RIGIDITY IMPARTED TO THE BACKBONE CONFORMATION BY D-AMINO ACID RESIDUES: AN NMR STUDY OF ENKEPHALIN ANALOGS

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ABSTRACT

A comparison of 2D-NOESY spectra of [D-Ser^2]-leucine-enkephalin-threonine, [D-Ala^2]-leucine-enkephalinamide and leucine-enkephalinamide under identical experimental conditions has revealed that the backbone in the synthetic analogs having D-amino acid is relatively more rigid than leucine-enkephalinamide. This is a consequence of the replacement of Gly^2 by a D-amino acid residue in the peptide chain and is manifested in the observation/absence of interresidue NOESY cross peaks between NH and C$^\alpha$H of the preceding residue.

INTRODUCTION

OLIGOPEPTIDES normally exist in solution as an equilibrium blend of low energy conformers. Backbone conformational constraints can limit the number of such conformers in peptides[1] and hence in bioactive peptides, this could help in the selection of an active conformer. This is achieved by non-covalent modification that includes the incorporation of D-amino acids[2], N-methyl amino acids[3] and α-methyl amino acids[1]. The most frequently utilized and successful among these have been D-amino acids.

Enkephalins, the endogenous peptides (i.e. leucine/methionine enkephalins, (H_2N-Tyr^1-Gly^2-Gly^3-Phe^4-Leu^5-Met OH) are endowed with analgesic properties of morphine. The observation of similarity in the biological activity of enkephalins and morphin resulted in extensive studies delineating structure-function relationship[4–10] and the development of synthetic analogs[11,12] with greater potency and lesser addiction. The incorporation of D-amino acid residue (D-Ser or D-Ala in position-2) has resulted in more potent and receptor stereospecific enkephalins[13]. The enhanced potency has so far been believed to be due to the relatively greater stability of Tyr-D-Ser/D-Ala-peptide bond to peptidases[14]. Is this the only reason for greater potency? Conformational modification on the incorporation of D-amino acid could also be another possible source for enhanced biological activity and hence, calls for detailed investigations.

In this paper we have demonstrated from a comparative 1D and 2D NMR study of leucine enkephalinamide, [D-Ser^2]-leucine-enkephalin threonine and [D-Ala^2]-leucine-enkephalin amide that the backbone of leucine-enkephalinamide is relatively more flexible than that of its [D-Ala^2]- and [D-Ser^2] analogs. It is borne out from the present investigations that D-amino acid residues impart rigidity to the backbone of these synthetic analogs.

MATERIALS AND METHODS

Leucine enkephalinamide and its analogs [D-Ser^2] and [D-Ala^2] were purchased from Sigma. The peptides (2.5 mg) were dissolved in 0.5 ml of 99.98% DMSO-d_6. Proton magnetic resonance spectra were recorded on Bruker AM-500 MHz FT-NMR spectrometer. The chemical shifts were measured relative to DMSO peak and later converted to TSP (DMSO = TSP-2.6 ppm).

2D COSY and NOESY spectra were recorded with data matrix of 512×2048 and J-resolved with 128×4 K. The time domain data was multiplied by phase-shifted sine and sine square bell functions before Fourier transformation. 1D spectrum of [D-Ser^2]-leucine-enkephalin threonine is shown in figure 1. The assignment of resonances is made from the COSY experiment and NMR parameters are obtained from the J-resolved spectrum.

RESULTS AND DISCUSSION

The conformation of the backbone of oligopeptides[15] is governed by the torsion angles ϕ, ψ and ω (figure 1). Torsion angle ω is 180° because of the trans-orientation of the peptide bond. The magni-

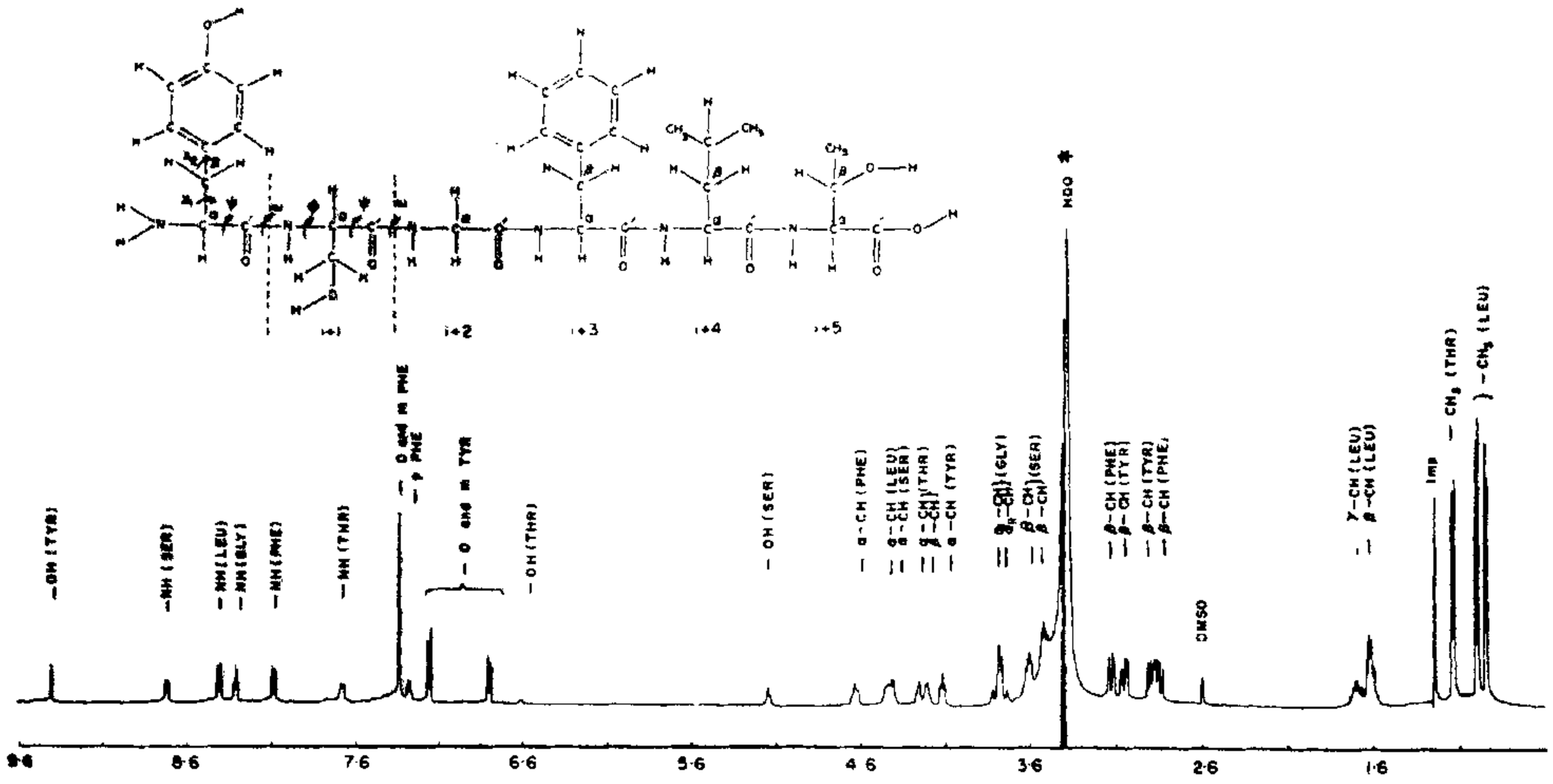

Figure 1. 500 MHz Proton spectrum of [D-Ser^2]-leucine-enkephalin-threonine in DMSO-d_6 (containing a trace of water) at 300 K. Chemical shifts are relative to TSP. The assignment of resonances has been made from 2D-COSY experiment.

tude of ϕ and ψ is manifested in various three bond coupling constants such as $J_{C^{\alpha}H\text{-}NH}$, $J_{^1HNC^{\alpha}\text{-}^{13}CC'}$, $J_{^{13}C'\text{-}NC^{\alpha}\text{-}^1H}$ and $J_{^1H\text{-}C^{\alpha}\text{-}C'\text{-}^{15}N}$ respectively. The observed value of J could reflect either the presence of a single rigid conformer or the time average value from the various low energy conformers. In addition, a single value of J corresponds to four different values of ϕ due to the nature of Bystrov curves[16] which correlate J with ϕ. Therefore the translation of J into conformation of the molecule requires the establishment of either a rigid conformer or an equilibrium blend of low energy conformers. This can be achieved by temperature variation studies as well as from pH and solvent variations[15].

The characteristics of the secondary structure of oligopeptides are also manifested in the NOESY cross peaks which reflect the dipolar coupling correlation and the chemical exchange between various protons[17]. NOE between the NH protons of the adjacent amino acid residues of a polypeptide chain has been called d_2-connectivity, while those between the NH protons and $C^{\alpha}H$ and $C^{\beta}H$ protons of the preceding residue, respectively, as d_1- and d_3-connectivities[18]. These connectivities help in the sequential assignment of resonances in peptides and proteins and also provide information about the secondary structure of the molecule[19]. Figures 2, 3

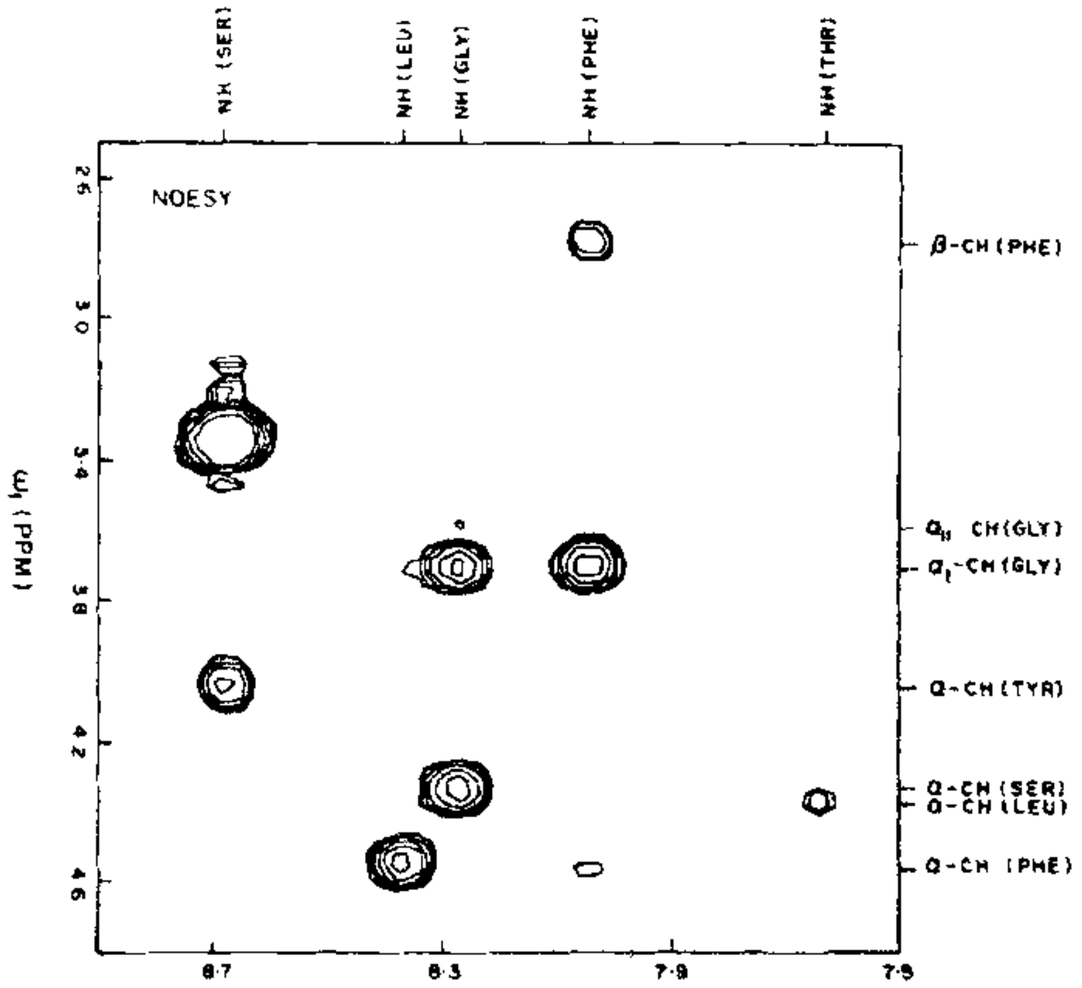

Figure 2. Expanded portion of 2D NOESY spectrum of [D-Ser^2]-leucine-enkephalin-threonine indicating the cross peaks between $C_i^{\alpha}H$, $C_i^{\beta}H$ and $N_{i+1}H$ protons. Mixing time used was 600 msec. The cross peaks at $\omega_2 = 8.74$ ppm and $\omega_1 = 3.35$ ppm is due to exchange of H_2O with NH of Ser. The cross peak at $\omega_2 = 8.09$ and $\omega_1 = 2.82$ is due to β-CH of Phe and –NH of Phe.

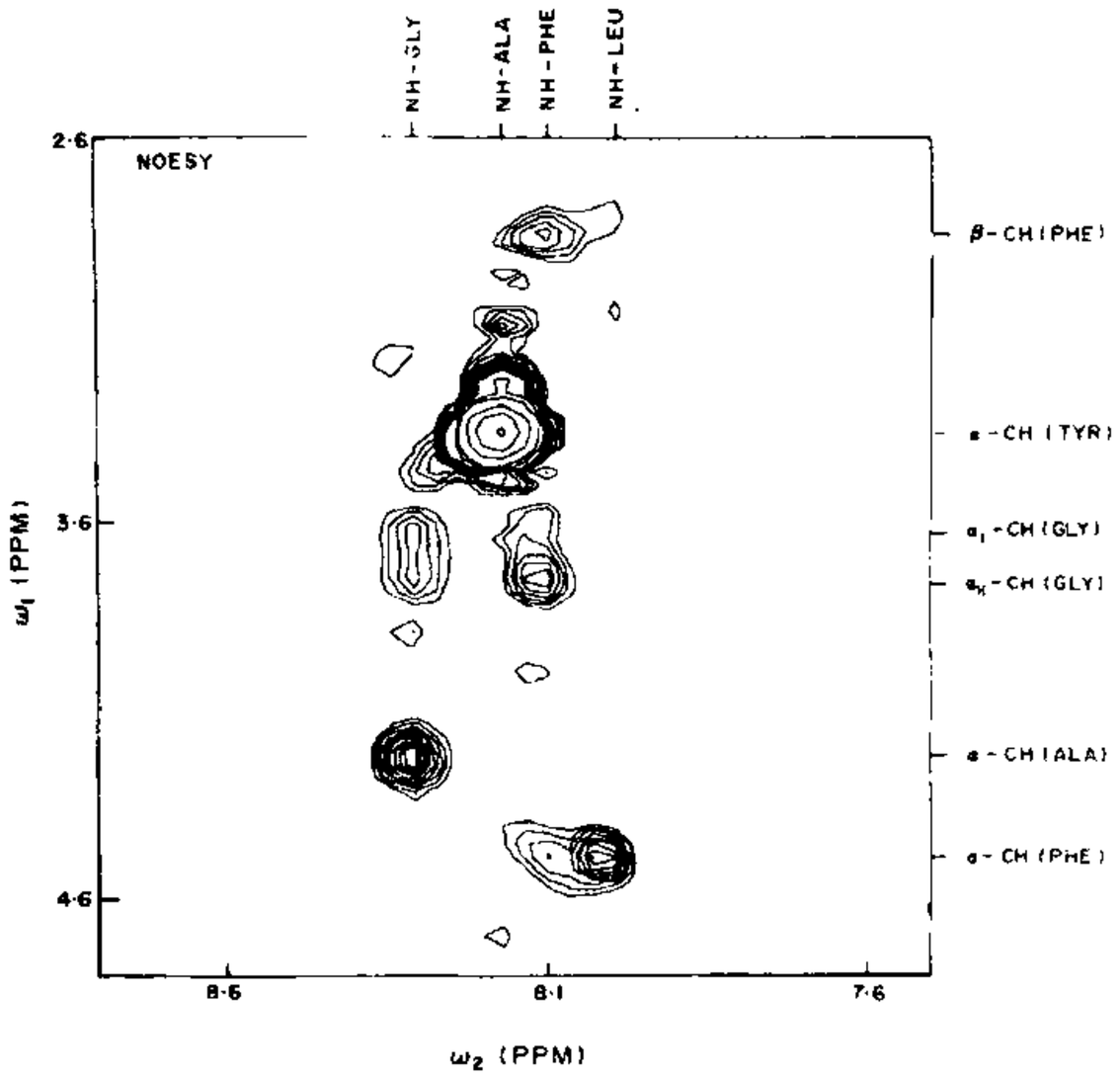

Figure 3. Expanded portion of 2D NOESY spectrum of [D-Ala^2]-leucine-enkephalinamide indicating the cross peaks between $C_i^{\alpha}H$, $C_i^{\beta}H$ and $N_{i+1}H$ protons. Mixing time used was 600 msec. The cross peak at $\omega_2 = 8.18$ ppm and $\omega_1 = 3.4$ ppm due to exchange of H_2O with NH of Ala. The peak at $\omega_2 = 8.13$ ppm and $\omega_1 = 2.92$ ppm is due to β-CH of Phe and NH of Phe.

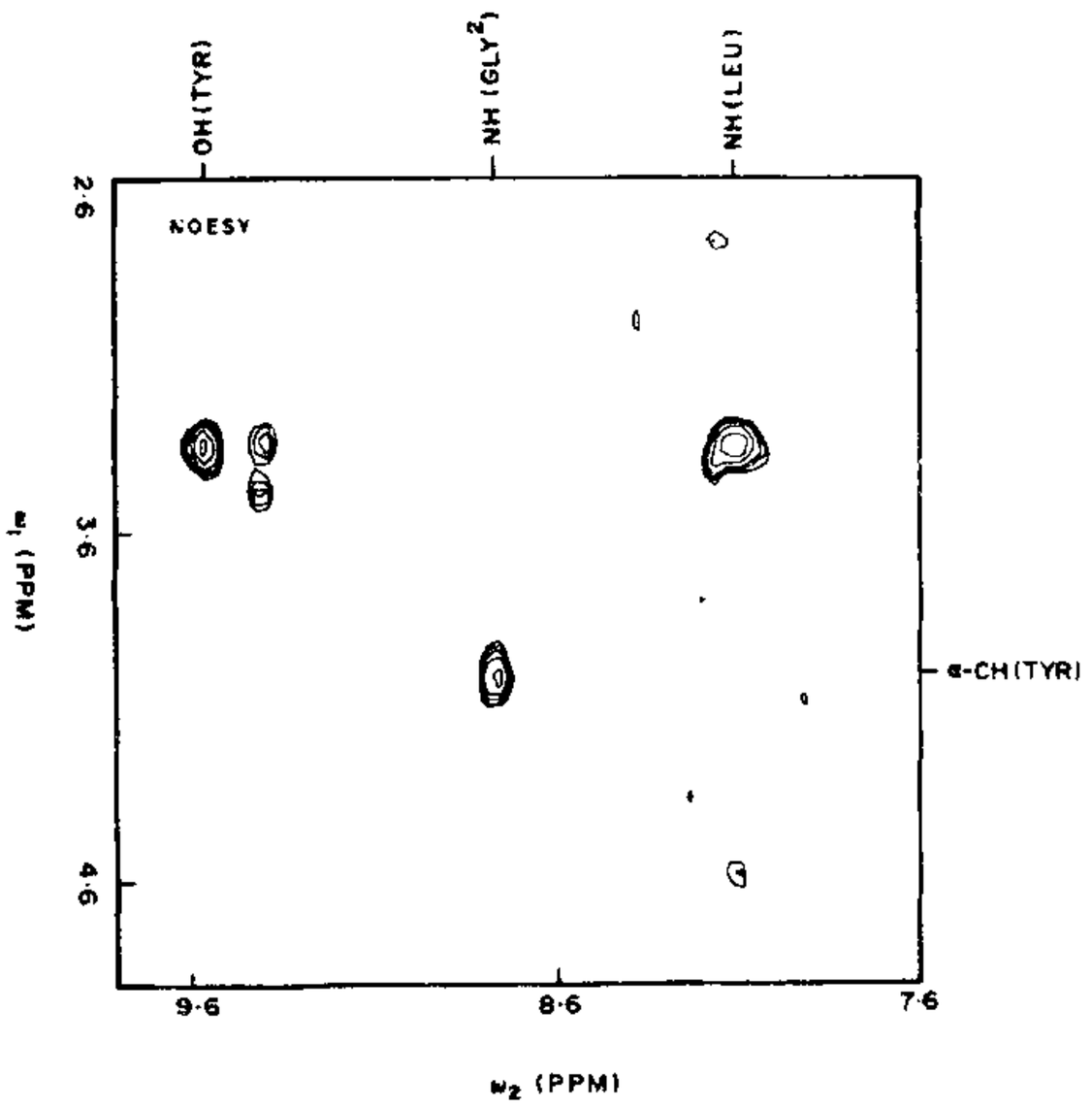

Figure 4. Expanded proton of 2D NOESY spectrum of leucine-enkephalinamide indicating the cross peak between $C_i^{\alpha}H$, $C_i^{\beta}H$ and $N_{i+1}H$ protons. Mixing time used is 600 msec. The cross peak at $\omega_2 = 9.6$ ppm and $\omega_1 = 3.4$ ppm due to exchange of H_2O with OH of tyrosine and the peak at $\omega_2 = 8.15$ ppm and $\omega_1 = 3.4$ ppm is due to exchange of leucine NH proton.

and 4 show the expanded portions of the NOESY spectra indicating cross peaks observed between the $C_i^{\alpha}H$ and N_{i+1} protons in [D-Ser^2], [D-Ala^2] and leucine-enkephalins respectively. A look at figures 2 and 3 indicates that all the interresidue NOESY cross peaks between $C_i^{\alpha}H$ and $N_{i+1}H$ are observed for [D-Ser^2]-leucine-enkephalin-threonine and [D-Ala^2]-leucine-enkephalinamide while in the case of leucine-enkephalinamide only a single cross peak between –NH of Gly^2 and the $C^{\alpha}H$ of Tyr is observed. The observation of such interresidue cross peaks in oligopeptides and proteins is a characteristic of β-structure[18–20]. In other words the distance between $C_i^{\alpha}H$ and $N_{i+1}H$ i.e. d_J is in the range of 2.2–2.4 Å and this reflects very limited conformational flexibility in the torsion angle. The absence of NOESY cross peaks between N_iH and $N_{i+1}H$ and $C_i^{\beta}H$ and $N_{i+1}H$ indicates the absence of short and long range ordering in the molecule.

The absence of a large number of interresidue NOESY cross peaks between $C_i^{\alpha}H$ and $N_{i+1}H$ in leucine-enkephalinamide (figure 4) suggests either different molecular correlation times τ_c or a different conformational preference[21]. Since the experimental conditions such as temperature, concentration and mixing times are identical and the molecules have similar molecular weights, it is expected that they will have identical correlation times. Therefore, the absence of NOESY cross peaks in figure 4 between $C_i^{\alpha}H$ and $N_{i+1}H$ in leucine-enkephalinamide clearly indicates relatively greater flexibility of the backbone relative to that in [D-Ser^2]- and [D-Ala^2]-analogs. This rigidity in the backbone is imparted due to the presence of D-amino acid residue in position-2 of enkephalins. Such backbone rigidity has also been deduced for [D-Ala^2]-methionine enkephalin from proton spin-lattice relaxation studies[22]. The influence of this backbone rigidity on the solution conformation of enkephalin analogs is being investigated in detail and the results will be reported elsewhere.

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