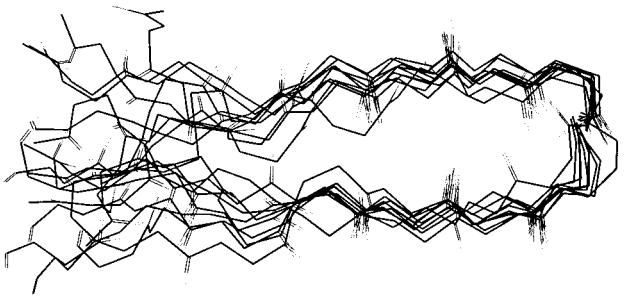

Figure 2. NOE-restrained dynamics results for **1**, using NOEs between protons on nonadjacent residues and angle restraints derived from coupling constants. A total of 500 random structures were annealed using the program DYANA (ref 12). The RMS deviation among backbone heavy atoms for the 10 best structures of **1** is 1.80 ± 0.38 Å; the RMS deviation improves to 1.03 ± 0.35 Å if the two outermost residues on each strand are omitted. The image was generated using Sybyl 6.6 (Tripos Inc., 1699 S. Hanley Rd., St. Louis, MO 63144).

reverse turn formation across this segment. In addition to the backbone NOEs shown in Figure 1, several interstrand side chain−side chain NOEs were observed for **1**. Side chain contacts implied by these NOEs included Tyr- -Ile, Tyr- -Val (upper strand), Phe- -Leu, and Phe- -Thr. The Tyr- -Val (upper strand) and Phe- -Thr contacts indicate that the strand segments of **1** display a right-handed twist, as is also observed for strands in parallel β-sheets of proteins.[11] Figure 2 shows an overlay of the 10 best structures from an NOE-restrained dynamics analysis of **1** with the program DYANA;[12] although the ends are frayed, the parallel β-sheet structure is well-developed for the residues nearer to the connector.

In addition to the fully assigned NOEs between nonadjacent residues discussed above, we observed five NOEs that could not be fully assigned because of imperfect resolution. In each case, the ambiguous NOE could be consistent with the proposed parallel β-sheet conformation. For example, the only strong ambiguous NOE involved Tyr $C_\delta H$ and either Ile $C_\beta H$ or Lys (upper strand) $C_\beta H$; the former assignment is consistent with the conformation of **1** proposed in Figure 1. (The remaining ambiguous NOEs were weak.)

α-Proton chemical shift data ($\delta_{\alpha H}$) provide further evidence that a parallel β-sheet conformation is significantly populated by **1** in aqueous solution. Participation in a β-sheet causes a residue's $\delta_{\alpha H}$ to shift downfield relative to the random coil position, and participation in an α-helix causes $\delta_{\alpha H}$ to shift upfield.[13] Figure 3 shows $\Delta\delta_{\alpha H} = \delta_{\alpha H}$ (observed) $- \delta_{\alpha H}$ (random coil) for each strand residue of **1** (random coil $\delta_{\alpha H}$ values from ref 13). Nearly all of the strand residues of **1** display $\Delta\delta_{\alpha H} > 0$. The large $\Delta\delta_{\alpha H}$ values ($>+0.1$) observed for most of the four innermost residues in each strand of **1** suggest that the parallel β-sheet conformation is particularly well formed for these inner residues. α-Carbon chemical shift data ($\delta_{\alpha C}$) are also sensitive to secondary structure,[14] and $\Delta\delta_{\alpha C}$ data have been used to characterize designed antiparallel β-sheets.[15] $\Delta\delta_{\alpha C}$ data for **1** support the conclusions derived from the $\Delta\delta_{\alpha H}$ data.[16]

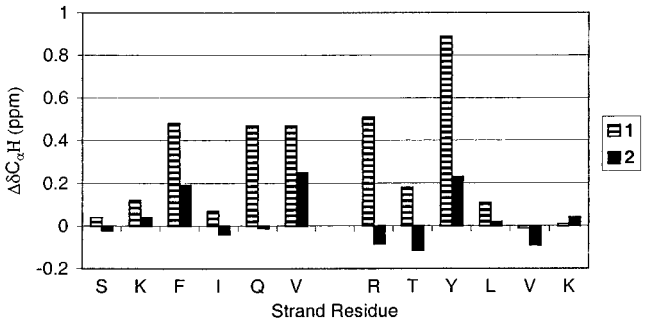

Figure 3. $\Delta\delta_{\alpha H}$ = (observed $\delta_{\alpha H}$ − random coil $\delta_{\alpha H}$) for strand residues of 2.5 mM **1** (D-Pro; striped) and 2.5 mM **2** (L-Pro; filled) in aqueous (9:1 H_2O:D_2O) sodium deuteroacetate buffer, pH 3.8 (uncorrected), 4 °C. (See ref 13 for origin of random coil values.) Chemical shifts were internally referenced to 2,2-dimethyl-2-silapentane-5-sulfonate (DSS).

The $\Delta\delta_{\alpha H}$ data in Figure 3 show that switching from D-Pro (**1**) to L-Pro (**2**) in the linker prevents parallel sheet interactions between L-strand residues. This conclusion is supported by our failure to detect any NOEs involving nonadjacent residues for L-Pro diastereomer **2**, by $\Delta\delta_{\alpha C}$ data for **1** and **2**,[16] and by a comparison of **1** and **2** via circular dichroism (CD).[16] Molecule **1** shows a minimum at ca. 217 nm and a maximum at ca. 200 nm, both of which are consistent with β-sheet CD signature.[17] In contrast, diastereomer **2** shows a minimum at 198 nm, which indicates a predominantly random coil state. A complete loss of parallel β-sheet formation upon replacing D-Pro with L-Pro was previously observed for small model systems in organic solvents.[5]

In summary, a combination of NMR and CD data shows that **1** adopts a two-stranded parallel β-sheet conformation in aqueous solution. As with other secondary structure model systems, folded and unfolded conformations appear to be in rapid equilibrium. Inverting the configuration of the Pro residue in the linker segment (**1** → **2**) has a profound effect on conformation, since **2** appears to be entirely random coil. Similar effects have previouly been observed for antiparallel β-sheet model systems in which Pro-Gly segments are used to link adjacent strands.[18] The ability to turn β-hairpin folding on and off via choice of Pro configuration has proven useful for probing the origins of antiparallel β-sheet stability,[19] and we anticipate that **1**, **2**, and related molecules will allow complementary exploration of parallel β-sheet stability.[20]

Supporting Information Available: Circular dichroism and α-carbon-13 NMR chemical shift data for **1** and **2** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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