

Solvent Effect on Biologically Peptide Analogues by using 2D-NMR Spectroscopy

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Abstract: The solvent dependence of the peptides, N-for-Met-Tyr-Ala-OMe and N-for-Leu-Tyr-Gly-OMe, at different concentration of DMSO-d₆ using H-1 and 2D-NOESY NMR is studied. The investigated peptides indicate that chemical shifts of the NH values lie between 6.33 and 6.85, where Gly NH is 6.33 ppm, Leu NH is 6.85 ppm and Tyr NH is 6.64 ppm. The differences of chemical shifts are significantly identified. From the results, it is observed that when solvent concentration is increased, Leu NH protons are in slow exchange situation and signal for NH proton appears as a doublet. This disappears at 100% DMSO-d₆ concentration. The NOESY spectrum of the peptides is obtained at 20mM concentration in DMSO-d₆. Based on the analysis of the spectra from solvent dependence and the NOESY experiments, intermolecularly hydrogen bond conformers such as ones are proposed for the peptides. The formation of hydrogen bond between the amide NH proton and DMSO-d₆ explains the downfield shift of the NH protons and upfield shift of the formyl H. The conformers explain a cross peak observed between the two groups in the NOESY spectrum of the peptides and these conformers support the conclusions arrived at from various NMR studies.

Keywords: Peptides, Conformational Studies, H-1 and 2-D NMR

I. Introduction

In the past two decades, increasing work has been devoted to the development of NMR methods which can be useful in many Biological applications. The detection and characterization of molecular interactions between a macromolecule and a ligand require different NMR techniques [1-3]. The finding that low molecular mass tripeptides are chemoattractants for neutrophils which also induce selective release of lysosomal enzymes. Foreexample, H-1 NMR studies of the most active member of this class of peptides N-Formyl-Met-Leu-Phe-OMe have suggested an extended, monomeric β -sheet conformation in DMSO-d₆ solution. A detailed understanding of the function of a biological macromolecule requires knowledge of its three-dimensional structure. For studies of the amino acid chain system, the primarily used NMR spectroscopy, which has become a powerful and versatile technique for the characterization of biomolecules and for the detection of molecular interactions. Among NMR studies, the Nuclear Overhauser Effect (NOE) is the result of cross relaxation between dipolar coupled spins as a result of spin-spin interactions through space and this is crucial for the determination of protein structure [4-5]. The cross peaks between the formyl H and methyl in DMSO-d₆ are between 3.00 – 4.00 Å for the investigated compounds. It uses the dipolar interaction of spins for correlation of protons. The intensity of NOE is proportional to the distance between protons of ligand. In the NMR spectroscopy, dipolar couplings are usually in the kHz range, and depend on the distance between the two spins and orientation of the internuclear vector with respect to the static magnetic field. However, due to fast overall tumbling of molecules in solution, the dipolar couplings are averaged to zero. Nevertheless, the dipolar coupling gives rise to spin-spin and spin-lattice relaxation. Hence, NOE is a result of cross-relaxation between spin and spin and is defined by the transition rates which involve spin flips of both spins [6-7]. The NOE allows transferring magnetization from one spin to another through space. The correlation between two protons depends on the distance between them, but normally a signal is only observed if their distance is smaller than 5 Å. The NOESY experiment correlates all protons which are close enough. It also correlates protons which are distant in the amino acid sequence but close in space due to the structure.

II. Experimental Technique

The chemotactic peptides N-for-Met-Tyr-Ala-OMe and N-for-Leu-Tyr-Gly-OMe were obtained from SIGMA Chemicals, USA and used as such for NMR studies. NMR samples were prepared by directly dissolving solid peptide in DMSO-d₆ and different molar concentrations of solutions were prepared. H-1 NMR spectra were recorded with WH 270 FT NMR Spectrometer at 270 MHz. The chemical shifts are downfield from TMS / TSP which served as the internal reference. The proton-proton NOE's can establish spatial proximity of hydrogen atoms in organic molecules [8]. NOE results are generally observable when the interproton distance is ≤ 3.0 Å. The NOESY experiments correlate all protons which are close enough. It also correlates protons which are distant in the amino acid sequence but close in space due to tertiary structure. The

NOESY pulse sequence starts as usual to provide chemical shift information in the FT domain. Then another 90° pulse transmit some of the magnetization to the Z axis and during the following mixing period, the non-equilibria Z compounds will exchange magnetization through NOE. After the laps of a brief period, during the mixing time, cross peaks will be observed in the spectra. In this work, NOESY spectra were recorded at 303 K and mixing time for peptide in the bound state was varied from 10 to 500 ms. Typical two-dimensional data sets consist of a data matrix of 1024 by 128 complex points, with 64 scans collected for each increment. The sweep width in both dimensions was 8012 Hz. However, the indirect dimension was zero filled during processing.

III. Result And Discussion

To understand the nature of the environment around the amide NH protons, the effects of polar solvent, DMSO-d₆, on the chemical shift values of the NH proton signals were studied. An idea about whether the NH protons are exposed to the solvent environment or not can be obtained by analyzing the spectra obtained for the peptide solutions of varied polarity. Table 1 lists the chemical shift values of the amide NH proton signals at different concentrations (percent volume/volume) of DMSO-d₆. The first column lists the percentage of DMSO-d₆ in the peptide solution in CDCl₃. The second, third and fourth columns list the chemical shifts of the Tyrosine, Methane/Leucine, and Alanine/Glycine NHs respectively. The fifth, sixth and seventh columns list the difference in chemical shifts of the Tyrosine, Methane/Leucine, and Alanine/Glycine NHs ($\Delta\delta$) for every percent increase in the DMSO-d₆ concentration. The last column represents the chemical shifts of the formyl hydrogen at different DMSO-d₆ concentrations.

N-for-Met-Tyr-Ala-OMe										
% DMSO-d ₆	Chemical Shifts			$\Delta(\delta T)$	$\Delta(\delta M)$	$\Delta(\delta A)$	Difference of Chemical Shifts			Formyl H
	Tyr NH	Met NH	Ala NH				$\delta T-\delta M$	$\delta T-\delta A$	$\delta M-\delta A$	
0	6.36	6.38	6.42	--	--	--	---	--	--	8.26
1	6.50	6.60	6.70	0.14	0.22	0.28	-0.08	-0.14	-0.06	8.23
2	6.60	6.80	6.90	0.10	0.20	0.20	-0.10	-0.10	0.00	8.17
3	6.79	6.94	7.14	0.19	0.14	0.24	0.05	-0.05	-0.10	8.15
4	6.85	7.10	7.20	0.06	0.16	0.06	-0.10	0.00	0.00	8.11
5	6.94	7.20	7.32	0.09	0.10	0.12	-0.01	-0.03	-0.03	8.08
6	7.08	7.30	7.40	0.14	0.10	0.08	0.04	0.06	0.06	8.06
7	7.17	7.40	7.50	0.11	0.10	0.10	0.01	0.01	0.01	8.02
8	7.19	7.30	7.42	-0.08	-0.10	-0.08	-0.02	0.00	0.00	7.96
9	7.19	7.39	7.50	0.00	0.09	0.08	-0.09	-0.08	-0.09	7.94
10	7.22	7.39	7.60	0.03	0.00	0.10	0.03	-0.07	-0.07	7.91
N-for-Leu-Tyr-Gly-OMe										
% DMSO-d ₆	Tyr NH	Leu NH	Gly NH	$\Delta(\delta T)$	$\Delta(\delta L)$	$\Delta(\delta G)$	$\delta T-\delta L$	$\delta T-\delta G$	$\delta L-\delta G$	Formyl H
0	6.38	6.40	6.42	--	--	--	--	--	--	8.18
1	6.50	6.60	6.64	0.12	0.20	0.22	-0.08	-0.10	-0.02	8.15
2	6.60	6.80	6.90	0.10	0.40	0.26	-0.30	-0.16	0.14	8.11
3	6.72	6.96	7.10	0.12	0.16	0.20	-0.04	-0.08	-0.04	8.07
4	6.88	7.18	7.30	0.16	0.22	0.20	-0.06	-0.04	0.02	8.04
5	7.00	7.33	7.50	0.02	0.25	0.20	-0.23	0.00	0.05	8.02
6	7.01	7.48	7.58	0.10	0.25	0.18	-0.15	-0.08	0.13	7.99
7	7.12	7.30	7.42	0.02	-0.02	0.24	0.00	-0.04	-0.04	7.92
8	7.18	7.50	7.58	0.06	0.20	0.16	-0.14	-0.10	0.04	7.90
9	7.20	7.50	7.60	0.02	0.00	0.02	0.02	0.00	-0.02	7.87
10	7.56	7.62	7.68	0.30	0.12	0.18	0.18	0.12	-0.06	7.85

Table 1: Chemical shifts of N-for-Met-Tyr-Ala-OMe and N-for-Leu-Tyr-Gly-OMe

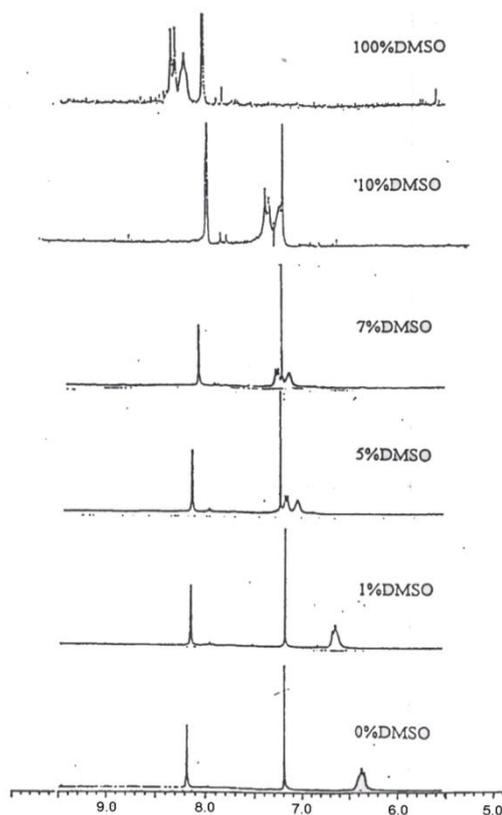


Fig. 1: Chemical shifts of N-for-Met-Tyr-Ala-OME

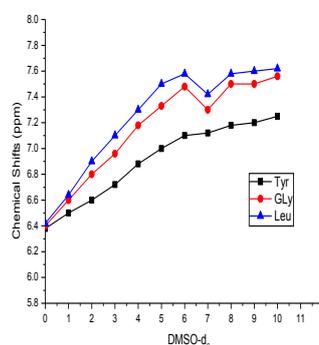
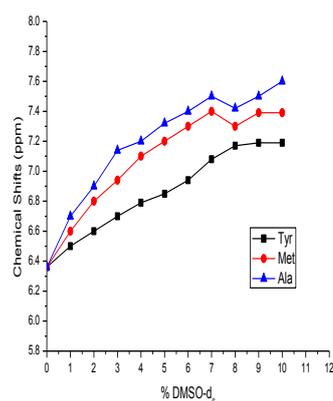


Fig. 2: The plots of δ vs % DMSO-d₆

Fig. 1 shows a plot of partial chemical shift values of the N-for-Met-Tyr-Ala-OME peptide Methane, Tyrosine and Alanine NHs Vs % of DMSO-d₆. It is observed that as the percentage of DMSO-d₆ is increased, the signal (at ~ 8.26 ppm) of the formyl hydrogen shifts slightly upfield, whereas the signal for all amino acids NHs shifts significantly downfield. Another observation that can be made from the figure is that at 0% DMSO-d₆ concentration, the proton signals for the Tyrosine NH is slightly more upfield than the signal for the other amino acids listed in Table 1. At 1% DMSO-d₆ concentration, the signals crossover and the proton signal of the Tyrosine NH is now slightly (0.01 ppm) downfield and further increase in DMSO-d₆ concentration, the proton signal of Tyrosine remains down field with respect to the other NH [9]. Therefore, the Tyrosine NH which was upfield with respect to allanine NH in CDCl₃ is shifted downfield with respect to glycine NH, on addition of DMSO-d₆. From columns six and seven of Table 1, it is observed that with an increase in DMSO-d₆ concentration, both glycine and alanine NH proton signals shift by similar values. DMSO-d₆ is a polar basic solvent and it can form a hydrogen bond with a hydrogen bond donor. In the peptide N-for-Met-Tyr-Ala-OME, the Tyrosine and alanine NH protons are the hydrogen bond donors [10-12]. DMSO-d₆, therefore, forms hydrogen bonds resulting in a decrease in electron density around the NH protons. This decrease in electron density results in deshielding of the alanine and Tyrosine NH protons. The deshielding causes a downfield shift of the alanine and Tyrosine NH proton signals. This downfield shift increases with increase in percentage of DMSO-d₆. The plots of chemical shift value (δ) vs % DMSO-d₆ are shown in Fig. 2. DMSO-d₆ being strong hydrogen bond acceptor solvent, bonds to the amide NHs. The formation of a hydrogen bond between the DMSO-d₆ and the amide NH protons, results in the downfield shift of the amide NH proton signal. Also, there was greater downfield shift of the Tyr NH proton signal in comparison to the other amino acids. This suggests that the Tyr NH is more expandable to add the DMSO-d₆.

CDCl₃ is proposed in order to explain the upfield shift of the formyl hydrogen between peptide molecules with increasing the percentage of DMSO-d₆. In this structure, the carbonyl of the formyl group is one of the hydrogen bond acceptors. This causes the carbonyl carbon of the formyl group to be more electronegative. Therefore, the shared pair of electrons between the formyl hydrogen and the formyl carbon is distorted towards the formyl carbon. As a result of this, the formyl hydrogen is deshielded. On further addition of DMSO-d₆, the hydrogen bond between peptide molecules break, and an intermolecular hydrogen bond between the DMSO-d₆ and the peptide molecule is formed [13]. This reduces the electronegative character of the formyl carbon. Therefore, the shared pair of electrons between the formyl hydrogen and the formyl carbon is

no longer distorted towards the formyl carbon. This change in electron distribution increases the shielding of the formyl hydrogen and causes an upfield shift of the formyl hydrogen signal.

Proton-Proton NOEs can establish spatial proximity of hydrogen atoms in organic molecules. NOESY spectrum of the peptide N-for-Met-Tyr-Ala-OMe was obtained at 20mM peptide concentration in CDCl₃. NOESY spectrum of the peptide shows the presence of cross peaks between the formyl H and OCH₃ methyl. In the NOESY spectrum of any compound, the presence of a cross peak between any two groups suggests that the distance between these groups is 3-4 Å⁰ [14-15]. Therefore, the distance between the formyl hydrogen and the OCH₃ methyl is 3-4 Å⁰. Based on the analyses of the spectra from solvent, temperature dependence, and the NOESY experiments, intermolecularly hydrogen bonded conformers such as the ones in Fig. 3 are proposed for the peptide N-for-Met-Tyr-Ala-OMe. The breaking of the hydrogen bonds is responsible for the shifts observed for the hydrogen signal. These conformers support the conclusion arrived at from the various NMR studies.

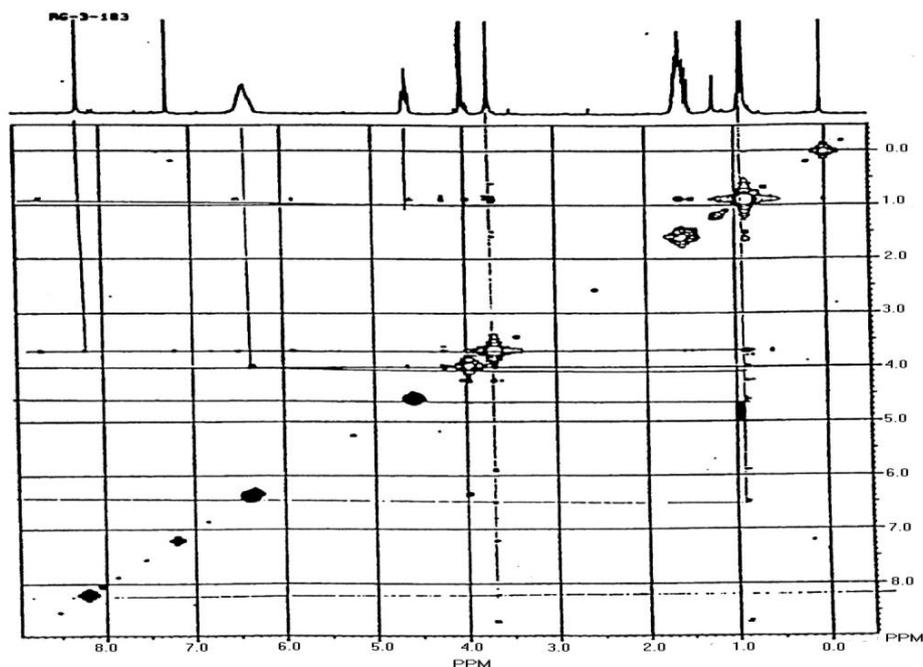


Fig. 3 NOESY Spectrum of N-for-Met-Tyr-Ala-OMe

On addition of DMSO-d₆, all the hydrogen bonds progressively break and a hydrogen bond between DMSO-d₆ and the NH of the peptide molecule is formed. The formation of the hydrogen bond between the amide NH proton and DMSO-d₆ explains the downfield shift of the NH protons and the upfield shift of the formyl hydrogen signal. When the concentration of the peptide is reduced and on increasing the temperature, the intermolecular hydrogen bonds are broken and the peptide molecule breaks free. This explains the upfield shift of the signal for the NH protons and the downfield shift of the formyl hydrogen signal with increase in DMSO-d₆. In the intermolecularly hydrogen bonded structure, the formyl hydrogen and the methyl of the methyl ester of leucine are close to each other. This conformer explains the cross peak observed between the two groups in the NOESY spectrum of the peptide.

IV. Conclusion

All amino acid NH protons of present investigated formyl peptides are the hydrogen bond donors. The formation of hydrogen bonds results in a decrease in electron density around the NH protons and this decrease in electron density results in deshielding of the NH protons. NOE results suggest that the intermolecular hydrogen bonds are broken and the peptide molecule breaks free when peptide concentration is reduced and the temperature is increased. This conformer explains the cross peak observed between the two groups in the NOESY spectrum of the peptide.

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