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NMR STUDIES ON ANGIOTENSIN II: HISTIDINE AND PHENYLALANINE RING STACKING AND BIOLOGICAL ACTIVITY

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The conformations of angiotensin II and the antagonist [Sar^1,Ile^8]angiotensin II in dimethylsulfoxide have been examined by high resolution proton magnetic resonance spectroscopy at 400MHz. The chemical shifts for the aromatic protons of the phenylalanine residue in angiotensin II are consistent with shielding and restricted rotation for this side-chain. The chemical shifts for the histidine C_2 and C_4 protons in angiotensin II also indicate shielding, whereas these same protons in the antagonist [Sar^1,Ile^8]angiotensin II do not demonstrate this shielding influence. These findings suggest a stacking interaction for the histidine and phenylalanine side-chains in angiotensin II which is important for activating angiotensin receptors.

The octapeptide angiotensin II (ANG II), Asp-Arg-Val-Tyr-Ile-His-Pro-Phe, has been implicated in the pathogenesis of hypertension and congestive heart failure, and knowledge of its conformation is important for the design and synthesis of improved antagonists. The angiotensin analog [Sar^1,Ile^8]ANG II antagonizes the effects of angiotensin at all known central and peripheral receptors. The conformational constraints produced by the replacement of the C-terminal aromatic side-chain in ANG II with a non-aromatic side-chain are not well understood in terms of the hormone-receptor interaction. From NMR studies on these peptides we suggest that a stacking interaction between the His and Phe side-chains in ANG II is an important aspect of the mechanism of activation of angiotensin receptors.

MATERIALS AND METHODS

ANG II and [Sar^1,Ile^8]ANG II were products of Peninsula Laboratories and contained a single peptide by reversed-phase

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HPLC(1). Proton magnetic resonance (PMR) spectra were obtained with a Bruker 400MHz spectrometer after dissolution of the peptides (5mg) in dimethylsulfoxide (DMSO). Assignments were made by comparison to the spectra for free Tyr, Phe and imidazole in DMSO, and after D_2O exchange of the peptides.

RESULTS AND DISCUSSION

The PMR spectra for ANG II and [Sar^1,Ile^8]ANG II in DMSO are shown in Fig. 1 and the chemical shifts for the aromatic protons of the side-chains of the Tyr, His and Phe residues are given in Table 1. By comparison to free Phe (singlet at 7.30ppm), the chemical shifts for the Phe residue in ANG II are characterized by doublet splitting and an overall upfield shift of the signals for this side-chain. These observations indicate that the Phe ring in ANG II is subject to a shielding influence and demonstrates restricted rotation. The shielding effect on the Phe ring is sufficiently large to invoke the possibility of a Π-Π^* type of stacking interaction with either the His or Tyr rings in ANG II. The chemical shifts for the aromatic Tyr protons are similar for both ANG II and [Sar^1,Ile^8]ANG II (Table 1), suggesting that this residue can be eliminated as the source of shielding of the Phe side-chain in ANG II. The signals for the His C_2 and C_4 protons in ANG II, on the other hand, demonstrate evidence of a shielding influence which could originate from a stacking interaction with the Phe ring. This interpretation is based on the observation that the His C_2 and C_4 protons in[Sar^1,Ile^8]ANG II are shifted downfield compared to ANG II and are apparently not subject to this shielding influence. Thus when the Phe ring is absent in an angiotensin analog, the shielding influence on the His side-chain is removed.

Based on the chemical shifts for the aromatic protons of His and Phe in both ANG II and [Sar^1,Ile^8]ANG II, it is suggested that a stacking interaction of the His and Phe rings

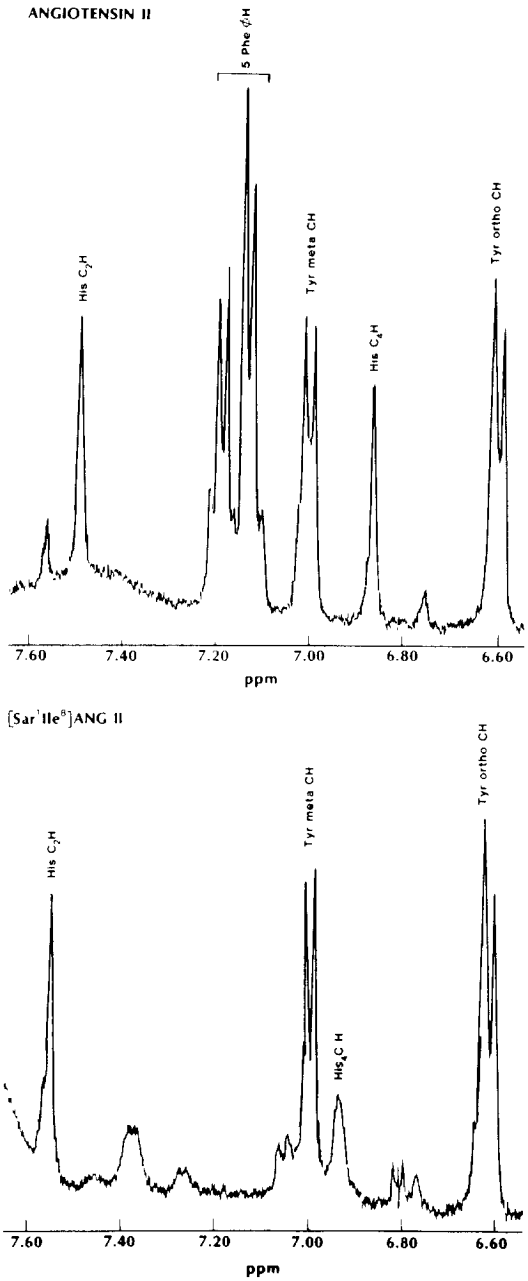

Figure 1. Proton magnetic resonance spectra (aromatic protons) at 400MHz for angiotensin II and [Sar^1,Ile^8]angiotensin II in dimethylsulfoxide.

exists in ANG II in DMSO. Although it is not known if DMSO represents a reasonable facsimile of the hydrophobic environment at membrane receptors, we suggest that this stacking interaction may be an important feature of the

Table I. Chemical shifts for the aromatic protons of ANG II and [Sar^1,Ile^8]ANG II in dimethylsulfoxide

	Tyr		His		Phe	
	meta	ortho	C_2	C_4		
Angiotensin II	7.00(d)	6.60(d)	7.47	6.86	7.18(d)	7.12(d)
[Sar^1,Ile^8]ANG II	7.00(d)	6.61(d)	7.54	6.94	--	

d=doublet. Chemical shifts for the aromatic protons of [Sar^1,Ile^8]ANG II also apply to [Ile^8]ANG II (Matsoukas and Moore, unpublished work).

receptor-bound conformation of ANG II. It also appears that when this stacking interaction is not present in ANG II analogs, these analogs are unable to activate the receptor mechanism and, as is the case for [Sar^1,Ile^8]ANG II, can demonstrate antagonist properties.

Stacking of the Tyr and Phe rings in vasopressin has been suggested to be an important feature of the vasopressin conformation which differentiates its biological activity from the structural analog oxytocin(2). The stacking interaction in vasopressin results in a 0.1ppm upfield shift for the aromatic proton signals of the Phe residue in water(2). Aromatic ring stacking interactions are commonly observed in a variety of biological structures including peptides, proteins and DNA. For the peptide hormones, the electronic nature of the stacking interaction and its significance in relation to the receptor environment are not well understood at the present time. The possibility should be considered that the overlap of p orbitals (and consequent exclusion of water) in a stacking interaction at the receptor may be responsible for driving the receptor response mechanism, possibly by producing a local condenser plate effect. The capacitance could be magnified if the receptor in turn contributed a third aromatic nucleus to the stacking interaction.

In conclusion, aromatic ring stacking in both ANG II and vasopressin has an important role in the mechanism of activation of their respective receptors. It may be no coincidence that a primary biological activity of both of these peptides is the induction of contraction of vascular smooth muscle.

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REFERENCES

1. Matsoukas, J.M., Scanlon, M.N., and Moore, G.J. (1984) J. Med. Chem. 27, 404-406.
2. Deslauriers, R., and Smith, I.C.P. (1978) Biochem. Biophys. Res. Commun. 40, 179-185.