Helical Structures

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Hybrid αγ Polypeptides: Structural Characterization of a C_{12}/C_{10} Helix with Alternating Hydrogen-Bond Polarity**

Prema G. Vasudev, Sunanda Chatterjee, Kuppanna Ananda, Narayanaswamy Shamala, and Padmanabhan Balaram**

The insertion of the higher homologues of the α-amino acids, specifically β, γ, and δ residues, into α peptide sequences results in hybrid structures with novel hydrogen-bonding possibilities.[1] Research in this area of hybrid polypeptides has been stimulated by the observation of novel helical structures in β oligopeptides in which the hydrogen-bond polarities are reversed. For example, the 12 helix (2.5_{12} or 2.5_1-*P*) in a $(\beta)_n$ sequence maintains the same directionality (C=O(*i*)···NH(*i*+3)) as the canonical 3_{10} helix in an $(\alpha)_n$ sequence[2] and may be formally considered as an expanded version of the latter. By contrast, the 14 helix (3_{14} or 3_1-*M*) in a $(\beta)_n$ sequence possesses a reversed hydrogen-bond polarity (NH(*i*)···C=O(*i*+2)).[3] Initial crystallographic results with hybrid αβ and αγ sequences suggest that the expanded analogues of the 3_{10} helix, namely, the C_{11} (αβ) and C_{12} (αγ) helices, can indeed be observed in short peptides.[4] Calculations suggest that helices with alternating hydrogen-bond polarities may indeed be stable structures in hybrid sequences.[1c,d] In this Communication, we report the C_{12}/C_{10} mixed hydrogen-bonding pattern in the crystal structure of the tetrapeptide Boc-Leu-Gpn-Leu-Aib-OMe (**1**; Boc: *tert*-butoxycarbonyl; Gpn: 1-(aminomethyl)cyclohexaneacetic acid (gabapentin); Aib: aminoisobutyric acid), which was serendipitously obtained during the synthesis of longer hybrid sequences. This novel helical conformation can be generalized as an $(\alpha\gamma)_n$ sequence. The sequence contains the stereochemically constrained γ residue gabapentin, which is an achiral β,β-disubstituted γ-amino acid. The presence of *gem*-dialkyl substituents on the Cβ atom limits the torsion angles about the Cγ–Cβ (θ_1) and Cβ–Cα (θ_2) bonds to approximately ±60°.[5]

[*] P. G. Vasudev, Prof. N. Shamala
Department of Physics, Indian Institute of Science
Bangalore, 560 012 (India)
Fax: (+91) 80-2360-2602
E-mail: shamala@physics.iisc.ernet.in
S. Chatterjee, K. Ananda, Prof. P. Balaram
Molecular Biophysics Unit, Indian Institute of Science
Bangalore, 560 012 (India)
Fax: (+91) 80-2360-0535
E-mail: pb@mbu.iisc.ernet.in

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Figure 1 shows the molecular conformation of tetrapeptide **1** in the crystal. The folded conformation is stabilized by two intramolecular hydrogen bonds. A C_{12} interaction is observed between the Boc C=O and Leu3 NH groups, while a C_{10} hydrogen bond with inverted polarity is observed between the Gpn2 NH and Leu3 C=O groups. In this structure, both of the α residues, Leu1 and Leu3, adopt the semiextended polyproline (P_{II}) conformation ($\phi \sim -60°$, $\psi \sim +120°$). This mixed C_{12}/C_{10} unit can readily be extended into a regular 12/10 helix with alternating hydrogen-bond directionalities, as shown in Figure 1c. The observed backbone torsion angles are very close to those computed for theoretical models.[1c]

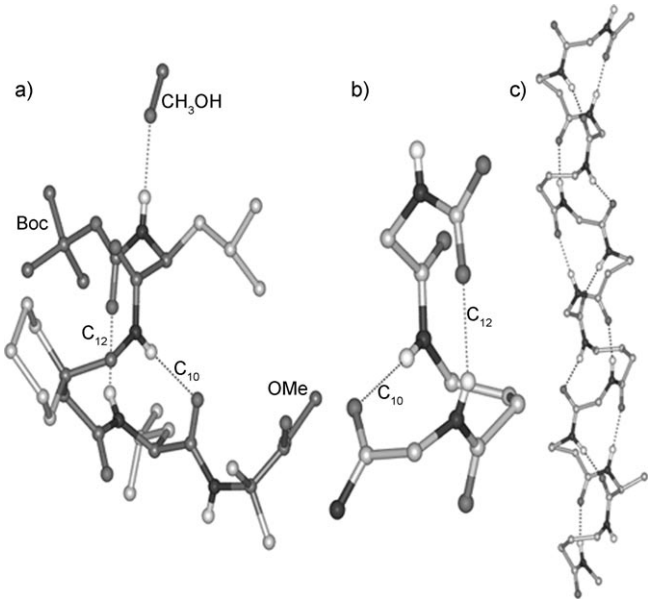

Figure 1. a) Molecular conformation of **1** in crystals. The Aib4 side chain, along with the CO(OMe) group, is disordered over two positions, with occupancies of 0.58 and 0.42. Only the major conformer is shown for clarity. The backbone torsion angles are: Leu1: $\phi = -71.9°$, $\psi = 121.1°$; Gpn2: $\phi = 87.8°$, $\theta_1 = 37.7°$, $\theta_2 = 45.1°$, $\psi = -129.3°$; Leu3: $\phi = -64.8°$, $\psi = 143.3°$; Aib4: $\phi = -56.8°$, $\psi = -30.7°$ ($\phi = 33.4°$, $\psi = -154.5°$ for the minor conformer). The C_{12} hydrogen-bond parameters are N···O = 2.93 Å, H···O = 2.17 Å, and N-H-O = 146.7°. The C_{10} hydrogen-bond parameters are N···O = 2.91 Å, H···O = 2.06 Å, and N-H-O = 166.7°. b) The backbone of the Leu1-Gpn2-Leu3 segment showing the C_{12}/C_{10} hydrogen bonds with opposite hydrogen-bond directionalities. c) The C_{12}/C_{10} mixed helix generated by extending the conformation observed in crystals.

The structure of peptide **1** illustrates some important conformational features of hydrogen-bonded turns in hybrid

αγ sequences. Figure 2 compares the conventional type I (type III) and type II β-turn conformations of αα segments[6] with the C_{12} turns observed in the αγ peptides. It is evident

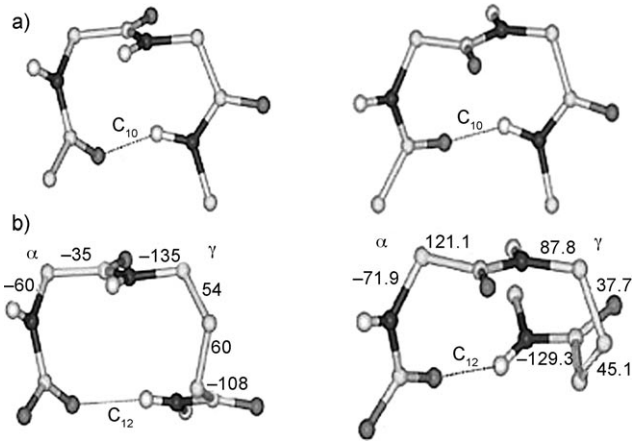

Figure 2. a) Type I/III (left) and type II (right) β turns in α polypeptides. b) C_{12} turns characterized in αγ hybrid peptides of Gpn: average conformation of αγ helical C_{12} turn (left) and the C_{12} turn observed in the Leu1–Gpn2 segment of peptide **1** (right).

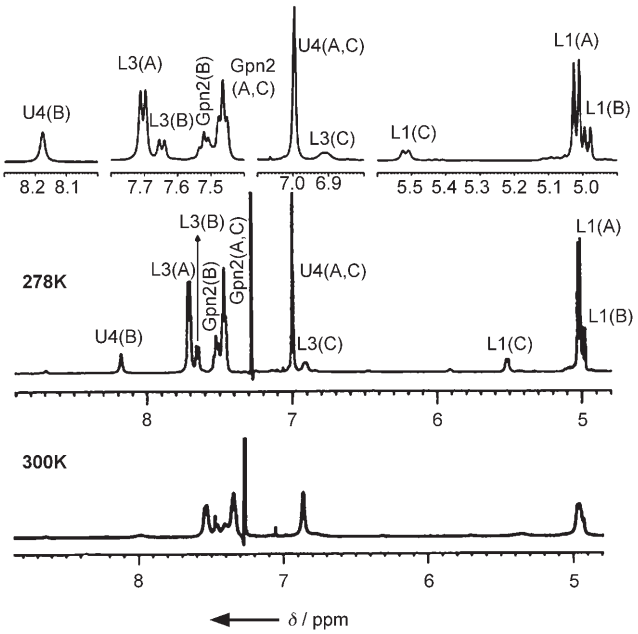

Figure 3. 500 MHz ^{1}H NMR spectrum of peptide **1** at 300 K (lower panel) and 278 K (middle panel). The top panel shows an expanded view of the amide NH resonances of peptide **1** in $CDCl_3$ at 278 K. A–C refer to the three distinct conformational species.

that αγ turns can also be classified into two categories. In the "helical turns", the γ residues adopt a conformation (ϕ=−135°, θ_1=54°, θ_2=60°, ψ=−108°)[5b] that will permit the formation of a successive γα turn. This structure is an expanded version of the helical type III β turn, which is repeated to generate a 3_{10} helix in all-α polypeptides.[2,7] Interestingly, the C_{12} αγ turn observed in tetrapeptide **1** is formally analogous to the type II β turn in an αα segment. The two types of αγ C_{12} turns illustrated in Figure 2 are formally related by an approximately 180° flip of the central peptide unit, a feature that also relates the type I and type II β turns in α peptides.[8] The structure in Figure 1c illustrates the fact that the αγ analogue of the type II β turn can be accommodated into the hybrid C_{12}/C_{10} $(\alpha\gamma)_n$ helix. By using internal hydrogen-bonded turns as the repeating structure in helices, the αγ C_{12} helix would have a two-residue repeating unit, while the C_{12}/C_{10} helix would have a three-residue repeating unit. If torsion angles were used as a descriptor, both types of αγ helices would be considered as $(\alpha\gamma)_n$ repeat units.

The αγα segment in tetrapeptide **1** could, in principle, adopt two distinct conformations, both of which are stabilized by two intramolecular hydrogen bonds. These are the C_{12}/C_{10} structure described above and the C_{12}/C_{12} structure, which has been previously characterized in the tetrapeptide Boc-Aib-Gpn-Aib-Gpn-OMe.[4c] Distinct conformational states may indeed be characterized in solution and possibly in polymorphic crystal forms. The amide ^{1}H NMR resonances of peptide **1** in $CDCl_3$ at 300 K are broad (Figure 3), in contrast to the sharp well-resolved ^{1}H NMR resonances observed for the side-chain protons in the region between 0 and 4 ppm (not shown). Cooling of the sample to 278 K results in the appearance of multiple resonances, with as many as three distinct species being observed. The estimated populations are 66% of A, 22% of B, and 11% of C. Exchange cross-peaks observed in two-dimensional ROESY experiments confirm that multiple resonances arise from slowly interconverting conformational states of the peptide.

Figure 4 summarizes the conformational regions that are likely to be populated in these αγ sequences. The Gpn residue is predominantly restricted to *gauche* conformations about the Cγ–Cβ (θ_1) and Cβ–Cα (θ_2) bonds, so the residue conformation may be represented in a two-dimensional ϕ–ψ plane. The conformational diagrams in Figure 4 illustrate the

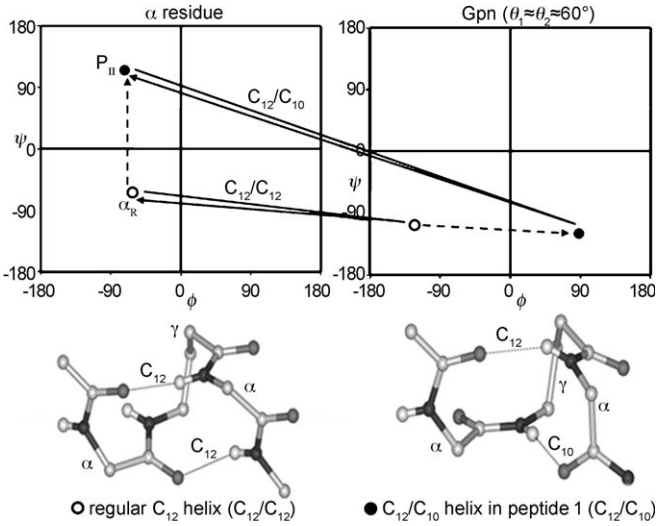

Figure 4. Representation of individual residue conformations in two-dimensional ϕ–ψ planes for the α and γ residues. For α residues, the normal Ramachandran space is shown. For γ residues, the values of θ_1 and θ_2 are both *gauche* and have the same sign.

structural transitions necessary for interconversions between the C_{12}/C_{10} and C_{12} helical structures. In the αγα segment, the α residue must transit from the helical region of ϕ–ψ space (α_R) to the polyproline region (P_{II}); this requires an approximately 180° rotation about the dihedral angle ψ. The γ residue must transit from a ϕ value of −130° in the C_{12} helix to a ϕ value of +87° in the C_{12}/C_{10} helical conformation. Although these conformational transitions require only rotation about single bonds, appreciable barriers may be anticipated, which result in the observation of slowly exchanging species, even at a temperature as high as 278 K. It is clear that the C_{12}/C_{10} structure is conformationally fragile in solution. In this short peptide sequence, nevertheless, the trapping of this unusual, incipient helix suggests that the construction of helices with alternating hydrogen-bond polarity can be readily achieved in longer sequences.

The results described above establish that αγ sequences containing stereochemically constrained residues can serve not only to characterize new helical folds in polypeptides but also to provide model systems to investigate folding transitions and conformational interconversions in solution. The designed structures, with hydrogen-bonding patterns unprecedented in proteins and all-α peptide sequences, provide an entry to new families of foldamers.[9]

Experimental Section

Tetrapeptide **1** was prepared by coupling Boc-Leu-Gpn-OH to Leu-Aib-OMe by using *N*,*N*′-dicyclohexylcarbodiimide (DCC)/1-hydroxybenzotriazole (HOBt) as the coupling reagent. The dipeptides were obtained by conventional procedures. The final peptide was purified by reversed-phase medium-pressure liquid chromatography (C_{18}, 40–60μ) and high-performance liquid chromatography on a reversed-phase C_{18} column (5–10 μ, 7.8–250 mm) with methanol/water gradients. The peptide was characterized by electrospray-ionization mass spectrometry (ESI MS) on an HP-1100 mass spectrometer and by complete assignment of the 500 MHz ^{1}H NMR spectra. NMR experiments were carried out on a Bruker AV 500 spectrometer. All processing was done by using BRUKER XWINNMR software.

Single crystals of peptide **1** ($C_{31}H_{56}N_4O_7$, $M_w = 596$; crystal size: $0.3 \times 0.23 \times 0.04$ mm^3) were obtained by slow evaporation from a methanol/dioxane mixture. The peptide crystallized in orthorhombic space group $P2_12_12_1$ with a molecule of methanol in the asymmetric unit. X-ray intensity data were collected at room temperature on a Bruker AXS SMART APEX CCD diffractometer ($Mo_{K\alpha}$ radiation, $\lambda = 0.71073$ Å, ω scan). The structure was solved by direct methods by using the SHELXS-97[10a] program and was refined against F^2, with the full-matrix least-squares methods by using the SHELXL-97 program.[10b] The unit-cell dimensions and refinement parameters were $a = 10.854(1)$, $b = 18.385(1)$, $c = 20.038(1)$ Å; $V = 3998.6(4)$ Å^3; $Z = 4$; $\rho_{calc} = 1.04$ g cm^{-3}; $\mu = 0.07$ mm^{-1}; $F(000) = 1360$; $2\theta_{max} = 53°$; $R_{int} = 0.05$; 7564 independent reflections; 4342 unique reflections; 2729 observed reflections ($F_o \geq 4\sigma|F_o|$); parameters = 443; $R_1 = 0.0767$ and $wR_2 = 0.1860$; GOF = 1.194; residual electron density $\Delta\rho_{max} = 0.47$ e Å^{-3}, $\Delta\rho_{min} = -0.19$ e Å^{-3}. The C-terminal CO(OMe) group, along with the side chain of the Aib4 residue, is disordered over two positions, which were refined with an occupancy ratio of 0.58:0.42. Hydrogen atoms were fixed geometrically in idealized positions and were refined as riding over the atoms to which they were bonded. CCDC 662233 contains 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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[1] a) I. L. Karle, A. Pramanik, A. Banerjee, S. Battacharjya, P. Balaram, *J. Am. Chem. Soc.* **1997**, *119*, 9087–9095; b) S. Chatterjee, R. S. Roy, P. Balaram, *J. R. Soc. Interface* **2007**, *4*, 587–606; c) R. S. Roy, P. Balaram, *J. Pept. Res.* **2004**, *63*, 279–289; d) C. Baldauf, R. Günther, H.-J. Hofmann, *J. Org. Chem.* **2006**, *71*, 1200–1208; e) C. Baldauf, R. Günther, H.-J. Hofmann, *Biopolymers* **2006**, *84*, 408–413; f) H. S. Choi, I. A. Guzei, L. C. Spencer, S. H. Gellman, *J. Am. Chem. Soc.* **2008**, *130*, 6544–6550.

[2] C. Tonlolo, E. Benedetti, *Trends Biochem. Sci.* **1991**, *16*, 350–353.

[3] a) D. Seebach, A. K. Beck, D. J. Bierbaum, *Chem. Biodiversity* **2004**, *1*, 1111–1239; b) R. P. Cheng, S. H. Gellman, W. F. DeGrado, *Chem. Rev.* **2001**, *101*, 3219–3232.

[4] a) M. A. Schmitt, H. S. Choi, I. A. Guzei, S. H. Gellman, *J. Am. Chem. Soc.* **2006**, *128*, 4538–4539; b) M. A. Schmitt, H. S. Choi, I. A. Guzei, S. H. Gellman, *J. Am. Chem. Soc.* **2005**, *127*, 13130–13131; c) K. Ananda, P. G. Vasudev, A. Sengupta, K. M. P. Raja, N. Shamala, P. Balaram, *J. Am. Chem. Soc.* **2005**, *127*, 16668–16674; d) for a recent report on the solution NMR spectrum, see: G. V. M. Sharma, V. B. Jadhav, K. V. S. Ramakrishna, P. Jayaprakash, K. Narsimulu, V. Subash, A. C. Kunwar, *J. Am. Chem. Soc.* **2006**, *128*, 14657–14668.

[5] a) K. Ananda, S. Aravinda, P. G. Vasudev, K. M. P. Raja, H. Sivaramakrishnan, K. Nagarajan, N. Shamala, P. Balaram, *Curr. Sci.* **2003**, *85*, 1002–1011; b) P. G. Vasudev, K. Ananda, S. Chatterjee, S. Aravinda, N. Shamala, P. Balaram, *J. Am. Chem. Soc.* **2007**, *129*, 4039–4048; c) for a definition of torsion-angle nomenclature in ω-amino acids see: A. Banerjee, P. Balaram, *Curr. Sci.* **1997**, *73*, 1067–1077.

[6] a) C. M. Venkatachalam, *Biopolymers* **1968**, *6*, 1425–1436; b) C. M. Wilmot, J. M. Thornton, *J. Mol. Biol.* **1988**, *203*, 221–232.

[7] a) I. L. Karle, P. Balaram, *Biochemistry* **1990**, *29*, 6747–6756; b) B. V. V. Prasad, P. Balaram, *CRC Crit. Rev. Biochem.* **1984**, *16*, 307–347.

[8] K. Gunasekaran, L. Gomathi, C. Ramakrishnan, J. Chandrasekhar, P. Balaram, *J. Mol. Biol.* **1998**, *284*, 1505–1516.

[9] a) R. Rai, P. Balaram in *Foldamers: Structure, Properties and Applications* (Eds.: S. Hecht, I. Huc), Wiley-VCH, Weinheim, **2007**, pp. 147–171; b) C. M. Goodman, S. Choi, S. Shandler, W. F. DeGrado, *Nat. Chem. Biol.* **2007**, *3*, 252–262; c) S. H. Gellman, *Acc. Chem. Res.* **1998**, *31*, 173–180.

[10] a) G. M. Sheldrick, SHELXS-97, a program for automatic solution of crystal structures, University of Göttingen, Göttingen, **1997**; b) G. M. Sheldrick, SHELXL-97, a program for crystal structure refinement, University of Göttingen, Göttingen, **1997**.

