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An Enhanced β Turn in Water

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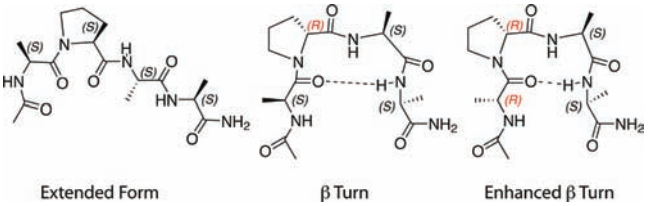
ABSTRACT



Aiming to design short linear peptides featuring strong intramolecular hydrogen bonds in water, a series of tetrapeptides based on the sequence Ac-Ala-Pro-Ala-Ala-NH_2 containing all possible combinations of L- and D-amino acids was synthesized. A regiospecific combination of heterochiral residues (DDLL or its mirror image LLDD) can be used to increase turn formation and stability within short peptides in water.

Establishing strong intramolecular hydrogen bonds in short linear peptides under aqueous conditions is a major challenge in peptide design. Such peptides can have a wide variety of applications, including in organocatalysis, green chemistry, and as templates for therapeutics and biomaterials.[1] However, the lack of covalent cyclization in linear peptides imposes an entropic penalty on the peptide backbone for folding. Consequently, short linear peptides usually adopt extended conformations in aqueous environments that feature intermolecular hydrogen bonds with water molecules.

Although some short peptide sequences are capable of adopting structured populations in an aqueous environment, the role of the local sequence in conformational bias is not straightforward.[2] Among the various strategies for designing linear peptides that form stable conformations in water, we and others have reported short linear peptides containing aromatic amino acids that adopt stable conformers in water.[3] In addition, incorporation of conformationally restricting Pro and D-amino acids can be used to induce turn structures within short peptides.[4] However, the ability of these amino acids to induce formation of intramolecular hydrogen bonds in water has been studied only to a limited extent. Moreover, a systematic comparison of hydrogen bond strength produced by different combinations of Pro and D-amino acids in model peptides in an aqueous environment is lacking. The objective of this study was to identify such combinations in linear peptides that are capable of inducing significant populations of folded conformations through intramolecular hydrogen bonding in water.

Backbone chirality significantly contributes to defining the conformational space of a peptide and is a driving force for the formation of certain types of β turns.[5] In addition to interactions including side chain–side chain, side chain–backbone, backbone–backbone, electrostatic and aromatic interactions, heterochirality can be used to promote turn formation and stability.[6] Indeed, heterochirality is a requirement for nucleating specific types of turns. For example, D-amino acids at the $i+1$ and $i+2$ positions increase the propensity of type II′ or type II turn formation, respectively.

We designed a series of tetrapeptides with the sequence APAA containing all possible combinations of D- and L-amino acids at each position (Figure 1). By varying the configuration of each residue in a tetrapeptide, there are 16

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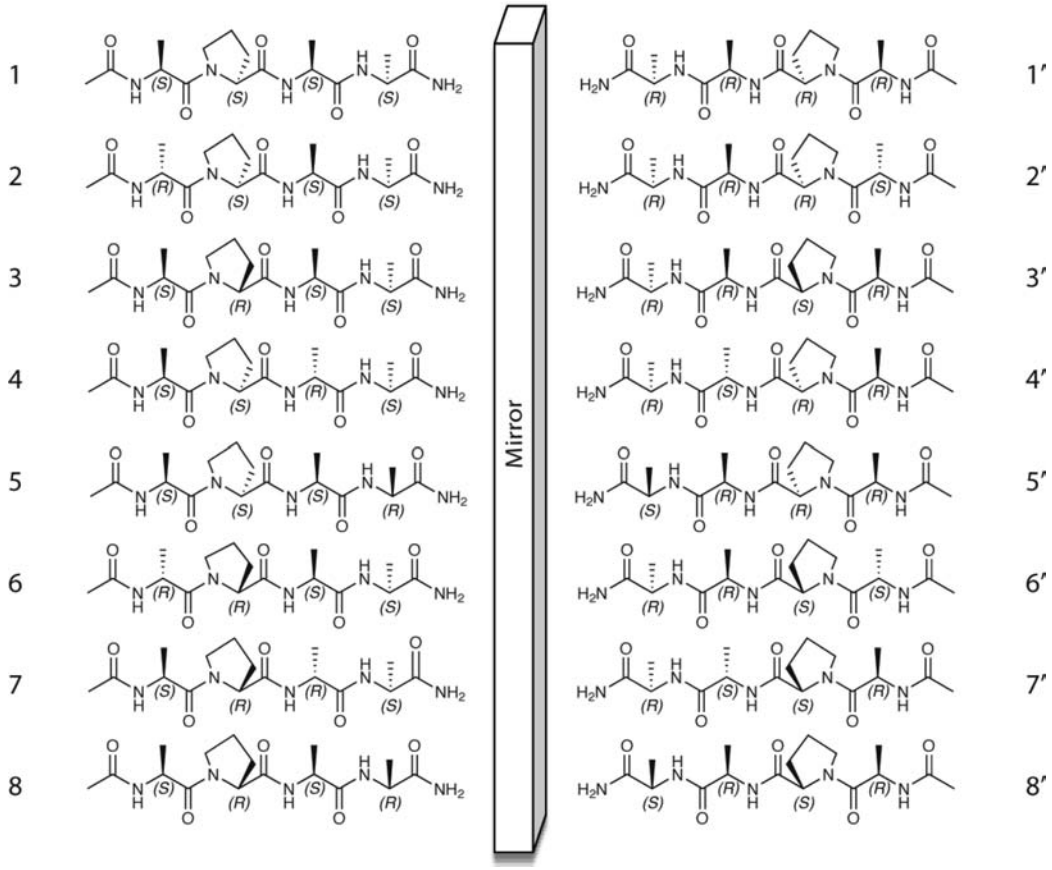

Figure 1. Based on the tetrapeptide Ac-Ala-Pro-Ala-Ala-NH_2, there are 2^4 (or 16) sequences with all combinations of L- and D-amino acids. We created a series of peptides containing at least one member from each pair (1−8) as well as two mirror-image pairs (6/6′ and 7/7′) and analyzed their ability to adopt folded hydrogen-bond-stabilized conformers in water.

possible combinations (8 mirror images). At least one representative from each of these eight pairs was used in the series of peptides studied herein. The XPXX motif is significant, as it has been observed in a variety of bioactive peptides, including opioid peptides such as endomorphins (YPWF and YPFF)[7] and Tyr-MIF1 (YPLG),[8] the contraceptive peptide kentsin (TPRK),[9] the immunodominant region (NPNA repeats) of the major surface protein in *Plasmodium falciparum*, and the DNA-binding motif SPKK. The number of bioactive peptides containing the XPXX motif is not surprising, considering the strong propensity of Pro at the $i+1$ position to induce β turns. Indeed, the XPXX motif in proteins promotes the formation of β turns, which serve as sites for receptor binding and antibody recognition and play an important role in protein folding.

We used Ala in the X position of the XPXX motif because its nonpolar yet nonbulky side chain is less likely to interfere sterically or electronically with hydrogen bonding. All of the peptides contained only four amino acids to eliminate the influence of residues flanking the tetrapeptide core. The peptides were acetylated and contained a C-terminal amide group and thus could potentially adopt conformations featuring 7-, 10-, or 13-membered hydrogen bonds. We performed H/D exchange, measured the amide temperature coefficients, and acquired CD and ROESY spectra in water to assess hydrogen bonding and to describe the conformational preferences of the peptides.

Amide chemical shifts depend on the distance between the proton and hydrogen bond acceptor.[10] Temperature coefficients, the variation in amide chemical shift with temperature, are widely used to predict hydrogen bonding and are considered to be far more reliable in water than in organic solvents.[10a] Values more positive than −4.5 ppb/K are generally accepted to suggest the presence of hydrogen bonds in proteins, and more negative values are expected for amides not involved in hydrogen bonding.[11] Temperature coefficients can be advantageous over H/D exchange rates as hydrogen bond predictors as they do not involve

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ambiguities arising from differential solvation of the amide backbone; however, they can be influenced by deshielding effects from surrounding residues and by a temperature-dependent loss of structure.[12]

The temperature coefficient data revealed distinct differences in the hydrogen bond strength within these peptides. The i+3 residues of **3** (Ac-Ala-D-Pro-Ala-Ala-NH_2) and **4** (Ac-Ala-Pro-D-Ala-Ala-NH_2) showed more positive temperature coefficients of −4.0 and −4.7 ppb/K, respectively (Figure 2). Notably, the i+3 residues of the mirror-image **6** (Ac-D-Ala-D-Pro-Ala-Ala-NH_2) and **6′** (Ac-Ala-Pro-D-Ala-D-Ala-NH_2) exhibited the most positive temperature coefficients (−2.5 ppb/K). Values in this range are typically only observed for amides that are hydrogen bonded in the globular environment of a protein or are influenced by ring-current effects.[11] Indeed, Ala residues found in a statistical coil structure exhibit temperature coefficients around −8.2 ppb/K,[13] as was observed for many other Ala residues in these peptides. The H/D exchange data of these peptides correlated well with the i+3 amide exchange rates, which were all slower than those of the i+2 residues (Figure S2 in Supporting Information). However, as anticipated, the H/D exchange rates did not follow an identical profile to that of temperature coefficients due to influence from backbone shielding effects.

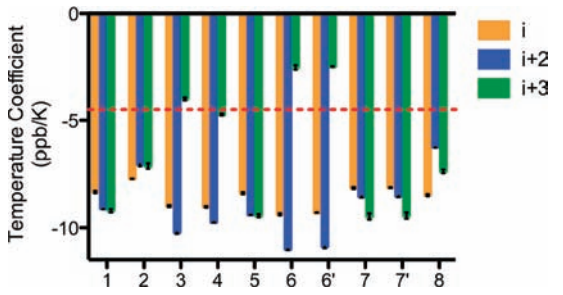

Figure 2. Amide temperature coefficients of the peptides. Temperature coefficients more positive than −4.5 ppb/K (dashed red line) indicate a significant population of folded conformers in aqueous solution.

We performed 2D ROESY experiments to characterize the conformational features of the peptides. Only **3**, **4**, **6**, and **6′** showed $d_{NN}(i,i+1)$ cross-peaks for the i+2 and i+3 residues, which is one criterion of turn formation in short peptides.[14] These four peptides also exhibited long-range cross-peaks in addition to their sequential $d_{NN}(i,i+1)$ cross-peaks. Notably, these four peptides all exhibited the most positive temperature coefficients for the i+3 amides. Moreover, the amide proton chemical shifts of these four peptides were better dispersed than the other peptides, which is also indicative of folded structures (Figure S1).[15] In addition to their exceptionally positive temperature coefficients, the long-range NOE data convincingly support the presence of a significant population of hydrogen-bond-stabilized turn structures, especially for **6** and **6′**. In **6** (and its mirror-image **6′**), the methyl group of i showed cross-peaks with the C-terminal amide protons and the methyl group of i+3 exhibited cross-peaks with the amide of i (Figure 3). In addition, a weak NOE was observed between the Hα of Pro and the amide of i+3. A concentration-dependent series of 1D spectra revealed that the chemical shifts of the amide protons do not change at various peptide concentrations, indicating that the NMR data reflect intramolecular interactions (Figure S7).

Accordingly, the CD spectra of **6** and **6′** indicated the presence of turn structures.[16] In **6**, a maximum was observed at 188 nm and a minimum was seen at 205 nm. By contrast, the mirror-image spectrum for **6′** exhibited a minimum at 188 nm and a maximum at 205 nm. Peptides containing the D-Pro-L-Xaa motif have been shown to adopt type II′ turns, and the L-Pro-D-Xaa sequence is well-known as a type II turn inducer.[4a,17] Collectively, these results suggest that **6** and **6′** adopt a significant population of type II′ and type II turns, respectively, in water. The long-range NOEs and CD spectra of the other peptides can be found in the Supporting Information.

In this systematic study, we evaluated the conformational preferences of XPXX peptides by incorporating all possible combinations of L- and D-amino acids. Of the 16 possible combinations, we only needed to evaluate eight peptides, as the **6**/**6′** and **7**/**7′** pairs confirmed that mirror-image peptides show nearly identical conformations and spectroscopic properties (similar H/D exchange rates, identical temperature coefficients and chemical shifts, and mirror-image CD spectra, Supporting Information).

Short peptides are considered to be fully solvated with minimum intramolecular hydrogen bonding interactions in water. However, we demonstrate that even small linear model peptides show a wide variety of conformational preferences based on the position(s) of D-amino acids. By systematically and sequentially changing the amino acid configuration, we discovered enhanced β turns that show tighter folds and stronger hydrogen bonding interactions than previously reported Pro-D-Xaa or D-Pro-Xaa motifs.[4a,c] Although the importance of heterochirality for turn formation is well-known, we show that a regiospecific combination of heterochiral residues can be used to increase turn formation and stability in water. Indeed, the peptides showing the most convincing evidence of turn formation in water contained heterochiral pairs (DDLL or LLDD). These results will add to the existing design principles used to create folded functional peptides in aqueous environments.

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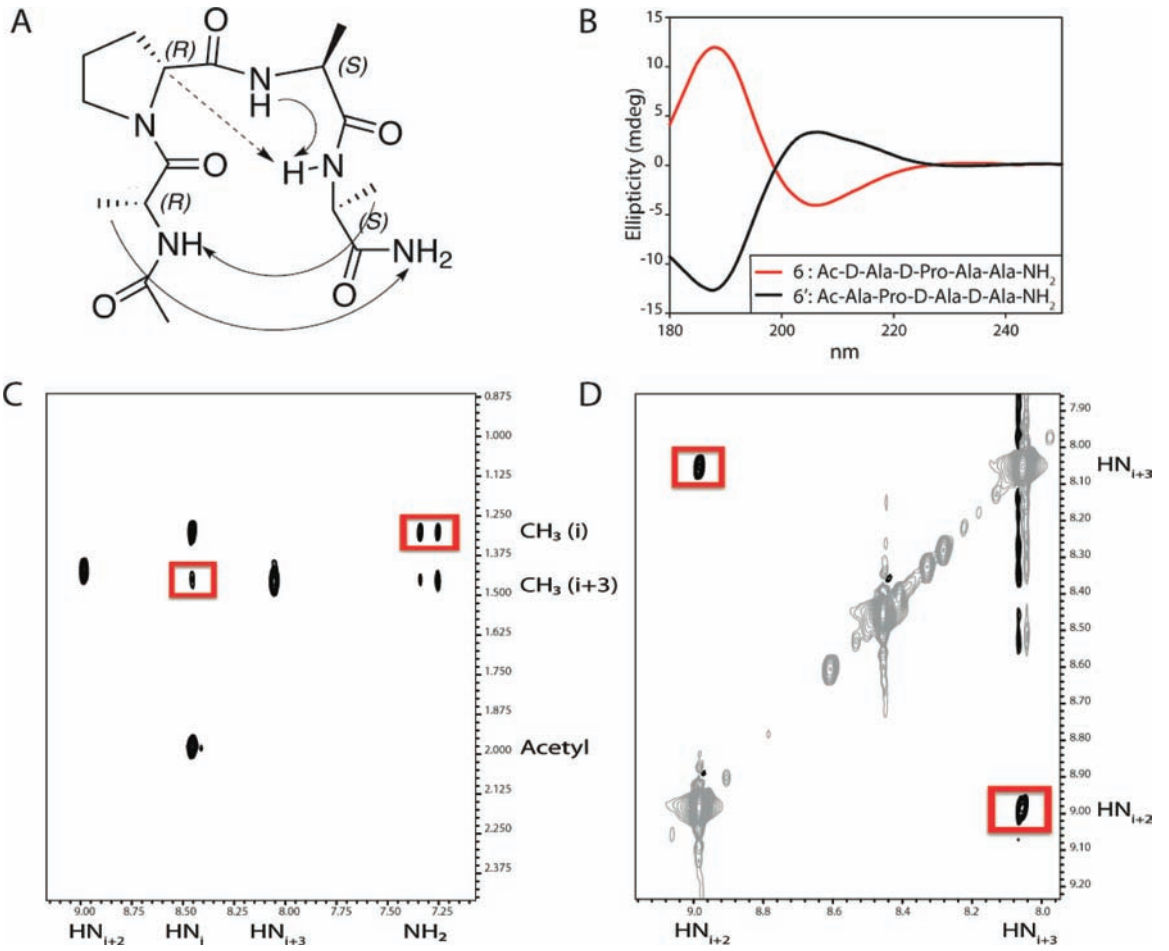

Figure 3. (A) Structure of **6** depicting the observed long-range NOEs. Solid arrows indicate strong or medium NOEs, and dashed arrows denote weak NOEs. (B) CD spectra of **6** and **6′**. (C,D) Portions of the ROESY spectrum of **6** with long-range NOEs highlighted in red. The ROESY spectra of **6** and **6′** are identical.

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Supporting Information Available. Experimental procedures, peptide purification properties, raw temperature coefficient and H/D exchange data, and additional CD and NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.