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CONFORMATIONAL PREFERENCE OF N-ACYL UREA CONTAINING VALINE RESIDUE IN DMSO d_6

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Abstract: The conformational analysis of N-{N - [(tert-butyloxy) Carbonyl] -L-valyl} - N, N -dicyclohexyl urea 1 in DMSO-d_6 has been performed using ^{1}H NMR spectroscopic studies. The data provide evidence for the occurrence of intramolecular hydrogen bonded NH of ureide proton. Two specific NOEs [NH (DCHU) <-----> $C^{\alpha}H$ (Val) and NH(Val) <----> $C^{\alpha}H$ (Val] prescribed the presence of ß-turn conformation of compound 1 in DMSO-d_6.

Interest in the conformational properties of Val containing model peptides have been stimulated by the widespread occurrence of this residue in the ß-sheet forming sequence in native proteins[1,2]. Amino acid compositions were analysed for the ß-sheets from the known crystal structure data of proteins. The relative ranking of propensity to form ß-sheet structure was found in the following order: Val >> Gly >Ile>Thr>Ala>Ser>Leu>Lys> Tyr>Phe>Asp>Glu. Among these amino acids, the relative probability to occur in the interfacial regions of ß-sheets were as follows: Val >>Leu>Ile>Phe>Ala>Asp>Gly. Thus the amino acid residue valine has the maximum tendency to occur in the ß-sheet structure. However, the propensity of Val taking ß-sheet conformation depends on the environment around the Val residues as inter residue packing play a dominant role in deciding the conformational preference of ß-sheet forming sequences[2]. For example, the conformation of compound 1 is found to be of ß-structure[3,4] in the solid state, whereas the other N-Acyl ureas of Ala and Leu takes C_9 folded conformation.[5] The conformational preference of Val is due to C^{β} branching in the sidechain of valine residue, which provides an ideal packing requirement for this residue to be present in adjacent strands of ß-sheet.[6] It will be worthwhile to analyse the conformation of 1 in the absence of intermolecular interactions. For this purpose the conformational analysis of 1 was carried out in DMSO-d_6, the solvent in which the peptide aggregation was known to be minimum .

The title compound 1 was synthesised by the procedure published elsewhere[4]. Difference NOE experiments were carried out in the sophisticated Instruments Facility of IISc, Bangalore. In this experiment, the perturbed and normal spectra recorded sequentially in different parts of the memory (8K each) were obtained by low power on-resonance saturation of a peak and by off-resonance shifting of the irradiation frequency

respectively. Typically 50 accumulations were utilised (acquisition time for each is 1.4s.) with a delay time of 5 s. between transients to facilitate the build up of initial equilibrium magnetization. The difference free induction decay was multiplied by a decaying exponential before Fourier transformation and an indentical filtering of the normal spectrum allowed quantitative NOEs. The assignments of the proton signal were performed using 2D COSY. The chemical shifts, temperature dependence for the two NH protons are listed in Table 1. The low dδ /dT value of NH of dicyclohexyl ureide moiety (0.0004 ppm/K) in DMSO-d_6 suggest that this proton is solvent shielded[7,8]. The NH of Val shows the temperature dependence of value 0.004 ppm/K. The high value of dδ/dT indicates that this proton is exposed to the solvent[7,8].

TABLE 1

NMR Parameters[a] for the NH groups of 1 in DMSO-d_6

	Val (1)	DCHU
δ (ppm)[a]	6.83	8.30
d δ /dT (ppm/K)	-0.004	-0.0004

[a]Values are expressed as ppm down field from TMS and the concentration of N-Acyl urea is 5 mM.

Representative difference NOE spectra obtained by irradiation of NH resonance are shown in Fig. 1. The observed NOEs are positive, suggesting that the rotational correlation times are short enough to be in the region $\omega\tau_c<<1$ at 270 MHz[9,10]. 8.3% NOE was observed for the $C^{\alpha}H$ (Val) proton on saturating NH of dicyclohexyl ureide moiety. On the otherhand only 6% of enhancement is observed in the reverse direction. This observation of slightly smaller NOE of NH on $C^{\alpha}H$ irradiation indicates alternative relaxation pathways for the NH protons. There is also substantial intraresidue NOE for NH and $C^{\alpha}H$ (Val) [4.2%].

A conformation, consistent with NMR data is shown in Fig.2. This structure involves one transanular intramolecular hydrogen bond formed between NH of ureide and Boc C=O groups. The molecule is depicted as intramolecular hydrogen bonded C_9 structure. The low temperature coefficient of NH (DCHU) protons is presumably a consequence of such

conformation. The inter-residue NOE between NH (DCHU) and $C^{\alpha}H$ (Val) and the intra-residue of $C^{\alpha}H$ (Val) indicate that these protons occur at a distance <3.5 A[11]. Among all the possible regular conformations of the peptides, only in type II ß-turn, the inter-proton distances of $d_{\alpha N}$ (2,4) and $d_{N\alpha}$(2,2) are 3.3 and 2.2 A respectively[11]. However, if the compound 1 takes ß-turn, then the NOE observed between ureide NH and $C^{\alpha}H$ (Val) should be of smaller magnitude than the intra residue NOE of NH (Val) and $C^{\alpha}H$ (Val) because of the expected closer proximity of the latter pair of protons.

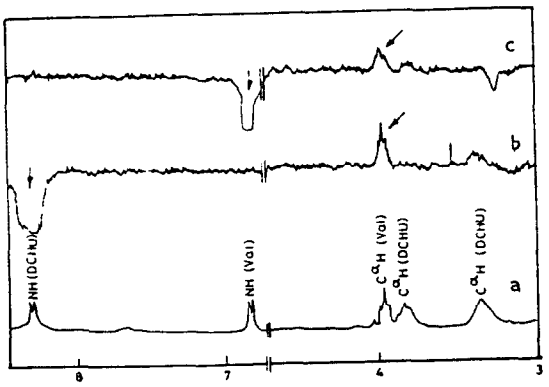
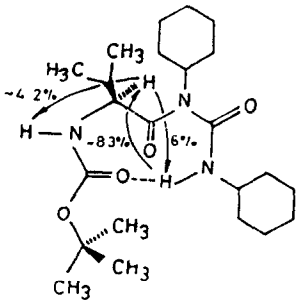


Fig. 1 (a) Partial 270 MHz spectrum of 1 in DMSo-d_6.
(b) Difference NOE spectra (X 16) obtained by saturating NH of dicyclohexyl ureide moiety. The positive NOE on $C^{\alpha}H$ (Val) is shown in the Fig.
(c) Difference NOE spectra (X 16) obtained by saturating NH of Val. The intraresidue NOE on $C^{\alpha}H$ (Val) is shown in the Fig.

Eventhough the NOE data agrees with the type II ß-turn the compound 1 is not entirely peptidic, potential energy caculations were carried out for the conformation depicted in Fig 2 (Similar to type II ß-turn conformation) and ß-structure conformation. Both the conformaotions of 1 were generated and the energy was minimized using the program AMBER[12]. The energy values for the the conformation similar to type II ß-turn and ß-structure were -22.20 and -20.27 Kcal/mol respectively. In the energy minimized turn conformation the distance between BOC-carbonyl group and NH(DCHU) moiety was 2.85A suggesting intra-molecular hydrogen bonding. This value clearly supports the low temperature coefficients observed for NH of DCHU moiety as intramolecular hydrogen bonded NH proton is found to have temperature coefficients < 0.002.[13]. The inter proton distances between NH(DCHU) and $C^{\alpha}H$ (Val) is 1.81 A for the energy minimized ß-turn conformation. This value agrees with the 8.3% NOE observation between these two protons. The inter proton distance between NH (Val) and $C^{\alpha}H$ (Val) in energy minimized ß turn bend conformation was 2.85 A. The slightly lower NOE observed for NH (Val)<----> $C^{\alpha}H$ (Val)

was clearly explained by this value. Stereo view of the energy minimized type II ß-turn conformation of compound 1 is depicted in Fig.3.



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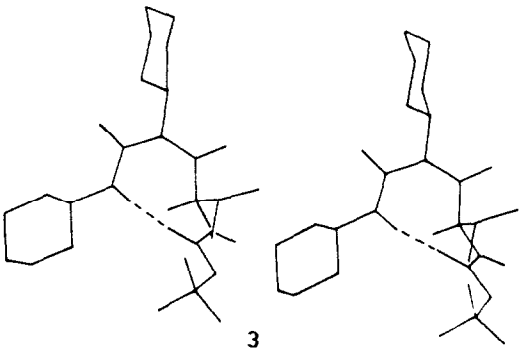

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Fig. 2. Proposed type II ß-turn conformation for N-Acyl urea 1. Magnitude of NOEs are indicated by arrows linking the two hydrogen atoms.

Fig. 3. Stereo view of the energy minimized conformation similar to type II ß-turn.

The results presented in this report provide evidences in favour of an type II ß-turn conformation for the compound 1 DMSO-d_6, indicating the conformation of the amino acid derivatives in solution is different from that observed in the solid state, where inter-molecular interactions play a dominant role.

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References

1. Chothia, C.; Janin, J. Proc. Natl. Acad. Sci (USA) 1981, 78, 4146.
2. Chothia, C.; Janin, J. Biochemistry, 1982, 21, 3955.
3. Jayakumar, R. "Conformational studies of acyclic peptides by NMR and crystal X-ray diffraction techniques" Ph.D. thesis 1989, I.I.T. Madras.
4. Toniolo, C.; Valle, G.; Crisma, M.; Moretto, V.; Isdebski, J.; Pelka, J.;Schneider, C.H. Helv. Chim. Acta, 1990, 73, 626.
5. Sudharshanakumar, Srinivasan, S.; Jayakumar, R. Int. J. Peptide Protein Res 1992, 39, 285.
6. Lee, B.K.; Richards, F.M.J. Mol. Biol. 1971, 55, 379.
7. Renugopalakrishnan, V.; Khaled, M.A.; Rapaka, R.S.; Urrey, D.W. Biochem, Biophys. Acta, 1978, 536, 421.
8. Von Dreele, P.H.; Rae, I.D.; Scheraga, H.A. Biochemistry 1978, 17, 956.
9. Glickson, J.D.; Gorden, S.L.; Pitner, T.P.; Agresh, D.G.; Walter, R. Biochemistry 1976, 15, 5721.
10. Bothner-By, A.A.; Magnetic Resonance in Biology; Shulman, R.G., Ed.; Academic Press New York, 1979, pp. 177-219.
11. Wuthrich, K.; Billeter, M.; Braun, W. J.Mol. Biol. 180, 175.
12. Weiner, P.K.; Kollman, P.A. J. Comp. Chem. 1981, 2, 287.
13. Iqbal, M.; Balaram.P. Biochemistry. 1981, 20, 4866.