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Structural Insight into Hybrid Peptide ε-Helices

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Unique ε-helical organizations (11-helices) from β,γ-hybrid peptides composed of chiral β³-amino acids along with achiral 3, 3- or 4,4-dimethyl substituted γ-amino acids are disclosed.

The protein secondary structures stabilized by the 11-membered H-bonds are relatively rare compared to the more frequent 7(γ)-, 10(β-) and 13(α)-membered H-bonds.[1] Repeated 10-membered and 13-membered intramolecular H-bonds in a polypeptide sequence leads to the 3_{10} and α-helix structures, respectively.[2] Indeed, the 11-helix polypeptide conformation was proposed by the Bragg, Kendrew and Perutz by projecting the H-bonds from N to C-terminal direction along the helix [NH(i)←OC(i+2)][3] and subsequently the structure was challenged by Corey and Pauling by predicting correct structure of α-helix (13-helix) with the H-bonds from C to N-terminal direction [CO(i)→NH(i+4)].[4] Through comprehensive literature survey of proteins and peptides structures, Toniolo, Balaram and colleagues recently reported the existence of 11-membered H-bond turns or "ε-turns".[5] In comparison to α, β and γ-turns, the ε-turns displayed the reversal of H-bonding directionality (N to C-terminal). Apart from its rare presence in the reverse turn segments, continuous 11-membered H-bond stabilized helical structures have not been observed in the protein and peptide structures.

In spite of their backbone conformational flexibility, oligomers of β- and γ-amino acids and hybrid peptides (α/β and α/γ) composed of α, β and γ-amino acids have been shown to adopt diverse helical organizations with different H-bond pseudocycles.[6] The potential of diverse helical structures of α/β and α/γ-hybrid peptides have been explored to design inhibitors for protein-protein interactions, antimicrobials and biomaterials.[6,7] In addition to α/β and α/γ-hybrid peptides, through systematic theoretical analysis, Hofmann and colleagues reported potential helical organizations from β,γ-hybrid peptides.[8] Among various helix types, β,γ-hybrid peptides are able to adopt 13-helix conformation as that of native α-helix with regular H-bonding directionality (C to N terminal direction). Interestingly, theoretical studies also suggested that 11-helix from β,γ-hybrid peptides with downstream H-bonding (N to C terminal direction) is also one of the most stable helices in the series. Recently, Gellman and colleagues experimentally proved the 13-helix conformation of β,γ-hybrid peptides composed stereochemically constrained γ-amino acids.[9] In continuation, Aitken and colleagues demonstrated the 9/8 ribbons and 13-helices from β,γ-hybrid peptides constituted with stereochemically constrained β-amino acids.[10] Further, Koksch and colleagues have demonstrated the incorporation of β,γ-peptide fragments into coiled coil sequence.[11] Additionally, Balaram and colleagues observed the 13-membered H-bonds in β,γ,α-tripeptides.[12] In continuation, Sharma and Kunwar showed the mixed 11/13 helix with alternating H-bond orientation composed of C-linked carbo-β and γ-amino acids.[13] However, there is no experimental evidence on the possibilities of 11-helix structures in β,γ-hybrid peptide foldamers.

We have been involved in designing various helical foldamers composed of α,γ-hybrid peptides. Both theoretical and experimental studies revealed the stable 12-helical conformations from α,γ-hybrid peptides.[8,14] In addition to the 12-helix, recently we reported 15/17-helix conformation in α,γ-hybrid peptides composed of achiral Aib (α-aminoisobutyric acid) and Aic (4-aminoisocaproic acid) residues.[15] Inspired by the conformational behavior of α,γ-hybrid peptides, we sought to examine structural properties of β,γ-hybrid peptides composed of achiral γ-amino acids. Here we are reporting the continuous 11-helical conformations in solution from β,γ-hybrid

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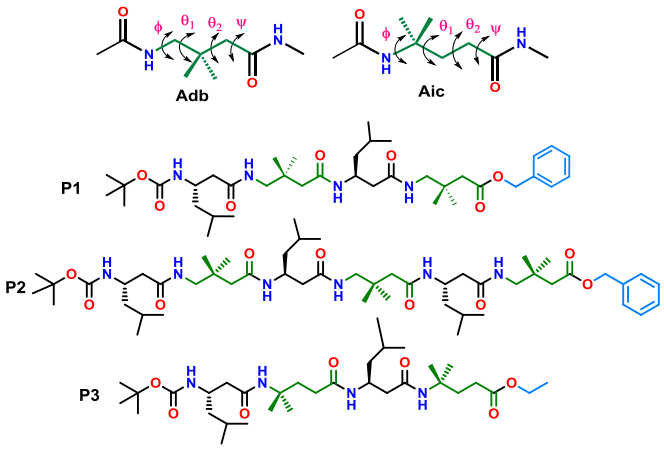
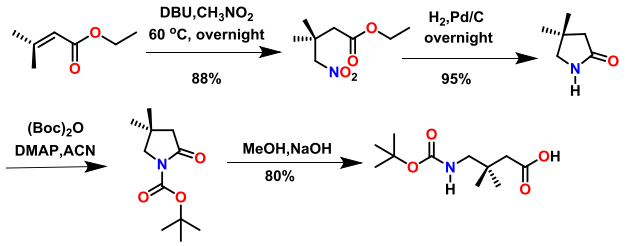
peptides composed of the chiral β-amino acid (β-Leu) and achiral 3,3(Adb)- and 4,4(Aic)-dimethyl substituted γ-amino acids.

The three peptides investigated are two 4-mers (**P1** and **P3**) and one 6-mer (**P2**). The sequences of these peptides are shown in the Scheme 1.



Scheme 1: Chemical structures of Aic(4-aminoisocaproic acid, doubly homologated Aib), Adb (4-amino-3,3-dimethyl butanoic acid) and sequences of α,γ-hybrid peptides.

The 4,4-dimethyl substituted γ-amino acid (Aic) was synthesized as reported earlier.[16] The 3,3-dimethyl γ-amino acid (4-amino-3,3-dimethylbutanoic acid, Adb) was synthesized through the Michael addition of nitromethane to the dimethyl acrylate followed by the reduction of nitro group through catalytic hydrogenation. The schematic representation of the synthesis of Adb is shown in the Scheme 2. The free amine of the γ-lactam was protected with Boc group and hydrolyzed using NaOH to get *N*-Boc-Adb. All hybrid peptides were synthesized by the conventional solution phase chemistry using EDC/HOBt as coupling agents and final peptides were purified by column chromatography and reverse phase HPLC.



Scheme 2: Synthesis of N- Boc protected 3,3-dimethylbutanoic acid (Adb)

As crystal structures provide an unambiguous information on the solid state conformation of peptides, we made several attempts to obtain single crystals of peptides **P1**-**P3**. However, we are unable to obtain single crystals of these hybrid peptides. Conversely, the higher solubility of **P1**-**P3** in nonpolar solvents like $CDCl_3$ as well as in polar solvents like CD_3OH inspire us to investigate their conformational behavior in solution using 2D NMR spectroscopy. A well-dispersed ^{1}HNMR spectrum of peptide **P1** in $CDCl_3$ (5 mM) at room temperature suggests the existence of well-defined secondary structure in solution. The amino acid type and sequential connectivity of the residues were established using ROESY and TOCSY spectra. The ROESY spectrum revealed weak NH↔NH NOEs between NH(1)↔NH(2), NH(3)↔NH(4) and strong interactions between $C^{\alpha}H(1)$↔NH(2), $C^{\beta}H(1)$↔NH(2), $C^{\alpha}H_2(2)$↔NH(3), $C^{\beta}H(3)$↔NH(4). In addition, strong NOEs between $C^{\gamma}H_2(2)$↔NH(3) was also observed. The DMSO titration studies revealed that all amide protons were involved in intramolecular H-bonding except the one at C-terminus (Adb4 NH) which was exposed to solvent. Besides the strong sequential $C^{\alpha}H_2(i)$↔NH(i+1) NOEs, hexapeptide **P2** showed the weak NH(1)↔NH(2), NH(5)↔NH(6) and medium $C^{\beta}H(1)$↔NH(2) and strong $C^{\gamma}H_2(2)$↔NH(3), $C^{\beta}H(3)$↔NH(4), $C^{\gamma}H_2(4)$↔NH(5), $C^{\beta}H(5)$↔NH(6) NOEs. Further, the DMSO titration experiment suggested that all amide NHs are involved in the intramolecular H-bonding except C-terminal NH of Adb6. To understand the effect of positional variation of dimethyl constraint in γ-amino acid towards the folding propensity of β,γ-hybrid peptides, we subjected tetrapeptide **P3** to 2D NMR. Similar to **P1** and **P2,**

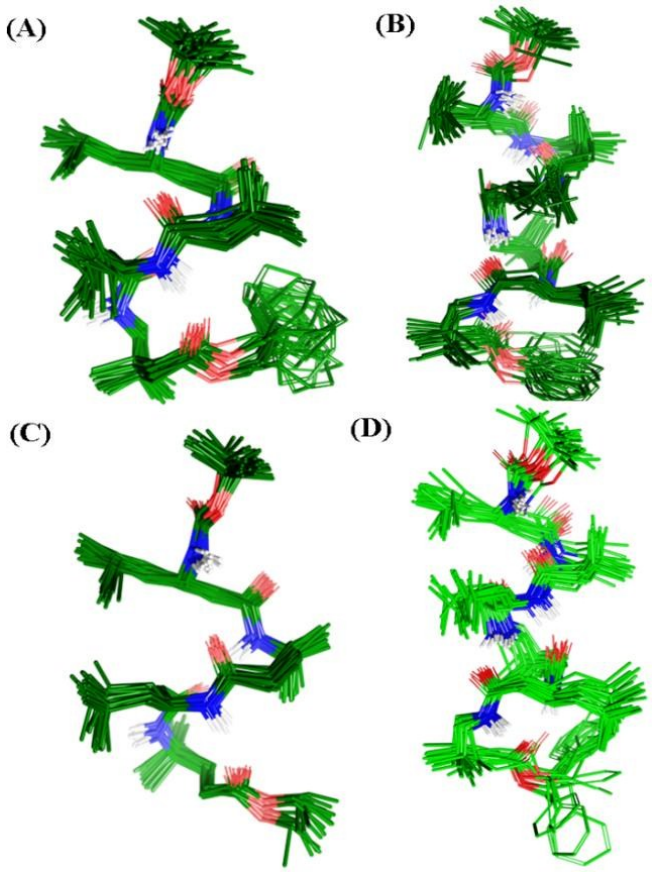


Figure 1: Solution NMR structures of peptides in $CDCl_3$ (A) **P1**, (B) **P2,** (C) **P3** and (D) **P2** in CD_3OH. Peptides displayed 11-membered hydrogen-bonded pseudo-cycles in the downstream direction (N to C terminal) between NH(i)···CO(i+ 1) residues.

peptide **P3** with 4,4-dimethyl substituted γ-amino acid (Aic) displayed weak NH(1)↔NH(2) as well as medium $C^{\beta}H(1)\leftrightarrow NH(2)$ and $C^{\beta}H(3)\leftrightarrow NH(4)$ NOEs and strong sequential $C^{\alpha}H\leftrightarrow NH$ in the ROESY spectrum. Similar to **P1** and **P2** small change in the chemical shifts of amide NHs observed during the DMSO titration except C-terminal amide proton (Aic4 NH) is suggesting its participation in intramolecular H-bonding. Using NOE and H-bonding restraints, the solution structures of **P1**, **P2,** and **P3** were generated and the superposition of 10 lowest energy minimized structure are shown in Figure 1. Strikingly, all three β,γ-hybrid peptides adopted 11-helical (ε-helix) conformation in solution.

Further, we examined the conformations of the peptides **P2** and **P3** in CD_3OH to understand the stability of 11-helix in polar solvents. Both **P2** and **P3** showed well resolved ^{1}H NMR in CD_3OH (5 mM). The ROESY experiments revealed weak NH(1)-NH(2), and strong $C^{\beta}H(1)\leftrightarrow NH(2)$, $C^{\beta}H(3)\leftrightarrow NH(4)$ and $C^{\beta}H(5)\leftrightarrow NH(6)$ NOEs in **P2** and weak NH(1)-NH(2), strong $C^{\beta}H(1)\leftrightarrow NH(2)$ and $C^{\beta}H(3)\leftrightarrow NH(4)$NOEs in **P3** similar to the NOEs observed in $CDCl_3$, suggesting similar conformational behaviour of the peptides **P2** and **P3** in CD_3OH. The superposition of 10 lowest energy minimized structures of the peptide **P2** using NOE and H-bonding restraints is shown in the Figure1D.

Along with NMR, we invoked the infrared absorption spectroscopy to understand H-bonding of peptides in $CDCl_3$. All peptides displayed the NH stretching frequency (amide A) in the range of 3300 cm^{-1} to 3310 cm^{-1} in the IR spectra suggesting the involvement of amide NHs in H-bonding with the exception of C-terminal NH group. The absorption band of this free C-terminal NH group is very weak. The concentration dependent IR investigations (See ESI Figure S33) suggested negligible change in NH stretching frequencies expect the change in intensity, indicating their involvement in the intramolecular H-bonds.[17]

Analysis of the NMR structures indicates that the helices are stabilized by NH(*i*)···CO(*i+1*) H-bonds. In addition, the directionality of the H-bonds are reversed when compared to the native α-helix. The torsion angles are tabulated in the Supporting Information. The hydrogen bond distances were found to be in the range of 1.8 to 2.5 Å. The torsion angle θ adopted *gauche*$^+$ (*g*$^+$) conformation along C^{β}-C^{α} in β-residues and ϕ, θ and ψ showed values with +++ sign. The backbone conformation of γ-residues in 11-helix is quite different from that of 13-helix in βγ-hybrid peptides as well as 12- or 15/17-helix in αγ-hybrid peptides. The γ-residues displayed *gauche*$^+$ (*g*$^+$) and gauche$^-$ (*g*$^-$) local conformations along C^{β}–C^{γ} and C^{α}–C^{β} bonds, respectively. The ϕ, θ_1, θ_2 and ψ variable displayed the +, +, - and + respectively. In addition, the torsion angle ω adopted regular *trans* geometry. The torsion values of γ-residues are consistent with reported theoretical values. Our findings reported here are nicely correlates with the Hofmann's predictions expect the torsion angle parameters of β-residues.[8] More importantly, accommodation of stereochemically unfavorable *g+*, *g-* along the C^{γ}-C^{β} and C^{β}-C^{γ} in γ-residues is quite interesting. The N to C terminal H-bonding direction observed in the 11-helix of β,γ-hybrid peptides resembles the 14-helix in β-peptides. The Aib and other achiral dialkyl-substituted α-amino acids have been widely used to control the α-peptide conformation by limiting the accessible conformational space through Thorpe–Ingold effect.[18] This conformational restriction of Aib residues promote helical conformation in α-peptides. In continuation, incorporation of Aib residue as a guest into the β-peptide 14-helix leads to transformation of 14-helix into 12-helix.[19] It is interesting to note that insertion of homologated analogue of Aib, β^3HAib, into a β-peptides prevent formation of 14-helix confroamtions.[20] Our report indicates that achiral 3,3-dialkyl substituted as well as 4,4-dialkyl γ-amino acid in the hybrid sequence could be used to access the otherwise inaccessible conformational space.

In conclusion, we have demonstrated the unique 11-helix conformations of β,γ-hybrid peptides composed with β-leucine and 3,3- and 4,4-dimethyl substituted γ-amino acids in solution. The helical structures are stabilized by the continuous 11-membered (*i→i+1*) H-bonds in the reverse direction, which is in sharp contrast with the α-helix. Overall, the new helix type from β,γ-hybrid peptides reported here will open up new possibilities to design new peptide foldamers.

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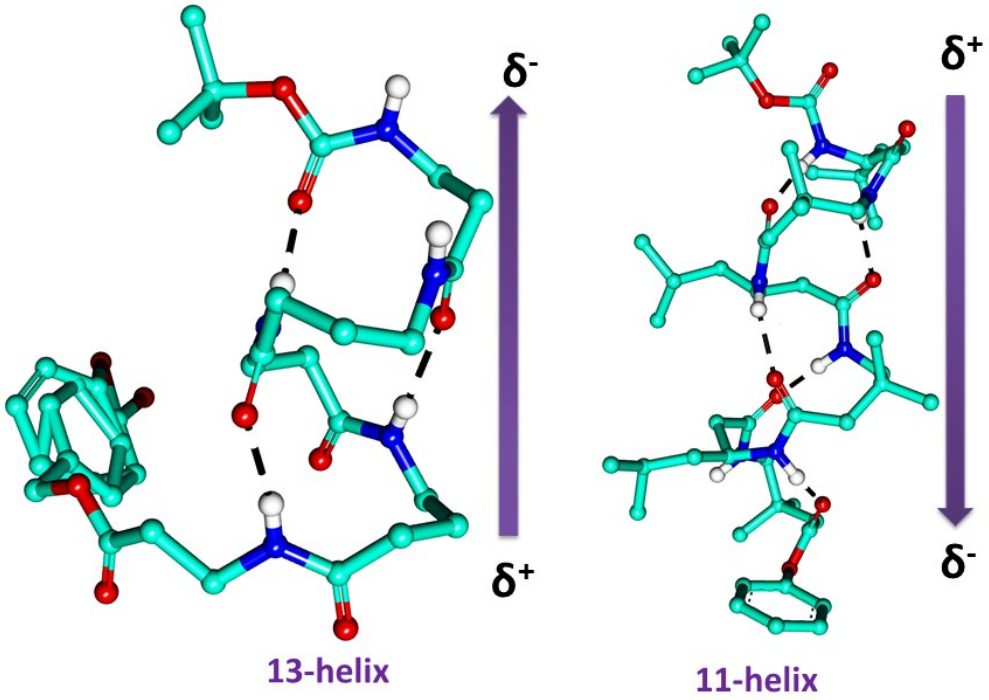

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