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Design of Helical Peptides:

Solution Conformation of Boc-Gly-Δ^ZPhe-Leu-Δ^ZPhe-Ala-NHMe

And Boc-Val-Δ^ZPhe-Phe-Ala-Leu-Ala-Δ^ZPhe-Leu-OMe

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ABSTRACT

Two model peptides have been synthesized, a pentapeptide 1 (Boc-Gly- Δ^ZPhe-Leu-Δ^ZPhe-Ala-NHMe) and an octapeptide 2 (Boc-Val-Δ^ZPhe-Phe-Ala-Leu-Ala-Δ^ZPhe-Leu-OMe). The conformations have been investigated in chloroform and dimethylsulfoxide by one and two dimensional NMR techniques. Assignments of amide protons' resonances have been made and intramolecularly hydrogen bonded NH resonances have been identified using solvent and temperature dependence of NH chemical shifts. The results in $CDCl_3$ have implicated that in the pentapeptide out of five NH groups first two NH groups belonging to Gly (1) and Δ^ZPhe (2) residues are solvent exposed while rest are solvent shielded suggesting a 4 --->1 intramolecular hydrogen bonding pattern. However, in the octapeptide first three NH groups corresponding to Val(1), Δ^ZPhe(2) and Phe(3) are solvent exposed while rest are shielded suggesting a 5 --> 1 hydrogen bonding pattern. Presence of consecutive N_i H <--> N_{i+1}H nuclear Overhauser effects (NOEs), diagnostic of helical conformation has been confirmed by difference NOE in $CDCl_3$ and NOESY techniques.

Consistent with the data, a 3_{10} helical conformation for the pentapeptide and an α-helical for the octapeptide in $CDCl_3$ have been proposed. In $(CD_3)_2SO$, although the major conformation in solution is a 3_{10} helix for the pentapeptide and an α-helix for the octapeptide, a few evidences like presence of some isolated C_iH <---> N_{i+1}H NOEs are obtained that point out towards some conformational heterogenity. These results suggest that in highly polar solvent the folded conformations are not very stable.

INTRODUCTION

Secondary structure is a dominant factor affecting the binding characteristics of most biologically active peptides. Many naturally occuring peptides have been implicated to adopt turn containing structures, essential for their bioactivity, although an unambiguous evidence from direct studies of such peptides is still lacking[1]. However, some reports have appeared recently, indicating that even small linear peptides may exist in solution in stable secondary structures[2]. Conformationally constrained peptides are of great merit in understanding structure function relationship and developing potent, and selective, analogues of bioactive peptides. Several backbone modifications and inclusion of unusual amino acids in peptides have been carried out to achieve this objective[3]. The use of α,β-dehydroamino acids in synthesising peptide analogues with preferred secondary structures is a promising new method. Model acyclic peptides containing dehydrophenylalanine (Δ^ZPhe) and dehydroleucine (Δ^ZLeu) have shown a strong tendency to adopt β-turn structures, in apolar solvents[4-8]. X-ray differaction studies have firmly confirmed this observation[9-13]. We have recently shown that a linear hexapeptide peptide containing two dehydrophenylalanines, in ΔPhe^Z-X-X-ΔPhe^Z arrangment, adopts a 3_{10} helical conformation, at least in apolar solvents[4].

In our continuing effort to elucidate general rules for designing secondary structural motifs, in particular helices, using α,β-dehydro amino acids, we have chosen to examine the conformational preferences of model peptides containing -Δ^ZPhe-X-Δ^ZPhe- and -Δ^ZPhe-X-X-X-Δ^ZPhe systems. In this report we describe the synthesis and ^{1}H NMR studies on a pentapeptide, Boc-Gly-Δ^ZPhe-Leu-Δ^ZPhe-Ala NHMe, and an octapeptide Boc-Val-Δ^ZPhe-Phe-Ala-Leu-Ala-Δ^ZPhe-Leu-OMe, in $CDCl_3$ and $(CD_3)_2SO$, using NH accessibility and nuclear Overhauser effects to probe the molecular conformations. The choice of X residues was influenced largely by NMR considerations and by their propensities for occuring in helical conformations.

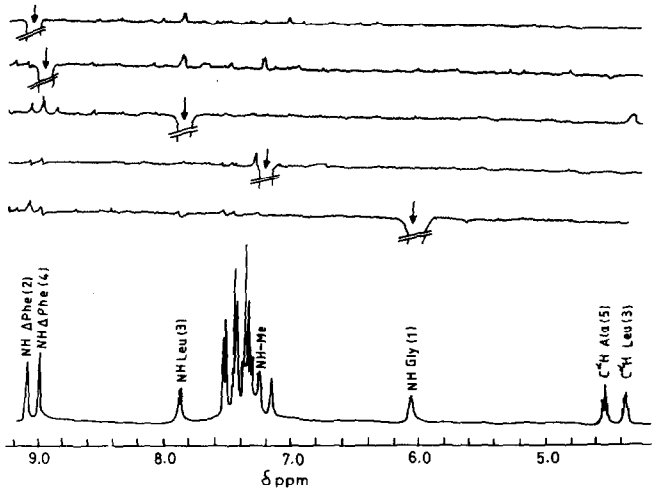


Fig. 1 500 MHz ^1H NMR spectrum of pentapeptide 1 in $CDCl_3$ with traces of $(CD_3)_2SO$. Difference NOE spectra obtained on irradiation of various NH groups are shown.

RESULTS AND DISCUSSION

Boc-Gly-Δ^ZPhe-Leu-Δ^ZPhe-Ala-NHMe 1.

Assignment of Resonances. Fig. 1 shows the 500 MHz ^1H NMR spectrum of the pentapeptide in $CDCl_3$ with traces of $(CD_3)_2SO$. Assignment of the resonances other than amide protons was straightforward and was done of the basis of chemical shifts and splitting pattern. Assignment of the five amide protons and their respective N_iH to $C^{\alpha}H$ connectivities were established on the basis of difference NOE spectra[14] (Fig. 1). The derived assignments are marked in Fig. 3 and listed in Table 1. Broad peak at ~6.0 δ was assigned to urethane [Gly (1)]NH by virtue of its high field position in $CDCl_3$[15]. Δ^ZPhe NH groups have usually been observed as low as ~9.0 δ[4]. Hence the peak at 8.92 δ was assigned to two Δ^ZPhe NH groups (integration of the peaks is for two protons). This peak however resolved into two peaks in $CDCl_3$ solution with traces of $(CD_3)_2SO$ (Fig 1). The

distorted quartet at ~7.3 δ could only be assigned to methyl amide NH group. Finally, NH of Leu was recognised at 7.8 δ since its irradiation resulted in observation of NOE on peak corresponding to $C^{\alpha}H$ of Leu. It was not possible to determine exact chemical shift of Ala (5) NH group due to its overlap with aromatic protons.

In $(CD_3)_2SO$, $C^{\alpha}H$ <-->N_iH connectivities were established using a two dimensional correlated spectrum[16] (Spectrum not shown). The chemical shifts assigned to various NH groups are summarized in Table 1. Due to the lack of corresponding $C^{\alpha}H$ protons two Δ^{Z}Phe NH resonances could not be assigned by the COSY spectrum. Hence assignments were made by monitoring their chemical shift positions in $CDCl_3$-$(CD_3)_2SO$ mixtures (Fig. 3a).

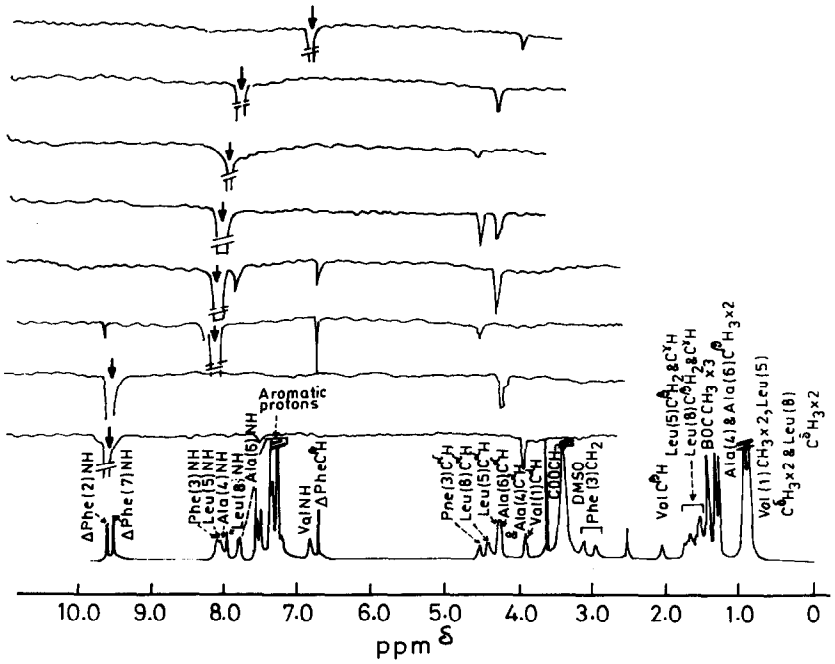


Fig. 2 500 MHz ^{1}H NMR spectrum of octapeptide 2 in $CDCl_3$. Difference NOE spectra obtained on irradiation of various NH groups are shown.

Table 1 NMR Parameters for peptides 1 and 2

NH Resonance (ppm/^{0}K)	δNH (ppm)		dδ /dT x 10^3
	$CDCl_3$	$(CD_3)_2SO$	
Pentapeptide			
Gly (1)	5.88	7.18	5.9
Δ^ZPhe (2)	9.0	9.72	5.8
Leu(3)	7.86	8.22	3.1
Δ^ZPhe (4)	8.92	9.58	3.08
Ala (5)	a	7.8	3.4
NHMe	7.3	7.64	2.6
Octapeptide			
Val (1)	4.99	6.8	5.7
Δ^ZPhe (2)	7.86	9.6	4.3
Phe (3)	7.46	8.11	5.0
Ala (4)	7.28	8.02	3.0
Leu (5)	7.31	8.08	3.0
Ala (6)	7.81	7.81	3.2
Δ^ZPhe (7)	8.50	9.50	3.1
Leu (8)	7.42	7.94	3.2

Delineation of Hydrogen-bonded NH Groups

The possible involvement of NH groups in intramolecular hydrogen bonding was probed using three criteria (i) temperature dependence of NH chemical shifts in $(CD_3)_2SO$[17], (ii) solvent dependence of NH chemical shifts in $CDCl_3$-$(CD_3)_2SO$ mixtures[18] and (iii) effect of adding paramagnetic radical, tetramethyl piperidine 1-oxyl (TEMPO) on line widths of NH resonances in $CDCl_3$[17].

The temperature dependence of chemical shifts in $(CD_3)_2SO$ is linear for all the NH groups. The temperature coefficients ($d\delta/dT$) are summarized in Table 1. Four backbone NH resonances belonging to Leu (3), Δ^ZPhe(4), Ala (5) and methylamide residues exhibit low $d\delta/dT$ values (<0.003 ppm/^{0}K), characteristic of solvent shielded (intramolecularly hydrogen-bonded) NH groups. Two resonances (Gly (1) NH and Δ^ZPhe(2)NH) have high $d\delta/dT$ valves (>0.005 ppm/^{0}K) indicative of their exposure to the solvent. Solvent dependence of NH chemical shifts in $CDCl_3$-$(CD_3)_2SO$ mixtures (Fig 3a) shows that an increase in the concentration of the strongly hydrogen bond accepting solvent, $(CD_3)_2SO$ results in significant downfield shift of Gly (1) and Δ^ZPhe(2)NH resonances while Leu(3), Δ^ZPhe(4) and methylamide NH groups appear to be insensitive to the change. Similar behavior is observed on addition of increasing concentration of the nitroxide radical (TEMPO) in $CDCl_3$ solutions (Fig. 3 b). The resonance line widths of Gly(1) and Δ^ZPhe(2)NH are broadened appreciably while the rest of the backbone amide proton resonances remain unperturbed.

The results summarized above establish that resonances Gly(1) and Δ^ZPhe(2) may be assigned to fully exposed NH groups. The behaviour of the remaining four backbone NH resonances is characteristic of solvent-shielded/intramolecularly hydrogen bonded NH groups. Involvement of last four NH groups in intramolecular hydrogen bonding hereby suggests a 4--->1 hydrogen bonding pattern characteristic of a 3_{10} helical conformation for the pentapeptide.

Nuclear Overhauser Effects.

Difference NOE spectra were recorded in $CDCl_3$ with traces of $(CD_3)_2SO$ so as to separate the two Δ^ZPhe NH resonances which overlap in pure $CDCl_3$ (Fig. 1). The NOEs observed on irradiation of various NH groups are summarized in Table 2. A positive NOE of 4.2% is observed on Δ^ZPhe(2)NH on irradiation of Gly(1) NH. Successive interresidue NOEs of the type N_iH <---->$N_{i+1}H$ were observed on irradiation of various NH groups. Only

Ala(5) NH could not be monitored in this experiment due to its merger with aromatic protons. Such NOEs are diagnostic of helical conformations with backbone torsional angles, ϕ_i~-50^0 and ψ_i~-50^0[19]. Based on observed NOEs and the other NMR data, a 3_{10} helical conformation can be proposed for the pentapeptide in $CDCl_3$.

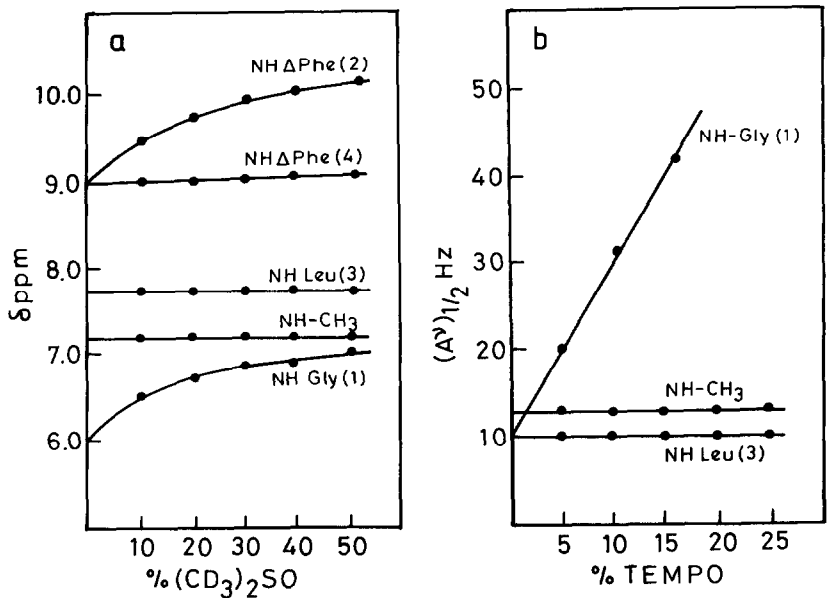


Fig. 3 (a) Solvent dependence of NH chemical shifts in pentapeptide 1 in $CDCl_3$-$(CD_3)_2SO$ mixtures.

(b) Dependence of NH resonance line widths in pentapeptide 1 on concentrations of TEMPO in $CDCl_3$.

In $(CD_3)_2SO$, the NOE studies were done by carrying out a two dimensional, magnitude mode NOESY experiment[20] (spectrum not shown). The NOEs observed are summarized in Table 2. NOESY cross peaks are observed between Leu(3)NH & Δ^ZPhe(4)NH and Δ^ZPhe(4)NH & Ala(5)NH. It was difficult to characterize other N_iH<-->$N_{i+1}H$ NOEs that might be present, since most of the NH resonances are crowded in the region of aromatic

protons. In addition appreciable interresidue NOEs of the type $C_iH<-->N_{i+1}H$ are also observed between Leu(3)C^{α} H<-->Δ^{Z}Phe(4) NH (3.8%) and Gly(1) C^{α} H<-->Δ^{Z}Phe(2)NH (4.2%). Observation of these NOEs suggests ψ values of ~120^0 at these residues[19]. This implicates that in highly polar solvent $(CD_3)_2SO$, along with the existence of a major population of helical structures, there exists a substantiative population of partially extended structures.

Table 2

NOEs observed in pentapeptide

	$CDCl_3$		$(CD_3)_2SO$	
Resonance Irradiated	Resonance Observed	% NOE	Pairs of Resonances	Volume of NOESY cross peaks
Gly(1)NH	$C^{\alpha}H2$ Gly	4.2		
ΔPhe(2)NH		3.3		
ΔPhe(2)NH	Leu(3)NH	3.8	Ala NH	
	$C^{\alpha}H$ Phe	5.2	Phe NH	1.8
Leu (3) NH	$C^{\alpha}H$ Leu	2.2	ΔPhe (4) NH	3.8
	ΔPhe(2)NH	2.1	$C^{\alpha}H$ Leu	
	ΔPhe(4)NH	3.5		
ΔPhe(4)NH	Leu(3)NH	4.8	$C^{\alpha}H_2$ Gly	4.2
	Ala(5)NH	5.1	ΔPhe(2) NH	
H-CH_3	Ala NH	5.1	Leu NH	4.5
	N-CH_3	4.1	$C^{\alpha}H$ Leu	

Boc-Val-Δ^{Z}Phe-Phe-Ala-Leu-Ala-Δ^{Z}Phe-Leu-OMe 2

Assignment of Resonances

Fig. 4 illustrates a two-dimensional correlated spectrum of the octapeptide in $CDCl_3$. Derived assignments are listed in Table 1. Six expected connectivities between NH and $C^{\alpha}H$ resonances [Val (1), Phe (3), Ala (4), Leu (5), Ala (6) and Leu (8)] are clearly identified. Val (1) NH (Urethane) is readily recognized at 4.99 δ by virtue of its high field position[15] and its coupling to $C^{\alpha}H$ which in turn is coupled to $C^{\beta}H$ coupled to $C^{\gamma}H_3$ of Val (Fig 4). Phe (3) spin system is characterized by the $C^{\beta}H_2$ (3.2 δ), which permits

identification of $C^{\alpha}H$ (4.42 δ) and NH (7.46 δ) resonances. By virtue of $C^{\beta}H_3$ (1.44 δ) <---> $C^{\alpha}H$ (4.16 δ) connectivity, one of the Ala NH is identified at 7.81 δ and the other at 7.28 δ by virtue of $C^{\beta}H_3$ (1.54 δ)<---> $C^{\alpha}H$ (4.35 δ) connectivity. However specific assignments to two Ala NH resonances could only be made by difference NOE studies. Spatial proximity is observed between Ala NH resonating at 7.28 δ and Phe $C^{\alpha}H$ resonating at 4.42 δ, and thus the peak at 7.28 δ was ascribed to Ala (4) NH and at 7.81 δ to Ala (6) NH. Similarly two Leu spin systems were recognized as $C^{\delta}H_3$ (0.85 δ)<--->$C^{\gamma}H$(1.6 δ)<---->$C^{\beta}H_2$ (1.75 δ)<--->$C^{\alpha}H$ (4.3 δ)<--->NH (7.31 δ) and $C^{\delta}H_3$ (0.9 δ)<--->$C^{\gamma}H$ (1.6 δ)<---->$C^{\beta}H_2$ (1.8 δ)<--->$C^{\alpha}H$ (4.78 δ)<--->NH (7.42 δ). Once again the distinction between two Leu residues was made by means of NOE studies. The Leu NH resonating at 7.31 δ is found in proximity of Ala (4)NH (7.28 δ) and Ala (6) NH (7.81 δ) and hence the peak at 7.31 δ is assigned to Leu (5) NH and the one at 7.42 δ to Leu (8) NH. Two Δ^{z}Phe NH resonances were readily recognized as two broad singlets most downfield (7.86 δ and 8.50 δ) of all other signals. However the signal at 7.86δ showed NOE to Val $C^{\alpha}H$ (3.9 δ) and was therefore assigned to Δ^{z}Phe(2) NH. Subsequently singlet at 8.50 δ was assigned to Δ^{z}Phe (7)NH.

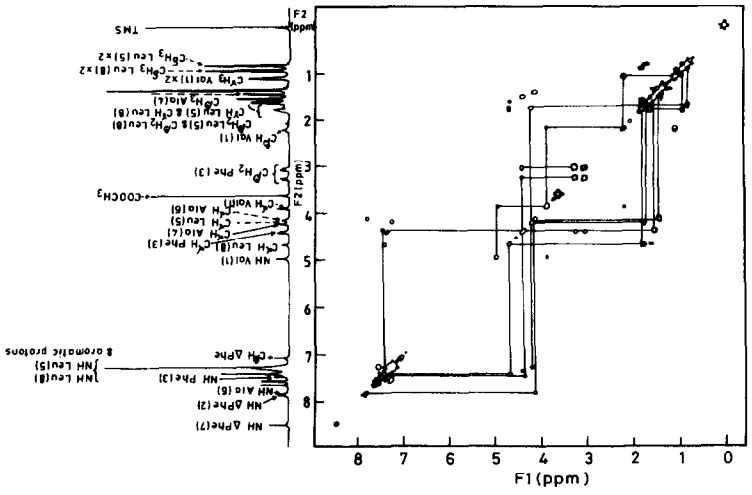


Fig. 4 500 MHz COSY spectrum of octapeptide 2 in $CDCl_3$.

Similarly, in $(CD_3)_2SO$ the assignments were made using a COSY spectrum (Spectrum not shown). Chemical shift position of only the backbone amide protons is altered in this solvent. Derived assignments are listed in Table 1.

Delineation of hydrogen bonded NH Groups

Solvent titrations in $CDCl_3$-$(CD_3)_2SO$ and temperature dependence in $(CD_3)_2SO$ experiments were conducted in order to explore the extent of exposure of various backbone NH groups to the solvent. Out of eight backbone NH groups, the first three NH groups Val (1), Δ^ZPhe(2) and Phe (3) show appreciable downfield shift with increasing concentration of $(CD_3)_2SO$ (up to 40% v/v). The effect on other NH groups is however not significant. Even the difference in chemical shifts in two solvents ($\Delta\delta$) is larger (~2.0 δ) for first three NH groups in contrast to other amide protons for which the $\Delta\delta$ values are of the order of 0 to 0.8. This implicates the solvent exposed nature of first three NH groups and solvent shielded nature of other remaining NH groups. Temperature coefficients ($d\delta/dT$) for all eight backbone amide protons are listed in Table 1. Temperature coefficient values (>.003 ppm/^{0}K) for Val (1), Δ^ZPhe(2) and Phe (3) NH groups are characteristic of fully exposed NH groups. NH resonances of Ala(6), Δ^ZPhe (7) and Leu (8) residues exhibit low $d\delta/dT$ values (<.003 ppm/^{0}K), characteristic of completely shielded NH groups while Ala (4) NH and Leu (5) NH exhibit moderate $d\delta/dT$ values (~0.0035 ppm/^{0}K), thereby implying their partial exposure to the solvent.

Above results indicate that in $CDCl_3$ first three NH groups of the octapeptide are exposed to the solvent while the rest remain inaccessible to the solvent, suggesting their involvement in intramolecular hydrogen bonding. In $(CD_3)_2SO$, however, at least two hydrogen bonds involving Ala(4) & Leu (5) NH groups are not completely stable, resulting in partial unfolding of helical structures.

Nuclear Overhauser Effects

Spatial proximity of various spin systems in the octapeptide was probed using a two dimensional NOESY experiment in $CDCl_3$[20]. Fig. 5 illustrates a magnitude mode NOESY spectrum of the octapeptide in $CDCl_3$. As expected consecutive N_iH<--->$N_{i+1}H$ NOEs are seen between the eight backbone NH groups, further confirming a helical conformation for the octapeptide[19]. In $(CD_3)_2SO$ however few $C^{\alpha}{}_iH$ <--->$N_{i+1}H$ NOEs

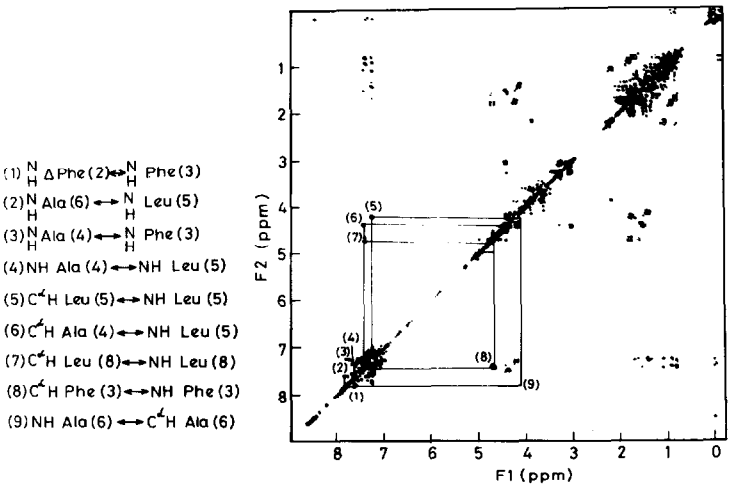
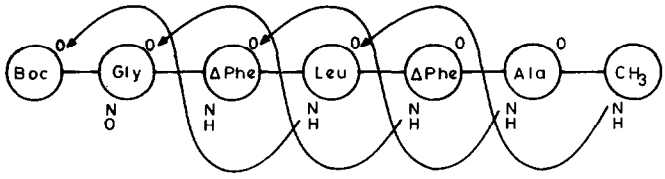


Fig. 5 500 MHz NOESY spectrum of octapeptide 2 in $CDCl_3$.

become significant between Leu (3) $C^{\alpha}H$ <---> Ala (4) NH and Ala (4) $C^{\alpha}H$ <---> leu (5)NH, suggesting that the octapeptide backbone which is folded into a complete helix (ϕ_i~-50^0 and ψ_i~-50^0) in $CDCl_3$, unfolds at Ala (4) and Leu (5) residues in $(CD_3)_2SO$ such that the backbone torsional angle ψ_i achieves a values of ~120^0 at these residues resulting in observation of $C^{\alpha}{}_iH$ <---> $N_{i+1}H$ type NOEs[19].

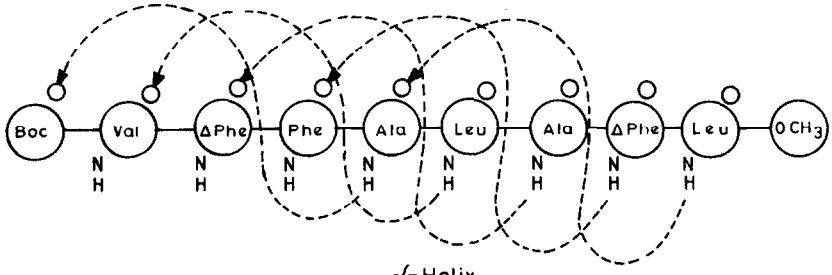
NMR results presented here clearly establish that the pentapeptide and the octapeptide adopt highly folded helical conformations in solution. The involvement of last four NH groups in intramolecular hydrogen bonding suggests a 4 -->1 intramolecular hydrogen bonding pattern. On the other hand in the octapeptide, NMR evidences are in favour of a

5-->1 hydrogen bonding pattern. The 4-->1 intramolecular hydrogen bonding in the pentapeptide and a 5-->1 hydrogen bonding pattern in the octapeptide diagnose a 3_{10} and α-helical conformation for these two peptides respectively (Fig. 6). For the ΔPhe containing peptides, formation of turn structures stabilized by intramolecular hydrogen bonds has been well examplified in our earlier studies[7-11, 21, 22].



3_{10} helix

Boc-Gly-ΔPhe-Leu-ΔPhe-Ala-$NHCH_3$



α-Helix

Boc-Val-Δ^ZPhe-Phe-Ala-Leu-Ala-Δ^ZPhe-Leu-OCH_3

Fig. 6 The conformations of pentapeptide 1 (top) and octapeptide 2 (bottom), compatible with NMR data in $CDCl_3$.

We have found that in model peptides[10,21] introduction of α, β dehydroamino acids produce conformational constraints. In small peptides, from dipeptide methylamides[21] to tetrapeptides[22], containing a Δ^ZPhe residue tend to favour a type II β-turn conformation, with Δ^ZPhe occupying the i+2 position of the turn. This behaviour of Δ^ZPhe[21-23] and Δ^ZLeu[10] is analogous to that of another well studied non protein amino acid residue, aminoisobutyric acid (Aib), which has also been shown to provide conformationally restricted peptides[24]. In peptides containing two Δ^ZPhe residues separated by at least one amino acid, helical structures are observed. For example, a hexapeptide containing -

Δ^{Z}Phe-X-X-Δ^{Z}Phe- system[4] and the pentapeptide containing -Δ^{Z}Phe-X-Δ^{Z}Phe- system reported in this study both favour a 3_{10} helical structure (Fig. 6).

Ciajolo et. al., have also reported a left handed 3_{10} helical conformation for the pentapeptide, Boc D Ala-ΔPhe-Gly-Δ-Phe-D Ala αCH_3[28]. However, as the length of the peptide increases α helical conformation appears to be the favoured one. Thus, for a heptapeptide with -ΔPhe-X-X-X-ΔPhe- system[5] and the octapeptide (Fig. 6) described in this study, in solution, an α helix seems to be more probable conformation. This transition from 3_{10} helix to α-helix appears to be related to the length of the peptide. For the same length of peptide, a 3_{10} helix makes an additional hydrogen bond due to a 4--->1 hydrogen bonding compared to a 5---->1 hydrogen bonding for an α helix. Short peptides, therefore, favour 3_{10} helical motif. A length of peptide is reached where the inherent increased stability of the α-helix compensates for the energy contribution of the single additional hydrogen bond formed in a 3_{10} helix, which perhaps is the main reason for the hepta- and the octa didehydrophenylalanine peptides described above to acquire an α helical structure in solution. This is in direct analogy with conformational behaviour of Aib containing peptides where crystal structure analyses of longer (6-20) residues Aib containing peptides have revealed mostly α helical structure for these peptides[25,26]. Although conformational preferences of Δ^{Z}Phe residues have not been investigated in as much detail as Aib, and some differences have been obtained, the similarities in conformational behaviours of these two residues in small linear peptides is noticeable. In conclusion, the introduction of Δ^{Z}Phe residue in peptides may be useful in designing peptides with preferred conformations such as β-turn and helices.

EXPERIMENTAL

The peptides were synthesized using solution phase methodology. All reactions were monitored by thin layer chromatography (TLC) in three solvent systems namely, A) $CHCl_3$:MeOH(9:1), B) BuOH:CH_3COOH:H_2O (4:1:1) and C) BuOH:CH_3COOH:Pyridine:H_2O(4:1:1:2). Purification of the peptides was carried by

reverse-phase high performance liquid chromatography (HPLC) on Water's μ Bondapak C_{18} column (3.9 mm x 300 mm) with gradient elution (50-95% MeOH in H_2O in 30 min, flow rate 0.8 ml min-1, detection 280 nm) on a Water's HPLC system. ^1H NMR spectra were recorded on a Bruker 500 MHz FT-NMR spectrometer fitted with Aspect 3000 computer at Tata Institute of Fundamental Research, Bombay and at Department of Oral Biology, University at Buffalo, New York.

Peptide Synthesis

Octapeptide 2 was synthesised according to the scheme outlined in Fig. 7. Boc-Gly-Δ^ZPhe azlactone, Boc Ala-Δ^ZPhe azlactone and Boc-Phe-Δ^ZPhe azlactone were synthesized on 20 mM scale using already reported procedure[27].

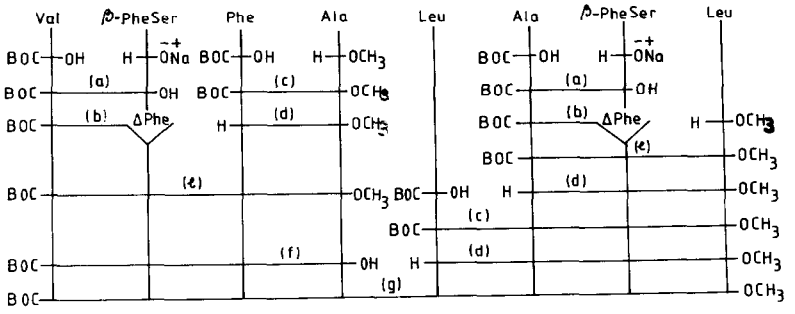


Fig. 7 Synthetic scheme for octapeptide 2

Boc-Ala-Δ^ZPhe-Leu-OMe 3

A solution of Boc-Ala-Δ^ZPhe azlactone (5.0 g, 15.8 mmol) in dichloromethane (DCM) (20 ml) was stirred at room temperature. Mixture of leucine methyl ester hydrochloride (3.2 g, 15.8 mmol) and triethylamine (TEA) (15.8 mmol) in DCM (20 ml) was added to the above stirred solution. The reaction mixture was stirred for 48 hrs. For workup, the solvent was removed in vacuo, the residue dissolved in ethyl acetate (50 ml) was washed successively with saturated $NaHCO_3$ solution, water, 5% citric acid solution, water, dried over anhydrous Na_2SO_4 and finally evaporated to yield the tripeptide in 50% (3.52 g)

yield. m.p. 145-148^{0}C; R_f(A) 0.78, R_f(B) 0.83, R_f(C) 0.79: ^{1}HNMR δppm($CDCL_3$, 60 MHz): 7.9 (1H, s, NH Δ^ZPhe), 7.4-7.1 (6H, m, aromatic protons & $C^\beta H$ Δ^ZPhe), 5.2 (1H, d, NH Ala), 4.7 (1H, m, $C^\alpha H$ Leu), 4.2 (1H, m, $C^\alpha H$ Ala), 3.8 (3H, s, OMe), 1.9-1.5(3H, m, $C^\beta H_2$ Leu & $C^\alpha H$ Leu), 1.4 (9H, s, Boc Me_3), 1.35 (3H, d, $C^\beta H_3$ Ala), 0.9 (6H, d, 2x $C^\delta H_3$ Leu).

Boc-Leu-Ala-Δ^ZPhe-Leu-OMe 4

The tripeptide Boc-Ala-Δ^ZPhe-Leu-OMe 3 (1.2 g, 2.7 mmol) was deprotected at its N-terminal using a mixture of TFA-DCM (1:1 v/v). To a precooled (-10^0 C) stirred solution of Boc-Leu.OH (0.6 g, 2.6 mmol) was added N-methyl morpholin (NMM) (0.3 ml, 2.6 mmol) and isobutylchloroformate (IBCF) (0.35 ml, 2.6 mmol). After 10 min. of stirring a solution of TFA salt (obtained above) and TEA (0.3 ml, 2.6 mmol) in THF (10 ml) was added. The reaction mixture was stirred at 0^{0}C for 2 hr and overnight at room temperature. Work up of reaction (same as for the tripeptide 3) afforded the tetrapeptide 4 in 80% yield: m.p. 160-161^{0}C: R_f(A) 0.73; ^{1}HNMR δppm($(CD_3)_2SO$, 270 MHz): 9.53 (1H, s, NH Δ^ZPhe), 8.33(IH, d, NH Ala), 7.98 (1H, d, NH Leu (4)), 7.56-7.38 (5H, m aromatic protons), 7.24 (1H, s, $C^\beta H$ Δ^ZPhe), 5.93 (1H, d, NH Leu(1)), 4.4 (1H, m, $C^\alpha H$ Leu (4)) 4.26 (1H, m, $C^\alpha H$ Ala (2)), 3.98 (1H, m, $C^\alpha H$ Leu (1)), 3.64 (3H, s, -COOMe), 1.61 (6H, m, $C^\beta H_2$ & $C^\gamma H$ of Leu (1) & Leu (4)), 1.36 (9H, & BocMe_3), 1.29 (3H, d, $C^\beta H_3$ Ala(2)), 0.88 (12H, m, $C^\delta H_3$ of Leu (1) & Leu (4))

Boc-Phe-Ala-OMe 5

To a precooled (0^{0}C and stirred solution of Boc-Phe-OH (3.0 g, 10 mmol) in THF (20 ml), N-hydroxysuccinimide (N-HOSu) (1.2 g, 10 mmol). After 10 min., DCC (2.0 g 10 mmol) was added to the stirred solution. After 30 min., a precooled solution of alanine methyl ester hydrochloride (1.7 g, 10 mM) and TEA (1.6 ml, 10 mmol) in THF (20 ml) was added and the reaction mixture was stirred for 1 hr at 0^{0}C and overnight at room temperature. Usual workup procedure afforded the desired dipeptide Boc-Phe-Ala-OMe as an amorphous white solid. Yield, 3.35 g (95%): R_f(A) 0.68, R_f(B) 0.72.

Boc-Val-Δ^ZPhe-Phe-Ala-OMe 6

The dipeptide Boc-Phe-Ala-OMe 5 (1.8 g, 5 mmol) was deprotected at its N-terminal using a mixture of TFA:DCM (1:1 v/v). The TFA salt was obtained in quantitative yield (1.98 g).

To a solution of Boc-Val-Δ^Z Phe azlactone (1.8 g, 5.2 mmol) in DCM (10 ml) was added a solution of the TFA salt (obtained above) and TEA (0.7 ml, 5.2 mmol). The resulting mixture was refluxed for 48 hr. The usual workup of the reaction afforded the tetrapeptide Boc-Val-Δ^ZPhe-Phe-Ala-OMe. Yield, 2.0 g (66%): m.p. 182^{0}C: R_f (A), 0.88; R_f (B), 0.92; R_f(C) 0.96: ^{1}HNMR δppm ($CDCl_3$, 270 MHz): 9.5 (1H, s, NHΔ^ZPhe); 8.4 (1H, d, NH Phe), 8.33 (1H, d, NH Ala), 7.62-7.45 (9H, m, aromatic protons) 7.26 (1H, s, CβHΔ^ZPhe), 5.95 (1H, d, NH Val), 4.6 (1H, m, CαH Phe), 4.26 (1H, m, CαH Ala), 4.12 (1H, t, CαH Val), 3.64 (3H, s, COOMe), 3.2 (2H, m, CβH2 Phe), 2.12 (1H, m, CβH Val), 1.38 (9H, s, Boc Me_3), 1.32 (3H, d, CβH_3 Ala), 0.92 (6H, dd, 2 x CγH_3 Val).

Boc-Val-Δ^ZPhe-Phe-Ala-Leu-Ala-Δ^ZPhe-Leu-OMe 2

Tetrapeptide 6 (Boc-Val-Δ^ZPhe-Phe-Ala-OMe) (1.5 g, 2.5 mmol) was converted to its free acid by the usual alkaline hydrolysis. Yield, 1.0 g (61%): m.p. 190-192^0 C R_f(A), 0.29; R_f (B), 0.78; R_f(C), 0.84. t-Butoxycarbonyl protection was removed from the N-terminal of the tetrapeptide 4 (0.4 g, 0.7 mmol) using a mixture of TFA:DCM (1:1 v/v). The TFA salt was obtained in quantitative yield.

To a precooled and stirred solution of the tetrapeptide free acid (0.4 g, 0.7 mmol) obtained above, in DMF (10 ml), 1-hydroxybenztriazole (HOBT) (0.11 g, 0.7 mmol) and DCC (0.14 g, 0.7 mmol) were added. After 30 min of stirring a precooled solution of TFA salt of tetrapeptide 4 (obtained above) was added to the reaction mixture. Reaction was stirred at 0^{0}C for 1 hr and overnight at room temperature. Usual workup afforded the crude octapeptide as pale yellow solid. Yield, 0.4 g (50%): m.p. 270-272^0 C: R_f(A), 0.9, R_f(B) 0.86, Rf(C) 0.89. The octapeptide was purified by HPLC using a gradient of methanol and water (Retention time 20 min). ^{1}H NMR of 2 in $CDCl_3$ is shown in Fig. 2.

Boc-Gly-Δ^ZPhe-Leu-Δ^ZPhe-Ala NHMe 1

The pentapeptide was synthesized on 20mM scale coupling Boc-Gly- Δ^ZPhe azlactone and Boc-Leu-Δ^ZPhe-Ala-NHMe using the same procedure as described above for Boc-Ala-Δ^ZPhe-Leu OMe. The pentapeptide 1 was obtained as a white solid. Recrystallisation from dry ethyl acetate- petroleum ether gave white needle shaped crystals. Yield, 65%. m.p. 205-207^0 C: R_f(A) 0.43, R_f(B) 0.94, R_f(C) 0.92, $[\alpha]^{26}_D$ - 40^0 (C, 0.95 MeOH). ^{1}H NMR in $CDCl_3$ is shown in Fig 1.

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REFERENCES

1. Hruby, V. J., Life Sciences, 1982, 31, 189.
2. Wright, P. E.; Lerner, R. A.; Houghten, R. A.; Rance, M.; Dyson, H. J. J. Mol. Biol., 1988, 201, 161-200.
3. Spatola, A. F. in `Chemistry and Biochemistry of amino acis, Peptides and proteins (Weinstein, B. ed.), 7, 267-357.
4. Chauhan, V. S.; Uma, K.; Kaur, P., Balaram, P. Biuopolymers, 1989, 28, 763-771.
5. Gupta, A.; Bhardwaj, A; Chauhan, V. S.; J. Chem. Soc. (Perkin Trans. 2) 1990, 1911-1916.
6. Bach, A.; Gierasch, L. M. Biopolymers, 1986, 25, 5175-5191.
7. Uma, K.; Chauhan, V. S.; Balaram, P. Int. J. Peptide Protein Res. 1988, 31, 349-358.

8. Uma, K.; Balaram, P.; Chauhan, V. S. Int. J. Peptide Protein Res. 1989, 33, 103-109.
9. Singh, T. P.; Haridas, M.; Chauhan, V. S.; Kumar, A.; Viterbo, D. Biopolymers, 1987, 26, 819-829.
10. Narula, P.; Patel, H. C.; Singh, T. P.; Chauhan, V. S.; Sharma, A. K. Biopolymers, 1988, 27, 1595-1606.
11. Singh, T. P.; Narula, P.; Chauhan, V. S.; Kaur, P. Biopolymers 1989, 28, 1287-1294.
12. Glowka, M. L. Acta Cryst. C. 1988, 44, 1639-1641.
13. Busetti, V.; Ajo, D.; Casarin, M. Acta Cryst. C 1984, 40, 1245-1248.
14. Wuthrich, K. Acc. Chem. Res. 1989, 22, 26.
15. Nagraj, R.; Balaram, P. Biochemistry, 1981, 20, 2828.
16. Aue, W. P.; Bartholdi, E.; Ernst, R. R. J. Chem. Phys., 1976, 64, 2229.
17. Kopple K. D.; Go A. and Pilipanskas P. R. J. Am. Chem. Soc., 1975, 97, 6830.
18. Pitner, T. P.; Urry, D. W. J. Am. Chem. Soc. 1972, 94, 1399.
19. Wuthrich, K., in ^{1}NMR of Proteins and Nucleic Acids, Wiley, New York, 1986.
20. Kumar, A.; Wagner, G.; Ernst, R. R.; Wuthrich, K. J. Am. Chem. Soc., 1981, 103, 3654.
21. Kaur, P.; Uma, K.; Balaram, P.; Chauhan, V. S. Int. J. Peptide Protein Res. 1989, 33, 103.
22. Uma, K.; Chauhan, V. S.; Kumar, A.; Balaram, P. Int. J. Peptide Protein Res. 1988, 31, 349.
23. Bhandari, K.; Chauhan, V. S. Biopolymers, 1991 (communicated).
24. Toniolo, C.; Bonora, G. M.; Bavoso, A.; Benedetti, E.; DiBlasio, B., Parone, V.; Pedone, C. Biopolymers 22, 205-215.
25. Karle, I. L.; Balaram, P. Biochemistry 1990, 29, 6747-6755.
26. Marshall, G. R.; Hodkin, E. E.; Langs, D. A.; Smith, G. D.; Zabrocki, J.; Laplawy, M. T. Proc. Natl. Acad. Sci. USA 87, 487-491.
27. Bergmann, M., Schmidt, V.; Mickley, A. Z. Physiol. Chem. 1930, 187, 264.
28. Ciajolo, M. R.; Tuzi, A.; Pratesi, C. R.; Fissi, A.; Pieroni, O. Biopolymers, 1990, 30, 911-920.