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Unconstrained Homooligomeric γ-Peptides Show High Propensity for C_{14} Helix Formation

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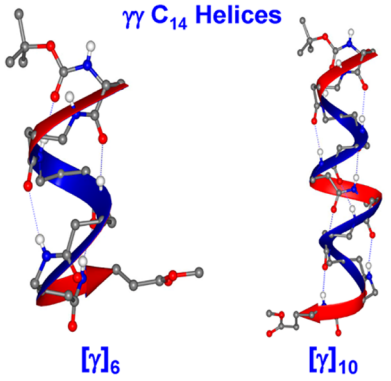
ABSTRACT



Monosubstituted γ^4-residues (γ^4Leu, γ^4Ile, and γ^4Val) form helices even in short homooligomeric sequences. C_{14} helix formation is established by X-ray diffraction in homooligomeric $(\gamma)_n$ tetra-, hexa- and decapeptide sequences demonstrating the high propensity of γ residues, with proteinogenic side chains, to adopt locally folded conformations.

The ability of polypeptides composed of α-amino acids to fold into helical structures was famously recognized in Pauling's proposal of the α-helix.[1] Helical structures have also been extensively characterized in short synthetic all-α-amino acid containing peptide sequences, especially those containing conformationally constrained residues such as α-aminoisobutyric acid (Aib).[2] Polypeptides formed from β and γ amino acids have additional atoms inserted into the peptide backbone, resulting in an expansion of available conformational space.[3] Early work by the groups of Seebach[4] and Hanessian[5] provided evidence for the formation of folded structures in oligo γ-peptides derived from the γ-residues obtained by homologation of ^{L}Ala, ^{L}Val, and ^{L}Leu. These reports suggested that helix formation may indeed be a property of peptides derived from backbone-expanded γ-analogues of α-amino acids.[6] The successful characterization in crystals of helical structures in oligopeptides containing cyclically constrained β amino acids by the Gellman laboratory[7] and extensive investigations of multiply substituted β and γ residues by the group of Seebach[8] launched the field of "foldamers".[9] The exploding literature on β and γ peptides in recent years has focused largely on sequences derived from constrained amino acids, in which cyclization involving backbone atoms or multiple substitution limits the range of accessible

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conformations.[7−13] Experimental and theoretical investigations on peptides containing α, *β*, and *γ* amino acids have provided a wealth of information on diverse helical hydrogen bonding patterns in hybrid polypeptides.[13−16] During the course of investigations of hybrid α*γ* peptides, we have examined the sequences of the types [Aib-γ^4(*R*)-Val]$_n$ and [Aib-γ^4(*R*)Val-γ^4(R)Val]$_n$ and successfully characterized the C_{12} helix in $(\alpha\gamma)_n$ and $C_{12}/C_{14}/C_{12}$ helix in $(\alpha\gamma\gamma)_n$ sequences by X-ray diffraction.[17] To our surprise, we also obtained helical structures in crystals of the peptides, Boc-[Leu-γ^4(R)Val-Val]$_n$-OH (n = 1, 2). Together with the report of Bandyopadhyay et al., these observations suggested that *γ* residues obtained by backbone homologation of the genetically coded α-amino acids may indeed have a significantly higher intrinsic tendency to fold into helical structures, as compared to their α-amino acid counterparts.[16a,17b] At first glance, it would appear that this proposition is counterintuitive, given the greater range of conformational possibilities in the former. Persuaded by the weight of accumulating evidence on the helix promoting ability of *γ*-residues having a single substituent at the C*γ* position, we turned to a systematic investigation of the homooligopeptides derived from *γ*-residues. We present here the structural characterization in crystals of folded C_{14} helical structures of 4, 6, and 10 residue homooligomeric *γ*-peptides and examine the development of helices in solution as a function of chain length. The facility with which helical structures are formed in homooligomeric *γ*-peptides is without precedent in the structural chemistry of homooligomers derived from the proteinogenic amino acids. The homooligomeric sequences Boc-[γ^4Val]$_n$-OMe (n = 2−6, 8), Boc-[γ^4Leu]$_n$-OMe (n = 4), and Boc-[γ^4Ile]$_n$-OMe (n = 6, 10) (Figure 1a) were synthesized by a solution-phase procedure purified by HPLC and characterized by ESI-MS and 500 MHz ^{1}H NMR. Single crystals were obtained for the peptides Boc-[γ^4(*S*)Leu]$_4$-OMe **1S**, Boc-[γ^4(*R*)Leu]$_4$-OMe **1R**, Boc-[γ^4(*R*)Ile]$_6$-OMe **2**, and Boc-[γ^4(*R*)Ile]$_{10}$-OMe **3**. (Note that homologation of the L-α-amino acid (*S*-configuration) yields the *R*-*γ*-amino acid).[6] Figure 1 shows the molecular conformations determined in crystals for the enantiomeric tetrapeptides **1S** and **1R**. In both cases, folded conformations stabilized by two 4→1, C=O$_i$···H−N$_{i+3}$ (C_{14}) hydrogen bonds are obtained. The incipient C_{14} helix formed in these homooligomeric, unconstrained *γ*-peptides is left handed in **1S** and right handed in **1R** (Figure 1C, D) (backbone torsion angles of peptides **1S** and **1R** are listed in Table S1 in the Supporting Information).

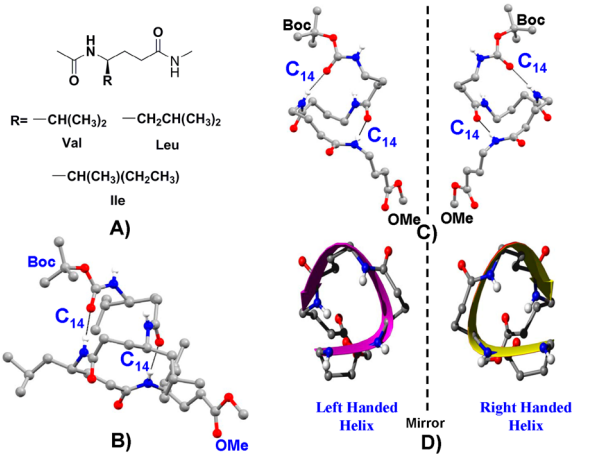

Figure 1. (A) Monosubstituted unconstrained γ^4-residues used in the present study. (B) Molecular conformation in crystals of the peptide Boc-[γ^4(*R*)Leu]$_4$-OMe **1R**. (C) Views of the enantiomeric tetrapeptides, Boc-[γ^4(*S*)Leu]$_4$-OMe **1S** (left) and Boc-[γ^4-(*R*)Leu]$_4$-OMe **1R** (right). (Side chains are not shown for clarity.) (D) Projection down helix axis of peptides **1S** and **1R** showing the handedness of the incipient C_{14} helices.

The formation of a well folded helical structure in crystals of a short tetrapeptide sequence, in the absence of any backbone conformational constraints, is surprising. Thus far, the analogous incipient 3_{10} helix (C_{10} helix) has been characterized only in short α-peptide sequences, containing conformationally constrained amino acids, Aib being the most notable.[2] Figure 2 illustrates conformations observed in crystals for the hexapeptide Boc-[γ^4(R)-Ile]$_6$-OMe **2** and the decapeptide Boc-[γ^4(R)Ile]$_{10}$-OMe **3**. Both peptides reveal well-formed, right-handed C_{14} helices over the entire length of the peptide chain. Four intramolecular C_{14} hydrogen bonds stabilize helical folding in the hexapeptide **2**, while eight hydrogen bonds are observed in

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the decapeptide **3**. Relevant backbone torsion angles in peptides **2** and **3** are listed in Table S2 (Supporting Information). Inspection of the values obtained for residues 1−8 of the decapeptide **3** suggests that *gauche*−*gauche* conformations about the central C−C bonds ($C^{\gamma}-C^{\beta}$ and $C^{\beta}-C^{\alpha}$) of the γ residues are a characteristic of a continuous C_{14} helical conformation. While values of the torsion angle $\theta_1(C^{\gamma}-C^{\beta})$ are *gauche* in all the structures reported here, the value of $\theta_2(C^{\beta}-C^{\alpha})$ is *trans* for the penultimate residues, namely residue 3 in **1S** and **1R**, residue 5 in peptide **2**, and residue 9 in peptide **3**. The average backbone torsion angles determined for the C_{14} helical structures obtained from the main body of the helices are as follows: $\phi = -132.6(13.3)°$, $\theta_1 = 57.1(3.8)°$, $\theta_2 = 68.2(2.8)°$, $\psi = -143.7(11.3)°$. Helical parameters determined from the crystal structure of the decapeptide Boc-[γ^4(*R*)Ile]$_{10}$-OMe (**3**) are as follows: *n* (residues/turn) = 2.52(2.5); *d* (height/residue) = 2.02 Å; *p* (pitch) = 5.09(5.5) Å, and *r* (radius) = 2.87(2.9) Å. These experimental observations are in good agreement with the previously reported values for C_{14} helices from theoretical calculations[14] and from four examples of C_{14} helices characterized in short γ-peptides containing constrained amino acids, with preorganized backbone conformations (values shown in parentheses above).[11] Helical parameters of all the peptides are given in the Supporting Information.

The formation of intramolecularly hydrogen-bonded structures in homooligomeric γ-peptides is also substantiated by infrared (IR) spectroscopic studies in dilute solution (2 mM). The concentration-dependent study was carried out for a model peptide **9** over the range of 0.1 to 10 mM to distinguish the effect of peptide association from intramolecular interactions. Figure 3A shows the N−H bond stretching for the homooligomeric sequences Boc-[γ^4(*R*)Val]$_n$-OMe. In the dipeptide, the intense band at 3438 cm^{-1} may be assigned to free N−H groups, while the broader hydrogen bonded N−H band at 3311 cm^{-1} may arise from a population of C_9 hydrogen-bonded structures previously characterized in peptides containing the constrained γ-residue, gabapentin (Gpn).[18] Inspection of the spectra reveals that from the level of the tetramer to the octamer there is a steady increase in the intensity of the hydrogen bonded NH band, with a concomitant shift to lower frequency from 3345 to 3322 cm^{-1}. These observations suggest that lengthening of the peptide chain results in an elongation of the folded, intramolecularly hydrogen bonded structures and are consistent with a continuous growth of the C_{14} helix with chain length.

The NMR solvent titration experiments carried out in $CDCl_3$−DMSO mixtures for Boc-[γ^4(*R*)Val]$_n$-OMe (n = 6 and 8) reveal downfield shifts for only two N−H protons, γ^4Val(1) and γ^4Val(2) (see Figure S8, Supporting Information). Specific NOEs may also be used as a diagnostic for C_{14} helix formation in solution. Figure 3B shows partial ROESY spectra for the tetrapeptide and octapeptide in the Boc-[γ^4(*R*)Val]$_n$-OMe series. An interesting

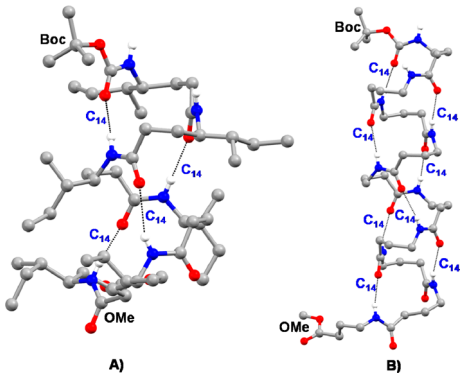

Figure 2. (A) Molecular conformation in crystals of the peptide Boc-[γ^4(*R*)Ile]$_6$-OMe **2**. (B) Molecular conformation in crystals of the peptide Boc-[γ^4(*R*)Ile]$_{10}$-OMe **3**. (Side chains are omitted for clarity.)

feature in both cases are the C^{γ}-H···H−N NOEs observed for the γ^4Val(1) C^{γ}-H proton. In the tetrapeptide, the C^{γ}-H of γ^4Val(1) shows inter-residue NOEs to the N−H groups of residues γ^4Val(2) (weak), γ^4Val(3) (strong), γ^4Val(4) (strong). A similar pattern of NOEs is observed for the γ^4Val(1) C^{γ}-H proton in the octapeptide. Notably, the $d_{\gamma Ni/i+2}$ NOE is more intense than the $d_{\gamma Ni/i+1}$ NOE. Furthermore, the $d_{\gamma Ni/i+3}$ NOE is also observed. Examination of the conformations of the incipient C_{14} helices in the crystal structures of **1R** (**1S**) reveals the following order of inter residue interproton distances: $d_{\gamma Ni/i+3}$ 3.40 Å (3.48 Å) < $d_{\gamma Ni/i+2}$ 3.62 Å (3.71 Å) < $d_{\gamma Ni/i+1}$ 4.24 Å (4.14 Å). The intramolecular $d_{\gamma Ni/i}$ NOE, which can serve as an internal intensity standard, corresponds to an interproton distance of 2.7 Å. A similar pattern of NOEs involving the residue 1 C^{γ}-H proton has also been observed in the Boc-[γ^4Leu]$_n$-OMe and Boc-[γ^4Ile]$_n$-OMe series. $C_{\gamma}H(i)$-NH(i+2) NOE [$d_{\gamma Ni/i+2}$] has been noted as a potentially important diagnostic for γ peptide C_{14} helices reported by Guo et al.[11] The present study suggests that $C_{\gamma}H(i)$-NH(i+3) NOE [$d_{\gamma Ni/i+3}$] is also an important indicator as the distance is indeed less than the $d_{\gamma Ni/i+2}$ value in the unconstrained C_{14} helices reported here.

While early studies on short oligomeric γ-peptides provided evidence for the formation of folded, intramolecularly hydrogen bonded structures, definitive crystallographic evidence for the formation of C_{14} helices has been limited to sequences containing conformationally constrained γ-residues. Indeed, Gellman et al. have emphasized the importance of subunit preorganization in promoting structural characterization of C_{14} helices.[11]

A well-known problem in the long history of peptide chemistry is the insolubility in organic solvents of the N and C terminus protected homooligomeric sequences composed of α-amino acids. The classic studies of Toniolo, Goodman and co-workers established a strong tendency for the sequences of the type Boc-[Xxx]$_n$-OMe (Xxx = Val, Leu, Ile, Met) to form extended strand structures, resulting in β-sheet formation.[19] An examination of the Cambridge

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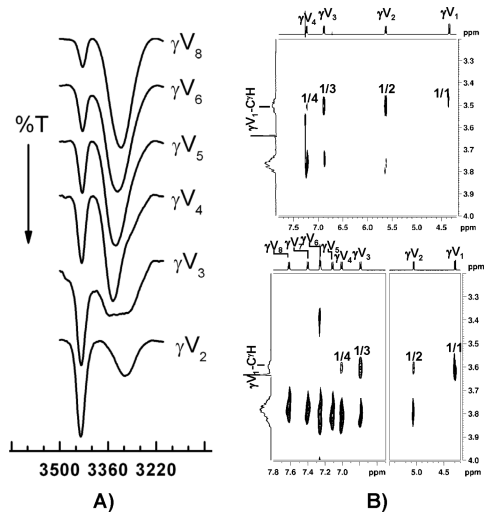

Figure 3. (A) N−H stretching band in the IR spectra of the peptides Boc-[γ^4(*R*)Val]$_n$-OMe (n = 2, 3, 4, 5, 6, 8) at 2 mM concentrations. (B) Partial ROESY spectra of the peptides showing the C$^\gamma$H↔NH region: Boc-[γ^4(*R*)Val]$_4$-OMe **6** (top), Boc-[γ^4(*R*)Val]$_8$-OMe **9** (bottom).

Crystallographic Data Centre database[20] reveals a complete absence of crystal structures of homooligomeric α-peptides even at the tetrapeptide level, with the sole exception of proline containing sequences.[21] An overwhelming majority of oligopeptide structures which lie in the size range six to twenty residues correspond to sequences containing structurally constrained residues, which limit the range of conformational choices available to the peptide backbone. During the course of synthesis of the γ-peptide oligomers, we were surprised by the high solubility of relatively long sequences in solvents like $CHCl_3$ and CH_2Cl_2. This observation suggested that, in contrast to α-peptides, the introduction of a CH_2−CH_2 unit into the backbone induces folding, thereby minimizing intermolecular hydrogen bonding which promotes sheet formation, resulting in insolubility. Crystal structures described in this report of two γ-tetrapeptides, one γ-hexapeptide and one γ-decapeptide, clearly demonstrate a strong tendency for C_{14} helix formation. The preponderance of helical structures in the solution state in poorly interacting organic solvents is also demonstrated by NMR and IR studies.

Why do γ-peptides containing the backbone expanded analogues of the common α-amino acids occurring in proteins show a significantly greater tendency to fold than their α-counterparts? Two factors merit consideration: (1) the expanded polypeptide backbone permits intramolecular hydrogen bonds with improved geometry, enhancing their energetic contribution. (2) The gauche, gauche conformation optimizes local van der Waals interactions in the folded structures. Theoretical analysis of the collapse of extended homopolymer chains into compact structures has provided insights into the understanding of heteropolymer chain folding, of which proteins are the prime example.[22] Extended hydrocarbon chains could, in principle, be considered as precursors of polyamide structures where amide or peptide bonds are randomly or periodically embedded along the chain. Recent studies on the simulation of hydrocarbon chain folding suggest that the formation of cylindrical or toroidal structures becomes significantly favorable for a C_{40} chain.[23] The last globally stable extended alkane may be observed only up to a C_{20} chain.[24] Polypeptides obtained from residues bearing an increasing number of methylene groups in the backbone may be viewed as being derived from the hydrocarbons, with insertion of amide groups at specific positions along the backbones. Strictly periodic insertion of amide groups yields homooligomeric structures, while hybrid backbones can be readily generated by patterned insertion of amide bonds.[13b,25] Even an oligomeric γ-tetrapeptide possesses as many as twenty atoms in the backbone. In the case of the γ-residues discussed in this paper, the substituent at the γ^4 position further promotes gauche conformations about the C$^\gamma$-C$^\beta$(θ_1) degree of torsional freedom. Weak dispersion interactions and intramolecular hydrogen bonds work in concert to promote chain compaction and helical folding in these homooligomeric γ-peptides. Taken together, these factors readily rationalize the facility with which unconstrained γ-residues fold into C_{14} helical structures, permitting definitive structural characterization, a feature unprecedented in the chemistry of α-peptides. The C_{14} helix may indeed be an attractive structure to consider for the sole example of a naturally occurring poly γ peptide, the poly-γ-glutamate produced by *Bacillus anthracis* and related organisms, examined by Rydon nearly half a century ago.[26]

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Supporting Information Available. Experimental details and characterization data for all the compounds including the crystal data and structural parameters for **1S**, **1R**, **2** and **3** (CIF); NMR solvent titration figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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