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Temperature Dependence of NH Chemical Shifts in Tyrosine N-Formyl Peptides By H-1 NMR Spectroscopy

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Abstract

In this paper the H-1 NMR studies on N-formyl peptides in $CDCl_3$ revealed the importance of folded conformations in biologically active analogues. The chemical shifts of NH groups in peptides at every temperature were measured in strongly hydrogen bond accepting solvents like $CDCl_3$ using H-1 NMR. The H-1 parameters for N-for-Phe-Gly-Leu-OMe peptide in organic solvent show intra molecular hydrogen bond between the Tyr NH and Gly COO group. This is found to be more active than parent compound that exists in DMSO-d_6 in an extended β-sheet conformation.

Key words: H-1 NMR Studies, Chemical shifts, Temperature dependence.

1. INTRODUCTION

The building block of a poly peptide chain is an. The conformation of amino acid residue, NH_2 - C α(H_1R) – COOH chain is better understood in terms of peptide unit which consists of the atoms between one C α and next C α atom. The major methods (approaches) of conformational analysis of peptides and proteins are X – Ray crystallography and NMR[1-4]. The X –ray crystallographic analysis provides comprehensive information on the structure of these molecules in the crystalline state and has played an important role in shaping our understanding of the state, the

peptides and their structure. However, X – Ray diffraction studies do not generally address the problem of conformational flexibility and dynamic inter-conversion between structures of comparable energy. Of the various spectroscopic techniques available for molecular conformational analysis in solution, NMR is the best tool to provide more ancillary information about peptide conformation [5]. H-1 NMR studies of peptides generally use chemical shifts (δ), relaxation times (solids) (T_1 and T_2), coupling constants (J_{AX}, J_{BX}, and J_{AB}) and rotamer populations to extract about molecular conformations and dynamics [4-6]. Some of the major goals of H-1 NMR studies of peptides are to identify the nature of the intra molecularly Hydrogen bonded, Nuclear Overhauser Effect (NOE) and To establish the nature of conformational equilibriums present in solution. So, Chemoattractants such as formyl peptides promote chemotaxis of neutrophils. As a result of the ligand-receptor interaction between the formyl peptide and neutrophil, formyl peptide finds application in the medical fields in the area of lesion detection. In an attempt to establish the nature of the receptor bond conformation of the chemotactic peptides, various stereochemically constrained analogues have been synthesized and studied [6]. H-1 NMR studies on N-for-Tyr-Cis-Ala-Leu-OMe in $CDCl_3$ revealed the importance of folded conformations in biologically active analogues [7]. Thus, H-1 NMR develops a correlation between peptide conformation and biological activity. The present study on this chemotactic peptide determines the conformer populations for the peptide residue chain.

2. EXPERIMENTAL TECHNIQUE

The chemotactic peptide N-for-Tyr-Cis-Ala-Leu-OMe was obtained from SIGMA Chemicals USA and used as such for NMR studies. Different molar concentrations of solutions were prepared in DMSO-d_6. H-1 NMR spectra were recorded with WH 270 FT NMR Spectrometer at 270 MHz. The chemical shifts are down-field from TMS / TSP which served as the internal reference [8]. The coupling constants and rotamer populations of (Tyr) peptide were obtained through computer simulation.

3. Results and discussion

The chemical shifts of NH groups in peptides at every temperature can be measured in strongly hydrogen bond accepting solvents like $CDCl_3$. The rates of variations of chemical shifts to the temperature variation, temperature coefficient ($\Delta\delta/\Delta T$) can be studied to differentiate solvent exposed and solvent shielded NH groups. The NMR spectra of the peptide N-For-Tyr-Cis-Ala-OMe in the range of 293 K to 333 K was obtained to understand the influence of temperature on the chemical shifts values of NH protons. Due to this, the nature of hydrogen bonding in the peptide can be deduced. The temperature was varied with 5 K degree intervals. The concentration was 20mM solution in $CDCl_3$ [9-10]. The NH chemical shifts from temperature dependence study for N-for-Tyr-Cis-Ala- -OMe are listed in Table 1.

Table. 1: NH Chemical Shifts of N-for-Tyr-Gly-Leu-OMe at Different Temperatures.

Temp in K	Chemical Shifts			Δ(δAla)	Δ(δCis)	Δ(δTyr)	Difference of Chemical Shifts Δ of			formyl (H)
	Ala (NH)	Cis(NH)	Tyr (NH)				(δC-δA)	(δA-δT)	(δT-δC)	
283	6.25	6.38	6.12	-	-	-	-0.10	0.12	-0.09	7.92
293	6.22	6.34	6.09	-0.02	-0.02	-0.02	0	0	0	7.96
303	6.18	6.30	6.16	-0.02	-0.02	-0.02	0	0	0	7.94
313	6.16	6.25	6.04	-0.02	-0.02	-0.03	0	0.01	-0.01	7.94
323	6.12	6.22	6.18	-0.02	-0.02	-0.02	0	0	0	7.98
333	6.06	6.19	6.24	-0.02	-0.01	-0.02	0.01	0	-0.01	7.98

The first column represents the temperature at which NMR spectra were obtained. Columns 2 to 4 give the NH chemical shift values of Ala, Cis, and Tyr respectively. The difference in chemical shifts of Tyr, Gly, and Leu are given in the other columns. The last column lists the chemical shifts for formyl hydrogen at different temperatures. The overlay of H-1 NMR of the temperature versus chemical shifts of Ala, Cis and Tyr are given in Fig 1 with necessary Table 2. From the Fig. 1, it is observed that as the temperature is increased, the chemical shift values of Tyr, Cis,

and Ala NH proton signals shift up-field. From the data in Table2, it is observed that as the temperature is increased, the value of Δδ for both Gly and Leu NH protons is approximately the same and this shows that the two proton signals are shifted to the same extent. However, the value of Δδ for Tyr is decreased and at last there is no change. This is due to the shielded NH group with solvent $CDCl_3$. From the Table 1, it is observed that below 263 K, the Cistine NH proton signal is down-field relative to the Alanine NH proton signal. With further increase in temperature, the two proton signals cross over and the Cis NH proton signal is up-field with respect to the Ala NH proton signal. At 303 K and above, the Cis NH proton signal, which was a doublet at temperature 303 K, coalesces to a broad singlet. Last at 333 K, the Alanine and the Cistine NH proton signals become indistinguishable. Also from the same Table, it is observed that the Tyr NH resonance does have relatively low values of chemical shifts than Cis and Ala NH signals. This argues that there is a partial shielding of the NH group in the Tyr from the solvent. In addition, with increase in temperature, there is a down-field shift of the Formyl hydrogen signal. The up-field of Cis NH and Ala NH proton signals implies that the hydrogen bonds in these peptides weaken with increase in temperature.

Table 2 NH Chemical Shifts of Tyr, Ala, and Cis at Different Temperatures.

Temperature	**Chemical Shifts**		
	Ala (NH)	**Cis (NH)**	**Tyr (NH)**
283	**6.48**	**6.47**	**6.32**
293	**6.46**	**6.45**	**6.3**
303	**6.44**	**6.43**	**6.28**
313	**6.42**	**6.41**	**6.25**
323	**6.4**	**6.39**	**6.23**
333	**6.38**	**6.38**	**6.21**

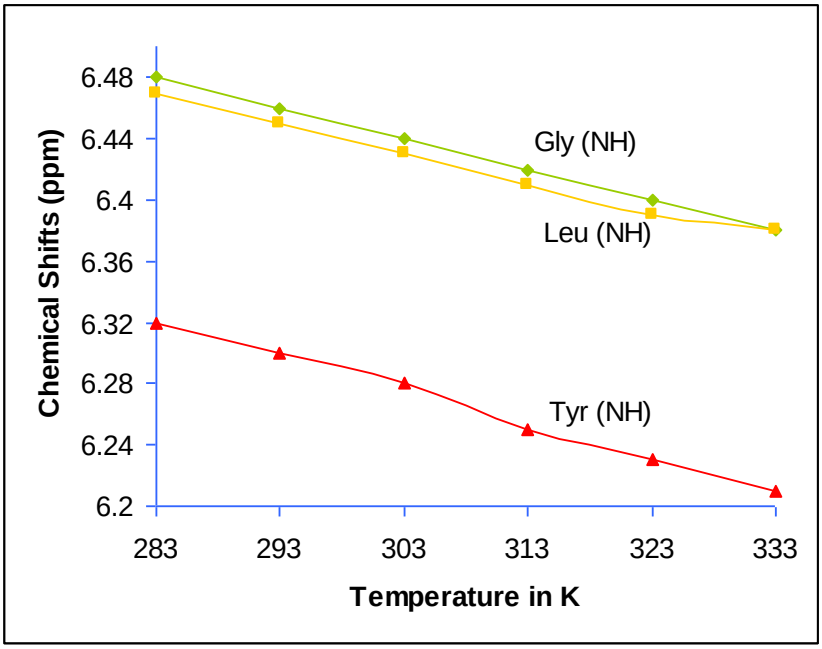

Fig 2.7 NH Chemical Shifts Vs Temperature

Table 3. Δδ /ΔT Values of NH Groups in the Peptide N-for-Tyr-Gly-Leu-OMe

Temperature in K	Δδ /ΔT (ppm / K) Values of NH Groups in the Peptide N-for-Tyr-Gly-Leu-OMe		
	Gly (NH)	Leu (NH)	Tyr (NH)
283	0.002	0.002	0.002
293	0.002	0.002	0.002
303	0.002	0.002	0.003
313	0.002	0.002	0.002
323	0.002	0.002	0.002
333	0.002	0.001	0.002

The Δδ/ΔT values for all NH groups in N-for-Tyr-Cis-Ala- -OMe listed in the Table 3, are solvent exposed NH groups which are generally characterized by Δδ/ΔT values greater than 0.004 ppm/K (Purucell et al, 1973), while solvent shielded or intra molecularly hydrogen bonded NH groups are characterized by values less than 0.003 ppm/K. The temperature coefficient for Ala and Cis protons are -0.0075 and -0.008

ppm/K. The literature values for Ala and Cis are greater than 0.003 ppm/K. This observation implies that the Alanine and Cistine NH protons are involved in an intra molecular hydrogen bond. $CDCl_3$ is not a hydrogen bond acceptor solvent; therefore, the intra molecular hydrogen bond in the case of the peptide exists between two peptide molecules. The $\Delta\delta/\Delta T$ value for Tyr proton in the peptide is 0.0052 ppm/K and this suggests that the NH proton is free and not involved in intra molecular hydrogen bonding in $CDCl_3$ / DMSO-d_6. At the temperatures above 313 K, the two proton signals become indistinguishable [13-14]. The doublet for Cistine NH coalesces and the signals for the two NH protons appear as a broad signal. On increasing the temperature, the hydrogen bond to the formyl Carboxyl is broken. Also, at high temperatures, there is free rotation about the amide bonds. Thus, the Formyl hydrogen behaves more likely as an aldehydic proton rather than an alkene proton.

4. Conclusion

H-1 NMR studies on N-for-Tyr-Gly-Leu-OMe in DMSO-d_6 revealed the importance of folded conformations in biologically active analogues. It is important that the effect of conformation of the peptide on the peptide receptor interaction be well understood. H-1 NMR results in the temperature range of 283 K to 333 K were obtained to understand the influence of the temperature on the chemical shift values of NH protons. The H-1 parameters for this peptide in organic solvent show intra molecular hydrogen bond between the Tyr NH and Gly COO group. This is found to be more active than parent compound N-for-Phe-Gly-Leu-OMe that exists in DMSO-d_6 in an extended β-sheet conformation.

REFERENCES

[1] Abragam A., "*The Principles of Nuclear Magnetism*" Oxford, London (1961)

[2] Abraham R J., and Benstein J., Can. J. Chem. **11** (1966)471

[3] Balaram P., Indian J. Chem. **25B** (1985) 641

[4] Davis P J., Davis F B., and Blas S D., Life Sci. **80** (1982) 675

[5] Davis P J., Davis F B., and Blas S D., J. Clin. Invist. **71** (1983 a) 579

[6] Nielsen N C., Bildson H., and Jakobsen H. J., J. Magn. Reson. **75** (1988) 149

[7] Iqbal M., and Balaram P., J. Am. Chem. Soc. **103** (1981) 5548

[8] Pochetti G., Traniello S., Spisani A., *Biopolymers* **35** (1995) 347

[9] Selfer A M., Lauri G., Bartlett P A., Int. J. Pep. Protein Res. **148** (1996) 129

[10] Sharma P., Singh T P., Indian J Phys. **70B** (1996) 205

[11] Reddy M C., Reddy B P N., and Ramakrishnanaik J., Indian. J. Biochem & Biophys. **23** (1986) 786

[12] V R Reddy, P N Reddy, B P N Reddy and Y M Reddy, Bulletin of Pure and Applied science **14D** (1993) P 17-22

[13] V R Reddy, P N Reddy, B P N Reddy and Y M Reddy, Indian Jol. On Pure and Applied Physics **33** (1995) pp 357-361**.**

[14] V R Reddy, P N Reddy, B P N Reddy and Y M Reddy, Indian J. Of Pure and Applied Physics **35** (1995) pp 653-656