EVIDENCE FROM PROTON MAGNETIC RESONANCE DATA FOR THE STACKING OF AROMATIC AMINO ACIDS IN LYSINE-VASOPRESSIN: COMPARISON WITH OXYTOCIN DERIVATIVES AND RELATED DIPEPTIDES[1]

Roxanne Deslauriers* and Ian C.P. Smith**

Department of Biochemistry*,
University of Ottawa.

Biochemistry Laboratory**,
National Research Council of Canada,
Ottawa 7, Ontario, Canada.

Received May 28, 1970

ABSTRACT

Shifts of some of the aromatic resonances of tyrosine and phenylalanine in lysine-vasopressin relative to the values observed in monomers and in substituted oxytocins are indicative of stacking of the aromatic rings. Consideration of the nature and magnitudes of the shifts leads to a model for the stack. Similar but smaller shifts are found in the related dipeptides.

It is well known that in aqueous solutions nucleotides containing aromatic rings, such as adenosine and guanosine, tend to form intermolecular stacks with the planes of the aromatic moieties parallel (1-4). To a large extent this has been determined by proton magnetic resonance, making use of large diamagnetic anisotropy of the aromatic rings which induces changes in the chemical shifts of the nearby protons (5). This ring current effect has been invoked to explain the splitting of the methyl proton resonance in DNA (6,7) and the appearance of the proton resonances at fields

[1] N.R.C.C. Number

higher than that of tetramethylsilane in the PMR spectra of proteins (8-10).

We wish to report here an example of this phenomenon in the cyclic oligopeptide lysine-vasopressin. The simplicity of the compound, and the observed spectral shifts, afford a good example of the ring current effect in polypeptide systems containing phenylalanine and tyrosine, and should therefore be helpful when considering such effects in more complex systems.

EXPERIMENTAL

Lysine-vasopressin from hog pituitaries was a generous gift of the National Institute of Health, U.S.A. The compound was purified by chromatography on Sephadex; amino acid analysis showed it contained only the amino acids known to constitute lysine-vasopressin, in the correct proportions (Figure 1,11). The material as received had a quoted vasopressor activity of approximately 260 I.U./mg., assayed according to U.S. Pharmacopeia XV in the male rat treated with Dibenzyline anesthetized with urethane.

The substituted oxytocin derivatives were synthesized by Professor M. Manning, Medical College of Ohio, Toledo, and were loaned to us by Professor L. Benoiton of the University of Ottawa. Phenylalanine and tyrosine were products of Calbiochem.

The original solutions were lyophilized three times with D_2O to minimize H_2O and remove residual NH_4Ac. The lyophilized powders were dissolved in D_2O at pD 7.0.

PMR spectra were measured on a Varian HA 100 spectrometer, using hexamethyldisiloxane in an internal

capillary as a reference; and on a Varian HR 220 spectrometer, using the sodium salt of 3-(trimethyl silyl)-propanesulfonic acid (DSS) as an internal reference. Time averaging was done on a Varian C-1024 computer.

RESULTS AND DISCUSSION

Lysine-vasopressin contains two contiguous aromatic amino acids, phenylalanine and tyrosine. The aromatic rings of these amino acids can each make two single bond rotations relative to the hormone backbone (whose rigidity is determined by the presence of a disulfide bond between positions one and six) and are therefore not necessarily close to one another. If they did approach one another closely, one would expect either upfield or downfield shifts in their PMR resonances, depending on the relative orientations of the aromatic rings. Slight shifts in the resonances can also result from formation of the peptide and disulfide bonds (12) and therefore the oxytocin derivatives which contain tyrosine in a sequence similar to that in lysine-vasopressin, but lacking a contiguous phenylalanine, were used as controls.

The resultant 100 MHz spectra of the aromatic protons are shown in Figure 1. The tyrosine protons result in an AA'BB' quartet, the upfield doublet attributable to the protons ortho to the hydroxyl group. The phenylalanine aromatic protons result in a complex multiplet, whose structure depends on pH and solvent conditions. This multiplet structure has not yet been analyzed. The tyrosine PMR spectra of the oxytocin derivatives show no change in chemical shift relative to the monomer; an increase in the widths of the individual lines can be attributed to the slower

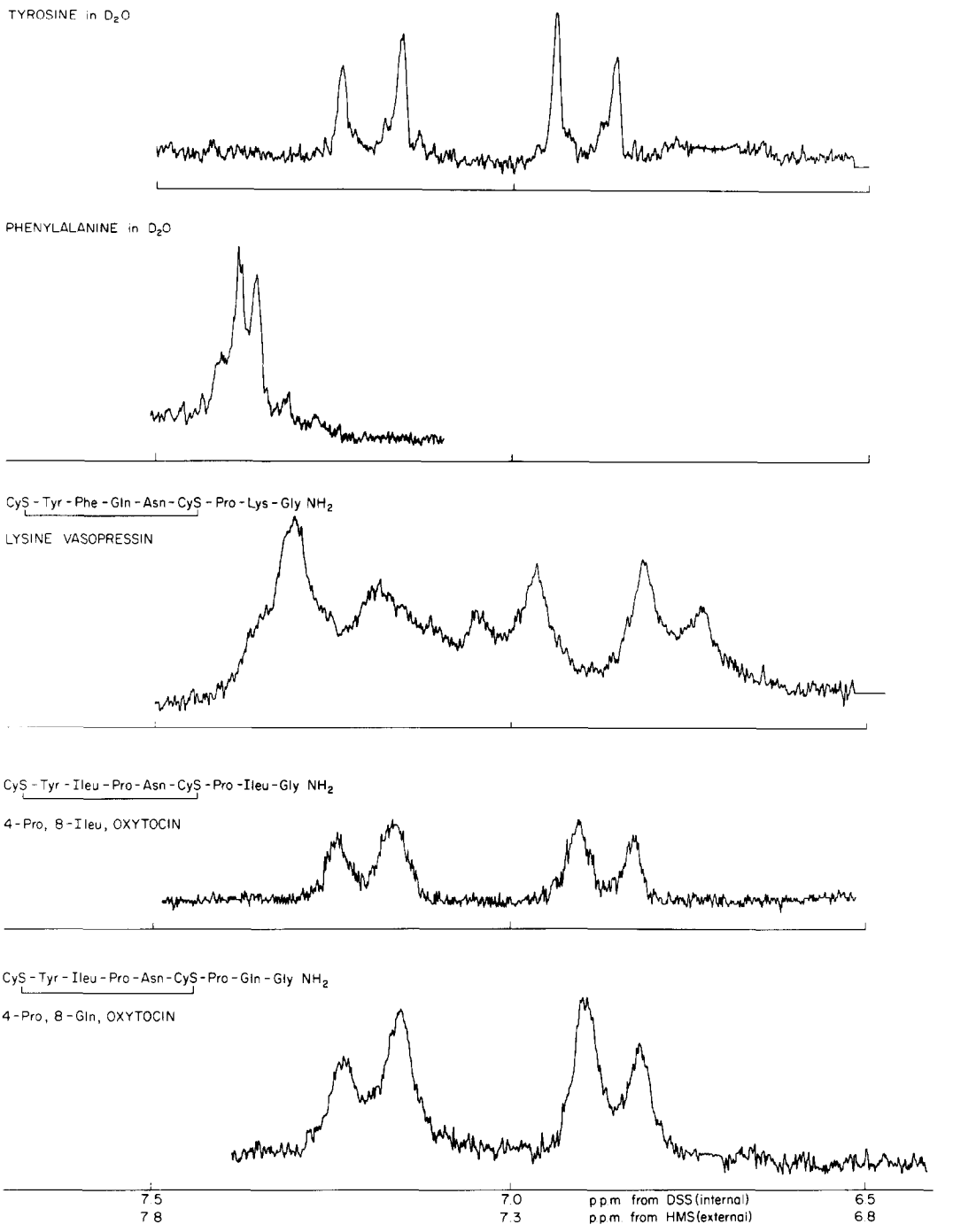

Figure 1: NMR spectra at 100 MHz of the aromatic protons of the compounds indicated. Samples were dissolved in unbuffered D_2O, as saturated solutions, and run at 25°C. All spectra are extensively time-averaged.

tumbling rate of the oligomer. Turning to the PMR spectrum of lysine-vasopressin in Figure 1, we see that all aromatic proton resonances are shifted upfield relative to their values in the monomers or the oxytocin derivatives, but some resonances are shifted considerably more than others. The line shapes and intensities can be seen better in the 220 MHz spectrum of Figure 2. In the phenylalanine resonance, a multiplet corresponding to two protons has been shifted upfield by 0.14 ppm, whereas the multiplet of intensity corresponding to three protons is shifted upfield by only 0.04 ppm. The resonance of the two tyrosine protons meta to the hydroxyl group are shifted upfield by 0.19 ppm, whereas the ortho proton resonances are shifted by only 0.12 ppm The relative chemical shifts were independent of temperature

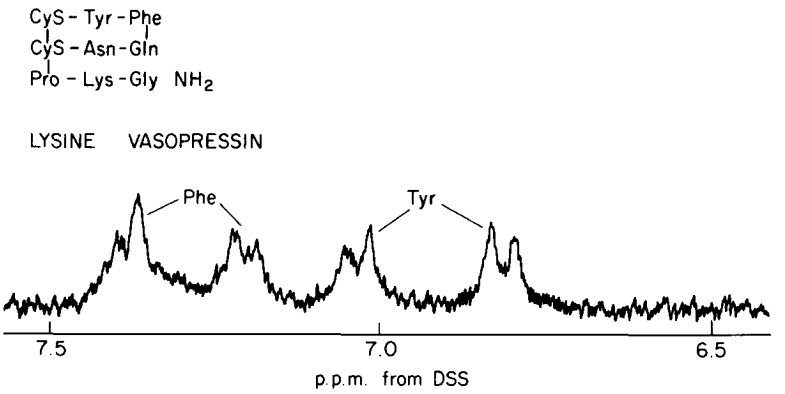

Figure 2: NMR spectrum at 220 MHz of the aromatic protons of lysine-vasopressin. The solution contained approximately 20 mg/ml lysine-vasopressin, 0.1M DSS, pD 7.2, 20°C.

over the range 8°-45°C. These data are consistent with a model in which the planes of the aromatic rings of tyrosine and phenylalanine are parallel, but with their twofold symmetry axes perpendicular, Figure 3. A more detailed

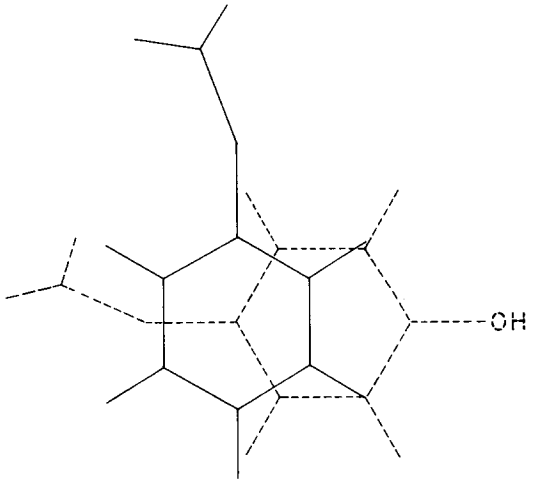

Figure 3. Possible stacking arrangement of phenylalanine and tyrosine in lysine-vasopressin.

model for the stacking will be possible when an analysis of the phenylalanine spectrum is completed.

The PMR spectra of the related dipeptides Tyr-Tyr, Phe-Phe, Tyr-Phe, Phe-Tyr show similar preferential shifts of aromatic resonances, but spectra due to both stacked and unstacked structures can be discerned. The relative proportions of stacked and unstacked structures depended upon solvent composition and acidity. The magnitudes of the shifts caused by stacking of the aromatic rings in these compounds are smaller, however. This is presumably because they lack the steric constraint imposed by the disulfide bond of lysine-vasopressin.

ACKNOWLEDGMENT

We are indebted to Professor R.R. Fraser, University of Ottawa, for the use of the HA 100, and to Dr. R.G. Shulman, Bell Telephone Laboratories, for the use of the HR 220. The

gifts of lysine-vasopressin from the National Institutes of Health, U.S.A., and oxytocin derivatives from Dr. L. Benoiton, are gratefully acknowledged.

REFERENCES

1.) S.I. Chan and J.H. Nelson, 1969, J. Amer. Chem. Soc., 91, 168.
2.) B.W. Bangerter and S.I. Chan, 1969, J. Amer. Chem. Soc., 91, 3910.
3.) A.D. Broom, M.P. Schweizer, and P.O.P. Ts'o, 1967, J. Amer. Chem. Soc., 89, 3612.
4.) M.P. Schweizer, A.D. Broom, P.O.P. Ts'o, and D.P. Hollis, 1968, J. Amer. Chem. Soc., 90, 1042.
5.) C.E. Johnson and F.A. Bovey, 1958, J. Chem. Phys., 29, 1012.
6.) C.C. McDonald, W.D. Phillips, S. Penman, 1964, Science 144, 1234.
7.) C.C. McDonald, W.D. Phillips, and J. Lazar, 1967, J. Amer. Chem. Soc., 89, 4166.
8.) C.C. McDonald and W.D. Phillips, 1967, J. Amer. Chem. Soc. 89, 6332.
9.) H. Sternlicht and D. Wilson, 1967, Biochemistry, 6, 2881.
10.) K. Wüthrich, R.G. Shulman, and T. Yamane, 1968, Proc. Nat. Acad. Sci. U.S. 61, 1199.
11.) V. du Vigneaud, M.F. Bartlett, and A. Jöhl, 1957, J. Amer. Chem. Soc., 79, 5572.
12.) A. Nakamura and O. Jardetzky, 1967, Proc. Nat. Acad. Sci. U.S. 58, 2212.