^{1}H NMR STUDIES OF [N-MeAib^{1}, Tyr(Me)^{4}]ANGII AND [N-MeAib^{1},Tyr(Me)^{4},Ile^{8}]ANGII IN DIMETHYLSULFOXIDE BY NUCLEAR OVERHAUSER EFFECT (NOE) ENHANCEMENT SPECTROSCOPY: *cis–trans* ISOMERISM OF THE HIS-PRO BOND*

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The conformational properties of the Sarmesin analogues [N-MeAib^{1},Tyr(Me)^{4}]ANGII and [N-MeAib^{1},Tyr(Me)^{4},Ile^{8}]ANGII in hexadeutero-dimethylsulfoxide were investigated by Nuclear Overhauser Effect (NOE) Enhancement Studies. *Cis-trans* isomers (ratio 1 : 6) due to restricted rotation of the His-Pro bond were observed. Interresidue interactions between the His C_{α} proton and the two Pro C_{δ} protons revealed that the major isomer was the *trans*.

Angiotensin II (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe; ANGII)** is an important component of the renin-angiotensin system, and has a direct involvement in blood volume and blood pressure homeostasis and in the etiology of certain forms of hypertension[1,2]. Knowledge of the conformation of angiotensin II and its analogues is required in order to understand the intricacies of the hormone–receptor interaction and to facilitate the design of therapeutically useful analogues. In this regard, angiotensin antagonists such as Sarmesin[3–5] (Sar-Arg-Val-Tyr(Me)-Ile-His-Pro-Phe) are of particular interest because blockade of ANGII action is desirable in the treatment of hypertension and congestive heart failure. The conformation of ANGII has been the subject of a large number of physicochemical and spectroscopic investigations[6–17]. The biologically active conformation of interest is likely to be that present as the hormone approaches the hydrophobic environment of the membrane receptor, rather than that found in aqueous media. Therefore solvents with intermediate polarity such as dimethylsulfoxide (DMSO) are potentially useful environments for studying the bioactive conformation. One of the more useful techniques

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** Abbreviations are according to the IUPAC–IUB Commission on Biochemical Nomenclature, 1984.

for determining the proximity of specific side-chains and thereby providing insights into the overall shape of the molecule, is the Nuclear Overhauser Effect (NOE) method utilized in NMR spectroscopy. This technique, which measures through-space interactions between neighboring nuclei, allows for a penetrating insight into certain aspects of peptide conformation. NOE studies in water often fail for molecules of the size of ANGII irrespective of the internuclear distances involved, because the tumbling rate for these solutes is close to that at which the maximum possible NOE passes through zero[18]. However the use of solvents of higher viscosity such as dimethylsulfoxide allows a stronger build up of the Nuclear Overhauser Effect, which is the fractional change in intensity of one NMR line when another resonance is saturated.

With the intent of understanding interresidue proton–proton backbone interactions in angiotensin II and consequently the interaction of this molecule with its receptor, we have examined the solution conformation of the Sarmesin analogues [N-MeAib^1, Tyr(Me)^4]ANGII and [N-MeAib^1, Tyr(Me)^4, Ile^8]ANGII in DMSO by NOE enhancement studies. In particular we have focused our attention on interactions between the His and Pro protons, in order to elucidate the configuration of the His-Pro peptide bond. These analogues were selected because their sharp Tyr(OCH_3) singlet at $\delta \sim 3{\cdot}60$ as well as the three sharp methyl singlets of the N-terminal N-MeAib (Aib : α-amino-isobutyric acid) residue at $\delta \sim 2{\cdot}2$ (for the N—CH_3 group) and $\delta \sim 1{\cdot}10-1{\cdot}12$ (for the two C—CH_3 groups) provide distinct probes in a clear area of the peptide NMR spectrum, for studying the spatial relationship between the Tyr^4 ring and the N-MeAib^1 moiety.

EXPERIMENTAL

[N-MeAib^1, Tyr(Me)^4]ANGII and [N-MeAib^1, Tyr(Me)^4, Ile^8]ANGII were synthesized by the solid phase technique using methods which have been described in detail previousy[5]. Cleavage of the peptide from the Merrifield resin by HF and purificaton by reversed-phase HPLC afforded the TFA salt of the peptide. The purified peptide (10 mg) was neutralized by passage through a column (1·5 × 3·0 cm) of carboxymethylcellulose (Whatman CM23) cation exchange resin. The peptide (10 mg) was first applied to the column in 0·01M ammonium acetate at pH 5 (5 ml) and then eluted with 0·5M ammonium acetate at pH 8 (10 ml). The effluent obtained at pH 8 was lyophilized three times and 5 mg of the product was dissolved in 0·5 ml of hexadeutero-DMSO and two drops of D_2O were added. Argon was bubbled through the sample for 5 min before the NMR tube was sealed.

NMR experiments were carried out using a Bruker 400 MHz NMR spectrometer. Data acquisition and data processing were controlled by an Aspect 3000 computer equipped with an array processor using 1987 DISNMR software. The chemical shifts were reported relative to the residual proton signal of hexadeutero-DMSO at 2·50 ppm with respect to tetramethylsilane. A total of 64 scans were accumulated to obtain a good signal-to-noise ratio. The methods used were similar to those described in detail previously[19].

One-dimensional NOE enhancement measurements were carried out in the difference mode using multiple irradiation. Each of the selected lines was irradiated 50 times for 100 ms (total

irradiation time 5·0 s). Other irradiation times (0·2, 0·5, 1, and 3 s) were also employed in some experiments to monitor the NOE buildup. A total of 1 000 scans for each line was required, and total relaxation time was 2 s. Under the experimental conditions which we have used for the NOE experiments (low power, different τ preirradiation times, saturation of control areas) spin diffusion and partial saturation were visibly minimized for the interactions under discussion. NOE enhancements were determined as the point increase in signal size per proton after saturation of a functionally distinct proton.

RESULTS AND DISCUSSION

One important feature in assessing the conformation which is assumed by ANGII and analogues in solution is the configuration of the His[6]-Pro[7] peptide bond[20]. The positioning of the aromatic rings in relation to each other is also of interest. Recent NMR studies in dimethylsulphoxide have indicated proximity of the histidine and phenylalanine rings[21]. This interaction is compatible with the presence of

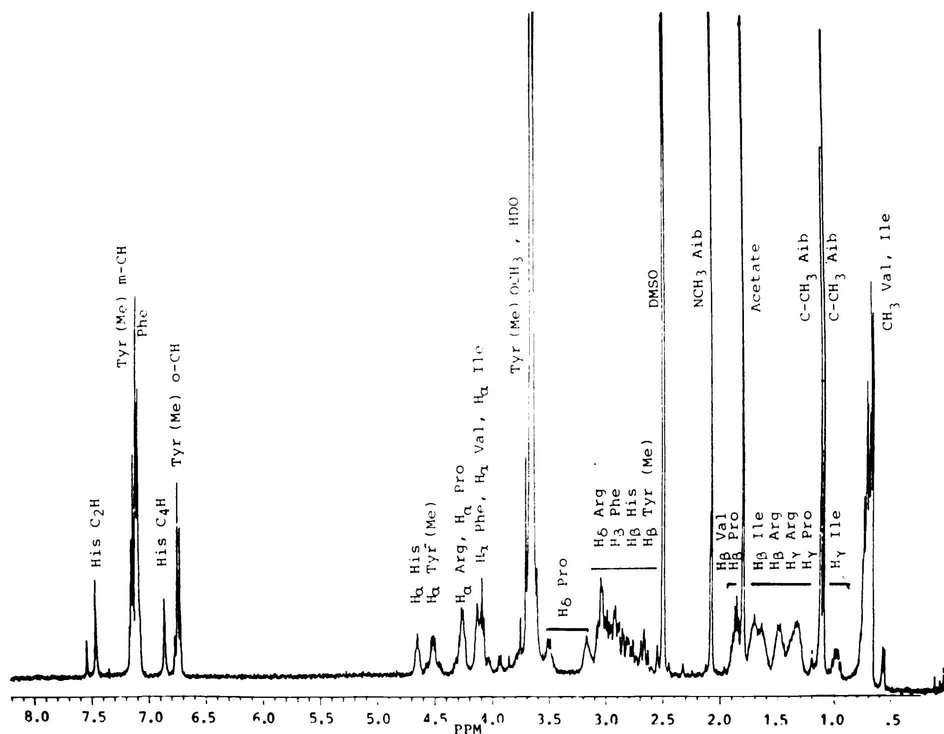

FIG. 1

^{1}H NMR spectrum for [N-MeAib^{1}, Tyr(Me)^{4}]ANGII at 400 MHz in hexadeuterodimethylsulfoxide after D_2O exchange

a γ-turn[8] and a *trans* His-Pro peptide configuration for the C-terminal tripeptide (His-Pro-Phe)[22].

In the present study the one-dimensional NMR spectra of [N-MeAib[1], Tyr(Me)[4]]-ANGII and [N-MeAib[1], Tyr(Me)[4], Ile[8]]ANGII in hexadeutero-DMSO showed a complex downfield region with broad overlapping NH resonances indicating fast exchange. To simplify the C_α proton and aromatic regions and to study intra-residue and interresidue proton–proton interactions, the NMR experiments were carried out after the NH's were exchanged with D_2O. *Cis–trans* isomers (ratio 1 : 6)

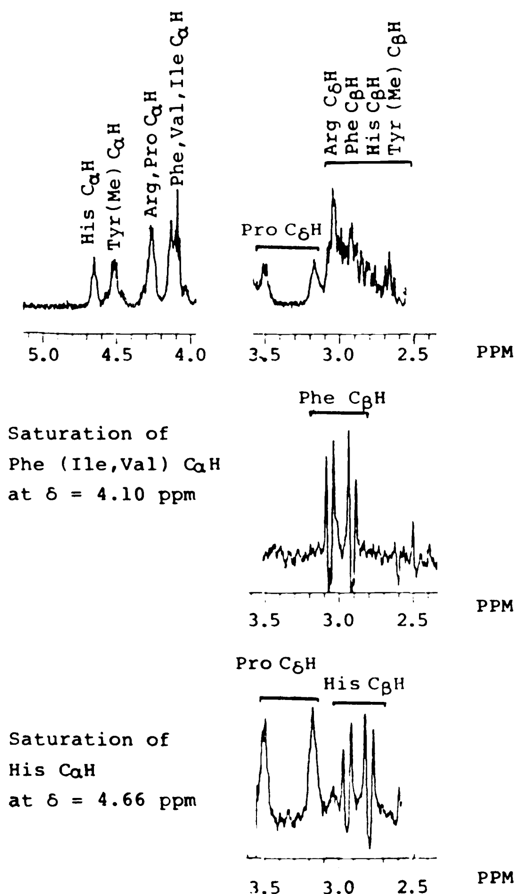

FIG. 2

^{1}H NMR NOE difference spectra for [N-MeAib[1], Tyr(Me)[4]]ANGII (acetate form) in hexadeuterodimethylsulfoxide

due to restricted rotation of the His-Pro bond were observed based on the relative intensities of the His C_2 and C_4 proton resonances (Fig. 1). Assignment of all backbone and side-chain proton resonances was possible by combined NOE and COSY experiments. Under the selected experimental conditions, interresidue interactions between the His C_α and Pro C_δ protons were observed, indicating a *trans* configuration for the His-Pro peptide bond. The NOE difference spectra for [N-MeAib^1, Tyr(Me)^4]ANGII resulting after saturation of the His C_α, Tyr(Me) C_α and Phe-(Val, Ile) C_α protons, show enhancements of the vicinal β protons revealing their pattern (AB quartet) and exact position in the crowded aliphatic region of the reference spectrum.

Thus, the NOE difference spectrum which resulted after saturation of the His C_α proton at $\delta = 4.66$ ppm, showed an AB quartet at $\delta = 2.86$ attributable to the two vicinal His C_β protons. Similarly, the NOE difference spectra which resulted after saturation of the Tyr(Me) C_α proton at $\delta = 4.52$ and the Phe C_α at $\delta = 4.11$, show an AB quartet for the vicinal Tyr(Me) and Phe C_β protons at $\delta \sim 2.70-3.00$. However the NOE difference spectrum resulting after saturation of the His C_α proton also shows two strong resonances at $\delta = 3.15$ and 3.48 due to enhancement of the two Pro C_δ protons (Fig. 2). Both Pro C_δ protons are almost equally affected, revealing close proximity and equidistance of both of these protons from the His C_α proton.

This interaction has also been observed in [N-MeAib^1,Tyr(Me)^4,Ile^8]ANGII. The NOE difference spectrum resulting after saturation of the His C_α proton at $\delta = 4.68$ show similarly two strong resonances at $\delta_1 = 3.65$ and $\delta_2 = 3.32$, due to enhancement of the two Pro C_δ protons. Under the experimental conditions used, interresidue interactions between the methyl groups of Tyr^4 and Aib^1 with other groups were not observed. The expected intraresidue interactions between the OCH_3 proton(s) and the *ortho* proton of the Tyr^4 residue as well as between the NCH_3 and the CCH_3 of the N-MeAib^1 residue were present for both analogues.

The present findings suggest that both analogues contain the same γ-turn bend at the C-terminal tripeptide as it has been suggested[8] for ANGII and confirm the presence of a predominantly *trans* configuration for the His^6-Pro^7 bond. This bend allows for a clustering of the aromatic rings (Tyr, His, Phe) which is an important feature of the charge relay system, believed to be present in ANGII and certain analogues[23,24]. However, the NMR evidence does not provide any indication that the rings of the present analogues are close enough to produce interresidue NOE effects, suggesting a more relaxed overall structure for these two N-MeAib analogues. This is in accord with the biological activities of these analogues, which are quite low ($pA_2 = 5-6$) compared to Sarmesin ($pA_2 = 7.7$) (ref.[5]).

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