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^{1}H NMR Spectroscopic Signatures of an Intraresidue Hydrogen Bonded C_5 - Structure

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Abstract : Using 1D and 2D ^{1}H NMR techniques, conformational analysis of model peptides **1** and **2** revealed that the Thr residue is conformationally restricted to a fully extended C_5 - structure, stabilized by an intraresidue hydrogen bond. Systematic investigation provided distinctive ^{1}H NMR spectral parameters for this conformation across a $C^{\alpha}H$ bearing chiral proteinogenic residue. © 1997 Elsevier Science Ltd.

Ideally, the fully extended intraresidue hydrogen bonded five - membered ring, the C_5 - structure, is characterized by $\phi \simeq \psi \simeq \pm 180°$ in the Ramachandran map.[1] This pentagonal structural motif proposed about three decades ago, represents a distinct class of intramolecularly hydrogen bonded secondary structures, in polypeptides and proteins.[2] Despite the importance of small intramolecularly hydrogen bonded structures, systematic spectroscopic characterizations of this element of secondary structure across chiral proteinogenic residues have never been reported.[3] In this communication, for the first time we wish to describe the conformational characterization and the ^{1}H NMR spectroscopic signatures of this element of secondary structure in 'model' peptides Boc - Ile - Thr - NH_2, **1** and Boc - Leu - Thr - NH_2, **2**.

The relevant ^{1}H NMR parameters of NH resonances in **1** and **2** are summarized in the Table. The similarity of ^{1}H NMR spectral behaviour of **1** and **2** in $CDCl_3$ and DMSO - d_6 may indicate similarities in their conformations (data not shown). The evidence for the involvement of the NH group in an intramolecular hydrogen bond was probed using temperature and solvent dependence of NH chemical shifts.[4] In $(CD_3)_2SO$ the extremely low temperature coefficient ($d\delta$ / dT) values ($\leq$ 0.0023 ppm/K) of Thr NH in **1** and **2** clearly suggest that Thr NH is strongly solvent shielded most probably due to their involvement in an intramolecular hydrogen bonding. Whereas, Ile / Leu NH groups showed significantly high $d\delta$ / dT values ($\geq$ 0.0066 ppm/K) suggestive of freely solvent accessible NH groups. Further, a large downfield shift ($\geq$ 2.16 ppm) of Ile / Leu NH was observed on adding increasing amounts of $(CD_3)_2SO$ to a peptide solution in $CDCl_3$, which is indicative of solvent exposed NH groups. In marked contrast, the Thr NH in both **1** and **2**, exhibits a very small downfield shift ($\leq$ 0.52 ppm) under similar conditions, a characteristic of strongly solvent shielded, intramolecularly hydrogen bonded NH group ($\Delta\delta_{NH}$, Table 1).

Table 1 : A Summary of Relevant ^1H NMR Parameters* of **1** and **2**.

Parameters	1		2	
	Ile	**Thr**	**Leu**	**Thr**
δ_{NH} [a]	4.95	6.94	4.89	7.01
δ_{NH} [b]	7.11	7.46	7.22	7.38
$\Delta\delta_{NH}$	2.16	0.52	2.33	0.37
$J_{HN\text{-}C^{\alpha}H}$ [a]	6.4	8.2	5.9	8.3
$J_{HN\text{-}C^{\alpha}H}$ [b]	8.6	8.6	8.2	8.6
$d\delta / dT$ [b] $\times 10^{-3}$	8.7	2.3	6.6	1.9

* δ values are expressed as ppm downfield from internal reference TMS. A peptide concentration of ~ 3 mg / 0.5 ml was employed. J values in Hz. **a** : in $CDCl_3$; **b** : in $(CD_3)_2SO$; $\Delta\delta_{NH} = \delta_{NH}{}^{b} - \delta_{NH}{}^{a}$

The observed high $^3J_{HN\text{-}C^{\alpha}H}$ values of 8.2 - 8.6 Hz for Thr residues, in both $CDCl_3$ and $(CD_3)_2SO$ for **1** and **2**, are arguably consistent with their ϕ values close to -140 ± 10° indicative of an extended conformation.[5] Interestingly, corresponding ^3J values observed for Ile / Leu do not suggest the presence of an extended conformation across these residues.

To rule out the involvement of Thr O^{γ} into an intramolecular hydrogen bond, we considered the $^3J_{H^{\alpha}\text{-}H^{\beta}}$ coupling constant and obtained the χ_1 value.[6] The observed significantly low $^3J \leq 2$ Hz for Thr residue indicated the χ_1 value close to ± 85 ± 10°. The positive χ_1 value established for the Thr side chain on the basis of observed $C^{\alpha}H \leftrightarrow C^{\beta}H$ and $C^{\alpha}H \leftrightarrow C^{\gamma}H_3$ characteristic NOEs (data not shown), a usually preferred conformation which tends to avoid the unfavorable steric interactions. This χ_1 value positions the O^{γ} significantly away from the Thr NH group and is unlikely to account for the observed shielding behavior. The interpretation is fully consistent with the results of the analysis of the geometry of the Thr side chain observed in a fully extended β - sheet conformation in non - homologous proteins, which clearly revealed that the Thr $O^{\gamma}H$ is unlikely to make a good hydrogen bond geometry to a peptide unit unless otherwise mediated through a water or solvent molecule.[7]

The observation of significantly low sensitivity of the change in the chemical shifts of the Thr NH with respect to temperature ($d\delta$ / dT) and varying compositions of $CDCl_3$ - DMSO - d_6 solvent mixtures and detectable diagnostic NOEs (data not shown) at high field NMR for the peptide of this size strongly indicate the presence of an ordered intramolecularly hydrogen bonded conformation. A proposed backbone conformation, fully consistent with NMR parameters, characteristic NOEs and molecular modeling is shown in Figure 1.

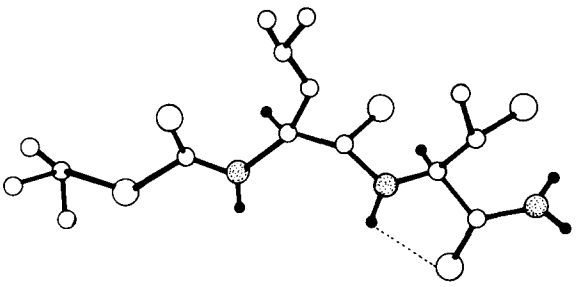

Figure 1 : Proposed conformation (ball & stick model) of **2** as determined from NMR data. Dotted line indicates the hydrogen bond across Thr residue. Amide and C^{α} hydrogens are indicated by filled circles.

The ϕ values determined for the Ile / Leu and Thr residue are incompatible with an extended β - sheet and reverse γ - turn conformations, respectively.[4a,8c] Further, the interpretation was supported by the non - observation of characteristic NOEs associated with a fully - extended β - sheet and a reverse γ - turn conformation.[8]

Unfortunately, the ^1H NMR characteristics of peptides containing the sole element of C_5 secondary structure across a chiral protein amino acid residue have never been reported. Therefore, we are inclined to suggest that the simultaneous observations of i) $\simeq 8 \leq {}^3J_{HN\text{-}C^{\alpha}H} \leq 9$ values ii) involvement of the amide N_iH in an intramolecular hydrogen bond *e.g.* low $d\delta$ / dT values ≤ 0.003 ppm/K and iii) non - observation of consecutive - $C^{\alpha}_{i-1}H \leftrightarrow N_iH$ and $C^{\alpha}_iH \leftrightarrow N_{i+1}H$ - NOEs may be taken as the characteristic of a C_5 - structure across a $C^{\alpha}H$ bearing residue. The intraresidue $N_iH \leftrightarrow C^{\alpha}_iH$ NOE may or may not be observed. These criteria indeed result in ϕ values close to $-140 \pm 10°$ in which the amide NH and the CO groups of the same residue can proximate to form an intraresidue hydrogen bond as expected in a locally extended C_5 - conformation.

This communication provides distinctive ^1H NMR spectroscopic signatures for the smallest possible intramolecularly hydrogen bonded C_5 - structure across a Thr residue suggesting that the presence of this element of secondary structure in short linear, unconstrained peptides containing proteinogenic residue(s) may not be rare. These results are conclusively at variance with the available experimental results accumulated to date on derivatives and peptides containing achiral non - proteinogenic, $C^{\beta, \beta'}$ - symmetrically disubstituted glycine residues associated with the C_5 - structure.[3] The remarkable stability of the structure, in both polar as well as apolar solvents, would provide its contribution in maintaining the tertiary structure of proteins and polypeptides containing this element of secondary structure. The transient existence of the C_5 - structure may also be visualized during early events in protein folding and is likely to substantiate the "framework model" of protein folding. Finally, the tendency of chiral protein amino acids bearing C^{β} stereogenic center to adopt specific compact, intramolecularly hydrogen bonded, folded backbone conformation in the absence of any other constraints, may be a useful alternative for the design of peptidomimetic molecules incorporating such folding patterns with oriented side chains.

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