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Synthesis and Structural Investigations of Functionalizable Hybrid β-Hairpin

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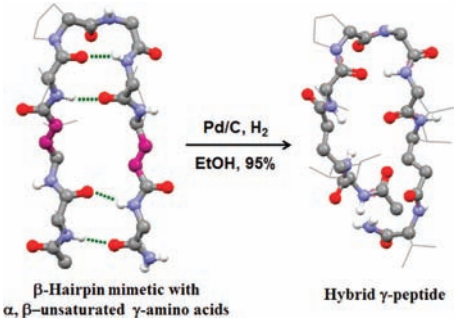
ABSTRACT



The solution and solid state conformations of a designed β-hairpin containing functionalizable α,β-unsaturated γ-amino acids at the antiparallel β-strands and a single step transformation to its saturated γ-peptide analogue are studied.

The β-Hairpin is a simple structural motif that consists of antiparallel β-sheets and a reverse turn. Many proteins often constitute a β-hairpin scaffold for biomolecular recognition.[1] The β-hairpin scaffolds have been used to structurally mimic the epitopes of antibodies and cytokine receptors.[2] They have been used as inhibitors for protein−protein and protein−nucleic acid interactions, which have proven difficult for small molecule drugs[3] as well as synthetic vaccine design.[4] Naturally occurring β-hairpin scaffolds have proven their potential as antimicrobial and antiviral candidates.[5] In addition, β-hairpins are also being used to obtain self-assembling biomaterials with gel and hydrogel properties.[6] Moreover, designed β-hairpins have been exploited as chiral catalysts in a variety of organic reactions.[7] The

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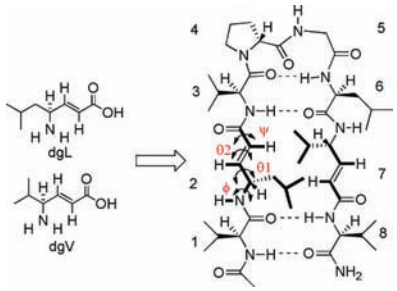
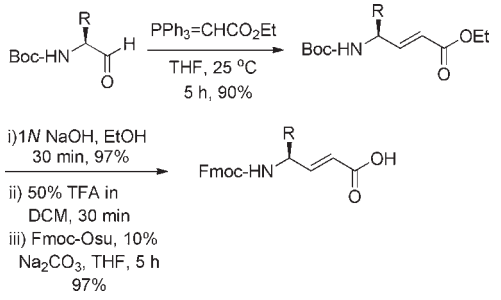


Figure 1. Model hybrid β-hairpin containing α,β-dehydro γ-amino acids (**dg**) at facing positions of the antiparallel β-strands.

Scheme 1. Synthesis of Vinylogous Amino Acids



remarkable and widespread properties of β-hairpins have attracted synthetic chemists and structural biologists to design stable β-hairpins using natural and non-natural amino acids.[3a,8] Unlike α-helical peptides, where the structure is stabilized by intramolecular 13-atom H-bonds, the β-strand requires a structural context for stabilization. The design of β-hairpins with stabilized β-strands is a challenging task. Recent advances that improved β-hairpin stabilities outside the protein context, thus far, have the sequences ^{D}Pro-Xxx (Xxx is Gly, Ala, Pro, Aib etc.), Asn-Gly, Aib-Gly, or Aib-^{D}Ala at the turn segment.[3a,8a,8b,9] The β-strand residues that modulate the β-hairpin stability depend upon their intrinsic β-sheet propensities through both cross strand and diagonal side chain−side chain interactions. The antiparallel β-strand stabilities are further improved by incorporating Trp-Trp residues at opposite faces.[10] Herein, we are reporting the design, synthesis and structural characterization of a stable hybrid octapeptide β-hairpin mimetic (Ac-Val-dgL-Val-^{D}Pro-Gly-Leu-dgV-Val-NH_2) **P1** inserting α,β- dehydro γ-amino acids at the antiparallel β-strands

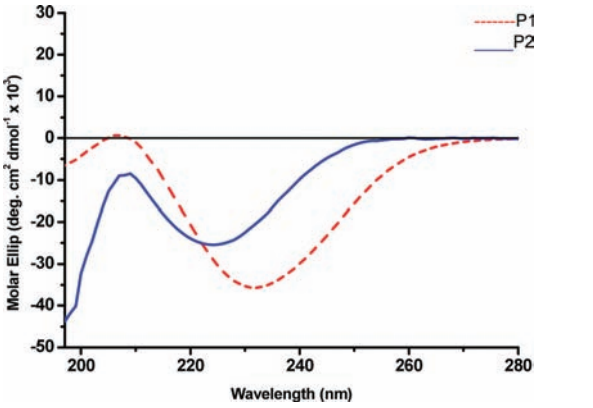


Figure 2. Circular dichroism (CD) spectra of vinylogous hybrid β-hairpin **P1** and **P2** in methanol.

using ^{D}Pro-Gly as a turn segment (Figure 1) and its single step transformation to γ-peptide analogue (**P2**).

Vinylogous amino acids (insertion of −CH=CH− between $C^{\alpha}H$ and CO, α,β-unsaturated-γ-amino acids) have been frequently found in many peptide natural products.[11] In their preliminary research, Schreiber and colleagues have described the formation of hybrid sheets and helical type of structures by the peptides with vinylogous amino acids.[12] Indeed inspired by the structural diversity of the unsaturated amino acids,[13,14] we sought to investigate their conformational behavior in the hybrid β-hairpin mimetic. Insertion of vinylogous amino acids into β-hairpin structures is of considerable interest in the design of analogues of biologically active peptides, as they have been used as substrates in many organic reactions including 1,4-conjugate addition,[15] epoxidation,[16] Diels−Alder reaction[17] and catalytic hydrogenation[18] to derive functional and saturated γ-amino acids. The *E*-vinylogous amino acids **dgL** and **dgV** were inserted at facing positions 2 and 7, respectively, as shown in Figure 1. The vinylogous octapeptide **P1** was synthesized using solid phase method on Rink amide resin. The corresponding vinylogous amino acids, Fmoc-(*S,E*)-dgL−OH and Fmoc-(*S,E*)-dgV−OH, were synthesized using Wittig reaction[12,14] starting from Boc-protected amino aldehydes as shown in Scheme 1. The ^{1}H NMR of **P1** (3 mM) in CD_3OH

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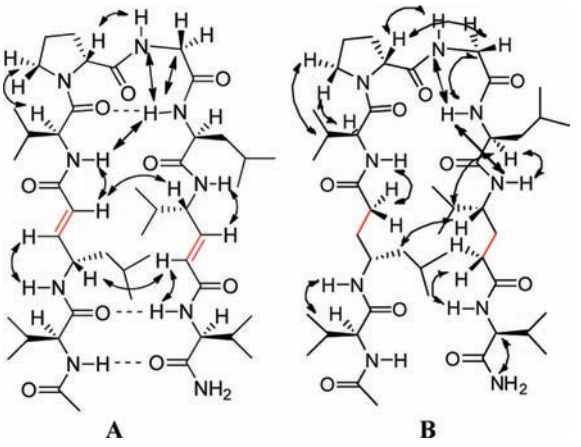

Figure 3. Observed NOEs of the peptides (A) **P1** and (B) **P2** in the ROESY are schematically shown by double headed arrows.

reveals that the wide dispersion of backbone NH and $C^{\alpha}H$ along with vinylic protons chemical shifts signaling a well structured peptide in solution. The circular dichroism (CD) spectrum of **P1** (110 μM) in methanol is shown in Figure 2. We speculate that the red-shift of the CD minima at 234 nm may be due to the conjugated enamides of vinylogous residues. As anticipated, the 2D NMR (TOCSY and ROESY) analysis showed the characteristic turn and the cross-strand NOEs of antiparallel β-strands confirming a β-hairpin structure in solution. The critical NOEs observed in the ROESY spectrum are illustrated in the schematic diagram shown in Figure 3A. The characteristic sequential $NH\leftrightarrow C^{\alpha}H$, cross-strand $NH\leftrightarrow NH$ and $C^{\alpha}H\leftrightarrow C^{\gamma}H$ NOEs of facing vinylogous residues observed in the ROESY spectrum are given in the Supporting Information. The strong $C^{\alpha}H\leftrightarrow C^{\gamma}H$ NOEs between the interstrand vinylogous residues in **P1** indicating the stable and the well folded antiparallel β-sheet character in solution. Further, the deuterium exchange of amide NHs was carried out in CD_3OD. Strikingly, it was found that the slower exchange rate of dgL2NH and dgV7NH even after 4.5 h, indicating that the NHs are being shielded from the solvent by the extended

Table 1. Backbone Torsional Variables of Vinylogous Hybrid Octapeptide β-Hairpin (**P1**)

residues	ϕ	θ_1	θ_2	ψ	ω
Val1	−118			114	178
dgL2	−118	132	174	174	−179
Val 3	−141			103	178
^{D}Pro4	61			−130	178
Gly5	−75			−10	178
Leu6	−75			124	−178
dgV7	−130	123	179	176	−173
Val8	−137			130	

antiparallel β-sheets of β-hairpins held together by strong intermolecular H-bonding. However, we did not observe notable intermolecular NOEs between the β-hairpins.

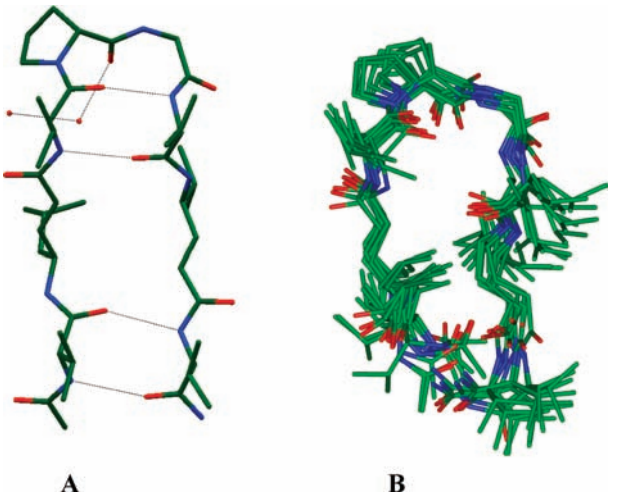

Figure 4. (A) Crystal structure of β-hairpin (**P1**). Hydrogens are not showed for clarity. All four intramolecular hydrogen bonds are represented in dotted lines. Two water molecules O10 and O11 connected through the O5 (Pro4) are also shown. (B) Solution conformation of the peptide **P2** in methanol. The ensamble of ten low energy structures are calculated based on the observed NOEs (shown Figure 3). Hydrogens are omited for clarity.

Single crystals of **P1** obtained in methanol solution after slow evaporation yield the structure shown in Figure 4A. Indeed, hybrid peptide **P1** adopts a well folded β-hairpin conformation in crystals. The torsional variables are given in the Table 1. Examination of the torsional angles at ^{D}Pro-Gly segment reveals that it adapts a type II′ β-turn conformation (Table 1). The characteristic hydrogen bond parameters are given in the Supporting Information. The α-residues adapted characteristic conformations of a β-sheet. Both vinylogous amino acids dgL(2) (ϕ = −118°, θ_1 = 132°, θ_2 = 174° and ψ = 174°) and dgV(7) (ϕ = −130°, θ_1 = 123°, θ_2 = 179° and ψ = 176°) accommodated into the β-sheets with a very similar characteristic backbone

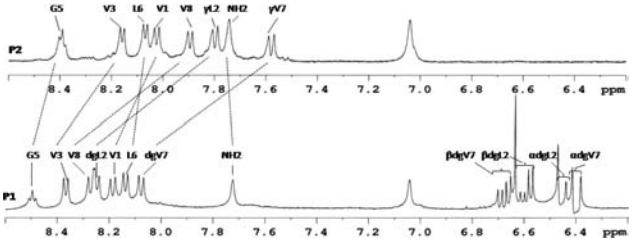

Figure 5. Assigned partial ^{1}H NMR spectra of **P1** and **P2**. A well dispersed amide and vinylic protons of **P1** are shown in the bottom spectrum. The disappearance of vinylic protons and the reshuffled amide protons of saturated peptide **P2** are shown in top spectrum. The sequential assignment of all amide protons were carried out using 2D NMR (TOCSY and ROESY).

conformations (Figure 1). The local *s-cis* conformation is observed in both enamides of the vinylogous residues. The

torsional angles θ_2, ψ and ω all have extended geometry with a value approximately (±)180°. The extended conformation of vinylic $-C^{\beta}=C^{\alpha}-$ and $-C^{\alpha}-CO-$ (*s-cis*) of enamide facilitates the accommodation of vinylogous residues into the backbone conformation without disturbing the canonical β-hairpin structure. Interestingly, the values of ϕ and θ_1 of vinylogous amino acids have values very similar to the ϕ and ψ of α-residues. In addition, the β-hairpin conformation in crystal is further stabilized by four cross strand intra molecular hydrogen bonds (Figure 4A). Both dgL2 and dgV7 are not involved in intramolecular hydrogen bonding as they are incorporated at the non-hydrogen bonding positions of antiparallel β-strands.

Further, we examined the feasibility of the transformation of an unsaturated β-hairpin to its saturated γ-peptide analogue (**P2**, Ac-Val-γLeu-Val-^{D}Pro-Gly-Leu -γVal-Val-NH_2) using catalytic hydrogenation. The pure **P1** was subjected to catalytic hydrogenation using 10% Pd/C in ethanol. The reaction was monitored by MALDI-TOF and HPLC. Noticeably, complete conversion of **P1** to **P2** was observed in 6 h. The pure hybrid γ-peptide was isolated in 95% yield. The transformation of vinylogous hybrid β-hairpin to its saturated analogue proceeds very smoothly. The completely assigned ^{1}H NMR of **P1** and **P2** (amide region) is shown in Figure 5. The redistribution of amide NHs and the complete disappearance of all vinylic protons is observed in hybrid γ-peptide analogue. Surprisingly, the upfield chemical shifts are observed for all β-strand residues (Figure 5). In contrast to **P1**, saturated **P2** shows the anomalous CD spectrum with negative maxima at 200 and 225 nm (Figure 2). We made several attempts to crystallize **P2** in different solvents; however, we could not able to get diffractable quality crystals. Further, to understand the conformational behavior of **P2** in solution, we subjected hybrid γ-peptide to 2D NMR analysis. The NOEs observed from 2D NMR (ROESY) are schematically shown in Figure 3B. Interestingly, in contrast to **P1**, we did not observe any characteristic cross-strand NH↔NH and CH↔CH interactions in **P2**. However, the sequential NOEs of 5NH↔6NH (weak) and 6NH↔7NH (medium) indicating that the lack of proper β-strand registry of hairpin in solution. This may be due to the conformational freedom around the saturated $C^{\alpha}-C^{\beta}$ bond of γ-residues. Interestingly, a strong NOE between the side chain of γ-Leu2 to $C^{\gamma}H$ of γ-Val7 is observed. Using the experimentally deduced NOEs for **P2**, the ensamble of structures are obtained by distance restrained molecular dynamic simulations (Insight II 2005, Accelrys Inc.).[19] The overlay of 10 low energy conformers of NMR calculated structures are shown in Figure 4B. The NMR model reveals that the saturation of vinylic double bond introduces the additional freedom in the backbone and breaks the proper strand registry of β-hairpin. Overall, the hybrid γ-peptide **P2** adapted a folded β-hairpin conformation with poor β-strand registry in solution. A well-folded β-hairpin structure of hybrid vinylogous peptide **P1** transformed to a distorted β-hairpin conformation of **P2** after the catalytic hydrogenation.

In conclusion, we have demonstrated the effective incorporation of functionalizable vinylogous amino acids into the β-hairpin without disturbing overall fold of the molecule. In addition, we have described a single step conversion of vinylogous hybrid peptide into its saturated analogue. The hybrid γ-peptide adapted a distorted β-hairpin conformation with poor strand registry in solution. Appropriate geometry for the proper interstrand registry of the β-hairpin peptide **P1** is dictated by the double bonds of vinylogous γ-residues. This geometrical constrain is lacking in **P2**, which leads to the nonregistry of interstrands, altering its conformation. This present study envisages that the choice of these α,β-unsaturated γ-amino acids at appropriate positions could be used to design a well-folded β-hairpin with proper strand registry.

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Supporting Information Available. Experimental procedures, compound characterization, and crystallographic information. This material is available free of charge via the Internet at http://pubs.acs.org.

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