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Proton Magnetic Double-Resonance Study of Angiotensin II (Asn^1Val^5) in Aqueous Solution Employing Correlation Spectroscopy. Assignment of Peptide NH Resonances and Transfer of Saturation from Water†

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ABSTRACT: Complete assignment of the peptide NH resonances of angiotensin II (Asn^1Val^5) (AII′) in aqueous solution has been accomplished by double irradiation of α-CH absorptions. The Karplus equation was employed to calculate the dihedral angles ϕ of specific HNCH fragments from corresponding peptide NH–α-CH coupling constants. These data exclude the α helix, conventional β turn, γ turn, random coil, and the structure proposed by Weinkam and Jorgensen (*J. Amer. Chem. Soc. 93*, 7038 (1972)) as the preferred solution conformation of AII′ in water. Although the data are consistent with an order–disorder equilibrium, additional information is required for a definitive conformational analysis. The data suggest that the solution conformation of AII′ differs from its conformation at the receptor site. Transfer of saturation from the H_2O hydrogens indicates that the Arg^2 peptide NH hydrogen exchanges with the solvent at a significantly more rapid rate than do the other peptide NH hydrogens.

Angiotensin II (Asn^1Val^5) (AII′),[1] an octapeptide hormone with amino acid sequence $Asn^1Arg^2Val^3Tyr^4Val^5$-$His^6Pro^7Phe^8$, has a broad range of biological activity (Peach, 1972; Page and McCubbin, 1968; Fisher, 1971; Needleman *et al.*, 1972). Conformational analysis of AII′ by proton magnetic resonance (pmr) spectroscopy requires that resonances be identified with specific hydrogens of this peptide. An extensive pmr study of AII′ (Glickson *et al.*, 1972a, 1973) included the assignment of all the CH resonances observed in the spectrum of AII′ in D_2O solution.

Of particular significance to conformational studies are the NH resonances observed in spectra of AII′ in H_2O solution. The cis and trans Asn primary amide NH resonances and the four proton Arg guanidino NH peak were assigned by straightforward comparison with other compounds (Glickson *et al.*, 1972a, 1973). However, the intense absorption of the solvent

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[1] Abbreviations used are: AII′, angiotensin II (Asn^1Val^5); pmr, proton magnetic resonance.

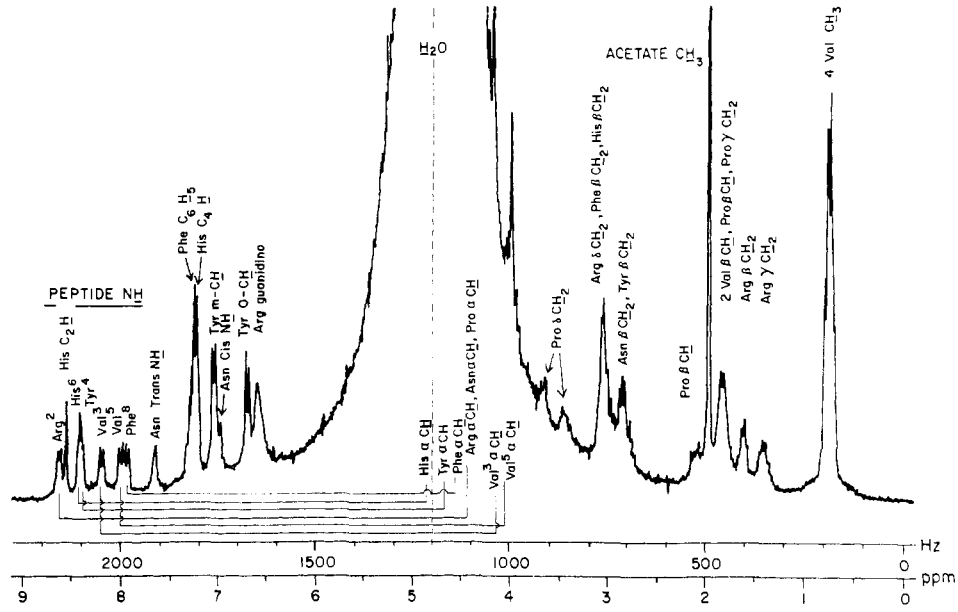

FIGURE 1: 250-MHz pmr spectrum of 7.6% (w/v) AII′ in H_2O at pH 3.0 and 30°. Lines indicate resonances of coupled protons as demonstrated by double-resonance experiments.

hindered assignment of the peptide NH peaks by conventional spin decoupling from corresponding α-CH resonances. The Phe NH peak was assigned by characteristic displacement of this resonance when the C-terminal carboxyl group was titrated. Rapid exchange with the solvent, expected for an N-terminal peptide group, yielded a tentative assignment of the Arg peptide NH peak. Subsequently, Bleich *et al.* (1973) arrived at a similar assignment of the terminal peptide absorptions and identified the remaining peptide NH resonances on the basis of the slow exchange of the Val hydrogens and displacement of the His resonance in the pH region of imidazole titration.

This assignment is, however, only tentative and incomplete. The Val^3 and Val^5 NH peaks remain to be distinguished, and the assignment of Arg, Tyr, and His peptide NH resonances needs to be confirmed. Acid–base catalysis could produce anomalous rapid exchange with the solvent of a peptide hydrogen other than Arg. Furthermore, a conformational change in the region of imidazole titration produced displacement of many resonances other than those associated with the His residue (Glickson *et al.*, 1972a, 1973; Vine *et al.*, 1973). For these reasons we undertook a definitive assignment of all the peptide resonances.

Two methods are available for this purpose. Decoupling could be performed in a nonprotic solvent and then the positions of the peptide peaks could be tracked as this solvent was progressively enriched with H_2O (Glickson *et al.*, 1972b). Alternatively, one can perform the decoupling experiment directly in H_2O by carefully avoiding saturation of amplifiers in the spectrometer (Dadok *et al.*, 1972a; Von Dreele *et al.*, 1972). Because positions of α-CH peaks in H_2O were already known from previous studies in D_2O (Glickson *et al.*, 1972a, 1973), the latter approach was chosen. The spectrometer employed in this study offered the additional advantage of measuring spectra by the correlation method—a technique which offers the signal enhancement advantages of Fourier transform nmr spectroscopy, but circumvents the serious dynamic range problem, which hampers employment of the latter technique to measure spectra of samples in H_2O solution (Dadok *et al.*, 1972b,c, 1973).

Materials and Methods

AII′, a generous gift from Dr. Werner Rittel of Ciba Pharmaceuticals (Basel, Switzerland), was used without further purification. The pH was adjusted with HCl and NaOH. The 250-MHz spectrometer and the decoupling procedure have been previously described (Dadok *et al.*, 1970, 1972a–c; Dadok and Sprecher, 1973; Von Dreele *et al.*, 1972). Each spectrum was the sum of 20 scans measured by the correlation method. Chemical shifts were measured relative to the methyl resonance of sodium 2,2-dimethyl-2-silapentane-5-sulfonate, the internal standard added after completion of the experiment.

Results and Discussion

The spectrum of AII′ in H_2O appears in Figure 1. The indicated assignments, except those of the peptide NH resonances, which appear at the extreme low-field end of the spectrum (7.8–8.8 ppm), were all previously obtained (Glickson *et al.*, 1972a, 1973). A strong methyl resonance, originating from acetate buffer introduced during purification of the hormone, appears at 1.98 ppm. The intense water resonance dominates the center of the spectrum. Buried beneath this peak are the eight α-CH resonances, whose positions are also indicated. The pH (3.0) was judiciously chosen to optimize resolution of all the α-CH peaks that are coupled to peptide NH absorptions. Overlap of Arg, Asn, and Pro α-CH peaks occurred at this pH, but of these, only the Arg peak was coupled to a peptide NH.

Figure 2 displays the peptide NH peaks on an expanded scale. Also shown is the sharp C_2H resonance, which emanates from the imidazole group of His. When only the resonance frequency was applied (Figure 1a), each of the peptide peaks was a doublet as a result of spin–spin coupling with the vicinal α-CH hydrogen. Two of these doublets overlapped to give an apparent triplet centered at 8.45 ppm. Application of a second rf at the resonance frequency of a particular α-CH hydrogen effectively decoupled the resonance of the vicinal peptide NH hydrogen. Thus, when the Val^5 α-CH resonance was irradiated

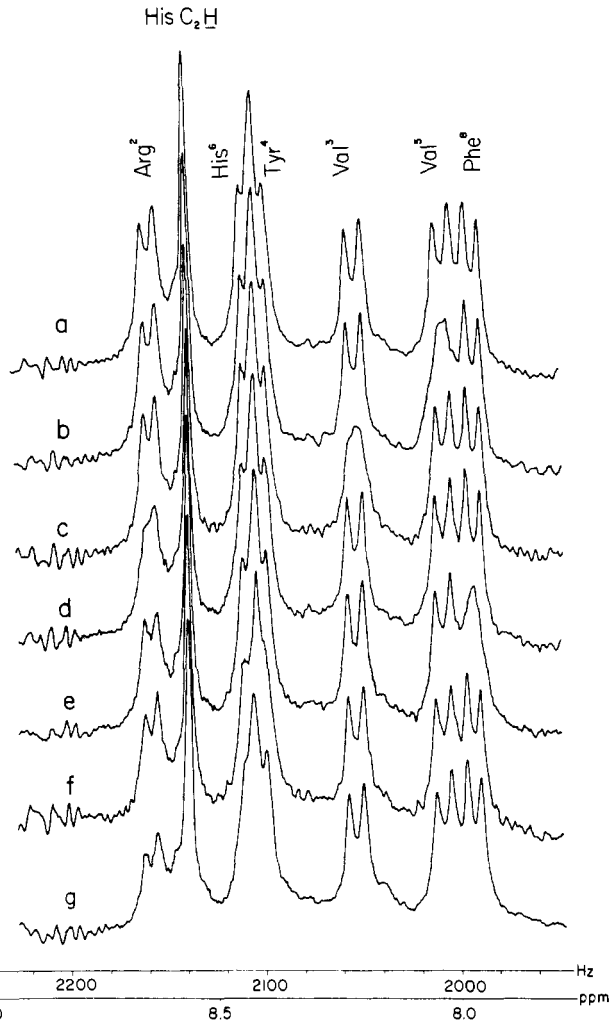

FIGURE 2: Peptide NH resonances of AII′ in H_2O with decoupling radiofrequency power applied at f_2, the resonance frequencies of α-CH resonances: (a) f_2 off, (b) f_2 = 1005.4 Hz, Val^5, (c) f_2 = 1029.4 Hz, Val^3, (d) f_2 = 1104.4 Hz, Arg^2, (e) f_2 = 1133.4 Hz, Phe^8, (f) f_2 = 1161.4 Hz, Tyr^4, and (g) f_2 = 1208.9 Hz, His^6.

(Figure 1b), the doublet at 8.04 ppm collapsed to a singlet. In this manner the Val^5 and all the other peptide NH peaks were unambiguously identified (Figure 2).

Figure 3 shows the peptide NH region of the spectrum before and after application of saturating radiofrequency power at the resonance frequency of H_2O. Significant transfer of saturation to the Arg peptide NH was observed, *i.e.*, the relaxation time associated with exchange of water protons and Arg peptide NH protons was short relative to their spin–lattice relaxation time. Rapid exchange of the Arg peptide NH hydrogen was also manifested in broadening of its resonance (Glickson *et al.*, 1972a, 1973) and rapid exchange with D_2O (Bleich *et al.*, 1973). The inductive effect of positive charges of the proximal α-amino group and guanidino group are believed to cause this effect.

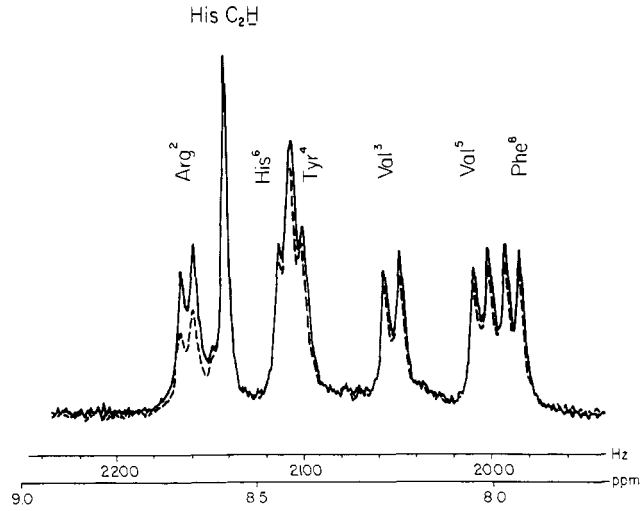

FIGURE 3: Peptide NH region of the pmr spectrum of AII′ during double irradiation of the water resonance. Diminution of the intensity of the Arg^2 resonance is caused by transfer of saturation from water protons.

The complete assignment of AII′ NH resonances is summarized in Table I. It is gratifying to note that it is in full agreement with the tentative proposals previously made (Glickson *et al.*, 1972a, 1973; Bleich *et al.*, 1973). Table I also contains the peptide NH–α-CH coupling constants (J) and dihedral angles ϕ for the HNCH fragment (Marshall *et al.*, 1973a). The latter quantities were calculated from the equation theoretically derived by Barfield and Karplus (1969)

$$J = A\cos^2\theta + B\cos\theta + C\sin^2\theta \qquad (1)$$

TABLE I: Summary of AII′ NH Resonance Data in H_2O, at 30° and pH 3.0.

Assignment	Chemical Shift (ppm)	Coupling Constant (Hz)	HNCH Dihedral Angles (ϕ), deg
Peptide NH's			
Arg^2	8.64	6.5 ± 0.3	−159 ± 3, −81 ± 3, 60 ± 10
Val^3	8.20	7.9 ± 0.3	−147 ± 2, −93 ± 2
Tyr^4	8.41	7.2 ± 0.5	−154 ± 5, −86 ± 5
Val^5	8.02	8.0 ± 0.4	−146 ± 4, −94 ± 4
His^6	8.43	6.0 ± 0.5	73 ± 13, 47 ± 13, −164 ± 4, −76 ± 4
Phe^8	7.96	7.3 ± 0.3	−153 ± 3, −87 ± 3
Asn′ primary amides			
Cis	7.02		
Trans	7.68		
Arg^2 NH—$C(NH_2)$⋯⋯NH_2^+	6.63		

where $\theta = |\phi - 60°|$ and A, B, and C are constants empirically estimated as 7.9, −1.55, and 1.35 Hz, respectively, by Ramachandran *et al.* (1971).

It has been noted (Glickson *et al.*, 1972a, 1973; Marshall *et al.*, 1973a) that these data exclude the α helix, conventional β turn, γ turn (Printz *et al.*, 1972; Nemethy and Printz, 1972), and the structure proposed by Weinkam and Jorgensen (1972) as major contributors to the conformational equilibrium. An order–disorder equilibrium, as proposed by Fermandjian *et al.* (1971, 1972a,b), may be consistent with the data. A β turn in which the corner residue (Tyr) assumes an orientation opposite to that normally assumed (Printz *et al.*, 1972) would produce coupling constants comparable to those observed only if substantial conformational averaging with particular disordered structures is assumed. However, potential energy calculations (Nemethy and Printz, 1972), X-ray data (Marshall *et al.*, 1973c), and deuterium-exchange kinetics data (Bleich *et al.*, 1973) all argue against such a structure. It has also been noted that these coupling constants indicate a difference between the conformations of AII′ in free solution and at the receptor site (Marshall *et al.*, 1973b).

Additional evidence is required for a definitive determination of the solution conformation of AII′. The number of structures significantly contributing to the conformational equilibrium must also be determined. Completion of the assignment of all the resonances in the pmr spectrum of AII′ provides a very powerful tool for answering these questions. The assignment also provides a method for monitoring in a highly specific manner interactions of AII′ with other molecules and relating these interactions to the biological activity of this hormone.

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