First Observation of an α-Helix in α,β-Dehydrooligopeptides: Crystal Structure of Boc-Val-ΔPhe-Ala-Leu-Gly-OMe

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De novo design of peptides and proteins has assumed considerable interest in recent year.[1−6] α,β-Dehydro residues, in particular, α,β-dehydrophenylalanine (ΔPhe), are being considered as one of the important conformational constraints in *de novo* design.[7] These residues have been found to occur naturally in peptides from microbial sources.[8−10] Their presence in peptides confers increased resistance to enzymatic degradation[11] and has led to the design of highly active analogues of bioactive peptides.[12,13] β-Bend[14] structures are stabilized in short peptides containing single ΔPhe residues.[15−18] In longer peptides containing one or more ΔPhe residues the 3_{10}-helical conformation has been mostly observed.[19−22] Recently a novel flat β-bend ribbon structure was observed in a dehydropentapeptide.[23] However, α-helices in dehydrophenylalanine oligopeptides have not been observed so far. Here we report the crystal structure of the pentapeptide Boc^0-Val^1-ΔPhe^2-Ala^3-Leu^4-Gly^5-OMe, exhibiting, for the first time, a right-handed α-helical conformation in dehydrooligopeptides. To the best of our knowledge the present pentapeptide represents the shortest α-helix seen in model peptides. The present example further confirms the versatility of ΔPhe residues in defining peptide conformation.

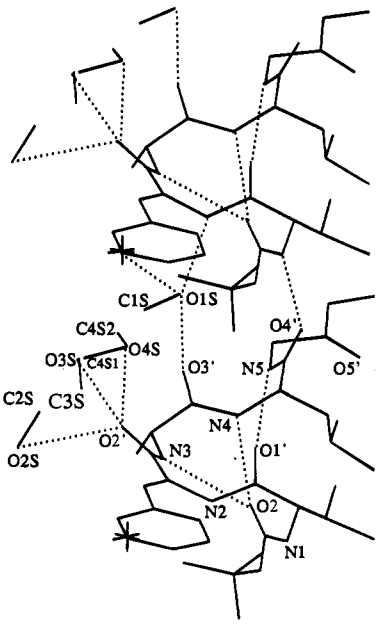

Figure 1. View of the α-helical peptide Boc-Val-ΔPhe-Ala-Leu-Gly-OMe perpendicular to the helix axis. The dotted lines represent the hydrogen bonds. Two molecules related by unit translation along the *x* direction are shown. C1S−O1S is a methanol molecule linking the two helical peptide molecules. The disordered methanol molecules hydrogen bonded to O2′ are also shown.

The molecular structure[24] of the pentapeptide Boc^0-Val^1-ΔPhe^2-Ala^3-Leu^4-Gly^5-OMe is illustrated in Figure 1. The peptide molecule exhibits two consecutive α-helical turns, one involving the fragment Boc^0-Val^1-ΔPhe^2-Ala^3-Leu^4 and the other involving Val^1-ΔPhe^2-Ala^3-Leu^4-Gly^5, which results in a right-handed α-helical conformation for the pentapeptide. In addition to the α-turns we also observe a β-turn centered around Val^1-ΔPhe^2 residues. These turn conformations are stabilized by appropriate intramolecular N−H···O hydrogen bonds (Table 1). The carbonyl oxygen of the Boc group acts as acceptor for N−H of both the Ala(3) and Leu(4) residues. A similar situation recognized in a molecular dynamics simulation of an α-helical

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(24) Experimental: The pentapeptide Boc-Val-ΔPhe-Ala-Leu-Gly-OMe ($C_{31}H_{47}N_5O_8$·2CH_3OH, MW = 681.8) was synthesized by using standard procedures.[22] Boc-Val-ΔPhe-Ala-OH was coupled to the TFA salt of Boc-Leu-Gly-OMe using dicyclohexylcarbodiimide (DCC) and *N*-hydroxybenzotriazole (HOBt) in dimethylformamide. The mixture was stirred for 4 h at 0 °C and then overnight at room temperature. For workup, the precipitated dicyclohexylurea was filtered off and the solvent was removed *in vacuo*. The residue was dissolved in ethyl acetate, washed successively with saturated $NaHCO_3$ solution, water, and 5% citric acid solution, dried over anhydrous Na_2SO_4, and finally evaporated to yield the desired pentapeptide. The pentapeptide was crystallized twice from CH_3OH/H_2O solution to get the pure compound: mp 93−95 °C; R_f ($CHCl_3$/MeOH, 9:1) = 0.5, R_f (CH_3CN/H_2O, 4:1) = 0.94. Colorless crystals were grown by slow evaporation of a peptide solution in aqueous methanol at 4 °C. It was observed that the peptide crystals are fragile and lose their crystalline nature when exposed to air. Hence a crystal mounted in a quartz capillary along with a drop of crystallizing solution was used for X-ray diffraction experiments. The crystals belong to orthorhombic space group $P2_12_12_1$, a = 10.197(2) Å, b = 10.869(2) Å, c = 35.261(7)Å , Z = 4, V = 3907.8 Å^3, D_{calcd} = 1.159 g/cm^3. X-ray intensity data were collected on a CAD4 diffractometer using Cu Kα (λ = 1.5418 Å) radiation. The structure was solved by direct methods using the computer program SHELX86.[34] Least-squares refinement (SHELX93)[34] on F_o^2 using 3304 unique reflections ($\theta \leq 60°$) resulted in an agreement factor $_wR2$ = 19.34% and goodness of fit parameter S = 1.028. The conventional agreement factor R1 based on 1808 reflections with $|F_o| \leq 4\sigma|F_o|$ is 5.95%. Hydrogen atoms were fixed on the basis of stereochemical criteria and were used only in structure factor calculations. One partially occupied water molecule and some disordered methanol solvent molecules were also located in a difference Fourier map.

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Table 1. Important Relevant Torsion Angles for Boc-Val-ΔPhe-Ala-Leu-Gly-OMe

residue	i	ϕ_i	ψ_i	ω_i	$\chi_i^{1,1}$	$\chi_i^{1,2}$	$\chi_i^{2,1}$	$\chi_i^{2,2}$
Boc[a]	0			176.0(6)				
Val	1	−57.9(8)	−36.8(8)	179.6(6)	−59.8(8)	175.5(6)		
ΔPhe	2	−60.2(9)	−23.2(10)	175.4(6)	−6.2(10)		−23.4(15)	161.9(10)
Ala	3	−95.3(8)	−35.0(9)	178.6(6)				
Leu	4	−68.5(9)	−32.2(10)	−178.5(7)	−65.9(8)		−68.7(9)	168.9(7)
Gly	5	74.3(10)						

[a] $\theta' = -177.7(6)$.

Table 2. Intramolecular and Intermolecular Hydrogen Bonds Observed in the Solid State Structure of Boc-Val-ΔPhe-Ala-Leu-Gly-OMe

donor (D)	acceptor (A)	distance D···A (Å)	distance H···A (Å)	angle D···H−A (deg)	sym
N3	O2	3.021(8)	2.260(8)	147.6(6)	x,y,z
N4	O2	3.102(7)	2.266(7)	164.0(7)	x,y,z
N5	O1o′	2.912(8)	2.110(8)	155.4(8)	x,y,z
N1	O4o′	2.853(7)	1.995(7)	176.8(8)	$x+1,y,z$
N2	O1Sa	2.879(7)	2.053(7)	160.5(7)	$x+1,y,z$
O1S	O3′	2.907(8)			x,y,z
O1S	O1Wa	2.730(8)			$x-1,y,z$
O2S[a]	O2′	3.149(10)			x,y,z
O3S[a]	O2′	3.147(10)			x,y,z
O4S[a]	O2′	2.776(9)			x,y,z

[a] O1S, O2S, O3S, and O4S are the oxygen atoms of methanol molecules with occupancies 1.0, 0.5, 0.5, and 0.3, respectively. O1W is a water molecule with occupancy 0.3. Further details can be seen in supporting information.

peptide suggests that, in the process of folding/unfolding of an α-helix, many of the carbonyl oxygen atoms share the feature of being involved in 4 → 1 and 5 → 1 hydrogen bonds simultaneously.[25] The average (ϕ,ψ) values for the first four residues are (−70.5°, −31.8°), which compare well with the values observed for α-helical conformations in peptides and proteins.[26,27] At Gly(5) the helix gets unwound ($\phi = 74.3°$), a frequent feature observed in helical peptides.[20,28]

The peptide crystal contains significant amount of solvent molecules which are highly disordered.[24] These solvent molecules interact with the peptide molecules through intermolecular hydrogen bonds and play a major role in crystal packing. One of the methanol molecules (O1S−C1S) mimics an additional amino acid residue which interlinks the pentapeptide molecules related by unit translation along the x direction, thereby stabilizing long helical rods in the crystal. The OH group of this methanol donates a hydrogen bond to the carbonyl oxygen of the Ala(3) residue and accepts a hydrogen bond from the amide N−H of the ΔPhe(2) residue, related by unit translation along the x direction. Water molecules have been observed to play such roles in some tripeptide crystals.[29] The amide N−H of the Val(1) residue donates a hydrogen bond to the carbonyl oxygen of the Leu(4) residue, related by unit translation along the x direction. The way in which adjacent molecules interact along the x axis is schematically represented in Figure 2. O2′ does not participate in any intramolecular hydrogen bonds; instead it forms hydrogen bonds with some disordered methanol molecules. The interaction of O2′ and O3′ atoms with external solvent molecules renders this originally apolar pentapeptide molecule somewhat amphiphilic. It is observed that in Aib (α-aminoisobutyric acid)-rich peptides the penetration of water

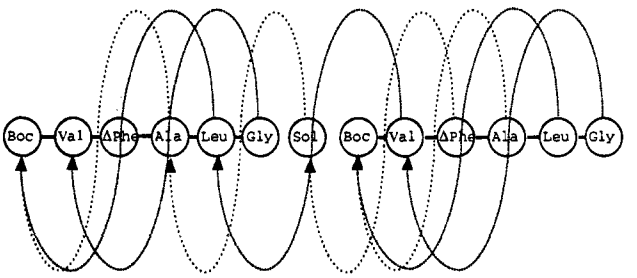

Figure 2. Schematic representation of the crystal packing for the pentapeptide Boc-Val-ΔPhe-Ala-Leu-Gly-OMe. Two pentapeptide molecules are represented with a circle for each residue. Sol is a methanol molecule mimicking an additional amino acid residue. Thin dotted lines indicate 4 → 1 hydrogen bonds, and thick dotted lines indicate 5 → 1 hydrogen bonds.

molecules into the helix backbone turns apolar helices amphiphilic.[30] It appears that the exposure of the crystal to air makes the loosely bound solvent molecules escape from the crystal lattice, which may result in disturbing the crystal packing, thereby breaking down the crystalline nature of the sample.[24] O5′ does not participate in any hydrogen bonds.

It is well established that, in general, short peptides up to seven residues long, containing Aib, tend to fold as 3_{10}-helical structures in the solid state whereas longer Aib peptides show significant α-helical content.[28,31,32] Pavone *et al.*[33] found that the mixed sequence (Aib-L-Ala)$_n$ exists as a 3_{10}-helix for $n = 3$ and a mixed $\alpha/3_{10}$-helix for $n = 4$, establishing a length-dependent $3_{10} \rightarrow \alpha$ helix transition in these peptides. Since the conformational behavior of the ΔPhe residue is considered similar to that of Aib, the existence of an α-helix in the present peptide may be of significance in studying the stability of the 3_{10}-helix versus the α-helix. The present structure is also interesting because it is the first report of an α-helix in ΔPhe oligopeptides and because only five residues are sufficient to stabilize two consecutive α-turns. However, at the same time, it also becomes clear that, in order to understand the role of peptide chain length and the number and positioning of ΔPhe residues, more studies will have to be undertaken.

Supporting Information Available: Details of crystal data and structure refinement, atomic fractional coordinates, thermal parameters, bond distances and angles, torsion angles, and packing diagram for Box-Val-ΔPhe-Ala-Leu-Gly-OMe (6 pages); observed and cultured structure factors (9 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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