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Thermodynamic Analysis of Autonomous Parallel β-Sheet Formation in Water

John D. Fisk, Margaret A. Schmitt, and Samuel H. Gellman*

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

Received February 8, 2006; E-mail: gellman@chem.wisc.edu

Protein folding patterns are dominated by two regular substructures, α-helix and β-sheet. The origins of conformational stability for these secondary structures, a subject of intense interest, can be explored with peptides that form a helix or sheet in the absence of a tertiary context. Design rules for medium-length peptides that form autonomous α-helices in aqueous solution were delineated in the 1980s,[1] and comparable achievements for autonomous antiparallel β-sheets were reported in the 1990s.[2] In both cases, the development of strategies for determining folded populations has allowed thermodynamic analysis of secondary structure formation.[3]

An autonomously folding *parallel* β-sheet (in contrast to an autonomous antiparallel β-sheet or α-helix) cannot be created exclusively from α-amino acid residues because the N-terminus of one strand in a parallel β-sheet does not lie near the C-terminus of a neighboring strand. Many groups have explored nonpeptide units that promote parallel sheet interactions, most commonly by linking C-termini with a short turn-forming diamine.[4] We have previously reported that the D-prolyl-1,1-dimethyl-1,2-diaminoethane (D-Pro-DADME) unit supports formation of a two-stranded parallel β-sheet in water, as indicated by 2D NMR data.[5] Here we show that the thermodynamics of parallel β-sheet formation can be evaluated in such a model system. This accomplishment provides a foundation for exploring sequence−stability relationships in the parallel β-sheet structural manifold, which would fill a significant gap in our understanding of proteins and protein aggregates.

NMR chemical shifts provide the most reliable insight on folded populations among antiparallel β-sheet model systems,[3,6] and we therefore aimed for a chemical shift-based analysis of parallel β-sheet folding. Most autonomous helix or sheet systems are not fully folded under accessible conditions; therefore, chemical shifts for such systems are population-weighted averages of the contributions from the limiting folded and unfolded states. To analyze **1**, we have built upon the strategy previously developed for antiparallel β-sheets by constructing model compounds intended to provide empirical estimates of chemical shifts in the fully folded and fully unfolded states.[3b] The unfolded state is represented by diastereomer **2**; changing proline configuration from D to L completely disrupts parallel β-sheet formation.[5,7] The folded state is represented by cyclic molecule **3**, an analogue of **2** in which the N-termini of the strand segments have been linked via a succinyl-glycine unit. Cyclization is intended to enhance the propensity for parallel β-sheet formation in **3** relative to linear molecule **2**. Among antiparallel model systems, two-stranded β-sheet conformations have been stabilized by cyclization strategies involving either the backbone (capping a β-hairpin with a β-turn) or side chains (disulfide formation between terminal Cys residues).[3a,b,8] We focused on backbone cyclization because Cys disulfide cross-links are not compatible with parallel β-sheet secondary structure.[9] The succinyl-glycine linker in **3** allows formation of a 10-membered-ring H-bond [C═O(Succ-18) to H−N (Ser-2)], which is analogous to the H-bond common among β-turns.

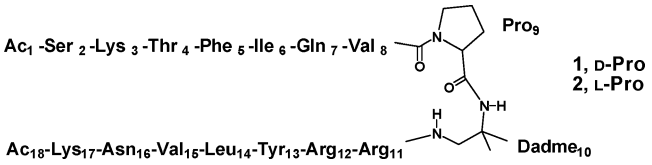

Figure 1. Chemical structures of linear compounds **1** and **2**. The numbering scheme was chosen to allow easy comparison between the linear and cyclic molecules.

NMR analysis indicated that **3** adopts the intended parallel β-sheet conformation in aqueous solution, and the data suggest that the extent of folding is greater for cyclic **3** than for linear analogue **1**. Four unambiguous interstrand NH- -C_αH NOEs were observed in the center of the intended β-sheet region of **3** (Figure 2); the Val-8/Arg-11 NOE, if present, would have been obscured by the residual solvent resonance. These NOEs are consistent with the expected parallel β-sheet hydrogen-bonding registry.[10] The lack of interstrand NH- -C_αH NOEs near the succinyl-glycine linker, however, suggests that this region is not as well folded as the rest of the molecule. A large set of side chain−side chain NOEs was observed for **3**, all consistent with the intended parallel β-sheet conformation. For linear molecule **1**, a comparable set of cross-strand NOEs was seen for only the eight strand residues nearest to the D-Pro-DADME unit (Phe-5 to Val-8 and Arg-11 to Leu-14), but not for the three residues at each strand terminus.[7] The difference in interstrand NOE patterns observed for **1** vs **3** suggests that the parallel β-sheet secondary structure encompasses nearly the entire strand length for **3**, but only the strand residues closer to the turn segment in **1**. The behavior of **1** is consistent with evidence that the termini of antiparallel β-sheet model systems tend to be unfolded ("frayed") in aqueous solution.[2] As intended, macrocyclization, in **3**, strongly discourages terminal fraying.

Downfield shifts in α-proton resonances ($\delta C_\alpha H$), relative to a random coil reference value, indicate participation in β-sheet secondary structure.[6] We use **2** to provide the "random coil" $\delta C_\alpha H$ values for assessment of folding in the strand regions of **1** and **3** because **2** shows no sign of folding (no NOEs between sequentially nonadjacent residues),[7] and the $\delta C_\alpha H$ values measured for **2** account for the effects of sequence context. Nearly all strand residues in **3** show $\Delta\delta C_\alpha H$ [$= \delta C_\alpha H(\mathbf{3}) - \delta C_\alpha H(\mathbf{2})$] $\geq +0.1$ ppm in aqueous solution at 287 K, which suggests extensive β-sheet formation along the entire length of each strand segment. This conclusion matches that reached from the interstrand NOEs observed for **3**. In contrast, the outermost residues of linear molecule **1**, Ser-2 to Thr-4 and Val-15 to Lys-17, display random coil-like $\Delta\delta C_\alpha H$ values. The strand segments nearer the linker in **1**, Phe-5 to Val-8 and Arg-11 to Leu-14, display $\Delta\delta C_\alpha H$ values consistent with β-sheet formation, although each $\Delta\delta C_\alpha H$ value is smaller than the corresponding value for **3**. Thus, parallel β-sheet structure is well-developed only for the segments of **1** near the linker, and this region is only partially folded. On the basis of these observations, we regard strand

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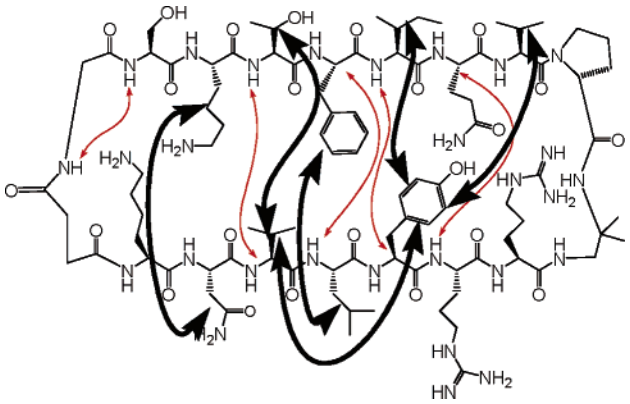

Figure 2. NOEs observed in **3** between residues nonadjacent in sequence. Red arrows indicate backbone−backbone NOEs. Bold arrows indicate multiple NOEs between side chain pairs (at least 3 NOEs for each pair).

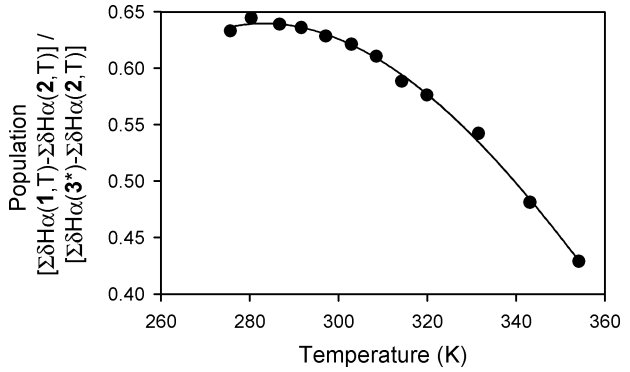

Figure 3. Change in folded population of **1** as a function of temperature, calculated from δCαH data by the method of ref 3a. See Supporting Information for details. *Data for **3** at 287 K and 50% TFE.

segments Phe-5 to Val-8 and Arg-11 to Leu-14 as the folded core of **1**, and we focus on this core in the analysis below.

We examined the effect of 2,2,2-trifluoroethanol (TFE) on $\Delta\delta C_\alpha H$ for **3** in an effort to determine whether **3** is fully folded in aqueous buffer. Addition of increasing proportions of TFE to aqueous solutions has been shown to induce progressively larger extents of antiparallel β-sheet folding in several designed peptides.[11] The $\Delta\delta C_\alpha H$ value for each strand residue of **3** became larger as the TFE content was raised from 0% to 30%,[7] suggesting that the parallel β-sheet conformation is not fully populated in purely aqueous solution. Further increases in TFE proportion to 40% or 50% had relatively little effect,[7] suggesting that β-sheet population is maximal at 30% TFE. We used $\delta C_\alpha H$ values obtained with **3** in 50% TFE to represent the fully folded state in our population analysis of **1**. Parallel β-sheet population at a particular residue of **1** in aqueous solution at a given temperature was estimated by interpolating the observed $\delta C_\alpha H$ value between the $\delta C_\alpha H$ value for the corresponding residue in unfolded reference compound **2** under the same conditions and the $\delta C_\alpha H$ for folded reference compound **3** at 287 K in 50% TFE. For each of the eight residues in the parallel β-sheet core of **1** we compared folded populations determined at 287 and 354 K in aqueous buffer.[7] The apparent population change is reasonably consistent across this set of residues, which suggests that the eight-residue core can be analyzed in terms of a two-state model, unfolded vs parallel β-sheet. We used the nonlinear fitting method developed by Searle et al.[3a] for van't Hoff analysis of two-state folding (Figure 3), which provided the following thermodynamic parameters for parallel β-sheet formation at 298 K: $\Delta H° = -1.1 \pm 0.1$ kcal/mol, $\Delta S° = -2.5 \pm 0.2$ cal/mol K, $\Delta C_p° = -73 \pm 2$ cal/mol K.[12] The thermodynamic signature for parallel β-sheet folding in **1** is qualitatively similar to that observed for a number of antiparallel β-hairpins in that β-sheet formation is enthalpically favorable and entropically unfavorable near room temperature.[3,13] This signature differs from that of a classical hydrophobic effect, but the observation of a significant and negative heat capacity change upon folding suggests that there is a hydrophobic contribution to the drive for folding, presumably from interstrand side chain−side chain interactions.

The results reported here lay the groundwork for thermodynamic analysis of the factors that control parallel β-sheet folding preferences. Such studies should provide fundamental insight on a structural motif that is very common in proteins and in protein aggregates associated with human diseases.[14]

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Supporting Information Available: Information concerning the synthesis of **3**; chemical shift and NOE information on **1**, **2**, and **3**; TFE titration data for **3** and the van't Hoff analysis of folding. This material is available free of charge via the Internet at http://pubs.acs.org.

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