Hybrid-Peptide Helices

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Promotion of Folding in Hybrid Peptides through Unconstrained γ Residues: Structural Characterization of Helices in $(\alpha\gamma\gamma)_n$ and $(\alpha\gamma\alpha)_n$ Sequences**

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The tendency of oligopeptides composed of γ-amino acids to fold into helical structures was first observed by the groups of Seebach and Hanessian in their studies of short γ peptide sequences in solution.[1,2] These observations, together with the reports on oligomeric β peptides, which emanated originally from the groups of Seebach and Gellman,[3,4] fuelled a great deal of interest in the design and formation of folded peptides ("foldamers"),[4c] incorporating the backbone-homologated analogues of the α-amino acids.[5] The incorporation of unsubstituted β-, γ-, and δ-amino acids into folded structures formed by host α-amino acid sequences has been extensively exploited in the design of polypeptide structures with hybrid backbones.[6] Crystallographic characterization of novel hydrogen-bonded helices in oligo β-peptides and in αβ, αγ, and βγ sequences has been possible in peptides containing stereochemically constrained β and γ residues, in which the additional degrees of backbone torsional freedom have been limited either by cyclization[7] or by incorporation of gem-dialkyl substituents.[8] Imposing a conformational bias by using constrained residues has been shown to greatly enhance the crystallizability of relatively long oligopeptides sequences that contain only α-amino acids, a feature that is best exemplified by extensive studies in the solid state of peptides that contain α-aminoisobutyric acid (Aib) or related α,α-dialkylated amino acids.[9]

Stimulated by the preliminary reports of folded structures in solution for peptides that contain unconstrained γ residues,[1,2] derived by homologation of the naturally occurring α-amino acids found in proteins, we embarked on a systematic survey of the conformational propensities of γ residues derived from the genetically coded amino acids.[10] Herein, we describe a mixed $C_{12}/C_{14}/C_{12}$ helical structure in an $(\alpha\gamma\gamma)_n$ sequence established in the crystals of the twelve-residue peptide Boc-[Aib-γ^4(R)Val-γ^4(R)Val]$_4$-OMe (**1**; Figure 1). We further demonstrate the ability of γ^4Val residues to promote helical folding in hybrid $(\alpha\gamma\alpha)_n$ sequences, even in the absence of conformationally constrained α residues, as illustrated by the crystal structures of the peptides Boc-Leu-γ^4(R)Val-Val-OH (**2**) and Boc-[Leu-γ^4(R)Val-Val]$_2$-OH (**3**; Figure 1). The helical structures of peptides **1**–**3** suggest a high propensity of γ-monosubstituted γ residues to favor local folded conformations.

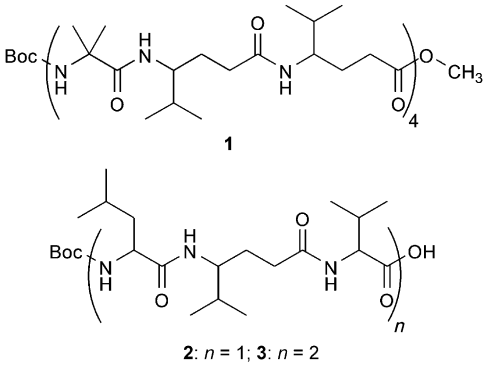

Figure 1. Structures of the peptides Boc-[Aib-γ^4(R)Val-γ^4(R)Val]$_4$-OMe (**1**), Boc-Leu-γ^4(R)Val-Val-OH (**2**), Boc-[Leu-γ^4(R)Val-Val]$_2$-OH (**3**).

The twelve-residue $(\alpha\gamma\gamma)_4$ peptide Boc-[Aib-γ^4(R)Val-γ^4(R)Val]$_4$-OMe (**1**) crystallized in the monoclinic space group $P2_1$, with three independent molecules in the crystallographic asymmetric unit. The backbone conformations of the three molecules show that the peptide folds into a continuous helix, stabilized by 4→1 intramolecular hydrogen bonds (Figure 2). The molecular conformation is best described as a backbone-expanded analogue of the classical 3_{10} helix,[11] observed in all-α peptides.[9c,12] The C_{12} hydrogen bonds (hydrogen bonds that result in the formation of a ring with 12 atoms) are formed over the αγ segments, while C_{14} hydrogen bonds occur over the γγ segments. In all three molecules, three C_{14} hydrogen bonds are observed over the first three γγ segments. The C-terminal γγ segment lacks the additional NH group necessary for C_{14} hydrogen bond formation. The three independent molecules differ in the number of C_{12} hydrogen bonds. Molecule 2 is a perfect $C_{12}/C_{14}/C_{12}$ helix with all seven anticipated C_{12} hydrogen bonds being observed (Figure 2). In both molecules 1 and 3, a single water molecule (O1W in molecule 1 and O3W in molecule 3) invades the backbone at a potential C_{12} hydrogen-bonding site. Such hydrated helical

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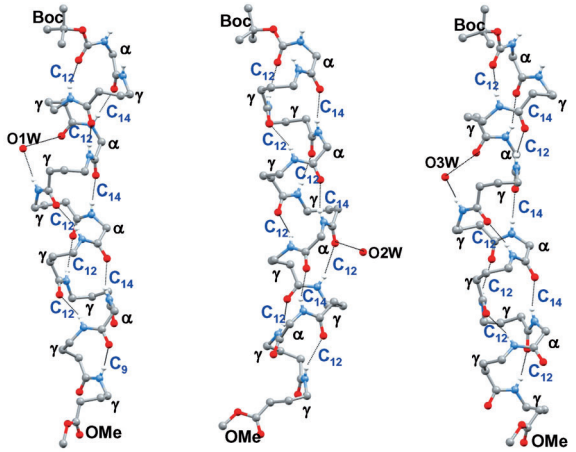

Figure 2. Conformation of three crystallographically independent molecules in the crystals of peptide Boc-[Aib-γ^4(R)Val-γ^4(R)Val]$_4$-OMe (**1**). Average hydrogen-bond parameters for C_{12} and C_{14} hydrogen bonds in peptide **1**: N···O = (3.00 ± 0.13) Å, H···O = (2.20 ± 0.14) Å, N–H···O = (156.0 ± 5.9) °.

structures have been commonly observed in α peptide helices.[13] In molecule 2, the γ^4(R)Val(6) CO group acts as an acceptor for both the intramolecular C_{12} hydrogen bonds and a hydrogen bond involving water molecule O2W. This result provides an example of helix hydration without any disruption of internal hydrogen bonds. Molecules 1 and 3 differ at the C terminal end. In molecule 1, γ^4(R)Val(11) formed a C_9 structure, which is an expanded analogue of C_7 hydrogen bond observed in proteins.[14] This C_9 hydrogen bond has been widely observed in peptides that contain the conformationally constrained γ residue gabapentin (Gpn).[8b] In contrast, a C_{12} hydrogen bond is formed in molecule 2, at the C-terminal end.

The backbone torsion angles are listed in Table 1. In all the three independent molecules, the Aib residues adopt the anticipated right-handed (α_R) helical conformation. 22 of the 24 independent γ^4(R)Val residues adopt *gauche–gauche* conformations about the C^γ–C^β (θ_1) and C^β–C^α (θ_2) bonds with the following average values of torsion angles: θ_1 = (53.6 ± 4.6)° and θ_2 = (65.1 ± 4.4)°. The two exceptions are the C-terminal γ^4(R)Val(12) residues in molecules 1 and 2, which have distorted θ_2 values of 102.3° and 143.6°, respectively. These distortions from the *gauche* conformation are undoubtedly a consequence of compensating intermolecular interactions in crystals. For γ residues, a convenient conformational representation in two-dimensional ϕ-ψ space can be made for the helical *gauche-gauche* conformations about the C^γ–C^β (θ_1) and C^β–C^α (θ_2) bonds.[10] 22 of the 24 γ^4(R)Val residues have averaged ϕ and ψ values of ϕ = (−124.1 ± 7.1)° and ψ = (−136.2 ± 21.4)°.

Helical structures in $(\alpha\gamma\alpha)_n$ sequences have been characterized in the tripeptide Boc-Leu-γ^4(R)Val-Val-OH (**2**) and hexapeptide Boc-[Leu-γ^4(R)Val-Val]$_2$-OH (**3**). In both cases, the crystals were obtained for the N-protected peptide acids (Figure 3). In the case of the hexapeptide, two crystallographically independent molecules with very similar conformations are observed. Table 2 lists the backbone torsion angles of both the peptides. In the case of tripeptide Boc-Leu-γ^4(R)Val-Val-OH, two intramolecular C_{12} hydrogen bonds are observed: (Boc) CO···HN [Val(3)] and [Leu(1)] CO···HO (OH). In the case of the hexapeptide, the αγααγα sequence results in two αγ C_{12} hydrogen bonds each at the N- and C-terminal segments, while the central αα segment forms the C_{10} hydrogen bond. In both the tripeptide and hexapeptide, the C-terminal carboxylic acid groups adopt the unusual *anti* conformation, resulting in the formation of an intramolecular CO···HO hydrogen bond, which is an oxy analogue of the CO···HN hydrogen bond in polypeptide secondary structures.[15] While the *syn* conformation predominates in the crystal structures of carboxylic acids, *anti* conformations have occasionally been found in the solid state.[15]

The formation of folded helical conformations with intramolecular hydrogen bonds in these unconstrained short peptides was unanticipated. All five independent γ^4(R)Val

Table 1: Backbone torsion angles of Boc-[Aib-γ^4(R)Val-γ^4(R)Val]$_4$-OMe (**1**).[a]

Molecule	Residues	Backbone torsion angles [°]			
		ϕ	θ_1	θ_2	ψ
Molecule A	U (1)	−57.4			−41.0
	γ^4(R)V (2)	−129.5	43.5	65.7	−146.9
	γ^4(R)V (3)	−131.2	57.0	59.9	−127.6
	U (4)	−61.9			−33.2
	γ^4(R)V (5)	−115.3	57.7	68.3	−168.8
	γ^4(R)V (6)	−115.8	59.0	65.0	−136.2
	U (7)	−68.6			−27.6
	γ^4(R)V (8)	−122.2	48.5	66.5	−146.3
	γ^4(R)V (9)	−130.0	54.8	61.9	−123.8
	U (10)	−62.2			−35.0
	γ^4(R)V (11)	−114.7	59.5	74.6	−83.9
	γ^4(R)V (12)	−110.3	67.8	102.3	117.3
Molecule B	U (1)	−69.5			−27.3
	γ^4(R)V (2)	−127.4	53.1	61.8	−145.3
	γ^4(R)V (3)	−130.3	56.2	63.1	−128.2
	U (4)	−63.1			−34.6
	γ^4(R)V (5)	−121.8	47.5	62.8	−145.3
	γ^4(R)V (6)	−131.8	60.6	57.9	−127.6
	U (7)	−69.0			−32.5
	γ^4(R)V (8)	−126.8	48.8	69.6	−151.1
	γ^4(R)V (9)	−121.7	55.7	64.7	−137.6
	U (10)	−63.6			−26.6
	γ^4(R)V (11)	−125.8	48.7	63.9	−101.5
	γ^4(R)V (12)	−99.0	52.3	143.6	136.3
Molecule C	U (1)	−66.8			−42.2
	γ^4(R)V (2)	−125.0	50.2	67.9	−144.6
	γ^4(R)V (3)	−130.6	57.8	58.8	−118.6
	U (4)	−61.3			−36.3
	γ^4(R)V (5)	−109.8	55.3	73.4	−167.3
	γ^4(R)V (6)	−122.9	56.7	62.3	−134.1
	U (7)	−60.2			−37.0
	γ^4(R)V (8)	−121.6	47.3	71.5	−150.1
	γ^4(R)V (9)	−124.7	53.3	64.3	−133.0
	U (10)	−62.9			−33.0
	γ^4(R)V (11)	−138.0	54.1	62.9	−107.0
	γ^4(R)V (12)	−113.4	53.2	66.0	−171.6

[a] U = Aib, γ^4(R)V = γ^4(R)Val. Note: Average value of estimated standard deviation of torsion angles for peptide **1** is 1.4°.

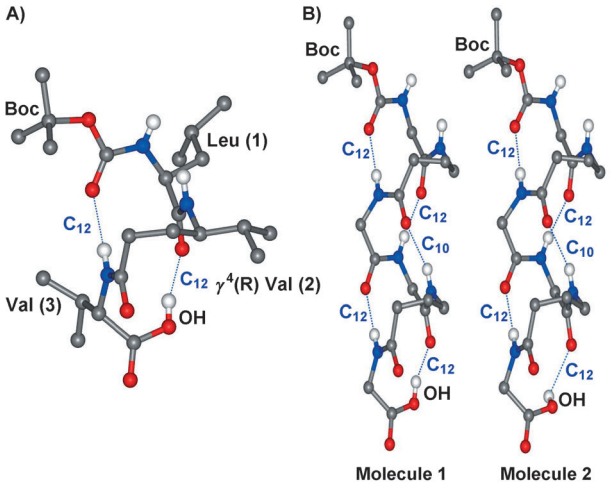

Figure 3. Conformation in crystals of A) Boc-Leu-γ^4(R)Val-Val-OH (**2**) and B) Boc-[Leu-γ^4(R)Val-Val]$_2$-OH (**3**, two molecules in the asymmetric unit without side chains). Average hydrogen-bond parameters for C_{12} hydrogen bonds in peptide **2**: N···O = (2.80 ± 0.28) Å, H···O = (1.96 ± 0.24) Å, N–H···O = (170.7 ± 8.1) °. Average hydrogen-bond parameters for C_{10} and C_{12} hydrogen bonds in peptide **3**: N···O = (2.89 ± 0.14) Å, H···O = (2.11 ± 0.09) Å, N–H···O = (158.2 ± 19.0) °.

Table 2: Backbone torsion angles [°] in the crystal structures of Boc-Leu-γ^4(R)Val-Val-OH (**2**) and Boc-[Leu-γ^4(R)Val-Val]$_2$-OH (**3**).[a]

Peptide	Residues	Backbone torsion angles [°]			
		ϕ	θ_1	θ_2	ψ
2	L (1)	−68.1			−33.3
	γ^4(R)V (2)	−127.0	53.9	57.9	−115.3
	V (3)	−57.4			−45.4
3, Molecule 1	L (1)	−70.6			−36.5
	γ^4(R)V (2)	−124.5	51.2	61.1	−124.7
	V (3)	−59.2			−33.0
	L (4)	−64.4			−24.3
	γ^4(R)V (5)	−128.3	49.3	58.8	−117.8
	V (6)	−65.3			−41.0
3, Molecule 2	L (1)	−70.0			−36.0
	γ^4(R)V (2)	−125.5	50.2	60.8	−127.6
	V (3)	−59.5			−31.6
	L (4)	−68.3			−23.2
	γ^4(R)V (5)	−128.4	50.4	59.5	−119.2
	V (6)	−64.3			−42.6

[a] L = Leu, γ^4(R)V = γ^4(R)Val, V = Val. Note: Average values of estimated standard deviation of torsion angles are for peptide **2**: 0.2° and for peptide **3**: 1.2°.

residues that were crystallographically characterized in these two structures adopt the *gauche–gauche* conformation about the backbone C^{γ}–C^{β} (θ_1) and C^{β}–C^{α} (θ_2) bonds. The average backbone torsion angles for the five residues are: $\phi = (-126.7 \pm 1.7)°$, $\theta_1 = (51.0 \pm 1.8)°$, $\theta_2 = (59.6 \pm 1.3)°$, $\psi = (-120.9 \pm 5.1)°$. Interestingly, there are no crystallographically characterized examples of an all-α-amino acid sequence composed entirely of conformationally unconstrained residues, which fold into short helices. The possibility that the γ^4(R)Val residue (and indeed, other related γ-amino acid residues derived from the protein amino acids) has an intrinsically high propensity to adopt local folded conformations must be considered. The hydrogen-bond parameters for peptides **1**–**3** are provided in Tables S1, S2, and S3 in the Supporting Information.

The three peptide crystal structures described herein show a strong tendency of γ^4Val residues to adopt local conformations, in which the monosubstituted trimethylene chain folds into a *gauche–gauche* conformation, compatible with nucleation of a continuous helical structure in oligopeptide sequences. While the sequence of the twelve-residue peptide **1** contains the conformationally constrained α-amino acid residue Aib, which strongly promotes helical folding, there are no specific promoters of polypeptide chain folding in the sequences of **2** and **3**. This result suggests that monosubstituted γ residues derived by homologation of amino acid residues (with the exception of Gly and Pro) in proteins may be readily accommodated into helical secondary structures. Indeed, monosubstituted γ residues may support the formation of folded helical structures to a substantially greater extent than their α- and β-amino acid counterparts. The ability to modulate backbone hydrogen bonding with hybrid sequences containing α, β and γ residues opens up the possibility to rationally design helical peptide scaffolds in which spatial orientation of the side chains, which project from the helical scaffold, can be systematically varied. This feature, together with the known proteolytic stability of peptides containing backbone-modified amino acids[16] may be exploited in the design of biologically active peptides.

Experimental Section

The amino acid Boc-γ^4(R)Val-OH was synthesized by previously described procedures. All peptides were prepared by conventional solution-phase procedures. The N-terminal end was protected with the *tert*-butyloxycarbonyl (Boc) group, and the C-terminal end was protected as a methyl ester. The Boc group was removed using 98% formic acid and a 1:1 mixture of trifluoroacetic acid (TFA) and CH_2Cl_2, whereas the methyl ester was cleaved by alkaline hydrolysis. Couplings were mediated by isobutylchloroformate (IBCF) and 1-hydroxy-1*H*-benzotriazole (HOBt; 1.01 equiv) for dipeptides and successive peptides with O-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU) and HOBt or 1-ethyl-3-(3-dimethyllaminopropyl)carbodiimide hydrochloride (EDC·HCl) and HOBt. All intermediates were identified by thin-layer chromatography (TLC) on silica gel (SiO_2, $CHCl_3$/MeOH = 9:1) and characterized by electrospray ionization mass spectrometry (ESI–MS), before they were used without further purification. The final peptides were obtained as pure products after washing with hexane–diethyl ether mixtures.

General reaction and workup procedures: The N-Boc-protected amino acid (1 equiv) was dissolved in a 4:1 mixture (50 mL) of CH_2Cl_2 and dimethyl formamide (DMF). The mixture was cooled in an ice–salt bath and N-methylmorpholine (NMM, 1 equiv) was added followed by the coupling agent (1 equiv). After stirring for 10 min, a pre-cooled solution of HOBT (1 equiv) in DMF was added. The reaction mixture was stirred for an additional 10 min and the pre-cooled free base (1 equiv) was added slowly. After 10–15 min, the pH value of the solution was adjusted to approximately 8 by adding NMM, and the reaction mixture was stirred over night at room temperature. After removing the solvent, the residue was dissolved in CH_2Cl_2 (100 mL) and washed successively with brine (3 × 50 mL), 10% aq. $KHSO_4$ (3 × 50 mL), 50% aq. $NaHCO_3$ (3 × 40 mL), and water. The combined organic layer was dried over anhydrous sodium

sulfate, and the solvent was evaporated in vacuo to obtain the desired product. Detailed procedures are provided in the Supporting Information. Physical parameters: mp (°C): (**1**) 218–219; (**2**) 184–185; (**3**) 119–120. ESI–MS (Da): $[M+H]^{+}_{obsd}$, $[M+Na]^{+}_{obsd}$, $[M+K]^{+}_{obsd}$, (M_{cal}),: (**1**) 1490.3, 1512.3, 1528.2, (1489.1); (**2**) 458.3, 480.3, 496.3, (457.3); (**3**) 797.2, 819.1, (796.5).

Appropriate single crystals of peptides **1**–**3** were obtained by slow evaporation from solvent mixtures. Peptide **1:** 6–8 mg of the peptide dissolved in 150 μL of hexane and 150 μL of dichloromethane; peptides **2** and **3**: 6–8 mg of the peptide dissolved in 200–300 μL of methanol and 2–3 drops of water. Peptide **1** crystallized in the monoclinic space group $P2_1$, with three peptide molecules and six co-crystallized water molecules in the crystallographic asymmetric unit. Peptides **2** (one peptide molecule in the asymmetric unit) and **3** (two peptide molecules in the asymmetric unit) crystallized in the orthorhombic space group $P2_12_12_1$, without any co-crystallizing solvent molecule. The relevant crystal data and structure refinement details of all the structures are provided in the Supporting Information. CCDC 909490 (**1**), 881185 (**2**) and 881186 (**3**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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