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Promotion of Sheet Formation in α-Peptide Strands by a β-Peptide Reverse Turn

Bayard R. Huck,[†] John D. Fisk,[§] and Samuel H. Gellman*[,†,§]

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

gellman@chem.wisc.edu

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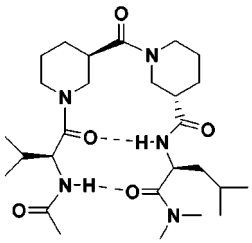
ABSTRACT



We show that a tetrapeptide with a heterogeneous backbone, i.e., with two different classes of amino acid residues, adopts a hairpin conformation in which each type of residue plays a different structural role. The α-residues at the ends form hydrogen bonds characteristic of antiparallel β-sheet secondary structure, while the central di-β-peptide segment forms a reverse turn. The configuration of the turn residues is critical to sheet formation.

Unnatural oligomers that adopt discrete conformations ("foldamers") are subjects of increasing interest.[1] β-Peptides, oligomers of β-amino acids, have received particularly intensive scrutiny.[1,2] All of the regular secondary structure types observed in conventional peptides and proteins ("α-peptides") have recently been documented in short β-peptides, including helices, sheets, and reverse turns. The β-peptide folding rules have been used to generate oligomers with specific biological activities.[3]

Most synthetic foldamers examined to date and the biofoldamers, α-peptides and RNA, have homogeneous backbones, i.e., they are built from a single type of monomer. Oligomers with heterogeneous backbones are also important subjects for conformational design and analysis. We are particularly interested in two versions of heterogeneous backbones: those in which different monomer types occur in a regular pattern[4] and those in which specific structural elements are created with different monomer types.[5] We describe a hetero-backbone foldamer of the latter type, an oligomer that adopts a hairpin shape in which the loop is composed of β-amino acids and the strands of α-amino acids.

The hairpin structural motif, in which two strands of β-sheet are connected by a short loop, is widespread among

[†] Department of Chemistry.
[‡] Graduate Program in Biophysics.

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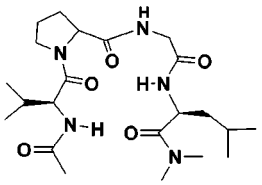
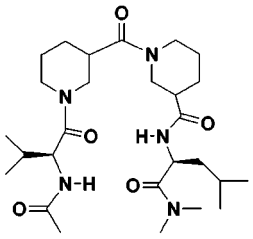
proteins.[6] We have shown that minimal α-peptide hairpins can be formed with just four residues: each terminal residue constitutes one strand, and the central two residues form the loop.[7] There is a strong interplay between the configurations of loop and turn residues in α-peptide hairpins; for example, all-L tetrapeptide **1** shows little or no hairpin conformation in methylene chloride, while D-proline-containing diastereomer **2** is very highly folded under the same conditions.[7] Here we examine hairpin formation in diastereomeric tetramers **3**−**6**, which have α-peptide strand residues identical to those in **1**−**2** but *β*-peptide loop segments.

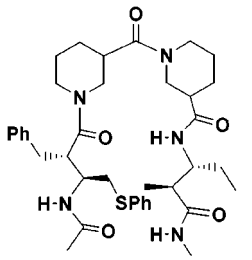


1, L-Pro
2, D-Pro



3, ***R*-Nip-*S*-Nip**
4, ***S*-Nip-*R*-Nip**
5, ***R*-Nip-*R*-Nip**
6, ***S*-Nip-*S*-Nip**

The loop segments in **3**−**6** are constructed from nipecotic acid (Nip) because we have previously shown that dinipecotic acid segments can function as loops in *β*-peptide hairpins (**7**).[8] We examined all possible stereoisomers for the dinipecotic acid segment in order to probe for correlations between the configurations of the loop and strand residues. Diastereomers **3**−**6** were prepared via standard solution-phase peptide coupling methods.



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Conformational analysis of **3**−**6** was carried out in methylene chloride. This relatively nonpolar solvent is well-suited for evaluating intrinsic conformational propensities of small oligoamides, because formation of one or two intramolecular hydrogen bonds provides a significant but not overwhelming drive for folding.[7−9] Valine and leucine were chosen as the "strand" residues because their alkyl side chains promote solubility in organic solvents. In addition, the side chains of Val and Leu lack polar groups that could compete for hydrogen bonding to the backbone amide groups in a nonpolar solvent, and they lack aromatic groups that could interfere with the use of amide proton NMR chemical shifts to detect intramolecular hydrogen bonding.[7−9] Among α-peptides, we have demonstrated that trends observed for minimal hairpins in methylene chloride (e.g., the L-Pro vs D-Pro effect in **1** vs **2**) hold up for larger hairpins in aqueous solution.[10]

Figure 1 shows N−H stretch region IR data obtained for 1 mM solutions of **3**−**6** in CH_2Cl_2 at room temperature (experiments described below indicate that there is no intermolecular hydrogen bonding under these conditions). The data indicate that the heterochiral loops (**3**−**4**) support hairpin formation while the homochiral loops (**5**−**6**) do not. Each of the spectra shows two maxima, one near 3420 cm^{-1} and the other in the range 3310−3325 cm^{-1}. Extensive precedent indicates that the 3310−3325 cm^{-1} bands arise from amide protons engaged in N−H- -O═C hydrogen bonds with favorable geometries (N−H- -O arrangement near linearity).[7−9,11] The ca. 3420 cm^{-1} bands arise from N−H

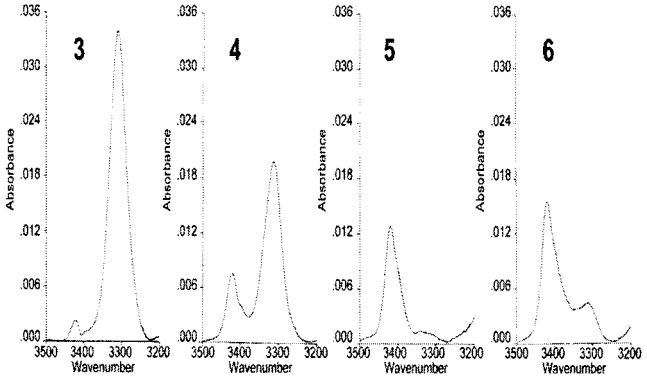

Figure 1. N-H stretch FT-IR data for 1 mM samples in CH_2Cl_2 at room temperature, after subtraction of the spectrum of pure CH_2Cl_2. From left to right: **3**, maxima at 3311 cm^{-1}; **4**, maxima at 3315 cm^{-1}; **5**, maxima at 3417 cm^{-1}; **6**, maxima at 3416 cm^{-1}.

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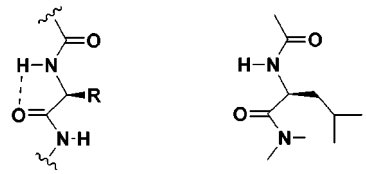
groups engaged in a "C_5" interaction, a weak intraresidue N−H- -O═C interaction with poor N−H- -O geometry.[11] (*N*-Acetylleucine-dimethylamide ($AcLeuNMe_2$), a reference compound for the C_5 interaction, displays a single band at 3429 cm^{-1} under these conditions.[12]) In the hairpin conformation, both N−H groups are involved in geometrically favorable hydrogen bonds, while C_5 interactions are expected in the absence of hairpin formation. Compound **3** (Figure 1) displays a large band at 3311 cm^{-1} and only a tiny band in the C_5 region, suggesting that this molecule exists almost



C_5 interaction **$AcLeuNMe_2$**

entirely in the hairpin conformation under these conditions. Compound **4** (Figure 1) also shows a dominant band at lower wavenumber, but the relative size of the C_5 band is larger for **4** than for **3**, which suggests that the *S*,*R* turn stereochemistry of **4** is not as effective as the *R*,*S* stereochemistry of **3** at promoting sheet interactions between L-α-residues. Both homochiral turns lead to dominant C_5 bands (**5**−**6**, Figure 1), which shows that neither of these turns is an effective promoter of sheet formation in attached α-residues.

^{1}H NMR data support the conclusions derived from the IR data and provide additional insight on the conformational behavior of **3**−**6**. Amide proton chemical shifts (δNH) offer two useful types of information. First, δNH values are very sensitive to hydrogen bond formation in nonpolar solvents, with increasing extent of hydrogen bonding signaled by increasingly downfield shifts. (Equilibration between hydrogen bonded and non-hydrogen bonded states is rapid on the NMR time scale, in contrast to the IR time scale, and the observed δNH values are population-weighted averages of the contributing states.) Second, the number of NH resonances indicates whether multiple amide rotamers are present. This issue arises because Xxx−Nip peptide bonds are unsymmetrical tertiary amides, the *E* and *Z* rotamers of which should be similarly populated in the absence of other conformational biases.[8,13] (In contrast, only the *Z* rotamer is significantly populated for most secondary amides.[13]) Interconversion of amide rotamers is slow on the NMR time scale at room temperature, and the existence of multiple amide rotamers is therefore implied when multiple resonances are observed for a single proton. The dispersion among the amide proton resonances of **3**−**6** renders these signals particularly useful for detecting rotamers.

Variable concentration studies indicated that δNH values for **3** were independent of concentration over the entire range studied (0.1−200 mM), and the δNH values for **4**−**6** were independent of concentration at or below 1 mM in CD_2Cl_2 at room temperature. For amide protons with δNH < 7, the chemical shift moved noticeably downfield as the concentration was raised above 10 mM, which suggests that intermolecular hydrogen bonding occurs at these higher concentrations. The NMR data discussed below were obtained with 1 mM samples to ensure that only intramolecular hydrogen bonding is detected.

Molecule **3** displayed two of the four possible tertiary amide rotamers. The population of the major rotamer was greater than 85%. The major rotamer NH resonances, Leu NH at 8.15 ppm and Val NH at 7.98 ppm, indicate that the NH groups are largely engaged in geometrically favorable N−H- -O═C hydrogen bonds, e.g, the 12- and 16-membered ring hydrogen bonds expected for the hairpin conformation. (The lone NH resonance of C_5 reference compound $AcLeuNMe_2$ occurs at 6.17 ppm under these conditions.[12]) The δNH data for **3** are consistent with the IR data obtained under comparable conditions (Figure 1), suggesting a very high population of the hairpin conformation.

δNH data for **4** indicated that all four of the possible tertiary amide rotamers are populated. One rotamer was dominant, and the other three displayed 10−50% of the major rotamer population. For the major rotamer of **4**, Leu NH = 8.13 ppm and Val NH = 7.43 ppm. These data suggest that the major rotamer is largely folded into a hairpin conformation, although the extent of hydrogen bonding at Val NH of the major rotamer appears to be significantly lower than the extent of hydrogen bonding at Val NH of **3**. Among the minor rotamers of **4**, there is one NH resonance at 7.53 ppm (Leu), and the remainder of the NH resonances fall between 6.1 and 6.4 ppm. These data indicate that none of the minor rotamers has a significant population of the hairpin (doubly hydrogen bonded) conformation.

δNH data for **5** and **6** indicate that neither of the homochiral dinipecotic acid turn segments supports hairpin formation. In both cases, NH resonance multiplicty shows that at least three amide rotamer forms are present. For **5**, one Val resonance occurs at 7.02 ppm, suggesting a moderate amount of internal hydrogen bonding; the other NH resonances appear between 6.2 and 6.8 ppm. For **6**, all NH resonances occur between 6.2 and 6.5 ppm. Thus, none of the rotamers populated for **5** or **6** has extensive cross-strand hydrogen bonding at both NH groups, as required for the hairpin conformation.

NOESY[14] analysis of **3** (10 mM in CD_2Cl_2, room temperature) provided further insight on the conformation in solution. Three NOEs were observed between protons on nonadjacent residues (Figure 2a): between Leu NH and a proton on C_2 of the (*R*)-nipecotic acid residue, between Leu NH and Val NH, and between the methyl of the N-terminal acetyl group and a methyl on the C-terminal dimethylamino group. The latter two correspond to the NH- -NH and C_αH- -C_αH NOEs that are characteristic of antiparallel β-sheet in proteins.[15] All three of these NOEs are consistent with the hairpin conformation drawn in Figure 2a; no NOEs incon-

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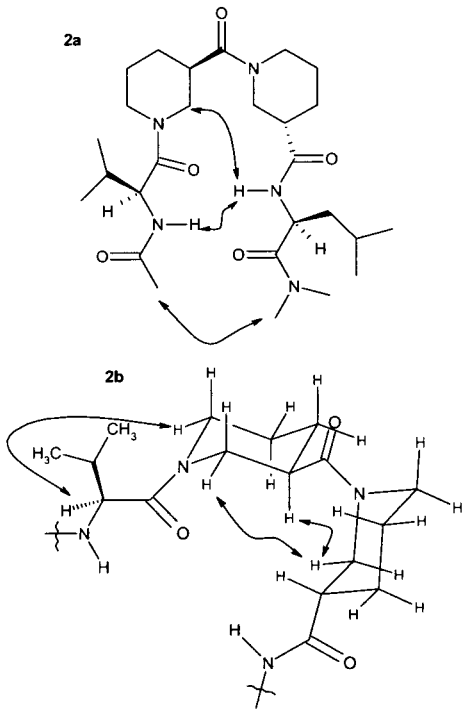

Figure 2. Selected NOEs observed in **3**: (a) NOEs between nonadjacent residues; (b) NOEs that define the turn configuration.

sistent with this conformation were detected. Three NOEs involving protons on the nipecotic acid residues verify that the two tertiary amide groups exist in the expected rotameric forms (Figure 2b). Specifically, an NOE between a proton on C_6 of the (*R*)-nipecotic acid residue and the Val $C_\alpha H$ show that the Val-Nip amide is *Z*, and NOEs between protons on C_2 and C_3 of the (*R*)-nipecotic acid residue and a proton on C_2 of the (*S*)-nipecotic acid residue shows that the Nip-Nip amide is *E*. (NOESY studies were not attempted for **4**−**6** because of the multiple rotamers.)

Our results show that the (*R*,*S*)-dinipecotic acid segment promotes antiparallel β-sheet interactions between attached L-α-amino acid residues; thus, the di-β-peptide unit replaces the loop region of the β-hairpin supersecondary structure, which is a common feature among conventional peptides and proteins.[6] The other heterochiral dinipecotic acid segment, (*S*,*R*), also allows hairpin formation, although the (*S*,*R*) segment is clearly inferior to the (*R*,*S*) segment as a hairpin promotor. Both homochiral dinipecotic acid segments preclude hairpin formation, which is consistent with our earlier observation that the homochiral dinipecotic acid β-peptide does not allow formation of 12-membered ring hydrogen bond that is necessary for hairpin folding.[8a] The relationship between loop residue configuration and hairpin formation in **3**−**6** mirrors that observed for tetramers **7**,[8] in which the strands are β-amino acid residues. This relationship stands in contrast, however, to the trend among Pro-Xxx loops in α-peptide hairpins, because only the Pro configuration is important in this case.[7]

Our findings suggest that heterochiral dinipecotic acid segments may find uses in two areas: development of β-turn mimics for biomedical applications[16] and creation of hairpin-shaped catalysts for asymmetric reactions.[17] In addition, our successful replacement of one component of an α-peptide hairpin with an unnatural unit suggests that more extensive substructure replacements may be possible (e.g., one helix in a helical bundle tertiary structure).[18]

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