Structural characterization of folded pentapeptides containing centrally positioned β(R)Val, γ(R)Val and γ(S)Val residues

Bhimareddy Dinesha a, Krishnayan Basuroyb, Narayanaswamy Shamalab,*, Padmanabhan Balaram a,*

a Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560012, India
b Department of Physics, Indian Institute of Science, Bangalore 560012, India

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ABSTRACT

A cylindrical pore of ~7.5 Å diameter containing a one-dimensional water wire, within the confines of a hydrophobic channel lined with the valine side chain, has been observed in crystals of the peptide Boc−α−Pro−Aib−Val−Alb−Val−OMe (1) (Raghavender et al., 2009, 2010). The synthesis and structural characterization in crystals of three backbone homologated analogues Boc−α−Pro−Aib−β(R)Val−Alb−Val−OMe (2), Boc−α−Pro−Aib−γ(R)Val−Alb−Val−OMe (3), Boc−α−Pro−Aib−γ(S)Val−Alb−Val−OMe (4) are described. Crystal structures of peptides 2, 3 and 4 reveal close-packed arrangements in which no pore was formed. In peptides 2 and 3 the N-terminus α-Pro-Aib segment adopted conformations closely related to Type II β-turns, while residues 2−4 form one turn of an αβ right-handed C11 helix in 2 and an αγ C12 helix in 3. In peptide 4, a continuous left-handed helical structure was observed with the α-Pro-Aib segment forming a Type III β-turn, followed by one turn of a left-handed αγ C12 helix.

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1. Introduction

The serendipitous observation that the peptide Boc−α−Pro−Aib−Leu−Alb−Val−OMe, formed a tubular structure in crystals, enclosing an empty pore of ~5.2 Å diameter, prompted us to investigate the analogue Boc−α−Pro−Aib−Val−Alb−Val−OMe (1). The peptide 1 crystallized in space group P63, with a cylindrical pore of ~7.5 Å diameter, coincident with the 6-fold screw axis. The channel, thus formed, had a completely hydrophobic lining encapsulating a wire of water molecules, which did not have any hydrogen bond interaction with the channel walls. The Val[3] residue is a part of the inner lining of the pore, with its isopropyl side chain protruding into the interior of the channel (Fig. 1a and b).1

A subsequent study of the pentapeptides Boc−α−Pro−Aib−Xxx−Alb−Val−OMe [Xxx=Ala, Phe], revealed that the hydrophobic pore encapsulating the water wire was observed only in the case of Xxx=Val (1).2 Crystal structure determination of four pentapeptides of this series, together with four independent enantiomers, established that this sequence consistently adopts a well-defined backbone conformation, stabilized by three intramolecular hydrogen bonds (Fig. 1c). The α-Pro-Aib segment forms a Type II β-turn structure followed by two successive Type III β-turn conformations over the segment Alb(2)-Xxx(3)-Alb(4). Residues 2, 3 and 4 adopt right-handed helical (αγ) conformations. These observations suggested that the nature and precise positioning of the residue 3 side chain was critical for the formation of porous crystals, with hydrophobic channels, since variation of the residue 3 side chain appeared to abolish pore formation, with the exception of βVal and γLeu. We therefore turned to exploring this effect of backbone variation1 on the molecular conformation and crystal packing in this peptide series. Stimulated by the growing interest in the structural chemistry of peptides containing β and γ amino acid residues,4 we synthesized the following analogues of peptide (1), Boc−α−Pro−Aib−β(R)Val−Alb−Val−OMe (2), Boc−α−Pro−Aib−γ(R)Val−Alb−Val−OMe (3), Boc−α−Pro−Aib−γ(S)Val−Alb−Val−OMe (4).

Crystallographic characterization of peptides 2−4, described in this report establish that well folded structures stabilized by intramolecular hydrogen bonds are indeed formed in the cases of backbone expanded peptide analogues. However, the crystals

* Corresponding authors. Tel.: +91 80 2260 2741; fax: +91 80 2260 0535 (P.B.); tel.: +91 80 2260 2602; fax: +91 80 2293 2856 (N.S.); e-mail addresses: shamala@physics.iisc.ernet.in (N. Shamala), pb@mbu.iisc.ernet.in (P. Balaram).

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formed reveal close-packed structures, with the absence of hydrophobic pores.

2. Results and discussion

Single crystals suitable for X-ray diffraction were obtained for peptides 2, 3 and 4. In the case of peptide 3 [Boc–α-Pro–Aib–γ-D-Pro–Val–Aib–Val–OMe], two different polymorphic forms were obtained and the structures determined in both the cases. Table 1 summarises the backbone torsional parameters for peptides 2–4. Corresponding values for the parent peptide 1 are also summarised. Table 2 summarises hydrogen bond parameters in the crystal structures of peptides 2–4. In all cases, the molecules crystallized in a close-packed fashion, in contrast to the case of 1, where crystals with large pores were formed (Fig. 1a and b).

The effect of insertion of backbone homologated residues on the folded structures of the peptides is considered below.

Table 1

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<th>a-Pro(1)</th>
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<th>Val(5)</th>
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</tr>
<tr>
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<td>−113.7</td>
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<tr>
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2.1. Molecular conformations of peptides

In crystals, peptide 2 [Boc–α-Pro–Aib–γ-D-Pro–Val–Aib–Val–OMe] crystallized in the monoclinic space group P2₁, with four independent molecules in the asymmetric unit named A, B, C, D. The backbone conformation of two of these molecules, A and B are illustrated in Fig. 2. Inspection of the backbone torsion angles and hydrogen bond parameters (Tables 1 and 2), establishes that in molecule A the Aib(2)–β-D-Pro(1)–Val(3)–Aib(4) segment is stabilized by two consecutive C11 hydrogen bonds. The consecutively formed hybrid φ turns correspond to a short stretch of a right-handed 310 helical structure, expanded by the insertion of an additional methylene group in the backbone at residue 3. In molecule B, three intramolecular hydrogen bonds are observed, with an additional C10 hydrogen bond stabilizing a Type II β-turn conformation for the α-Pro(1)–Aib(2) segment. The main conformational difference between molecule A and molecule B is observed at the N-terminus.
with D-Pro(1) adopting a \( \psi \) value (\( \psi \approx -160^\circ \)), molecules A, D; \( \psi \approx -140^\circ \), molecules B, C), which deviates significantly from that expected in an ideal Type II' \( \beta \)-turn (\( \psi \approx -120^\circ \)). Molecules A and D in the asymmetric unit of peptide 2 adopt very similar conformations, while molecules B and C are conformationally similar. Two co-crystallized water molecules are observed in the asymmetric unit of peptide 2.

The observed distortion at the N-terminus in the case of molecules A and D, is a consequence of an intramolecular hydrogen bond formed between (R)[\( \gamma^\delta \)]Val-[3]-Aib(4) C=O of molecule C of translated asymmetric unit along the z-axis (Fig. 3).

Depending on the crystallization conditions, peptide 3 crystallized in two polymorphic forms, both of which were orthorhombic (P2₁₂₁₂₁). In one case a co-crystallized water molecule was observed, while in the other a molecule of ethanol was obtained (Fig. 4). Both molecules adopted a very similar backbone conformations, with two consecutive \( \psi \) C₁₂ turns being formed at the Aib(2)-\( \gamma^\delta \)Val-[3]-Aib(4) segment. The torsion angles and the hydrogen-bonding pattern correspond to one turn of a right-handed \( \psi \) C₁₂ helix, which is a backbone expanded analogue of the classical 3_10 helix. \(^{26,27}\) Torsion angles at the N-terminus n-Pro(1)-Aib(2) segment deviate considerably from the values expected for ideal Type II' \( \beta \)-turns. Consequently, the C₁₀ hydrogen bond Boc(0) CO\cdots HN\( \gamma^\delta \)Val(3) is not formed. The overall backbone fold for the two independent molecules of peptide 3 is very similar to that obtained for molecules A and D in peptide 2. Comparison of peptides 2 and 3 with the parent peptide 1 establishes that the right-handed helical turn formed by the Aib(l)-Xxc(3)-Aib(4) segment is maintained, despite the expansion of the backbone at residue 3.

Peptide 4 [Boc-\( \gamma^\delta \)-Pro-Aib-\( \gamma^\delta \)Val-Aib-Alb-Val-OMe] crystallized in the triclinic space group P1. The molecular conformation is shown in Fig. 5. Three intramolecular hydrogen bonds, C₁₀, C₁₂ and C₁₅ stabilized the folded structure. Inspection of the torsion angles in Table 1 established that the molecule folds into a left-handed helix, with the Type III' \( \beta \)-turn at the n-Pro(1)-Aib(2) segment followed by two consecutive \( \psi \) C₁₂ turns. Such hybrid helical hydrogen bonding patterns are observed in peptides containing both \( \alpha \) residues and higher homologues. \(^{26,27}\) An interesting feature of peptide 4 is the switching of the turn type at the N-terminus n-Pro(1)-Aib(2) segment. While this segment adopted conformations very close to that Type II' \( \beta \)-turns in all the other examples, an almost ideal Type III' \( \beta \)-turn is observed in peptide 4.

2.2. Circular dichroism (CD)

Fig. 6 shows the far-UV CD spectra of the pentapeptides containing a central \( \gamma \)-valine residue at position 3 (3, \( \gamma^\delta \)Val; 4, \( \gamma^\delta \)Val). In the case of peptide 3 (\( \gamma^\delta \)Val) a negative band is observed at \( \approx 215 \) nm. In contrast, for peptide 4 (\( \gamma^\delta \)Val) a positive band is observed at \( \approx 215 \) nm, with a crossover point at \( \approx 203 \) nm. This is consistent with the X-ray diffraction results where a change in the handedness of the short helical fold is observed. In peptide 3 the N-terminus Type II' \( \beta \)-turn is followed by a right-handed \( \psi \) helix, while in peptide 4, a left-handed helical fold encompassing both the Type I \( \beta \)-turn segment and the two successive \( \psi \) C₁₂ turns is observed. While a detailed interpretation of CD spectra for short hydrid sequences may be premature, it is clear that the sense of twist of the helical backbone observed in crystals is indeed maintained in solution.

3. Conclusions

The structural results presented above for peptides 2 and 3 suggest that insertion of an additional methylene groups at the central position in this series of pentapeptides does not disrupt the folded, intramolecularly hydrogen bonded structure. The change of chirality at position 3 results in a switch in the handedness of the helix in peptide 4. The \( \beta \) amino acid residue in peptide 2 adopts an approximately gauche conformation about the \( C^\delta-C^6 \) bond. In peptides 3 and 4, the centrally positioned \( \gamma \)-amino acid residue adopts a gauche conformation about the \( C^\delta-C^6 \) and \( C^6-C^7 \) bonds. The tendency of \( \beta \) and \( \gamma \) residues bearing backbone substituents to adopt locally compact conformations, facilitates their incorporation into helical peptide backbones. While foldamer design has often focussed on studies of more severely constrained \( \beta \) and \( \gamma \) residues, \(^{26,27}\) it is clear that residues readily accessible, synthetically, from chiral \( \alpha \) amino acid residues can also be used in the design of folded
peptides, which mimic conformational features characterized in sequences comprising exclusively of $\alpha$-amino acid residues.

4. Experimental

4.1. General

All reagents were purchased from commercial sources and were used without further purification. Melting points were determined using Stuart melting point apparatus SMP10. Electrospray ionization mass spectrometry (ESI-MS) was done on a Bruker Daltonics Esquire-3000 instrument. Far-UV circular dichroism (CD) spectra were recorded on a JASCO J-715 spectropolarimeter. X-ray data were collected on Bruker AXS KAPPA APEXII CCD with MoK\textalpha{} ($\lambda=0.71073$ Å) radiation and Bruker AXS ULTRA APEXII CCD (rotating anode X-ray generator) with CuK\textalpha{} ($\lambda=1.54178$ Å) radiation. $^1$H NMR spectra were recorded on Bruker 500-MHz or 700-MHz spectrometers. Chemical shifts were measured in delta with TMS or residual solvent signal as a standard.
4.2. Synthesis of $\beta^3(R)$-valine

Literature procedures were followed with minor modifications. Briefly, Boc-L-Val-OH (10 mmol) was dissolved in anhydrous tetrahydrofuran (THF, 25 ml) and then cooled to $\sim -15^\circ C$. Triethylamine (Et$_3$N, 1.25 ml, 1 equiv) and ethyl chloroformate (ClCO$_2$Et, 1.25 ml, 1 equiv) were added to the solution. After 30 min, the triethylamine salt was filtered off and the filtrate was cooled to $\sim -15^\circ C$. The cooled filtrate was charged with diazomethane (CH$_2$N$_2$) until a rich greenish-yellow colour persisted. The mixture was then stirred for 5 h. After aqueous workup by successive washing with 10% KHSO$_4$ (3×50 ml), 5% NaHCO$_3$ (3×50 ml) and brine (30 ml), the organic layer was dried over Na$_2$SO$_4$ and concentrated under reduced pressure. The crude product was subjected to Wolff rearrangement as previously described. The diazoketone (10 mmol) was dissolved in THF (25 ml) with the
addition of 10% (v/v) H2O and then cooled to ~15 °C. A solution of silver acetate (1 mmol) in Et3N (11 mmol) was added, and the resulting mixture stirred for 3 h. The progress of the reaction was monitored by TLC (CHCl3/MeOH/ACO-H 40:2:1 (v/v)). The precipitated solid was filtered off, washed with 10% KHSO4, brine and dried over anhydrous Na2SO4. The solution was used for the next step without purification. Workup was by successive washing with 5% KHSO4 (2 ×, 1.87/2.24 (4H, m, D-Pro C H2), 2.38 (2H, d, b-Val C H2), 3.43 (2H, br, D-Pro C H2), 3.69 (3H, s, OCH3), 3.84 (1H, m, γ-HVal C H), 4.1 (1H, br, α-Pro C H), 4.48 (1H, q, Val C H), 6.26 (1H, br, γ-HVal C H), 6.79 (1H, br, α-Pro C H), 6.78 (1H, s, Aib NH), 6.61 (1H, br, b-Val NH), 7.46 (1H, s, Aib NH), 7.17 (1H, s, Aib NH), 6.79 (1H, s, Aib NH), 6.78 (1H, br, Val NH). Boc–b-Pro-Aib–γ(R)Val–b-Val–Val–OMe (4), 0.85/0.89/0.97 (12H, d, γ-HVal C H, Val C H), 1.4 (9H, s, Boc C H), 1.49/1.55 (12H, s, Aib C H), 1.88–2.04 (4H, m, b-Pro C H2), 1.9 (γ-HVal C H), 2.1 (2H, m, γ-HVal C H), 3.43 (2H, br, b-Pro C H), 3.69 (3H, s, OCH3), 3.84 (1H, m, γ-HVal C H), 4.1 (1H, br, d-Pro C H), 4.48 (1H, q, Val C H), 6.26 (1H, br, γ-HVal C H), 6.79 (1H, br, α-Pro C H), 6.78 (1H, br, Val NH), 7.46 (1H, s, Val NH). Boc–b-Pro-Aib–γ(R)Val–Val–Me (8), 0.85/0.89/0.97 (12H, d, γ-HVal C H, Val C H), 1.4 (9H, s, Boc C H), 1.49/1.55 (12H, s, Aib C H), 1.89–2.04 (4H, m, b-Pro C H2), 1.9 (γ-HVal C H), 2.1 (2H, m, γ-HVal C H), 3.49–3.58 (2H, m, b-Pro C H2), 1.9 (γ-HVal C H), 3.72 (3H, s, OCH3), 4.08 (1H, br, b-Pro C H), 4.45 (1H, q, Val C H), 6.36 (1H, s, Aib NH), 6.72 (1H, d, γ-HVal C H), 7.17 (1H, s, Aib NH), 7.96 (1H, d, Val NH), 7.51 (1H, s, Val NH). 

4.5. X-ray diffraction

Single crystals of the pentapeptides 2, 3 and 4 were obtained by slow evaporation using a range of solvent conditions. Colourless single crystals were grown by dissolving ~8 mg of peptides 2, 3a, 3b and 4 in acetonitrile/water mixture (200 µl/10 µl) in ethyl acetate/petroleum ether mixture (100 µl/100 µl), in ethanol (300 µl) and in ether (500 µl), respectively. Peptide 2 crystallized in the monoclinic space group P21, with four peptide molecules and two co-crystallized water molecules in the asymmetric unit. For peptide 3, polymorphic forms (ethyl acetate/petroleum ether and ethanol) were obtained. Form 3a crystallized with one peptide molecule and one water molecule in the asymmetric unit. Form 3b crystallized with one peptide molecule and one ethanol molecule in the asymmetric unit. Forms 3a and 3b both crystallized in the same orthorhombic space group P212121, whereas peptide 4 crystallized in the triclinic space group P1, without any co-crystallizing solvent. For peptides 2 and 4, X-ray data were collected on Bruker AXS KAPPA APEXII CCD with MoKα (λ = 0.71073 Å) radiation, and for peptides 3a and 3b X-ray data were collected on Bruker AXS ULTRA APEXII CCD (rotating anode X-ray generator) with CuKα (λ = 1.54178 Å) radiation. These data sets were collected in phi and omega scan type mode. For peptides 2 and 4, the structures were solved by using iterative dual space direct methods in SHELXD11 and for peptides 3a and 3b, the structures were solved by direct methods in SHELXS. After the initial solution methods all the structures were refined against F2 isotropically followed by full
matrix anisotropic least-squares refinement using SHELXL-97. The solvent molecules in peptides 2, 3a and 3b were located from difference Fourier maps. For peptide 2, all the hydrogen atoms were fixed geometrically in idealized positions and allowed to ride with the C or N atom to which each was bonded, in the final cycles of refinement. The hydrogen atoms of the co-crystallized water molecules in peptide 2 could not be located from the difference Fourier map. In the case of peptides 3a, 3b and 4 all the hydrogen atoms attached to N atoms and hydrogen atoms bonded to some other C atoms also were located from the difference Fourier map and suitable restraints were applied judiciously in order to get a chemically meaningful geometry.

The final R value for peptide 2 was $R_1 = 0.0770$ ($wR_2 = 0.2403$) for 6858 observed reflections with $F_o \geq 4\sigma(F_o)$ and for 1568 parameters. The final R value for peptide 3a was $R_1 = 0.0472$ ($wR_2 = 0.1509$) for 3790 observed reflections with $F_o \geq 4\sigma(F_o)$ and for 458 parameters. The final R value for peptide 3b was $R_1 = 0.0450$ ($wR_2 = 0.1409$) for 3634 observed reflections with $F_o \geq 4\sigma(F_o)$ and for 490 parameters. The final R value for peptide 4 was $R_1 = 0.0507$ ($wR_2 = 0.1467$) for 3251 observed reflections with $F_o \geq 4\sigma(F_o)$ and for 490 parameters. CCDC deposition numbers for the peptides are 848245 (2), 848246 (3a), 848247 (3b) and 848248 (4).

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References and notes