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Secondary Structure of the Cyclic Moiety of the Peptide Hormone Oxytocin and Its Deamino Analog*

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Abstract. The secondary structure of the cyclic moiety of oxytocin and deamino-oxytocin has been determined by nuclear magnetic resonance spectroscopy (220 MHz). Oxytocin, in a dimethylsulfoxide-methanol mixture, contains a β-turn involving the sequence -L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-. Deamino-oxytocin, in addition to the β-turn, contains a hydrogen bond involving the amide hydrogen of the tyrosine residue and the peptide carbonyl group of the asparagine residue, resulting in an antiparallel β-type conformation for the ring component. An initial attempt has been made to relate conformational features of the hormonal peptides to their biological activity.

Previous work utilizing nuclear magnetic resonance spectroscopy has defined the secondary structure of gramicidin S-A,[1, 3] valinomycin in dimethylsulfoxide,[2, 3] and the valinomycin-potassium complex,[2, 4] and has resulted in details of secondary structures of polymyxins B_1 and E_1[3, 5] and actinomycin D.[6] These studies, as well as those of hexapeptides,[7, 8] when supported by X-ray crystallographic data,[9–11] provide the basis for utilizing temperature dependence of chemical shifts to identify hydrogen-bonded amide protons. The resonance of a hydrogen-bonded amide proton, in contrast to one which is exposed to solvent, exhibits a decreased temperature coefficient of its chemical shift for the solvent systems utilized.

Characterization of conformational features by studying amide proton spectra goes beyond the identification of hydrogen bonding. The observation of a high field shifted resonance with a low temperature coefficient, in each case noted above, is indicative of a β-turn[3] (or β-fold).[12, 13] The β-turn involves a hydrogen bond from the peptide oxygen of residue i to the hydrogen on the peptide nitrogen of residue $i + 3$. In terms of nuclear magnetic resonance, the β-turn is further distinguished by a relatively small $J_{\alpha CH-NH}$ for residue $i + 1$ and a large $J_{\alpha CH-NH}$ for residue $i + 2$. High-field shifting of the amide proton resonance is due to shielding by the end peptide moiety of the β-turn. The end peptide moiety is derived from residues $i + 1$ and $i + 2$. Accordingly, in the nuclear magnetic resonance spectra, the β-turn is characterized by a high field shifted amide proton resonance with a low temperature coefficient for its chemical shift in conjunction with the proper coupling constants for residues $i + 1$

and $i + 2$ in the polypeptide sequence. These conclusions are entirely in accord with the original experience of Stern, Gibbons, and Craig[1] with gramicidin S-A.

In the present communication it will be noted that oxytocin, in the solvent system DMSO-d_6: MeOH (3:7, v/v), contains a β-turn involving the sequence -L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-. It will also be shown that the biologically highly active oxytocin analog [1-β-mercaptopropionic acid]-oxytocin (deamino-oxytocin)[14] possesses in the same solvent system, in addition to the β-turn, a hydrogen bond involving the peptide hydrogen of the tyrosine residue and the peptide oxygen of the asparagine residue. This results in an antiparallel β-type conformation for the ring moiety of deamino-oxytocin.

Materials and Methods. Oxytocin[15] and [1-β-mercaptopropionic acid]-oxytocin,[14] synthesized and highly purified in these laboratories, exhibited biological properties in accord with those reported earlier. Spectra were recorded using a Varian Associates HR-220 spectrometer. Sample concentrations were 6% on a weight basis in DMSO-d_6:MeOH (3:7, v/v). Internal reference was TMS. Temperature in the sample zone was controlled and known within $\pm 2°$.

Results. Proton resonance assignments, including those of the amide protons, for oxytocin and deamino-oxytocin in DMSO-d_6 have been reported by Johnson *et al.*[16] Starting with these assignments, spectra for the amide protons of deamino-oxytocin were obtained with increasing amounts of methanol. Changes in chemical shifts were followed until 70 per cent methanol was reached. This procedure allowed the assignments given in Figure 1. The features of

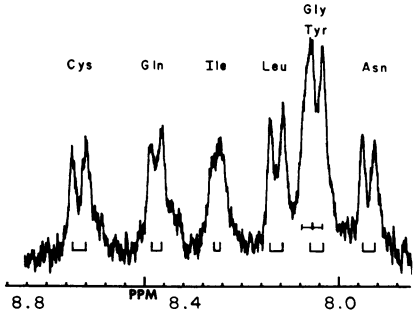

FIG. 1.—Nuclear magnetic resonance spectra, taken at 220 MHz, of the amide proton region of deamino-oxytocin in DMSO-d_6: MeOH (3:7 by volume) at 6°C. Note that the highest field resonance is that of the asparagine residue and that the coupling constant for the isoleucine residue is relatively small.

particular interest are the high-field position of the amide proton resonance of the asparagine residue and the small coupling constant of the amide proton resonance of the isoleucine residue. The remaining resonances all exhibit relatively large αCH-NH coupling constants.

Variation of amide proton chemical shifts as a function of temperature are plotted in Figure 2. For our present concern the significant features in this plot are the low sensitivity to temperature of the Asn and Tyr peptide proton chemical shifts. As shown previously with gramicidin S and the valinomycin-K^+ complex, this indicates that these peptide protons are involved in hydrogen bonding. Hydrogen-deuterium exchange studies utilizing CD_3OD were largely inconclusive but did show the asparaginyl peptide proton to exchange somewhat more slowly.

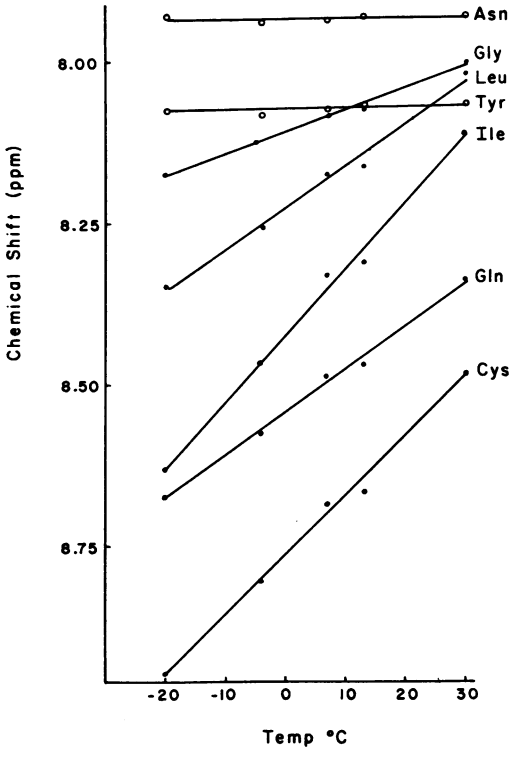

FIG. 2.—Temperature dependence of amide proton chemical shifts for deamino-oxytocin in DMSO-d_6:MeOH (3:7 by volume). Note that the chemical shifts of the protons of the asparagine and tyrosine residues are almost temperature independent over the temperature range studied.

Discussion. The high-field position of the amide proton resonance in deamino-oxytocin (Fig. 1) and its zero slope with changes in temperature (Fig. 2) suggests that the peptide proton of the asparagine residue participates in a β-turn. In the structure given in Figure 3, the peptide proton of the asparagine residue is hydrogen bonded and shielded by the end peptide moiety; the αCH-NH dihedral angle of the isoleucine residue (residue $i + 1$, see introduction) is less than 90°, as is indicated by the small coupling constant, and the αCH-NH dihedral angle of the glutamine residue (residue $i + 2$) with its significantly larger coupling constant[3, 17] is closer to 180°. This completes the characterization of the β-turn.

The low temperature coefficient of the amide proton resonance chemical shift of the tyrosine residue in connection with the large coupling constants for the amide proton resonances of the tyrosine and asparagine residues in deamino-oxytocin (Figs. 1 and 2) are explained by a second hydrogen bond involving the peptide carbonyl group of the asparagine and the amide hydrogen of the tyrosine residue. This completes the characterization of the secondary structure of the ring moiety of deamino-oxytocin (Fig. 3).

Closing of the ring structure is less certain. But, if we may assume that the handedness of the disulfide bridge as determined by circular dichroism studies in aqueous solutions[18–20] is the same for the DMSO-d_6:MeOH solvent system, then this conformational feature may be added. The large $J_{\alpha CH-NH}$ of residue 6 suggests a *trans* orientation and this has been assumed in the ring closure. There

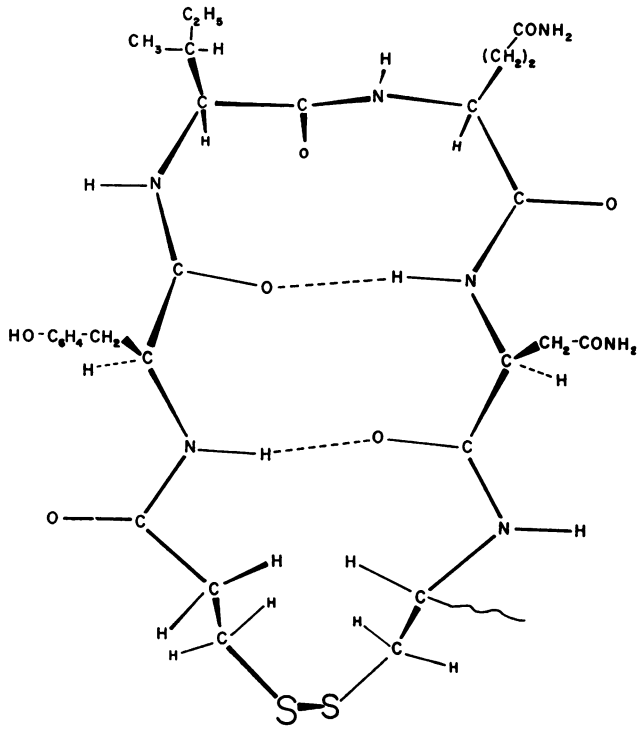

FIG. 3.—Secondary structure of the ring component of deamino-oxytocin in DMSO-d_6:MeOH (3:7 by volume).

remains for future analysis the detailing of the χ_1 and χ_2 angles of the residues in positions 1 and 6 of the molecule.

Oxytocin, in the same solvent system, appears to have the same conformation as its deamino analog; however, the tyrosyl amide proton resonance was quite broad so that the results with respect to the second hydrogen bond involving this amide hydrogen and the peptide carbonyl group of the asparagine residue are less certain. Oxytocin exhibits the β-turn as indicated in Figure 4, not only in DMSO-d_6:MeOH but also in DMSO-d_6.[21] In fact, several oxytocin analogs with structural modifications in positions 1, 2, 4, and 8 (deamino-oxytocin, deamino-2-alanine-oxytocin, deamino-4-valine-oxytocin, and deamino-8-alanine-oxytocin) were found to possess in DMSO-d_6 a high field shifted amide proton resonance of the asparagine residue, implying a β-turn as a conformational element.[22] Interestingly, 5-valine-oxytocin, a hormone analog with an exceedingly low order of biological activity[23, 24] appears to lack such a β-turn as indicated by the absence of the high-field amide proton resonance. In this context it will be recalled that replacements of the asparagine residue in oxytocin by other amino acid residues, irrespective of their nature, has always resulted in virtually inactive hormonal analogs.[25] If future studies confirm this result, it may be suggested that the β-turn is an essential conformational feature for highly potent

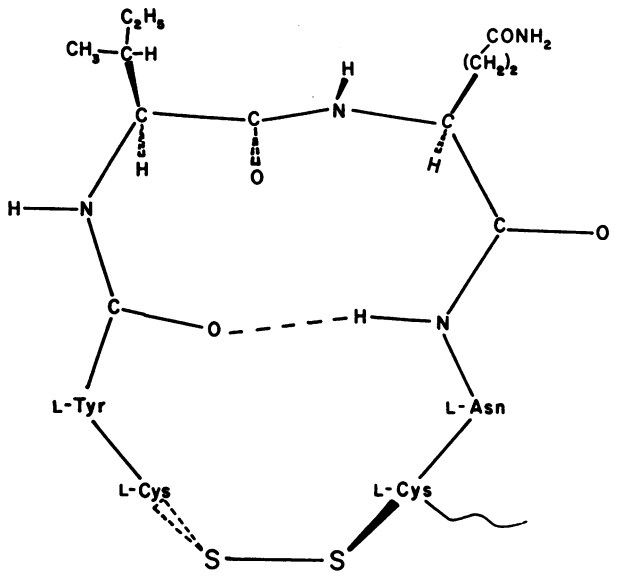

FIG. 4.—The β-turn of the ring component of oxytocin.

neurohypophyseal peptides. Perhaps we are now in a position to begin relating three-dimensional structure to biological activity.

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