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α/γ^4-Hybrid peptide helices: synthesis, crystal conformations and analogy with the α-helix†

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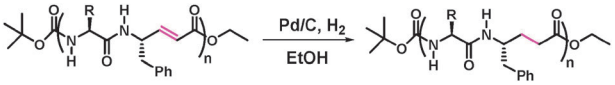
Synthesis, crystal conformations of α/γ^4-hybrid peptide helices containing proteinogenic amino acid side-chains, and the analogy with the α-helix are reported. Results suggest that α/γ^4-hybrid peptides adopted helical conformations with 12-membered H-bond pseudocycles in single crystals.

Designing synthetic protein structures from non-natural amino acids has immense importance not only to understand the protein folding but also from the perspective of medicinal chemistry. Significant progress has been achieved in this regard using the oligomers of β- and mixed sequences containing α/β hybrid peptides.[1,2] However, the research in the area of γ^4-peptides containing proteinogenic amino acid side-chains (γ^4-amino acids, double homologated α-amino acids) is lagging behind that of β- and hybrid β-peptides, this is in part probably due to the difficulty of obtaining stereochemically pure γ^4-amino acids. In addition, very little is known about the heterooligomers containing α- and γ^4-amino acids. The advantage of the hybrid peptides containing α- and backbone homologated amino acids is that a new class of helical structures can be generated by varying the amino acid sequence patterns.[3] Nonetheless, Seebach and colleagues[4] and Hanessian *et al.*[5] in their pioneering work recognized the 14-helical conformations from the oligomers of γ^4-amino acids. Herein, we report the synthesis, single crystal conformations of α/γ^4-hybrid peptides Boc-Aib-γ^{4}Phe-Leu-γ^{4}Phe-OEt (**P1**), Boc-Aib-γ^{4}Phe-Leu-γ^{4}Phe-Aib-dgF-OEt (**P2**) and Boc-Aib-γ^{4}Phe-Leu-γ^{4}Phe-Aib-γ^{4}Phe-OEt (**P3**), and the structural analogy with the α-helix.

Recently, we reported the utility of *E*-vinylogous amino acids in the construction of a stable and functionalizable hybrid β-hairpin and its direct transformation to the γ-peptide analogue.[6] We adopted a similar strategy for the synthesis of α/γ^4-hybrid peptides (Scheme 1). To understand the conformational properties of hybrid peptides with 1 : 1 alternating α- and γ^4-amino acids, the α/*E*-vinylogous hybrid peptide Boc-Aib-dgF-Leu-dgF-OEt (**D1**) (dgF = α,β-unsaturated γ-phenyl alanine) was synthesized in solution-phase using Boc-chemistry. The *E*-vinylogous amino acid dgF was synthesized using the Wittig reaction.[7] The pure peptide was directly transformed to its saturated α/γ^4-hybrid peptide analogue (**P1**) by catalytic hydrogenation in the presence of 20% Pd/C in ethanol. The reaction was monitored by MALDI-TOF and HPLC. The complete conversion of peptide **D1** to **P1** was achieved within six hours and the pure α/γ^4-hybrid peptide was isolated in 96% yield. The atomic resolution structures are very important in understanding the conformational properties of peptides; however, peptides containing γ^4-amino acids showed poor record of X-ray structures in the literature. Keeping this in mind, we attempted to grow the single crystals of α/γ^4-hybrid peptides in various solvent combinations. Single crystals of α/γ^4-hybrid peptide **P1** obtained from the slow evaporation of methanol solution yield the structure shown in Fig. 1A. Two molecules of **P1** were observed in the asymmetric unit with slight variation in the torsional values. Instructively, the tetrapeptide adopted a right-handed 12-helix [12-atom ring H-bonds of C=O (i)···H–N(i + 3), C_{12}] conformation in the crystalline state. Recently, Hofmann and co-workers have predicted the formation of the 12-helical conformation in 1 : 1 alternating α- and unsubstituted γ-amino acids using theoretical calculations.[8] In addition, Balaram and colleagues[9] and Gellman *et al.*[10] have shown the formation of a 12-helix in the α/γ-hybrid peptides containing 3,3-dialkyl γ-amino acids and cyclic γ-amino acids, respectively. However, mixed 10/12 helices have also been reported from the hybrid peptides containing carbo-γ-amino acids as well as 3,3-dialkyl γ-amino acids.[11,12] The crystal structure analysis of **P1** reveals that the two continuous twelve membered backward (1 ← 4) H-bonds, C=O(Boc, i)···NH(3, i + 3) [C=O···H–N dist. 2.10 Å, O···N dist. 2.95 Å and ∠O···H–N 161°] and C=O(1)···NH(4) [C=O···H–N dist. 2.07 Å, O···N dist. 2.90 Å and ∠O···H–N 156°], stabilize the helical conformation (Fig. 1A). The conformational analysis of γ-residues reveals that γ^{4}Phe2 adopted the *gauche*$^+$, *gauche*$^+$ (g^+, g^+, $\theta_1 \approx \theta_2 \approx 60°$) local conformation about the C_β–C_γ and C_α–C_β bonds, while the C-terminal γ^{4}Phe4



Scheme 1 Direct transformation of α/*E*-vinylogous hybrid peptides into α/γ^4-hybrid peptides.

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† Electronic supplementary information (ESI) available: Experimental details, NMR spectra, mass spectra and X-ray crystallography (for peptides **P1–P3**). CCDC 878071–878073. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c2cc32911e

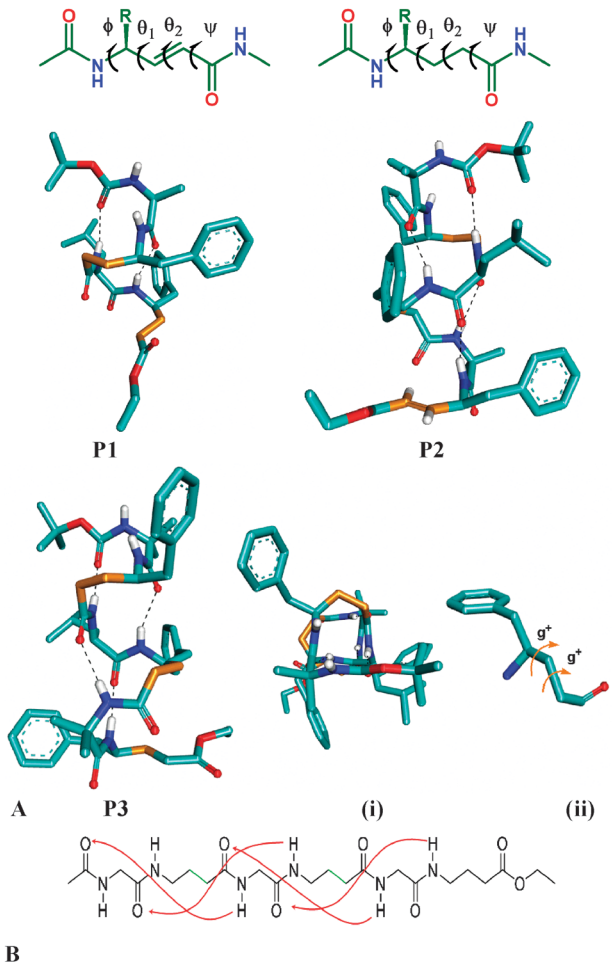

Fig. 1 (A) X-ray structures of **P1**, **P2** and **P3**, (i) top view of **P3** showing the distinct projections of amino acid side-chains, (ii) helix favouring the g^+, g^+ conformation adopted by the γ^4Phe residue in 12-helices. (B) Continuous 12-membered backward H-bonds observed in the hybrid peptides **P2** and **P3**.

residue displayed the *gauche*$^+$ (C_β–C_γ), *anti* (C_α–C_β) conformation due to the lack of a terminal H-bond donor (NH). To introduce the additional H-bond at the C-terminal of **P1** and also to understand the behaviour of *E*-vinylogous amino esters at the C-terminal, hexapeptide **P2** (Boc-Aib-γPhe-Leu-γPhe-Aib-dgF-OEt) was synthesized from **P1** through a 4 + 2 convergent strategy. It is worth mentioning that pure **P2** was crystallized within 30 min. in many solvents including acetone and ethyl acetate/hexane. Single crystals of **P2** obtained from the ethyl acetate/hexane solution yield the structure shown in Fig. 1A. The crystal structure analysis demonstrates that the 12-helical structure of **P2** is stabilized by four consecutive backward 1 ← 4 hydrogen bonds and γ^4Phe4 adopted the g^+, g^+ conformation. Surprisingly, the C-terminal vinylogous residue nicely accommodated into the helix through a 10 membered weak C–H···O H-bond between the dgF6 C_α–H and the carbonyl of γPhe4 (1 ← 3). The torsional angle of the vinylogous residue is 23° for θ_1 and the conjugated ester adopted the local *s-trans* conformation with the ψ value of 28° (Fig. 1).[7] Further, **P2** was transformed into a completely saturated analogue **P3** (Boc-Aib-γ^4Phe-Leu-γ^4Phe-Aib-γ^4Phe-OEt) by catalytic hydrogenation and its X-ray structure is shown in Fig. 1A. The analysis revealed that

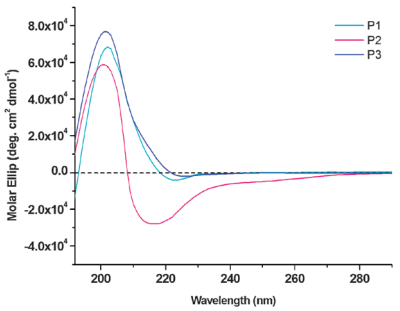

Fig. 2 Circular dichroism spectra of α/γ^4-hybrid peptides **P1**, **P2**, and **P3** in MeOH (200 μM). The CD signature showed similarity with β-peptide 12-helices.

similar to **P2**, peptide **P3** also adopted 12-helical structure in single crystals and the structure is stabilized by four consecutive C_{12} intramolecular 1 ← 4 H-bonds. In addition, due to the lack of a H-bond donor (NH) at the C-terminal, a weak 10 membered C–H···O H-bond between the acidic α-hydrogens and the C=O of the preceding γPhe residue (1 ← 3) was observed in all hybrid peptides. The H-bond parameters of all hybrid helical peptides (**P1–P3**) are tabulated in the ESI.† Circular dichroism spectra of all α/γ^4-hybrid peptide helices recorded in methanol solution (200 μM) are shown in Fig. 2. Recently, Gellman and colleagues demonstrated the CD signature for 12-helices containing cyclic β-amino acids.[13] Instructively, α/γ^4-hybrid peptide 12-helices **P1** and **P2** displayed a similar CD pattern to that of β-peptide 12-helices with CD maxima at ~205 nm and weak minima at 220 nm. In contrast, **P2** displayed a little distorted CD spectrum with the CD maxima at 204 nm and minima at 218 nm, this may be due to the interference of C-terminal α,β-unsaturated ester.

The crystal structure analysis of the α/γ^4-hybrid peptides **P1–P3** reveals that the α-residues adopted right handed helical conformations with average ϕ and ψ values being $-59 \pm 5°$ and $-38 \pm 5°$, respectively. The dihedral angles of γ^4-residues were measured by introducing two additional variables θ_1 (N–C_γ–C_β–C_α) and θ_2 (C_γ–C_β–C_α–C) as shown in Fig. 1A. The torsional angles of γ^4-residues in all hybrid peptides are tabulated in Table 1. The average ϕ and ψ values of γ^4-residues in the helix were found to be $-126 \pm 10°$ and $-118 \pm 10°$, respectively. Further, the top view of the α/γ^4-hybrid peptide helix **P3** signifies the projection of amino acid side-chains at four corners of the helical cylinder (Fig. 1A(i)). In comparison to the β- and γ-peptides, the distinct orientation of the amino acid side-chains was observed in α/γ^4-hybrid helical peptides.

The intriguing results of the hybrid peptides **P1–P3** encourage us to assess the hybrid 12-helical structure with respect to the

Table 1 Backbone torsional variables of α/γ^4-hybrid peptide (**P1**, **P2** and **P3**) 12-helices

Peptide	Residue	ϕ	θ_1	θ_2	Ψ
P1	γPhe1	-126.5 ± 3.5	50.1 ± 2.5	63 ± 3	-111 ± 4
	γPhe2	-109.5 ± 7.5	62.5 ± 0.5	±180	—
P2	γPhe1	-137.5 ± 0.5	61.5 ± 0.5	59	-115 ± 1
	γPhe2	-123 ± 2	49 ± 1	63 ± 1	-120 ± 3
	γPhe3	-113	24.5 ± 0.5	173 ± 1	29.5 ± 1.5
P3	γPhe1	-135	59 ± 1	61	-114 ± 1
	γPhe2	-122.5 ± 2.5	46.5 ± 2.5	63.5 ± 2.5	-126.5 ± 3.5
	γPhe3	-116 ± 3	55 ± 1	177.5 ± 0.5	24.5 ± 0.5

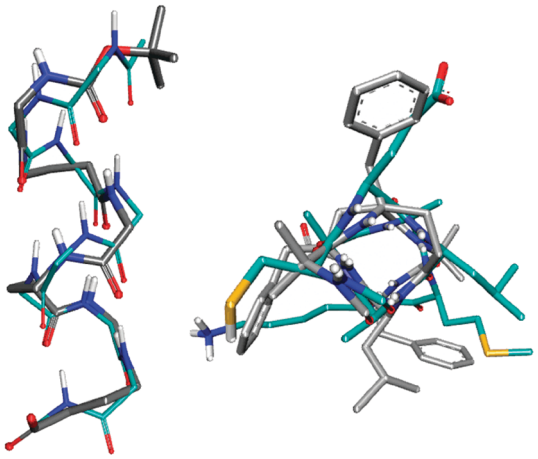

Fig. 3 Superposition of the backbone conformation of the α/γ^4-hybrid peptide (gray) on the α-helix (cyan). All the backbone atoms of the α/γ^4 hybrid peptide were used to overlay with the octapeptide of the α-helix, RMS = 0.81.

predominantly existing natural α-helix. The superimposition of the backbone conformations of **P3** with the α-helix of chicken egg white lysozyme[14] (PDB code: 1HEL, sequence: C-E-L-A-A-A-M-K, 6–13) is shown in Fig. 3. Instructively, the backbone conformation of hybrid hexapeptide **P3** is well correlated with the eight residue α-helix, except the H-bonding pattern. However, the internal H-bonding orientation and the macrodipole of α/γ^4-hybrid peptides are analogous to the α-peptide helix. The top view of the superimposed **P3** and the α-helix signifies the projection of the amino acid side-chains (Fig. 3). The backbone correlation and the side-chain projections of α/γ^4-hybrid peptide helices with respect to the α-helix suggest that these hybrid peptides can be exploited as mimics of α-peptide helices. Further, with the availability of broad side-chain diversity in both α-and γ^4-amino acids, these α/γ^4-hybrid peptides stand unique than the other α/γ-hybrid peptides.

In conclusion, we presented the facile transformation and the structural analysis of the α/γ^4-hybrid peptides containing backbone homologated γ^4-amino acids. Though there is a possibility of conceiving different types of H-bond pseudocycles, the atomic resolution data of a series of α/γ^4-hybrid peptides revealed the unprecedented 12-helical conformations. The solution conformations of these peptides and the analogy with other helices are under investigation. The conformational analysis and the unique side-chain projections of α/γ^4-hybrid peptide helices presented here may provide fundamental information for the design of functional foldamers.

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