Observation of Water-Mediated Helix-Terminating Conformation in a Dehydrophenylalanine Peptide: Crystal and Solution Structure of the Octapeptide Ac-ΔPhe-Val-ΔPhe-Phe-Ala-Val-ΔPhe-Gly-OMe[§]

K. R. Rajashankar,[†] S. Ramakumar,[†] R. M. Jain,[‡] and V. S. Chauhan*[,‡]

Contribution from the Department of Physics, Indian Institute of Science, Bangalore 560012, India, and International Center for Genetic Engineering and Biotechnology, NII Campus, Aruna Asaf Ali Marg, New Delhi 110067, India

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Abstract: We have synthesised and determined the solution conformation and X-ray crystal structure of the octapeptide Ac-ΔPhe[1]-Val[2]-ΔPhe[3]-Phe[4]-Ala[5]-Val[6]-ΔPhe[7]-Gly[8]-OCH_3 (ΔPhe = α,β-dehydrophenylalanine) containing three ΔPhe residues as conformation constraining residues. In the solid state, the peptide folds into (i) an N-terminal $3_{10}{}^{R}$-helical pentapeptide segment, (ii) a middle non-helical segment, and (iii) a C-terminal incipient $3_{10}{}^{L}$-helical segment. The results of ^{1}H NMR data also suggest that a similar multiple-turn conformation for the peptide is largely maintained in solution. Though the C-terminal helix is incipient, the overall conformation of the octapeptide matches well with the conformation of the hairpins reported. Comparison of the π-turn seen in the octapeptide molecule with those observed in proteins at the C-terminal end of helixes shows the structural similarity among them. A water molecule mediates the 5 → 2 hydrogen bond in the π-turn region. This is the first example of a water-inserted π-turn in oligopeptides reported so far. Comparison between the present octapeptide and another $3_{10}{}^{R}$-helical dehydro nonapeptide Boc-Val-ΔPhe-Phe-Ala-Phe-ΔPhe-Val-ΔPhe-Gly-OCH_3, solved by us recently, demonstrates the possible sequence-dependent conformational variations in α,β-dehydrophenylalanine-containing oligopeptides.

Introduction

One of the aims of contemporary protein research is the rational design of synthetic peptide mimics for structural motifs in proteins.[1] Incorporation of nonstandard amino acids with well-defined stereochemical and functional properties turns out to be an attractive approach to impose localized restrictions on the polypeptide chain.[2] In this direction, incorporation of α,β-dehydro residues has given rise to a possible method for generating various schemes of secondary structures. The steric constraints introduced by incorporating an α,β-dehydro residue in a peptide sequence force the backbone to fold into definite conformations.[3] Experimental and theoretical conformational studies have indicated that ΔPhe strongly favors the formation of β-turn structures in short peptides.[3] In longer peptides containing more than one ΔPhe residue, 3_{10}-helical structures are mostly observed.[3h,j] In this regard, conformational characteristics of ΔPhe residues are similar to those of α-aminoisobutyric acid (Aib), a well-known helicogenic non-protein amino acid residue.[1c] Recently, we have observed a novel β-bend ribbon structure in a pentapeptide containing two ΔPhe residues[3k] which may represent the versatility of dehydro residues in constraining the peptide backbone. But at the same time, it is also clear that the conformational consequences of the number and positioning of ΔPhe residues in peptide sequence is not yet well understood. As a part of our continuing research program on building polypeptide structural motifs for the *de novo* design of proteins using α,β-dehydro amino acid residues, we report here the synthesis and X-ray crystal structure of the dehydro octapeptide Ac-ΔPhe[1]-Val[2]-ΔPhe[3]-Phe[4]-Ala[5]-Val[6]-ΔPhe[7]-Gly[8]-OCH_3, which exhibits a helix-terminating conformation. Solution conformation studies provide the evidence that the solid state structure of the octapeptide is mostly maintained in solution as well.

Experimental Procedures

Synthesis. Peptide II was synthesised by standard solution phase methods. Amino acid couplings were performed by either mixed anhydride or dicyclohexylcarbodiimide–hydroxybenzotriazole procedures as described earlier.[4] Trifluoroacetic acid (TFA) was used to remove the N-terminal Boc group in peptide fragments. The ΔPhe residue was introduced using the literature procedure.[4] All reactions were monitored by TLC on precoated silica plates in at least two different solvent systems.[4] The peptide intermediates, a tetrapeptide, Boc-Val-ΔPhe-Phe-Ala-OH, and a tripeptide, Boc-Val-ΔPhe-Gly-OCH_3, were prepared from Boc-Val-ΔPhe-azlactone.[3f] The tripeptide

[†] Indian Institute of Science.
[‡] ICGEB.
[§] Abbreviations: ΔPhe, α,β-dehydrophenylalanine; peptide I, Boc-Val[1]-ΔPhe[2]-Phe[3]-Ala[4]-Phe[5]-ΔPhe[6]-Val[7]-ΔPhe[8]-Gly[9]-OCH_3; peptide II, Ac-ΔPhe[1]-Val[2]-ΔPhe[3]-Phe[4]-Ala[5]-Val[6]-ΔPhe[7]-Gly[8]-OCH_3; ROESY, rotating frame Overhauser spectroscopy.
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was deprotected at its N-terminal and coupled with the above tetrapeptide to yield a heptapeptide, Boc-Val-ΔPhe-Phe-Ala-Val-ΔPhe-Gly-OCH_3, which on deprotection and coupling with acetyldehydrophenylalanine gave the target octapeptide as a white solid. Recrystallization from ethyl acetate−petroleum ether afforded the octapeptide in pure form. The octapeptide eluted as a single peak in an HPLC run on a Waters C_{18} column (3.9 × 300 mm). UV detection, 280 nm, using a methanol−water gradient (70% methanol to 90% methanol in 35 min) at a flow rate of 1 mL/min. Yield 62%. Mp = 254 °C. $[\alpha]_D^{27} = -58°$ (c, 1.05 g/dL, CH_3OH). ^1H NMR (500 MHz, $CDCl_3$ with 1% of $(CD_3)_2SO$): δ 9.56 (1H, s, NH ΔPhe[1]), 9.25 (1H, s, ΔPhe[3]), 8.39 (1H, s, NH ΔPhe[7]), 7.93 (1H, br, NH Val[2]), 7.66 (1H, br, NH Phe[4]), 7.63 (1H, br, NH Ala[5]), 7.60 (1H, br, NH Gly[8]), 7.30−6.90 (20H, m, aromatic protons of ΔPhe[1], ΔPhe[3], ΔPhe[7], and Phe[4]), 7.00 (1H, br, NH Val[6]), 4.10 (1H, br, $C^\alpha H$ Phe[4]), 4.00 (2H, br, $C^\alpha H$ Val[6] and $C^\alpha H$ Ala[5]), 3.80 (1H, br, $C^\alpha H$ Val[2]), 3.70 (2H, br, $C^\alpha H$ Gly[8]), 2.80 (2H, m, $C^\beta H$ Phe[4]), 2.10 (1H, br, $C^\beta H$ Val[6]), 2.05 (3H, s, CH_3CO-), 2.00 (1H, br, $C^\beta H$ Val[2]), 1.21 (3H, d, $C^\beta H$ Ala[5]), 0.80 (6H, dd, $C^\gamma H_3$ Val[2]), 0.60 (6H, dd, $C^\gamma H_3$ Val[6]).

Spectroscopic Studies. ^1H NMR spectra were recorded on a Bruker 500 MHz FT NMR equiped with Aspect 3000 computer at Tata Institute of Fundamental Research, Bombay. All chemical shifts are expressed as δ (ppm) downfield from internal reference tetramethylsilane. Spectra were recorded at concentration of 10 mg/mL. Two-dimensional COSY and ROESY spectra were recorded using standard procedures.[5] Short mixing times (300−400 ms) were used in the ROESY experiments in order to minimize spin−diffusion effects.

X-ray Diffraction. The peptide ($C_{54}H_{62}N_8O_{10}\cdot 2H_2O\cdot C_3H_6O$, MW = 983.09) was crystallized by slow evaporation of the peptide solution in a 1:1 mixture of methanol−acetone at 4 °C over a period of 45 days. A crystal of size 0.2 × 0.15 × 0.6 mm mounted on a glass fiber was used for characterization and X-ray diffraction experiments on a CAD4 diffractometer. The cell parameters were determined by setting the angles of 25 accurately measured reflections. The crystal belongs to the orthorhombic space group $P2_12_12_1$ with a = 9.574(2) Å, b = 26.010(3) Å, c = 23.230(3) Å, V = 5784.364 Å^3, and Z = 4. Three-dimensional X-ray intensity data were collected using Cu Kα radiation (λ = 1.5418 Å) up to a Bragg angle θ_{max} = 70° with varying scan speeds in the $\omega-2\theta$ scan mode. The negligible variation in the intensity of two standard reflections monitored at regular intervals confirmed the electronic and crystal stability. Thus 5497 unique reflections were collected and corrected for Lorentz and polarization factors, and no absorption correction was made (μ = 5.74 cm^{-1}). Attempts to solve the structure by direct methods using SHELXS86[6] and MULTAN[7] were not successful. The structure was determined using molecular replacement methods by employing the program PATSEE.[8] Backbone atoms of four residues of a 3_{10}-helical search model, constituting a fractional power of 0.24, were successfully positioned in the unit cell of peptide II by a rotation−translation search. Partial structure expansion followed by a couple of weighted difference Fourier maps revealed the full structure. Least squares refinement was carried out using 4380 reflections having $|F_o| > 3\sigma(|F_o|)$ with anisotropic temperature factors for all the non-hydrogen atoms. It was observed that the C^γ atoms of the Val[2] residue are disordered between two of the three possible rotomeric positions, which leads to full occupancy for one C^γ position and half-occupancy for other two C^γ positions. Such disorder of Val side chains have been observed in crystals of Aib peptides.[9] The disordered C^γ atoms of the Val residue were refined isotropically. During the course of refinement two water molecules and one acetone molecule were located in the difference Fourier map. Solvent atoms having unusually high temperature factors were refined isotropically. All the hydrogen atoms fixed on the basis of stereochemical considerations were used only for structure factor calculations. The final agreement factors are R = 0.0998 and R_w = 0.0968.

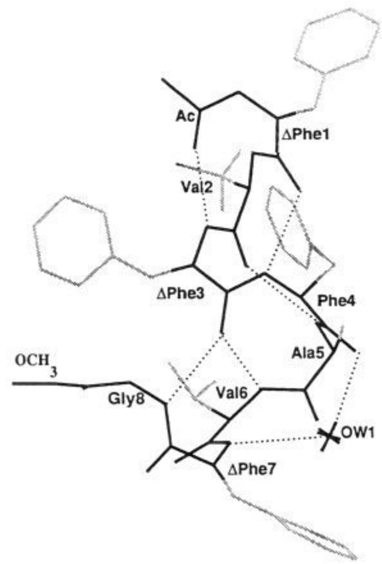

Figure 1. Molecular structure of peptide II. Intramolecular hydrogen bonds are indicated by dotted lines. The molecule consists of a N-terminal 3_{10}-helical pentapeptide segment, a π-turn in the middle region, and an incipient 3_{10}-helical segment at the C-terminus.

Table 1. Backbone Torsion Angles of Peptide II

residues	ϕ (deg)	ψ (deg)	ω (deg)
ΔPhe[1]	−48.0	−31.2	178.2
Val[2]	−56.1	−32.2	178.0
ΔPhe[3]	−58.7	−24.6	−179.8
Phe[4]	−77.4	−8.8	172.2
Ala[5]	−92.6	−14.0	−169.9
Val[6]	−123.8	18.7	179.2
ΔPhe[7]	64.8	32.8	168.9
Gly[8]	82.0		

Table 2. Hydrogen Bonds Observed in the Crystal and Molecular Structure of Peptide II

donor (D)	acceptor (A)	distance D- - -A (Å)	distance H- - -A (Å)	angle D−H- - -A	symmetry code
N3	O1	2.844	1.92	150.4	0
N4	O1′	2.968	2.02	155.7	0
N5	O2′	3.026	2.07	156.6	0
N6	O3′	3.274	2.32	156.2	0
N7	O1W	3.016	2.15	143.4	0
N8	O3′	2.947	2.00	155.0	0
O1W	O4′	2.704[a]			0
N1	O6′	2.837	1.84	169.2	1
N2	O7′	3.028	2.09	153.6	1
C1	O6′	3.329	2.36	148.2	1
C2G3	O7′	3.361	2.37	151.5	1
C8A	O5′	3.150	2.13	156.2	2
O2W	O1S	3.182[a]			0
C3S	O2W	2.923[a]			0
O2W	O1′	2.865[a]			0

[a] Hydrogen atoms of the donor were not located. Symmetry codes: (x, y, z): 1, ($-x + 2$, $y + 1/2$, $-z + 3/2$); 2, ($x + 1$, y, z).

Results

Crystal Structure. Figure 1 illustrates the conformation of the peptide II molecule in the solid state. The backbone dihedral angles and the hydrogen bonds observed in the solid state are listed in Tables 1 and 2. The solid state conformation of the peptide II molecule is best explained in terms of three peptide segments having definite conformational characteristics: (i) The

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backbone of the N-terminal pentapeptide segment Ac-ΔPhe[1]-Val[2]-ΔPhe[3]-Phe[4]-Ala[5]- is in right-handed 3_{10}-helical conformation, (ii) in the middle portion, Val[6] residue assumes non-helical backbone dihedral angles causing a significant change in the direction of propagation of the peptide backbone, and (iii) the C-terminal segment ΔPhe[7]-Gly[8]-OCH_3 assumes backbone torsion angles compatible with a left-handed 3_{10}-helix. Together, these three segments provide an example of a helix with a helix-terminating conformation and thus may serve as the basis for design of helix–turn–helix structural motifs.

From Table 1 and Figure 1 it can be seen that the N-terminus pentapeptide segment Ac-ΔPhe[1]-Val[2]-ΔPhe[3]-Phe[4]-Ala[5]- of peptide II adopts a 3_{10}-helical conformation. The backbone dihedral angles of ΔPhe[1], Val[2], and ΔPhe[3] are close to average values of 3_{10}-helical conformations as found in proteins and peptides.[10] However the dihedral angles of Phe[4] and Ala[5] depart from the average 3_{10}-helical conformation which is manifested as varying donor to acceptor distances in 4 → 1 hydrogen bonds (Table 2). Thus, the N-terminal pentapeptide segment is made up of three type III β-turns followed by one type I β-turn.[3a] Even though the type I β-turn is considered non-helical, minor adjustment of the dihedral angles would allow it to assume helical conformation as has been pointed out by others.[11] Also, based on the crystal packing of a simple peptide molecule, Boc-Val-Ser-$NHCH_3$, a model has been proposed recently for the interconversion of type I β-turn to helical structures and vice versa in proteins.[12] Though Phe[4] and Ala[5] residues of the N-terminal pentapeptide segment have backbone dihedral angles (−77.4°, −8.8° and −92.6°, −13.9°) representing a somewhat distorted type I β-turn, helical nature is maintained throughout. Thus, the N-terminus pentapeptide segment forms nearly two turns of a 3_{10}-helix, which corresponds to the search model positioned in the unit cell of peptide II using PATSEE as mentioned.

In peptide II the chain reversal takes place at the Val residue which assumes non-helical dihedral angles $\phi = -123.81°$ and $\psi = 18.76°$. This effects positioning the NH of the ΔPhe[7] residue away from the C=O of the Phe[4] residue, thereby breaking the 4 → 1 hydrogen bond pattern. This signals the end of the N-terminal helix. The helix termination and chain reversal are facilitated by a water molecule (OW1) which invades a helix hydrogen bond by donating a proton to O4′ and induces the chain reversal by accepting a proton from N7. This water-mediated 4 → 1 hydrogen bond probably leads to a stabilizing interaction between O3′ and N8 which forms a 6 → 1 hydrogen bond, characteristic of a π-turn. In addition, this water molecule introduces some perturbation in the helicity of Phe[4] and Ala[5] which is manifested in their dihedral angles (Table 1). It has been observed in proteins and peptides that the water-invaded helixes exhibit a bend in the helix axis.[13] However, in the present case, the penetration of the water molecule into the helix backbone terminates the helix and dramatically changes the backbone direction by facilitating a π-turn. The occurrence of π-turns in linear oligopeptides has been reported earlier,[14] but this structure provides the first example of a π-turn containing a water molecule. It is noteworthy that π-turns have been observed frequently at the C-terminal end of helixes in proteins.[11a,15]

The segment -ΔPhe[7]-Gly[8]-OMe at the C-terminus of peptide II has backbone dihedral angles (Table 1) compatible with a left-handed 3_{10}-helix. However, no 4 → 1 hydrogen bonds are observed, presumably because of the chain termination at Gly[8]. The carbonyl oxygens O7′ and O8′ are oriented in a direction appropriate to accept 4 → 1 hydrogen bonds, but the absence of hydrogen bond donating (i + 4) amino nitrogens prevent them from doing so. For Gly[8], though ψ is not defined uniquely, ϕ is nearly left-handed 3_{10}-helical. Thus, based on the backbone dihedral angles, the -ΔPhe[7]-Gly[8]-OCH_3 segment can be said to form an incipient helical conformation. The above three segments in peptide II put together may be seen to represent an incipient helix followed by a terminating signal.

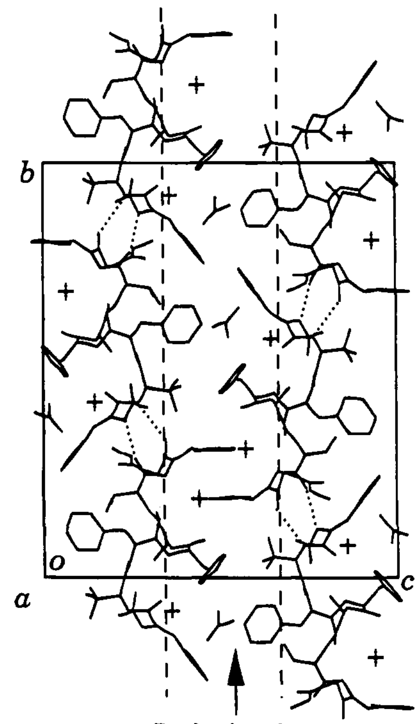

Figure 2. Crystal packing diagram for peptide II. View down the crystallographic a axis. The intermolecular N—H- - -O hydrogen bonds are shown by dotted lines. The predominantly aromatic slab can be seen at z = 0.5.

Crystal Packing. Figure 2 shows the packing of peptide II molecules in the solid state. The carbonyl oxygens which do not participate in helix hydrogen bonding take part in intermolecular hydrogen (Table 2) bond formation except O4′, which accepts a proton from the water molecule OW1. O5′ forms a O- - -H—C type of strong hydrogen bond with C8A of another molecule related by a unit cell translation along the x direction. There are many examples of C—H- - -O interactions seen in biomolecules.[16] O6′ and O7′ accept hydrogen bonds from amide nitrogens N1 and N2 of the molecule related by symmetry ($-x + 2$, $y + 1/2$, $-z + 3/2$). This results in a zigzag pattern of helixes in contrast to the continuous helical rods formed by head to tail hydrogen bonds as seen in peptide I[3e] and in many Aib

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Table 3. NMR Parameters for NH Protons in Peptide II

residues	$CDCl_3$ (ppm)	$(CD_3)_2SO$ (ppm)	Δ (ppm)	$d\delta/dT$ (10^{-3}ppm/K)	$J_{NHC\alpha H}$ ($CDCl_3$) (Hz)
ΔPhe^1	9.56	9.73	0.17	3.70	
Val^2	7.93	8.13	0.20	3.27	3.55
ΔPhe^3	9.25	9.65	0.40	2.30	
Phe^4	7.66	8.03	0.57	2.18	3.55
Ala^5	7.63	7.95	0.32	8.00	5.30
Val^6	7.00	8.35	1.35	4.30	7.10
ΔPhe^7	8.39	9.60	1.21	5.00	
Gly^8	7.60	8.25	0.65	2.50	

peptides.[13c] This probably shows the tendency of the peptide II molecule to be in a helical terminating conformation. The zigzag pattern of helixes seen in the present structure provides evidence for the versatility of crystal packing in accommodating molecules of irregular shapes such as peptide II in the crystal lattice. A predominantly aromatic slab,[17] consisting of Val^2, ΔPhe^3, Phe^4, and ΔPhe^7 residues, can be observed parallel to xy plane at $z = 0.5$. One acetone molecule and another water molecule OW2 are located in this aromatic slab. The acetone molecule forms one hydrogen bond with OW2, which in turn forms a hydrogen bond with O1′; O8′ does not participate in any hydrogen bonding.

Solution Conformation. ^1H NMR spectra in $CDCl_3$ were not well resolved. However, in $CDCl_3$ containing 1% DMSO-d_6, much better spectra with well-resolved peaks were obtained, and therefore, all the NMR experiments were carried out in this solvent system. Assignments were made by standard two-dimensional NMR techniques.[5] The position of NH resonances, the temperature coefficient [$d\delta/dT$ in $(CD_3)_2SO$ measured over a range of 300−340 K], and vicinal coupling constant $J_{C^\alpha HNH}$ values are summarized in Table 3.

It is observed that NH ΔPhe^3, NH Phe^4, and NH Gly^8 have low $d\delta/dT$ ($<3 \times 10^{-3}$ ppm K^{-1}) and are unperturbed with change in solvent composition, indicating that these NH's are not easily accessible to the solvent and may be involved in intramolecular hydrogen bonding.[18] Involvement of NH ΔPhe^3 and NH Phe^4 in intramolecular hydrogen bonding may be indicative of the presence of two $4 \rightarrow 1$ β-turns[3f] at the N-terminus tetrapeptide fragment Ac-ΔPhe^1-Val^2-ΔPhe^3-Phe^4. But high temperature coefficient values for Ala^5, Val^6, and ΔPhe^7 NHs suggest that $4 \rightarrow 1$ intramolecular hydrogen bonding pattern does not continue until the end of the peptide chain. On the other hand, NH Gly^8 appears to be involved in intramolecular hydrogen bonding,[19] for which there are two possibilities: the amide hydrogen of Gly^8 may be involved in an intramolecular hydrogen bond with the carbonyl of Ala^5 ($4 \rightarrow 1$) or with the carbonyl of Phe^4 ($5 \rightarrow 1$).

Spatial proximity of various spin systems in the octapeptide was probed by means of two-dimensional ROESY.[20] Figure 3 shows ROESY spectra for peptide II. Along with intra-residue NOEs, significant inter-residue NOEs are also observed. Continuous $d_{NN}(i,i+1)$ NOE cross peaks are observed in the peptide sequence except between NH Phe^4 and NH Ala^5 (Figure 3a). Presence of such continuous $d_{NN}(i,i+1)$ NOE cross peaks from NH ΔPhe^1 to NH Phe^4 are characteristic of ϕ and ψ values in the helical region[5] for N-terminal fragment Ac-ΔPhe^1-Val^2-ΔPhe^3-Phe^4-. This helical backbone is seen to be disrupted on going toward the C-terminal. In the ROESY spectra (Figure 3b), $d_{\alpha N}(i,i+1)$ NOEs between $C^\alpha H$ $Phe^4 \leftrightarrow$ NH Ala^5 and $C^\alpha H$ $Val^6 \leftrightarrow$ NH ΔPhe^7 are also observed. Such inter-residue NOEs are considered diagnostic of ψ_i value of $\sim 120 \pm 30°$, which result in a $C_i^\alpha H \leftrightarrow N_{i+1}H$ distance of ≤ 2.5 Å.[21] Presence of these NOEs, together with the fact that $d_{NN}(i,i+1)$ NOEs were not observed for NH Phe^4 and NH Ala^5, suggests that the helical backbone is disrupted in the middle, on going toward the C-terminal. At the same time, however, the involvement of NH Gly^8 in an intramolecular hydrogen bond as revealed by solvent titration experiments and the presence of both $d_{\alpha N}(i,i+1)$ and $d_{NN}(i,i+1)$ connectivities suggest a rigid backbone conformation[22] at the C-terminal segment, -Phe^4-Ala^5-Val^6-ΔPhe^7-Gly^8-.

Furthermore, medium range cross peaks between $C^\alpha H$ $Val^2 \leftrightarrow$ NH Phe^4 and $C^\alpha H$ $Phe^4 \leftrightarrow$ NH Val^6 [$d_{\alpha N}(i,i+2)$ type] are also observed in the ROESY spectra (Figure 3b). In addition, NOE cross peaks between $C^\alpha H$ $Val^2 \leftrightarrow$ NH Ala^5 and $C^\alpha H$ $Phe^4 \leftrightarrow$ NH ΔPhe^7 [$d_{\alpha N}(i,i+3)$ type] are observed (Figure 3b). Such $d_{\alpha N}(i,i+2)$ and $d_{\alpha N}(i,i+3)$ NOE cross peaks are diagnostic NOEs of 3_{10}-helical conformations wherein consecutive type III β-turns are present.[5,23] Since no such medium range cross peaks are observed between $C^\alpha H$ $Val^6 \leftrightarrow$ NH Gly^8 [($d_{\alpha N}(i,i+2)$] and $C^\alpha H$ $Ala^5 \leftrightarrow$ NH Gly^8 [$d_{\alpha N}(i,i+3)$], it is clear that type III β-turns are not continued until the end of the sequence. At the same time the presence of inter-residue $d_{\alpha N}(i,i+1)$ and $d_{NN}(i,i+1)$ connectivities in the C-terminal segment -Ala^5-Val^6-ΔPhe^7-Gly^8 suggest a turn conformation[24] for the C-terminal segment of peptide II. However, information regarding the type of turn may not be unambiguously inferred from NMR data.

The above conclusion regarding the backbone conformation of peptide II is further supported by the vicinal coupling constant $J_{C^\alpha HNH}$ values. $J_{C^\alpha HNH}$ values for NH Ala^5 (5.3 Hz) and NH Val^6 (7.1 Hz) are comparatively higher than for NH Phe^4 (3.53 Hz) and NH Val^2 (3.55 Hz). Low vicinal coupling constant values for NH Val^2 and NH Phe^4 suggest the torsion angle ϕ for these residues to be $\sim -60°$, indicating the presence of two type III β-turns (incipient 3_{10}-helix) at the N-terminal segment Ac-ΔPhe^1-Val^2-ΔPhe^3-Phe^4- of II,[25] whereas coupling constant values for NH Ala^5 and NH Val^6 suggest the torsion angle ϕ to be in the range $\sim -80°$ to $-100°$, deviating from torsion angle values for 3_{10}-helical backbone conformations or type III β-turns.[25] However, these ϕ values do not correspond to a completely extended backbone conformation ($\phi = 180°$) for the C-terminal fragment.[25,26] In conclusion, the temperature and solvent dependence studies along with NOE and coupling

Table 4. Rms Deviations (Å) Observed in a Least-Squares Superposition of the 19 Backbone Atoms within the π-Turn of Peptide II and Some Representative Proteins[a]

	peptide II (ΔF3−C8)	1ROP (L26−A31)	256B (L78−K83)	2MHR (M62−Y67)	1R69 (A21−T26)	2CRO (A21−V26)	2WRP (A29−L34)	1SBP (L145−D151)
peptide II	0.00	0.43	0.56	0.44	0.49	0.48	0.39	0.16
1ROP		0.00	0.32	0.13	0.31	0.26	0.23	0.50
256B			0.00	0.38	0.48	0.44	0.35	0.61
2MHR				0.00	0.33	0.27	0.23	0.51
1R69					0.00	0.19	0.26	0.59
2CRO						0.00	0.24	0.56
2WRP							0.00	0.45
1SBP								0.00

[a] Amino acids are indicated by single letter codes and proteins are indicated by their PDB (4) codes.

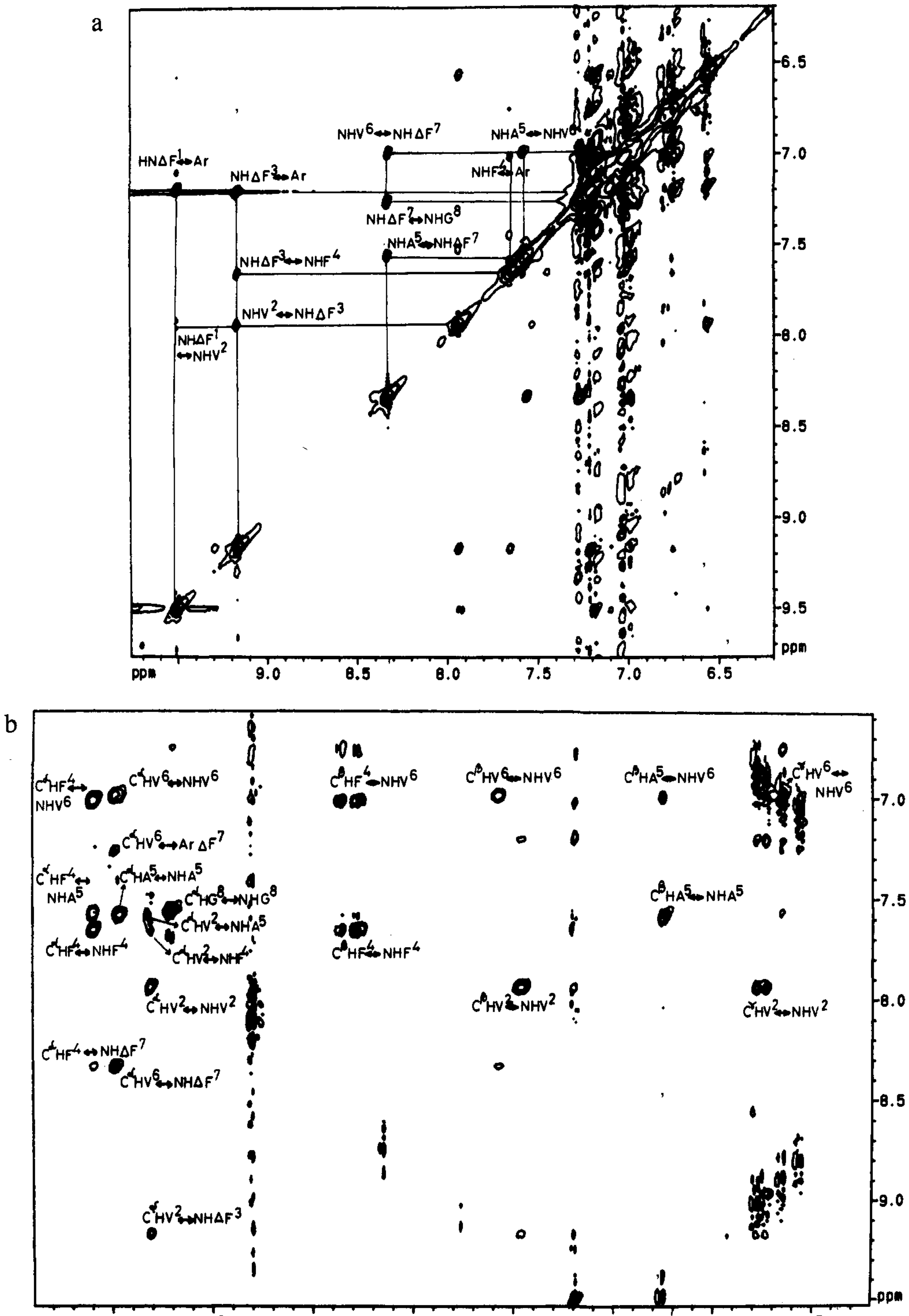

Figure 3. 500 MHz ROESY spectrum of peptide II in $CDCl_3$ [with few drops of $(CD_3)_2SO$] at room temperature. A spin-locking mixing period of 400 ms was used.

constant data suggest that backbone conformation of peptide II may be defined by multiple turns. Presence of two consecutive type III β-turns at the N-terminal segment Ac-ΔPhe^1-Val^2-ΔPhe^3-Phe^4- indicates an incipient 3_{10}-helical conformation, which may be followed by helix-terminating turn conformation at the C-terminal segment -Ala^5-Val^6-ΔPhe^7-Gly^8-OMe of peptide II.

Discussion. The crystal structure of the peptide II reveals that in the solid state the peptide molecule consists of a 3_{10}-helix, which is interrupted by a π-turn stabilized by a water molecule, resulting in an apparent chain reversal of the backbone. In a relatively nonpolar solvent condition, the overall structural features of peptide II are also maintained in solution. It may be emphasized here that solution studies were carried

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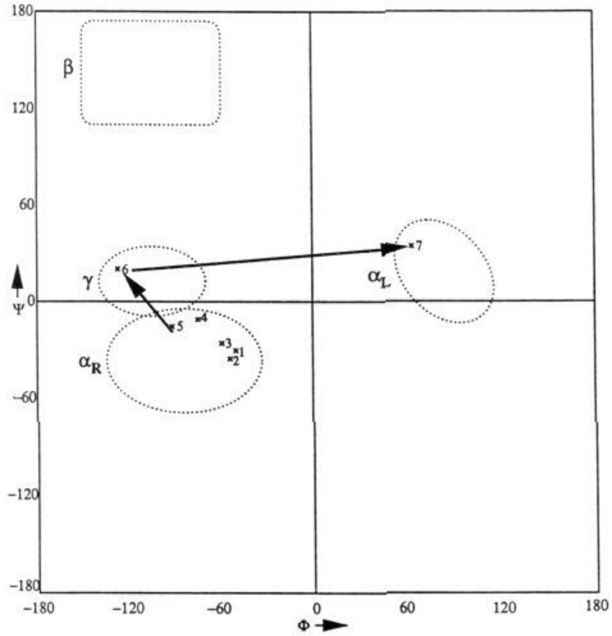

Figure 4. Ramachandran map depicting the backbone conformations of the residues of peptide II. Only the regions relevant for the discussion are shown following Effimov's nomenclature (see ref 31). The drift of the backbone torsion angles of the residues 4 and 5 toward the γ region can be noticed.

out only to probe whether the peptide backbone structural features seen in solid state are maintained in solution.

The terminating conformation observed for the peptide II matches well with the results obtained by Effimov from an analysis on the conformation of $\alpha-\alpha$ hairpins in proteins.[27] Even though peptide II has 3_{10}-helixes whereas $\alpha-\alpha$ hairpins consist of α-helixes, the comparison would be meaningful as α and 3_{10}-helixes span a contiguous region in conformational space (Figure 4) and can be converted into each other with a small change in torsion angles.[12,13c] Following Effimov's shorthand nomenclature[28] for describing the conformation of residues in a polypeptide chain, the conformation of peptide II can be represented as $\alpha_5\gamma\alpha_{L2}$, describing five residues in α_R region and one residue in γ-region followed by two residues in α_L region (Figure 4). This conformation is similar to "perpendicular exit" [27] from an α-helix, as seen in $\alpha-\alpha$ hairpins characterized by a $\alpha_n\gamma\alpha_L\beta$-polypeptide backbone conformation (n = no. of residues in the α-region). In peptide II, the $\alpha_5\gamma\alpha_L$ conformation is followed by another residue in the near α_L conformation instead of a residue having β-conformation. The absence of a residue in the β-conformation makes the loop short. On the whole there is a good agreement between the backbone conformational features in the turn region observed in peptide II and those found in $\alpha-\alpha$ hairpins in proteins.

As mentioned earlier, the π-turn is one of the modes by which the helix termination and chain reversal takes place in proteins.[11a,15] Hence it is approrpiate to compare the π-turn observed in peptide II with those found in proteins. We have considered seven examples from the Protein Data Bank (PDB)[29] for comparison purposes. It may be mentioned that an exhaustive survey of PDB to detect similar π-turns in other proteins was not carried out by us. The way in which the bend is derived from a straight 3_{10}-helix in the peptide II is very much similar to the development of a bend from an α-helix in the rop protein,[30] cytochrome b_{562},[31] myohemerythrin,[32] phage 434 repressor protein,[33] phage 434 cro protein,[34] trp repressor protein,[35] and sulphate binding protein.[36] By naming the residues within the π-turn, in sequences 1−6, the observed hydrogen bonding scheme in these proteins can be represented as $6 \rightarrow 1$, $4 \rightarrow 1$, and $5 \rightarrow 2$ (except ISBP, see below) characteristic of the so-called "paper clip" conformation.[14a] The conformational features observed in these protein π-turns are similar to those in peptide II. A least-squares superposition of 19 atoms within the ring formed by the π-turn in peptide II and the above-mentioned proteins was performed using a Fortran program SUPER.FOR developed in our laboratory.[37] The resulting maximum rms deviation was 0.56 Å with 256B and the minimum rms deviation was 0.16 Å with 1SBP, indicating the similarity of the π-turn observed in peptide II and those found in proteins. The π-turn in protein 1SBP needs special mention as it displays conformational and hydrogen bonding features exactly the same as in peptide II; even the $5 \rightarrow 2$ hydrogen bond is water mediated, and the water mediating this hydrogen bond occupies almost the same relative position (the resulting rms deviation for the superposition including the water is 0.18 Å). Figure 5 shows peptide II with a π-turn in 1SBP superposed. The π-turn observed in peptide II and in 1SBP shows how water molecules can be inserted in the turn regions without significant change in the backbone conformation of the residues forming the turn. In peptide II, among the 19 atoms superposed, O2′, which is hydrogen bonded to a water molecule (OW1) exhibits comparatively large deviation. These results suggest that the conformation observed in peptide II is not an artifact of crystal packing forces but may be representative of

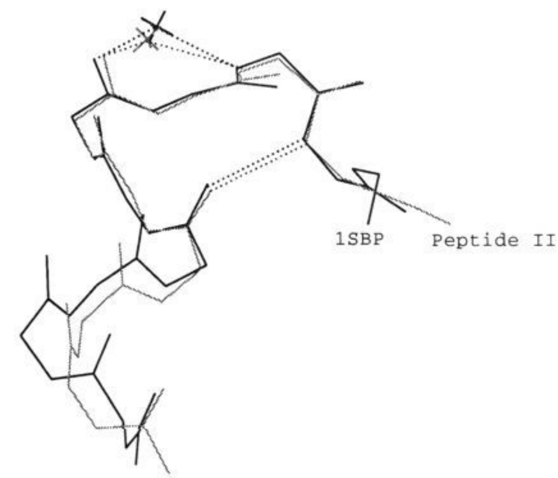

Figure 5. Diagram illustrating the least-squares superposition of the π-turn region of peptide II with the corresponding region in protein 1SBP. Dark lines represent 1SBP, and light lines indicate peptide II. The water molecule mediating the $5 \rightarrow 2$ hydrogen bond is also shown.

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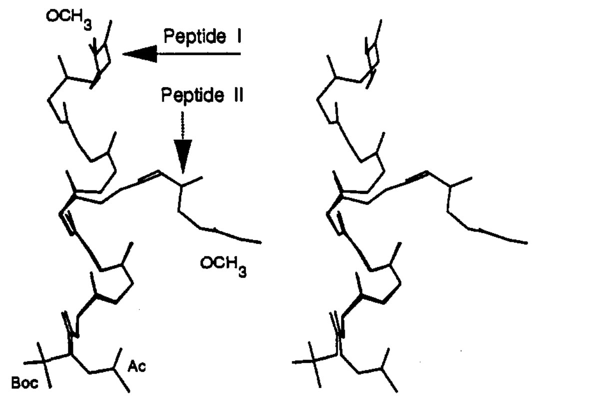
a fundamental mode of helix termination and chain reversal in proteins also.

In proteins, the π-turns terminating the helixes contain Gly or to a lesser extent Asn, a polar residue, in the fifth position (i.e., α_L conformation),[15b,27,38] and the reason suggested for this is the steric hindrance.[27] Interestingly, in peptide II, the corresponding position in the π-turn is occupied by an apolar and achiral ΔPhe[7] residue, thereby exhibiting a way to accommodate bulky residues in fifth position in the π-turn.

Of the two water molecules observed in the unit cell, only one (OW1) forms an integral part of the peptide II molecule while the other (OW2) is essentially used to fill the voids in crystal packing. The folding–unfolding, structure–function, and stability of polypeptide chain is highly influenced by the presence of water molecules.[39] Molecular dynamics simulation results also provide sufficient evidence for the suggestion that the water insertion into the helix hydrogen bond is a frequent feature of helix unfolding.[40] These results and the hydrogen bonding pattern, observed for the internal water molecule OW1 in peptide II, are suggestive of a critical role to OW1 in providing the peptide II molecule helix termination and turn formation. As observed in proteins,[39b] the water molecule OW1 in peptide II, anchors between the carbonyl oxygen O4′ and the amide nitrogen N7 by prying open the 4 → 1 helix hydrogen bond and probably encourages the formation of a π-turn. The present peptide provides the first example of a π-turn invaded by a water molecule, in linear oligopeptides, and suggests a role of the water molecule in turn formation.

Comparison with the Nonapeptide Boc-Val¹-ΔPhe²-Phe³-Ala⁴-Phe⁵-ΔPhe⁶-Val⁷-ΔPhe⁸-Gly⁹-OMe. As mentioned before, sequence comparison of peptide II with peptide I[3e] reveals the presence of the Val-ΔPhe-Phe-Ala- tetrad sequence in both the peptides, while comparison of their three-dimensional structures shows the conservation of the conformation in the regions of homologous sequence (in fact, this was the search model used with good reason for molecular replacement methods). Figure 6 shows the main chain atoms of peptide I and peptide II with the conserved tetrad sequence superposed. This reveals the similarity of three-dimensional structures for the conserved sequence, thereby proving the reproducibility of the *de novo* peptide design using dehydro residues. A least-squares superposition performed using the program SUPER. FOR[37] for the 16 backbone atoms of the conserved sequence resulted in an rms deviation of 0.297 Å which confirms the structure conservation. In peptide I, the conserved tetrad sequence is followed by a bulky residue Phe, the side chain of which probably forces the backbone to bend away from it, due to steric reasons, thereby maintaining the 3_{10}-helical nature, whereas in peptide II, the same conserved tetrad is followed by a less bulky Val residue which presumably allows the backbone to adopt a conformation that is not accessible for peptide I. These



Peptide I: Boc-Val^1-ΔPhe^2-Phe^3-Ala^4-Phe^5-ΔPhe^6-Val^7-ΔPhe^8-Gly^9-OCH_3

Peptide II: Ac-ΔPhe^1-Val^2-ΔPhe^3-Phe^4-Ala^5-Val^6-ΔPhe^7-Gly^8-OCH_3

Figure 6. Stereo diagram showing the backbone superposition of the conserved sequence in peptide I and peptide II. The rms deviation for the 16 backbone atoms of the conserved sequence superposed is 0.297 Å. Departure from helicity at the middle portion of peptide II can be seen clearly.

results demonstrate the possibility of introducing sequence-dependent conformational variations in dehydro oligopeptides, which may be used as effective tools in *de novo* design of protein structural features, in particular helix termination and chain reversal.

Conclusion. In the present report, we show the utility of the ΔPhe residue in designing a helix termination and chain reversal motif by X-ray structure determination of peptide II. We found that the backbone conformation observed in the solid state for peptide II is largely maintained in relatively nonpolar solvent conditions. A comparison of structural features of peptide II and peptide I highlights the structural variability that may be obtained by a suitable placement of ΔPhe residues in peptide sequences. The comparison of the octapeptide with α–α hairpins observed in proteins shows that in peptide II there is a definitive chain reversal after the first helix and a helical tendency after the chain reversal. To our knowledge, the present crystal structure is the first example of a π-turn stabilized by a water molecule in linear oligopeptides and provides possibility for the design of helix-terminating signals utilizing ΔPhe residues. Even though ΔPhe is a noncoded amino acid, the type of π-turn found in the octapeptide is similar to those seen in some proteins, indicating the compatibility of conformation that may be obtained by using ΔPhe residues. However more studies will have to be undertaken to fully explore the potential of dehydro residues in the *de novo* design of protein structural elements.

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