"Mirror Image" Reverse Turns Promote β-Hairpin Formation

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The design of peptides and proteins with well-defined folding patterns is a topic of wide interest at present.[1] Most residues in natural globular proteins occur in one of just a few types of secondary structure, α-helix, β-sheet, or β-turn.[2] Strategies for α-helix[3] and β-turn[4] promotion (e.g., choice of amino acid sequence) have been identified via study of minimal model systems for these secondary structures. Less is known about the promotion of β-sheet, perhaps because of a lack of good model systems for this type of secondary structure,[5] and because β-sheet in proteins, unlike the other secondary structures, can involve direct contact of residues that are far apart in the linear sequence. One type of β-sheet arrangement, the β-hairpin, involves local nonbonded contacts. A β-hairpin is a two-stranded segment of antiparallel β-sheet in which the strands are connected by a loop.[6] The shortest common loop contains two residues (i.e., a β-turn), and a minimal β-hairpin would therefore involve four residues and the formation of two intramolecular hydrogen bonds, as indicated below.[7,8] We report results for a series of depsipeptides that demonstrate that the relative configuration at the chiral centers along the backbone has a profound effect on the favorability of the β-hairpin folding pattern in these minimal model systems.

Our experiments were inspired by statistical surveys of β-hairpins in crystalline proteins, which reveal that two rare classes of β-turn, types I′ and II′, are commonly associated with β-hairpins.[9] The various turn types are defined by the sequence of ϕ and ψ torsion angles of the middle two of the four β-turn residues (i.e., the two residues of the minimal β-hairpin's loop).[10] Turn types I and II are most common in globular proteins; turn types I′ and II′, by definition, have "mirror image" torsion angles relative to types I and II. For L-amino acid residues, type I′ and II′ turns are believed to be destabilized by unfavorable intraturn nonbonded interactions.[4e] However, as Sibanda and Thornton have pointed out, the natural twist of type I′ and II′ turns, but not of type I and II turns, is compatible with the natural twist between adjacent strands of antiparallel β-sheet formed from L-amino acids.[9a]

Our studies are intended to determine whether the trends observed by Thornton et al.[9] can be correlated with the intrinsic β-hairpin-forming tendencies of short, flexible peptides in solution. We have focused initially on the behavior of depsipeptides **1–4** in organic solvents because these systems allow detailed analysis of folding behavior via two complementary spectroscopies, IR and NMR.[11] In **1–4**, β-hairpin folding involves the formation of two intramolecular hydrogen bonds; because there are no additional amide N–H groups in the molecules, N–H stretch IR data should be very sensitive to β-hairpin formation. Prolyl-glycolyl and prolyl-lactyl were chosen for the two-residue loop segments because these depsipeptide sequences have been shown to have a very high β-turn-forming tendency in organic solvents.[12] In the crystalline state and organic solution, acetyl-L-prolyl-L-lactyl methyl amide adopts a type I turn; the analogue containing a glycolate residue in place of lactate appears to interconvert between type I and type II conformations in solution.[12]

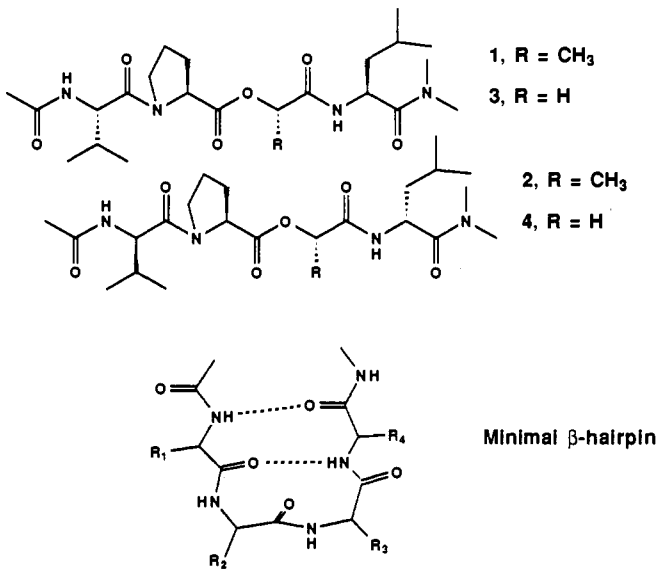

Figure 1 shows N–H stretch region IR data for 1 mM solutions of **1–4** in CH_2Cl_2. For **1** and **3**, strong bands are observed at 3422 and 3317/3306 cm^{-1}. On the basis of precedent, the highest energy band may be assigned to N–H involved in a weak intraresidue five-membered ring N—H···O=C interaction ("C_5 conformation"),[13] and the lower energy bands may be assigned to N–H involved in stronger N—H···O=C hydrogen bonds.[11,12] Variable concentration ^1H NMR data (Figure 2) indicate that the hydrogen bonding detected by IR in the 1 mM solutions is

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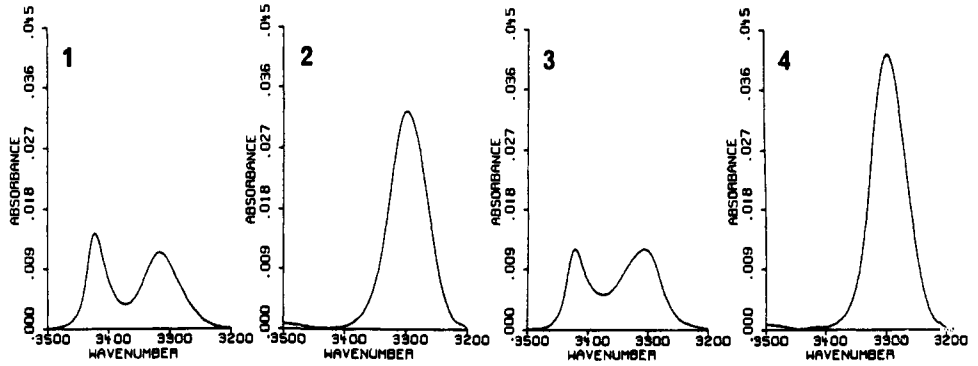

Figure 1. N–H stretch FT-IR data for 1 mM depsipeptide samples in CH_2Cl_2 at room temperature after subtraction of the spectrum of pure CH_2Cl_2. From left to right: **1**, maxima at 3422 and 3317 cm^{-1}; **2**, maximum at 3297 cm^{-1}; **3**, maxima at 3422 and 3306 cm^{-1}; **4**, maximum at 3295 cm^{-1}.

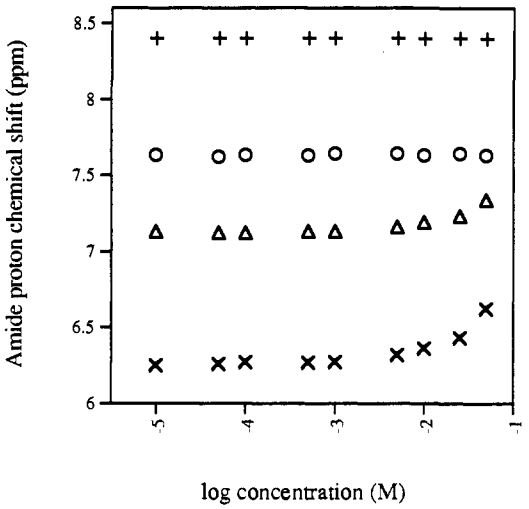

Figure 2. Amide proton NMR chemical shifts in CD_2Cl_2 at room temperature, as a function of the logarithm of depsipeptide concentration: (×), Val NH of **1**; (Δ), Leu NH of **1**; (+), Val NH of **2**; (○), Leu NH of **2**.

exclusively intramolecular. For **1**, both amide proton chemical shifts are independent of concentration at or below 1 mM; concentration dependence above 1 mM indicates the onset of aggregation (**3** behaved similarly[14]). At or below 1 mM, δ(Leu–NH) = 7.13, which is consistent with a substantial degree of intramolecular hydrogen bonding,[15] while δ(Val–NH) = 6.27, which is consistent with little or no hydrogen bonding. The combination of NMR and IR data suggests that the 10-membered-ring (β-turn) hydrogen bond is highly populated in **1** and **3**, but that the 14-membered-ring (β-hairpin) hydrogen bond is not significantly populated, under these conditions.

IR and NMR data indicate that the depsipeptides containing D residues at both termini (**2** and **4**) exist *completely* in the β-hairpin folding pattern in CH_2Cl_2 at room temperature, in contrast to their diastereomers containing all L residues (**1** and **3**). Only one N–H stretch band is observed for **2** and **4** (Figure 1), at 3297/3295 cm^{-1}, which indicates that both amide protons are engaged in strong N—H···O=C hydrogen bonds. Figure 2 shows that both δ(NH)s for **2** are concentration-independent between 0.01 and 50 mM (**4** behaved similarly[14]), which is consistent with a monomeric state throughout this range.[16] δ(Leu–NH) = 7.63 for **2**, modestly downfield from δ(Leu–NH) for the all-L isomer **1**, but δ(Val–NH) for **2** (8.40) is over 2 ppm downfield of the corresponding value for **1**. This large Δδ(Val–NH) supports the proposition that the 14-membered-ring (β-hairpin) hydrogen bond is fully populated in **2** but not in diastereomer **1**; analogous arguments hold for **4** vs **3**.[14] ROESY ^1H NMR experiments[17] provided further evidence for β-hairpin formation in **2** and **4**; a Leu–NH···Val–NH cross peak was observed for these depsipeptides,[14] but not for the all-L isomers **1** and **3**, in CD_2Cl_2. The NH···NH crosspeak was also observed for **2** (but not **1**, **3**, or **4**) in $(CD_3)_2S{=}O$.

Our data for model compounds **1**–**4** demonstrate that the sequence of α-carbon configurations along a four-residue sequence can profoundly affect the backbone's propensity to adopt a β-hairpin folding pattern. The behavior of **1** and **3** shows that the existence of a β-turn is not sufficient to promote β-hairpin formation. Our observations suggest that the correlation between "mirror image" β-turn conformations and β-hairpin occurrence in protein crystal structures is a manifestation of local folding propensities. These results have important implications for the design of peptides and proteins that adopt stable antiparallel β-sheet structure. We are currently determining whether judicious placement of D residues in small, predominantly-L peptides will promote β-hairpin formation in aqueous solution.

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Supplementary Material Available: Concentration-dependent ^1H NMR data for compounds **3** and **4** and ROESY data for compounds **2** and **4** (3 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(14) Relevant data may be found in the supplementary material.

(15) For 1 mM acetyl-L-prolyl-L-lactyl methylamide, which IR data indicate to be almost completely hydrogen bonded at the lone N–H, δ(NH) = 7.11 at 298 K; see ref 11c.

(16) The concentration independence of both δ(NH) values for **2** and **4** does not allow one to distinguish between a lack of aggregation and the formation of an extremely stable aggregate (dimer, trimer, etc.). However, because **1** and **3** do not aggregate below 1 mM, it seems unlikely that an aggregated state of **2** and/or **4** would have sufficient stability to be fully populated at 0.01 mM. For vapor pressure osmometry studies on peptides of similar sizes in chloroform solution, see: Sugawara, N.; Stevens, E. S.; Bonora, G. M.; Toniolo, C. *J. Am. Chem. Soc.* **1980**, *102*, 7044.

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