Insights on β-Hairpin Stability in Aqueous Solution from Peptides with Enforced Type I′ and Type II′ β-Turns

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Three motifs, α-helix, antiparallel β-sheet, and β-turn, account for most of the regular secondary structure observed in folded proteins.[1] Short peptides that adopt a defined secondary structure in solution have been used to examine the origins of α-helix[2] and β-turn[3] stability, but this approach has been difficult to implement for a β-sheet.[4] β-Hairpins, which contain two antiparallel β-strands linked by a short loop, constitute minimum increments of a β-sheet and therefore could provide a basis for probing antiparallel β-sheet stability.[5−9] Here, we show that selective D-residue incorporation in a family of 16-residue peptides induces β-hairpin folding, with a tight two-residue loop at a defined position, in aqueous solution. Comparison of these heterochiral peptides with the all-L diastereomers provides insight on the forces that favor the β-hairpin conformation.

Several short peptides have recently been shown to adopt β-hairpin conformations in aqueous solution,[5−8] but the use of these peptides as platforms for β-sheet analysis is problematic because the factors that specify the position and size of the loop have been unclear. The first reported autonomous hairpin, YQNPDGSQA (**1**), was designed by extrapolation from residues 15−23 of tendamistat, YQSWRYSQA.[5a] In **1**, the PDG segment forms a three-residue loop,[5a] but the original sequence in the native protein adopts a β-hairpin with a two-residue loop at the WR segment.[10] Searle et al. have found β-hairpin folding in MQIFVKNPDGTLTLEV-NH_2 (**2**),[7] a 16-residue peptide derived from the 17 N-terminal residues of ubiquitin, MQIFVKTLTGKTITLEV. In the native protein, these 17 residues adopt a β-hairpin with a three-residue loop across LTG.[11] Searle et al. intended to replace this natural loop with a tight, two-residue loop across PD; however, **2** formed a β-hairpin with a three-residue loop across PDG, which led to a non-native strand pairing.[7]

Our approach is based on statistical analysis of β-hairpins in folded proteins.[12] When the loop contains only two residues, these residues correspond to positions $i + 1$ and $i + 2$ of a β-turn. Thornton et al. found that the most common β-turn conformations, types I and II, are seldom associated with two-residue-loop β-hairpins, while rare "mirror image" β-turn conformations, types I′ and II′, are often observed in two-residue-loop β-hairpins.[12] Proline at the $i + 1$ position has long been known to promote β-turn formation,[3] but L-proline in this position strongly favors type I and II β-turns. Therefore, ^{L}PX segments should not be conducive to formation of tight β-hairpins, which is consistent with the unexpected three-residue "bulged" loops observed for **1** and **2**. In contrast, ^{D}PX segments, which favor type I′ and II′ turns, should stabilize β-hairpins with two-residue loops. We have previously shown that these predictions hold for tetrapeptides and analogous depsipeptides in organic solvents,[13] and the present studies probe β-hairpin formation in aqueous solution.[14]

We tested the predicted β-hairpin promotion by ^{D}PX segments with five 16-residue peptides, MQIFVKSXXKTITLKV-NH_2, derived from the N-terminal segment of ubiquitin. The sequences employed at XX, ^{D}P^{D}A, ^{L}P^{L}A, ^{D}P^{L}A, ^{D}PG, and ^{L}PG, were intended to replace the native three-residue loop (LTG).[11] Two other changes were made: (i) the residue preceding the loop was switched from threonine to serine, to minimize sequence redundancy; (ii) the penultimate residue was switched from glutamate to lysine, to increase net positive charge and thereby discourage aggregation. Analytical ultracentifugation indicated that none of these five peptides forms aggregates at 5.5 mM (24 °C).[15]

Figure 1 summarizes the long-range crosspeaks observed in ROESY[16] experiments for ^{D}P^{D}A in aqueous solution (24 °C), and representative ROESY data involving the side chains of Gln 2, Phe 4, and Thr 13 are shown in Figure 2. The interstrand

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S0002-7863(96)03653-0 CCC: $14.00

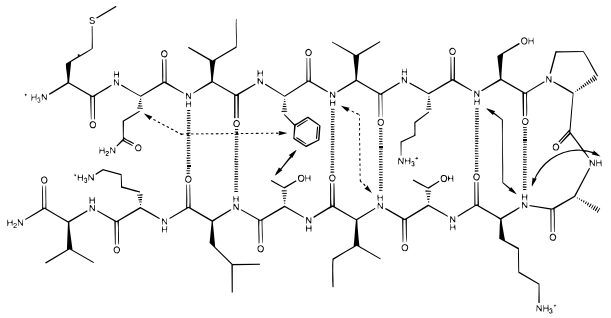

Figure 1. Summary of long-range NOEs observed for the peptide $^{D}P^{D}A$ in 9:1 H_2O/D_2O (proposed β-hairpin hydrogen bonds are shown). Solid arrows indicate strong NOEs, and dotted arrows indicate weak NOEs. Data were obtained on a Varian Unity 500 MHz spectrometer at 24 °C. Peptide concentration was 5.5 mM. H_2O/D_2O solutions were buffered to pH 3.8 with 100 mM sodium deuteroacetate. ROESY mixing time was 200 ms.

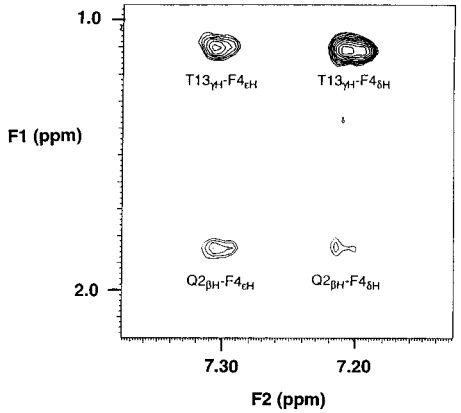

Figure 2. Representative ROESY data for $^{D}P^{D}A$ in 9:1 H_2O/D_2O (conditions defined in caption to Figure 1), showing NOEs involving the side chains of Q2 (β-hydrogens), F4 (*ortho*- and *meta*-hydrogens), and T13 (methyl group).

crosspeaks extend out to the middle of each strand (i.e., the fourth residue from the loop segment on each side). Similar evidence of β-hairpin formation was observed for ^{D}PG and $^{D}P^{L}A$.[17] In contrast, no long-range ROESY crosspeaks were observed for $^{L}P^{L}A$ and ^{L}PG in aqueous solution, which indicates that a "mirror image turn"[12] (presumably type I′, for $^{D}P^{D}A$, or type II′, for ^{D}PG and $^{D}P^{L}A$[3a]) is required for formation of the two-residue-loop β-hairpin.

α-Proton chemical shifts are sensitive to residue conformation, and the deviation of these chemical shifts from "random coil" values has been used to deduce secondary structure in peptides and proteins.[18−20] It is generally observed that β-sheet residues are downfield-shifted and α-helical residues are upfield-shifted relative to the random coil state. Figure 3 shows $\Delta\delta_{\alpha H}$(observed $\delta_{\alpha H}$ − random coil[19] $\delta_{\alpha H}$) values for $^{D}P^{D}A$ and $^{L}P^{L}A$. Most of

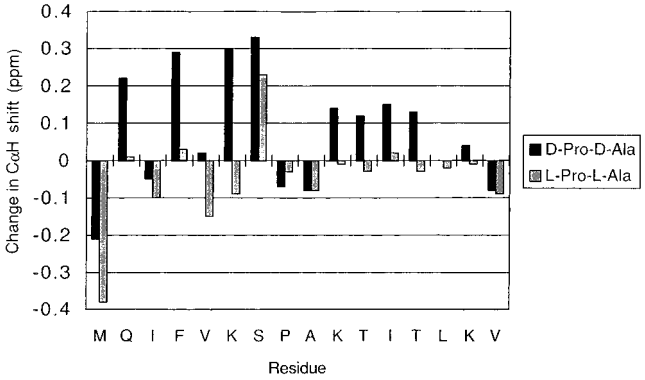

Figure 3. $\Delta\delta_{\alpha H}$ = observed $\delta_{\alpha H}$ − random coil $\delta_{\alpha H}$ for $^{D}P^{D}A$ and $^{L}P^{L}A$ in 9:1 H_2O/D_2O. (See ref 19 for origin of random coil values.) Chemical shifts were externally referenced to sodium 3-(trimethylsilyl)-propionate-2,2,3,3-d_4.

the residues of $^{D}P^{D}A$, other than in the loop and at the termini, are downfield-shifted by >0.1 ppm (a similar trend was observed for ^{D}PG and $^{D}P^{L}A$[17]), while the $\Delta\delta_{\alpha H}$ values for $^{L}P^{L}A$ are variable (a similar trend was observed for ^{L}PG[17]). Thus, the $\Delta\delta_{\alpha H}$ data support our conclusion that type I′ and type II′ β-turns promote formation of β-hairpins containing two-residue loops.

Short-range ROESY crosspeaks between the α-proton of one residue and the amide proton of the adjacent residue in the C-terminal direction [$d_{\alpha N}(i, i + 1)$] are commonly attributed to β-strand conformations.[21] These $d_{\alpha N}(i, i + 1)$ crosspeaks were observed for most residues, outside the loop region, of all five of our peptides; however, the $d_{\alpha N}(i, i + 1)$ NOEs were generally more intense for $^{D}P^{D}A$, ^{D}PG, and $^{D}P^{L}A$ than those for $^{L}P^{L}A$ and ^{L}PG. This consistent difference in crosspeak intensity provides further support for the conclusion that ^{D}PX segments promote β-hairpin formation.

What are the origins of β-hairpin stability in aqueous solution? Constantine et al. have proposed that interstrand hydrogen bonds provide the major stabilizing force for the β-hairpin conformation of **1** in aqueous solution.[5c,d] Searle et al. have suggested that side chain hydrophobic interactions are crucial for the β-hairpin folding of **2**.[7] Our results indicate that neither hydrogen bonding nor hydrophobic interactions nor a combination of these two factors is sufficient to induce β-hairpin folding of $^{D}P^{D}A$, ^{D}PG, or $^{D}P^{L}A$, since the all-L diastereomers could experience these same forces. Comparison among our five peptides demonstrates that the conformational proclivity of the backbone (which results from a combination of the torsional preferences of the covalent bonds, the steric repulsions experienced in alternative folding patterns, and the entropic factors) is at least as important as hydrogen bonding and hydrophobic interactions in stabilizing β-hairpin conformations. Further study of these and related peptides should continue to provide insight on the origins of β-hairpin stability.

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Supporting Information Available: Summary of long-range ROESY crosspeaks for ^{D}PG and $^{D}P^{L}A$ in aqueous solution and comparison of $\Delta\delta_{\alpha H}$ values for ^{L}PG, ^{D}PG and $^{D}P^{L}A$ (4 pages). See any current masthead page for ordering and Internet access instructions.

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(17) Supporting data may be found in the Supporting Information.

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