THE CONFORMATION OF OXYTOCIN IN DIMETHYLSULFOXIDE AS REVEALED BY CARBON-13 SPIN-LATTICE RELAXATION TIMES

Roderich Walter*, Ian C. P. Smith+, and Roxanne Deslauriers+

*Department of Physiology, Mount Sinai School of Medicine, New York, New York 10029, U.S.A.

and

+Division of Biological Sciences, National Research Council of Canada, Ottawa, Canada K1A 0R6.

Received March 20,1974

Summary: Information was obtained on rates of overall molecular reorientation and segmental motion of amino acid sidechains of oxytocin in dimethylsulfoxide by determination of spin-lattice relaxation times (T_1) at 25 MHz for carbon-13 in natural abundance in the hormone. The T_1 values of the α-carbons of amino acid residues located in the 20-membered ring of oxytocin are all about 50 msec. The overall correlation time for the hormone backbone was estimated to be 8.8×10^{-10} sec. The sidechains of Tyr, Ile and Gln undergo segmental motion with respect to the backbone of the ring. The T_1 value of the α-carbon of the Leu residue is greater than for any α-carbon in the ring, indicating an increased mobility of the backbone of the C-terminal acyclic peptide as compared to the ring. The β- and γ-carbons of the Pro residue undergo an *exo-endo* interconversion with regard to the plane formed by α-carbon, δ-carbon and N atom of the Pro pyrollidine ring. These data are discussed in light of results from other experimental and theoretical studies, including carbon-13 spin-lattice relaxation times for oxytocin in aqueous solution.

The preferred structure proposed by Urry and Walter (1) for oxytocin in solution has been subject to extensive investigation by proton NMR (2-6), carbon-13 NMR (7-9), conformational energy calculations (5,10,11) and model-building (11). We report here the use of ^{13}C spin-lattice relaxation time measurements to determine the degree of intramolecular motion of oxytocin in dimethylsulfoxide. In particular, we address ourselves to the suggestion of Brewster *et al.* (5) that in dimethylsulfoxide oxytocin may be in rapid equilibrium between several energetically favorable conformations. In addition to the β-turn, comprised of the sequence Tyr-Ile-Gln-Asn and closed by a hydrogen bond between the peptide

NH of the asparagine residue and the carbonyl oxygen of the tyrosine residue, as originally proposed for oxytocin by Urry *et. al.* (12), Brewster *et al.* consider as particularly favorable a conformer with an 8-membered hydrogen-bonded ring closed by the peptide NH of the asparagine residue and the oxygen of the carboxamide carbonyl of the glutamine sidechain (5). If such a conformer is present to a significant degree among the hypothetically possible structures, it could be expected that the intramolecular motion of the glutamine sidechain would be restricted, and that this would be reflected in the ^{13}C spin-lattice relaxation times, T_1 (13-16).

Materials and Methods: Oxytocin (purchased from Connaught Laboratories, Toronto, Canada) had an avian vasodepressor activity of 450 U/mg. A 100 mg sample was dissolved in 1.0 ml of deuterated dimethylsulfoxide ($(CD_3)_2SO$). Spectra were obtained on a Varian XL-100-15 spectrometer operating at 25.16 MHz. Fourier transform spectra were accumulated in a Varian 620-L computer. T_1 data (± 15%) were acquired using a $180°-\tau-90°-T_\infty$ pulse sequence, where T_∞ is at least five times the longest T_1 to be measured. Thirty values of τ from 10 - 600 msec were chosen. For each τ value 10,000 scans were taken.

Results and Discussion: The C-13 chemical shifts of oxytocin in $(CD_3)_2SO$ solution have been reported (7,8). The assignments for Tyr and Cys-1 α carbons were taken as in reference 8. T_1 values multiplied by N, the number of directly-attached protons, are shown for oxytocin in Figure 1. In the limit of extreme narrowing, the longer the NT_1 value, the more rapid the motion of the ^{13}C species. NT_1 values are not reported for the α-carbon of Gly, nor the β-carbons of Leu and Cys-6, because they were obscured by the resonances from the solvent for values of τ smaller than 100 msec.

The NT_1 values for the backbone carbons are ca. 53 msec. From

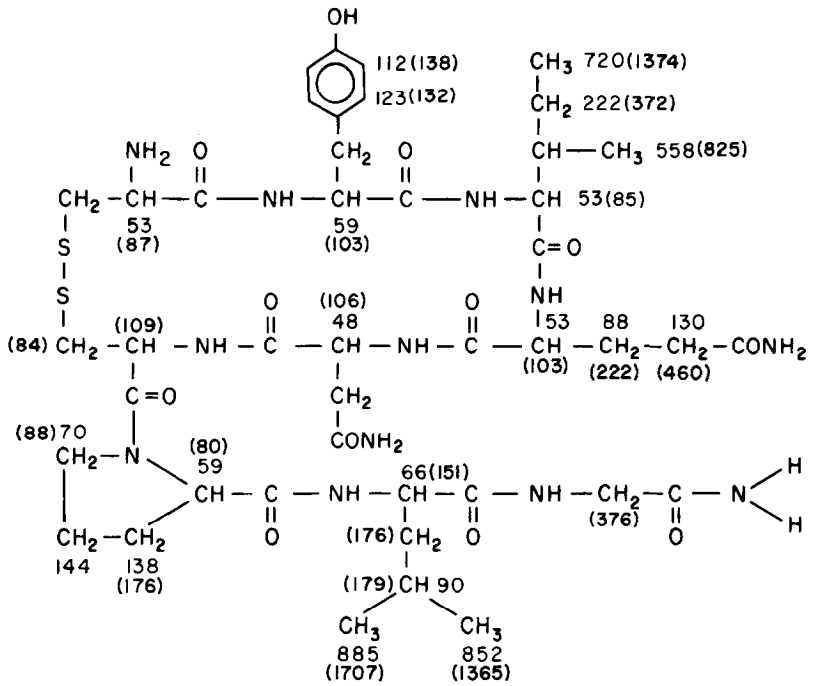

Figure 1

1) NT_1 values in msec observed in oxytocin in deuterated dimethylsulfoxide.
Numbers in parentheses are NT_1 values obtained in aqueous medium (see reference 14).

the dipolar contribution to the T_1 value (13) we calculate an overall correlation time (τ_c) of 8.8×10^{-10} sec assuming that the hormone tumbles isotropically in solution. Thus, oxytocin tumbles more slowly in $(CD_3)_2SO$ than in D_2O in which a value of 5×10^{-10} sec was calculated (14).

The sidechain of Tyr undergoes more motion than the backbone as judged from NT_1 values of the *ortho* and *meta* carbons of the ring portion which are twice that of the Tyr α-carbon. However, because we have no NT_1 value for the β-carbon, we cannot conclude whether the ring portion simply undergoes segmental motion or whether rapid rotation occurs about the aryl-C_β bond. In view of conformational

considerations of the preferred rotamer position of the aromatic sidechain of Tyr in the hypothetical "active" conformation of oxytocin (18) and the position of the Tyr when the hormone is in different solvents (e.g. 1, 19-21), it is noteworthy that we find in the present study a proportionally more mobile Tyr sidechain in $(CD_3)_2SO$ solution as compared to D_2O (14), taking in both cases the T_1 values of the α-carbons in the peptide backbone as reference. In D_2O, the T_1 value of the α carbon of Tyr is 103 msec and the NT_1 values for the *ortho* and *meta* carbons of the aromatic ring are ≃ 135 msec, whereas in $(CD_3)_2SO$, the T_1 value for the α carbon of Tyr is 59 msec and the *ortho* and *meta* carbons of the aromatic ring show NT_1 values of ≃ 117 msec.

The Ile residue undergoes segmental motion as judged from a four-fold increase in T_1 in going from the α- to the γ-carbon. The β-methyl group of the Ile residue is more restricted than the γ-methyl group, as has been observed in the free amino acid (17) and in oxytocin in aqueous solution (14).

The sidechain of the Gln residue also undergoes segmental motion; there is a factor of 1.7 in NT_1 values between the α- and β-carbons and a factor of 1.5 between the β- and γ-carbons. This type of behavior has also been observed for the Gln residue in Gly-Gly-Gln-Gly-Gly (16) and in oxytocin in aqueous solution, where differences in NT_1 values between the α-, β- and γ-carbons were slightly greater than a factor of 2 (14). Thus the T_1 values of the Gln residue give no indication that its sidechain is restricted in mobility as might be expected if the carboxamide carbonyl were hydrogen-bonded to the peptide NH of the Asn residue in oxytocin (5); in the solution model proposed earlier (1) the Gln sidechain exhibits optimal mobility by virtue of its occupying a corner of the 1→4 turn. Residues in these corners are thought to be important

for intermolecular interactions, rather than intramolecular stabilization; in fact, sidechains of residues in corner positions of the β-turns of oxytocin have been postulated to be critical for expression of hormonal activity (18).

Turning to the acyclic tripeptide, the T_1 value for the α-carbon of Pro indicates that this residue is restricted due to its peptide linkage to Cys-6 in oxytocin. The α- and δ-carbons of the Pro ring have similar mobilities, whereas the β- and γ-carbons interconvert very rapidly between *exo* and *endo* conformations with respect to the plane formed by the α-carbon, δ-carbon and N atom. Such rapid ring interconversions have been detected for Pro in a wide variety of peptides and peptide hormones (14,17,22,23). The α-carbon resonance of the Leu residue unfortunately overlaps that of the Cys-6 residue. The combined resonances have an overall T_1 value of 66 msec. Within the accuracy of our experiments no curvature in the plot of log peak intensity vs. τ was detected. Assuming, however, the T_1 value of the α-carbon of the Cys-6 residue to be similar to those of the other α-carbons in the 20-membered oxytocin ring (ca. 53 msec), a tentative T_1 value of 75 - 80 msec can be estimated for the α-carbon of Leu*. This would indicate that the backbone of the terminal tripeptide is more mobile in $(CD_3)_2SO$ than the backbone of the ring moiety.

*We have calculated that in the τ value range used, the observed T_1 value is approximately the average of the T_1 values of the constituent carbons, provided the ratio of T_1 values is less than 2.

In conclusion the ^{13}C T_1 values appear to provide a more sensitive conformational monitor than do the ^{13}C chemical shifts. In particular, for oxytocin in dimethylsulfoxide they show that a hydrogen-bond between the Gln and Asn residues is highly unlikely,

that the C-terminal tripeptide is less mobile relative to the cyclic portion and that the Pro residue is interconverting very rapidly between ring-puckered forms.

References

1. Urry, D.W. and Walter, R. (1971) Proc. Nat. Acad. Sci. U.S.A. *68*, 956.
2. Walter, R., Glickson, J.D., Schwartz, I.L., Havran, R.T., Meienhofer, J. and Urry, D.W. (1972) Proc. Nat. Acad. Sci. U.S.A. *69*, 1920.
3. Glickson, J.F., Urry, D.W. and Walter, R. (1972) Proc. Nat. Acad. Sci. U.S.A. *69*, 2566.
4. Feeney, J., Roberts, G.C.K., Rockey, J.H. and Burgen, A.S.V. (1971) Nature New Biol. *232*, 108.
5. Brewster, A.I., Hruby, V.J., Glasel, J.A. and Tonelli, A.E. (1973) Biochem. *12*, 5294.
6. Brewster, A.I. and Hruby, V.J. (1973) Proc. Nat. Acad. Sci. U.S.A. *70*, 3806.
7. Deslauriers, R., Walter, R. and Smith, I.C.P. (1972) Biochem. Biophys. Res. Commun. *48*, 854.
8. Brewster, A.I., Hruby, V.J., Spatola, A.F. and Bovey, F.A. (1973) Biochem. *12*, 1643.
9. Walter, R., Prasad, K.U.M., Deslauriers, R. and Smith, I.C.P. (1973) Proc. Nat. Acad. Sci. U.S.A. *70*, 2086.
10. Kotelchuck, D., Scheraga, H.A. and Walter, R. (1972) Proc. Nat. Acad. Sci. U.S.A. *69*, 3629.
11. Konig, B., Kabat, E.A., Katz, L., Levinthal, C. and Wu, T.T. (1973) J. Mol. Biol. *80*, 277.
12. Urry, D.W., Ohnishi, M. and Walter, R. (1970) Proc. Nat. Acad. Sci. U.S.A. *66*, 111.
13. Allerhand, A. and Komoroski, R.A. (1973) J. Am. Chem. Soc. *95*, 8228.
14. Deslauriers, R., Smith, I.C.P. and Walter, R., J. Am. Chem. Soc., in press.
15. Keim, P., Vigna, R.A., Marshall, R.C. and Gurd, F.R.N. (1973) J. Biol. Chem. *248*, 6104.
16. Keim, P., Vigna, R.A., Morrow, J.S., Marshall, R.C. and Gurd, F.R.N. (1973) J. Biol. Chem. *248*, 7811.
17. Deslauriers, R., Walter, R. and Smith, I.C.P. (1973) FEBS Lett. *37*, 27.
18. Walter, R., Schwartz, I.L., Darnell, J.H. and Urry, D.W. (1971) Proc. Nat. Acad. Sci. U.S.A. *68*, 1355.
19. Deslauriers, R. and Smith, I.C.P. (1970) Biochem. Biophys. Res. Commun. *40*, 179.
20. Craig, L.C. (1972) in *Structure-Activity Relationships of Protein and Polypeptide Hormones* (Margoulies, M. and Greenwood, F.C., eds.) part 2, p. 443, Excerpta Medica, Amsterdam.
21. Fric, I., Kodicek, M., Jost, K. and Blaha, K. (1973) in *Peptides 1972*, Proc. 12th Eur. Pept. Symp. (Hanson, H. and Jakubke, H.-D., eds.) p. 318, North-Holland, Amsterdam.
22. Deslauriers, R., Walter, R. and Smith, I.C.P. (1973) Biochem. Biophys. Res. Comm. *53*, 244.
23. Deslauriers, R., Garrigou-Lagrange, C., Bellocq, A-M. and Smith, I.C.P. (1973) FEBS Lett. *31*, 59.