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Characteristic Structural Parameters for the γ -Peptide 14-Helix: Importance of Subunit Preorganization

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Abstract

γ -peptides-14 Helix

We report crystallographic data for a set of homologous γ -peptides that contain a Boc-protected residue derived from the flexible gabapentin monomer at the N-terminus and cyclically constrained γ -residues at all other positions. The crystallized γ -peptides range in length from 3 to 7 residues. Previously only one atomic-resolution structure had been available for a short γ -peptide 14-helix. The new data provided here allow derivation of characteristic parameters for the γ -peptide 14-helix, and establish guidelines for characterizing 14-helical folding in solution via 2D NMR. In addition, the results suggest that the substitution pattern of a γ -residue has a profound effect on the propensity for 14-helical folding.

Keywords

γ -peptides; 14-helix; H-bond; backbone; NOE

The complexity of structure and function among biopolymers has inspired chemists to extrapolate to non-natural analogues that are intended to emulate the natural archetypes.^[1] β -Amino acid oligomers, for example, can adopt helix, sheet or reverse turn conformations comparable to the regular secondary structures found in proteins,^[2] and β -peptides display a wider range of secondary structure variation than do α -peptides.^[3,4] Elucidation of β -peptide folding rules has enabled function-directed design,^[5] which has encouraged exploration of higher amino acids as "foldamer" subunits.^[1] γ -Peptides that form discrete helix, sheet or reverse turn secondary structures have been reported,^[6–9] but the pace of γ -

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Dedicated to Prof. Ronald Breslow on the occasion of his 80th birthday

peptide exploration lags behind that of β -peptides, in part because of the difficulty of obtaining stereochemically pure building blocks. The γ -peptide 14-helix, defined by 14-atom ring $i, i+3$ C=O--H-N H-bonds, was identified through pioneering efforts of Hanessian et al.^[6] and Seebach et al.^[7] and represents the most thoroughly documented γ -peptide secondary structure. Nevertheless, just one atomic-resolution structure containing this helix has been reported.^[9,10]

Here we report a set of crystal structures that allow derivation of characteristic parameters for the γ -peptide 14-helix, which was not previously possible. In addition, the crystallographic data establish proton NOE patterns that are definitive for 14-helical folding in solution. Use of a conformationally constrained γ -amino acid (**2**)^[11] was crucial for the generation of these structural data.

We used solution-phase methods to prepare a γ -peptide series, **3–7** (Figure 1), which contain a Boc-protected residue derived from commercially available gabapentin (**1**) at the N-termini, and cyclically constrained residues derived from γ -amino acid **2** at all other positions. Stepwise synthesis of α -peptides typically proceeds from C-terminus to N-terminus, because carboxyl activation of α -amino acid derivatives in which the backbone nitrogen is part of an amide group (e.g., at the C-terminus of a peptide) often leads to epimerization via transient azalactone formation, while epimerization is suppressed when the amino group is part of a urethane (e.g., Boc). We find a complementary situation with γ -peptides constructed from **2**: the N-Boc derivative of **2** is highly prone to γ -lactam formation under standard coupling conditions, and this side reaction is suppressed when the backbone nitrogen is part of an amide.^[12] Therefore, γ -peptides **3–7** were constructed via stepwise extension starting from the N-terminus. A gabapentin residue was placed at the N-terminus because *N*-Boc-gabapentin is not prone to γ -lactam formation during coupling reactions.

γ -Amino acid **2** was selected as the principal component of these oligomers because protected forms are readily available from butanal and 1-nitro-cyclohexene,^[11] and because the ring constraint and stereochemistry are expected to promote a *gauche*⁺, *gauche*⁺ torsion angle sequence about the C _{α} -C _{β} and C _{β} -C _{γ} bonds, which computational analysis suggests to be conducive to 14-helix formation.^[13] In contrast, the diastereomeric building block with a *trans*-disubstituted cyclohexane ring^[11] is expected to favor a *gauche*⁺, *anti* torsion angle sequence. The synthetic route that provides **2** enables placement of a wide variety of side chains at the α -position, and it seems likely that the behavior observed with an ethyl group at this position will prove to be representative of other unbranched side chains.

In the crystal structures of **4–7** (Figure 2), the segments derived from **2** display the 14-helical conformation. All possible 14-atom ring H-bonds are formed within each of these segments. This data set permits us to derive robust averages for the backbone torsion angles of a γ -amino acid residue involved in a canonical 14-helix (Table 1). These average values correspond well to relevant torsion angles in the only previous crystal structure of a helical γ -peptide, a tetramer.^[9] Our averages do not include the C-terminal residue from **4–7**. The C-terminus itself is an ester in each case, and the lack of an H-bond donor at this position prevents incorporation of the C-terminal γ -residue into the 14-helical H-bond pattern. Similarly, the C-terminus of the previously described tetramer^[9] is an ester, and in this case the C-terminal γ -residue displays torsion angles that deviate dramatically from those required for the 14-helix: the (O=)CC _{α} -C _{β} C _{γ} (ζ) and C _{α} C _{β} -C _{γ} N (θ) torsion angles are *anti* rather than *gauche* for this residue. Among **4–7**, only minor backbone torsion angle variations are observed between the C terminal residue and other residues derived from **2**, a trend that presumably arises because the cyclic constraint of **2** confers strong conformational

preorganization, in contrast to the modest conformational preference provided by an acyclic stereochemical control strategy.^[9]

Standard methods for deriving helical parameters such as the number of residues per turn (n), rise per turn (pitch; p), rise per residue (d) and radius (r) require atomic-resolution structures containing segments of four contiguous helical residues.^[14] Hexa- γ -peptide **6** and hepta- γ -peptide **7** provide the first opportunities for such analysis among γ -peptides, with one four-residue segment in **6** and two such segments in **7**. The helical parameters deduced from these three segments are very similar to one another, as shown in Table 2.

The new structural data for segments derived exclusively from **2** allow us to identify to identify H—H distances that are expected to give rise to medium-range NOEs in the γ -peptide 14-helical conformation. In general, NOEs between protons that are distant from one another in terms of covalent connectivity, specifically, protons that are not from the same residue or from sequentially adjacent residues, provide strong evidence for population of compact conformations in solution. The crystallographic data indicate six types of H—H juxtapositions, involving protons on the backbone or on the first carbon of a side chain, that should give rise to non-sequential NOEs characteristic of the 14-helix (Figure 3 and Table 3). This analysis reveals that $C_{\gamma}H(i)-C_{\alpha}H(i+2)$, $C_{\gamma}H(i)-NH(i+2)$ and $C_{\gamma}H(i)-C_{\alpha(\beta')}H(i+2)$ NOE patterns should be particularly useful, since these H—H distances are between 2.5 and 3.5 Å in the crystal structures. (The designation " $C_{\alpha(\beta')}H$ " indicates a proton on the first carbon (β') of a side chain attached to the backbone α -carbon.) NMR data reported by Hanessian et al.^[6] for two γ -peptide hexamers include numerous NOEs of these types, which strongly supports 14-helix formation. Our crystallographic data identify three additional H—H distances between 4.0 and 4.5 Å, which might give rise to weak NOEs: $C_{\gamma}H(i)-NH(i+3)$, $C_{\beta}H(i)-NH(i+3)$ and $C_{\gamma(\beta')}H(i)-NH(i+2)$. (" $C_{\gamma(\beta')}H$ " indicates a proton on the first carbon (β') of a side chain attached to the backbone γ -carbon.) NMR data reported by Seebach et al.^[7,15] are largely consistent with the data in Table 3, but these studies also revealed NOEs that can now be recognized as *inconsistent* with the 14-helix. One hexa- γ -peptide displayed a strong $C_{\gamma(\beta')}H(i)-NH(i+3)$ NOE,^[7] but the crystallographic data indicate that this H—H distance is typically 5.3 ± 0.2 Å in the 14-helix (six measurements). Another hexa- γ -peptide displayed a medium-intensity $C_{\gamma}H(i)-C_{\alpha}H(i+3)$ NOE,^[15] but the crystallographic data indicate an H—H distance of 5.9 ± 0.2 Å (six measurements). The non-14-helical NOEs suggest conformational heterogeneity for these γ -peptides in solution.

The gabapentin residue at the N-terminus of **3–7** forms a nine-atom ring H-bond ($C=O(i)-H-N(i+2)$) in each crystal structure. Trimer **3** has a second C_9 H-bond, across the central residue, which suggests that this H-bond pattern is energetically reasonable for γ -amino acid residues derived from **2**. However, no C_9 H-bond is observed in the crystal structures of larger γ -peptides **4–7**, where 14-atom H-bonding becomes possible in the segments comprised of **2**. We interpret the dominance of C_{14} H-bonding among **4–7** as strong evidence that the residue derived from **2** has an intrinsic preference for the 14-helical conformation. In contrast, our data indicate that the gabapentin residue has a strong preference for the C_9 H-bond: even when the gabapentin residue could participate in a 14-helix, as in **4–7**, this residue consistently forms the shorter-range H-bond. Previously we have noted that adoption of compact and specific conformations by unnatural peptidic oligomers requires subunits that disfavor H-bonds between nearest-neighbor backbone amide groups.^[16]

Our conclusions regarding gabapentin conformational preferences are consistent with those suggested by crystal structures of short gabapentin-containing peptides, many of which feature the C_9 H-bonding pattern.^[17] This purely local gabapentin folding pattern does not

necessarily lead to a regular secondary structure, as illustrated by the crystal structure of a gabapentin tetramer, in which the torsion angles within the C₉ rings vary irregularly along the sequence.^[8g] In contrast, NMR analysis of γ -peptide oligomers containing γ^4 -amino acid residues bearing a bulky side chain suggest formation of a regular 9-helix.^[8h] Other γ -peptide conformations containing multiple C₉ H-bonded rings have been reported as well.^[8f,i,j]

Atomic-resolution data from a series of 14-helical γ -peptides have enabled us to generate definitive structural parameters for this secondary structure. In addition, the crystallographic data identify key backbone proton NOE patterns that should be manifested upon 14-helical folding in solution. These benchmarks are useful both for evaluating previous NMR studies^[6,7,15] and for guiding future conformational explorations. This level of analysis was not previously possible among γ -peptides; the conformational preorganization inherent in γ -residues derived from 2 presumably contributes to the high propensity of oligomers 3–7 to crystallize, and therefore to our success in acquiring multiple 14-helical structures. Atomic-resolution conformational analysis of foldamers containing preorganized β -amino acid residues has provided a foundation for structure-based designs of β - and α/β -peptides with specific functions,^[5,18] and the conformational insights provided here should have comparable value for function-based design of γ -peptides.

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Figure 1. Structures of γ -peptides **3–7** (arrows indicate H-bonds in the crystal structures of **3–7**).

(a)

(b) **(c)**

Figure 2. Crystallographic data. (a) Crystal structures of **3–7**. (b) Stereoview of the 14-helical segment of **7** (N-terminal gapapentin residue not shown). (c) View along the helix axis of the 14-helical segment of **7**.

- (a)** $C_{\gamma}H(i)$ to $C_{\alpha}H(i+2)$
- (b)** $C_{\gamma}H(i)$ to $NH(i+2)$
- (c)** $C_{\gamma}H(i)$ to $C_{\alpha(\beta')}H(i+2)$
- (d)** $C_{\gamma}H(i)$ to $NH(i+3)$
- (e)** $C_{\beta}H(i)$ to $NH(i+3)$
- (f)** $C_{\gamma(\beta')}H(i)$ to $NH(i+2)$

Figure 3.
Characteristic NOEs patterns in the crystal structures of γ -peptides **5–7**.

Table 1

Average Torsion Angles ^[a] from 14-Helical Segments

γ -Peptides	residues	ϕ	θ	ζ	ψ
4-7	gabapentin ^[b]	98.6	-67.2	-76.7	82.5
	γ ^[c]	-154.5	60.2	59.5	-126.8
<hr/>					
Seebach et al. ^[9]	γ ^[d]	140.4	-67.1	-54.6	133.6
	γ ⁽⁴⁾ ^[e]	109.1	174.6	-178.6	51.0

^[a] Backbone torsion angles in γ -residues are defined in Figure 1

^[b] Average backbone torsion angles of gabapentin γ -residues in **4-7**.

^[c] Average backbone torsion angles of γ -residues derived from **2** in **4-7**, excluding the C-terminal residue in each case (14 independent γ -residues from **4-7** were used to generate the torsion angle averages; see Supporting Information).

^[d] Average backbone torsion angles of the first three γ -residues in the tetra- γ -peptide crystal structure reported in ref. 9

^[e] Torsion angles of the C-terminal residue in the tetra- γ -peptide crystal structure reported in ref. 9

Table 214-Helical Parameters from Structures of **6** and **7**.

peptides	res/turn <i>n</i>	rise/turn <i>p</i> (Å)	rise/res <i>d</i> (Å)	radius <i>r</i> (Å)
hexamer 6	2.5	5.4	2.1	2.9
heptamer 7	2.6	5.5	2.1	2.9
	2.5	5.5	2.2	2.9
average	2.5	5.5	2.1	2.9

Table 3

Average H—H distances (Å) in crystal structures of **4–7** corresponding to medium-range NOE patterns expected to be characteristic of γ -peptide 14-helix formation in solution.

NOE type	# measurements	Distance (Å)
C $_{\gamma}$ H(i) to C $_{\alpha}$ H(i+2)	9	2.6 $\pm$ 0.4
C $_{\gamma}$ H(i) to NH(i+2)	9	2.7 $\pm$ 0.1
C $_{\gamma}$ H(i) to C $_{\alpha}(\beta)$ H(i+2)	9	3.4 $\pm$ 0.4
C $_{\gamma}$ H(i) to NH(i+3)	6	4.0 $\pm$ 0.1
C $_{\beta}$ H(i) to NH(i+3)	6	4.4 $\pm$ 0.2
C $_{\gamma}(\beta)$ H(i) to NH(i+2)	9	4.0 $\pm$ 0.1