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# CONFORMATIONAL STUDIES OF CARBA-ANALOGS OF OXYTOCIN

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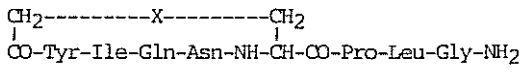
## Introduction

Substitution of $CH_2$ for a sulfur atom of the oxytocin disulfide bridge has a significant effect on biological properties of the resulting analogs, depending on the location of the sulfur exchanged (for review see (1)). We have investigated the conformational characteristics of a number of carba analogs (the structures of some of them are shown below) by $^1H$ NMR and CD spectroscopy and by UV fluorescence.




I, X=$CH_2$-S-S  II, X=$CH_2$-$CH_2$-S  III, X=$CH_2$-$CH_2$-$CH_2$  IV, X=$CH_2$-S-$CH_2$

## Results

**$^1H$ NMR spectroscopy:** We found that the analogs could be divided into two groups depending on the presence or absence of sulfur in position 6. The first group contains analogs having sulfur in position 6 (I and II) while the other group has a $CH_2$ group instead (III and IV). Comparison of chemical shift values (determined in DMSO solution) with the values of a similar amino acid residue in model tetrapeptide TFA-Gly-Gly-Xaa-Ala-OMe revealed the following similarities: The first group (sulfur in position 6) displays more shielded amide protons of asparagine (0.4 ppm) and glycine (0.1 to 0.2 ppm), the $\alpha$-proton of isoleucine (0.3 ppm), and also significantly more deshielded is one of the $\beta$-protons of tyrosine. The second group displays a deshielded NH proton of tyrosine (0.2 to

0.3 ppm), a shielded NH proton of isoleucine (0.6 to 0.8 ppm), and a more shielded $\alpha$-proton of asparagine (0.1 to 0.2 ppm). The difference in the chemical shifts of $\beta$-protons of tyrosine is greater in the first group (0.52 and 0.65 ppm) than in the second group (0.38 and 0.43 ppm). These two groups also differ in the sensitivity to the change of configuration of the amino acid in position 2. Introduction of a D-amino acid into a compound of the first type leads to a large upfield shift of the glutamine NH (0.9 to 1.0 ppm), a downfield shift of the asparagine NH (0.35 to 0.6 ppm), isoleucine $\alpha$-proton ( 0.1 ppm) and glutamine $\alpha$-proton (0.1 to 0.2 ppm), and to a small upfield shift of the $\alpha$-proton of asparagine (0.05 to 0.1 ppm). A larger upfield shift is observable in one $\beta$-proton of the aromatic amino acid in position 2 (0.5 ppm). Analogs of the second type display a deshielded $\alpha$-proton of the aromatic amino acid (~0.1 ppm) and an amino acid in position 6 (~0.25 ppm). The change of configuration in position 2 leads in both groups of analogs to an upfield shift of all but $\alpha$-CH protons of isoleucine and to a downfield shift of NH of the amino acid in position 6 (with the exception of deamino-oxytocin). The difference in chemical shifts of $\beta$-protons of the aromatic amino acid is strongly reduced by configurational change (from 0.38-0.7 to 0.12-0.25 ppm). This is accompanied by the change of $\alpha$-$\beta$ coupling constants (3-4 and 10-12 Hz for all-L-analog to 5-8 and 6-9 Hz for D-diastereoisomers) of the aromatic residue.

Two groups of analogs may also be recognized according to the temperature dependences of the backbone NH chemical shifts. Analogs of the first group have a quite small temperature coefficient for the proton in position 2 (-1 to -2 ppb/deg) and a positive coefficient for the asparagine NH proton (+4 to +6 ppb/deg). Analogs of the second type have a low value of the temperature coefficient for amides in position 3 and 6. The change of the aromatic amino acid configuration does not change the temperature coefficients in the analogs of the second group, while changing it dramatically in the first group: the glutamine NH proton is now temperature independent (slightly positive). However, there are differences inside the first group between II and I, the first analog having temperature-independent amides of residues in position 6 and 9.

A very important feature of the spatial structure of carba-analogs may be the interaction of cystine sulfur in position 6 with the residue in position 2, which was suggested on the basis of their CD spectra (2) and, recently, on the basis of the downfield shift of tyrosine aromatic proton resonances (3) in analogs containing sulfur in position 6. That the interaction is not limited only to the tyrosine residue and that phenylalanine can participate in it as well is evidenced by the downfield shift (~0.06 ppm) of aromatic protons in /Phe$^2$/dCOT-1 in comparison to the shift of phenylalanine in the model tetrapeptide, or in

/D-Phe$^2$/dCOT-1.

In the analogs of the first type the intramolecular interaction of the aromatic residue with the sulfur in position 6 may be responsible for the more compact structure in which the aromatic side chain influences the amino acids in positions 4 and 5. The positive temperature coefficient of the asparagine amide proton together with its upfield shift in the analogs of the first type may be explained by hydrogen bonding interaction of this proton with $\pi$-electron cloud of the aromatic residue; this interaction is made possible by the above mentioned sulfur-aromatic residue interaction. The conformation of II is significantly more similar to that of I than to those of III or IV. The increased flexibility of the carba-joint allows the formation of more intramolecular hydrogen bonds. Our interpretation of the presented data still needs further refinement and should be taken as tentative only.

**CD spectroscopy:** The results obtained with analogs provide further support for the existence of a tyrosine - $S^6$ interaction. CD spectra do not contain features which would directly reflect the interaction in question but they indicate the conformational situation in which the interaction is possible. The middle part of the CD spectrum ranging from 210 to 240 nm appears to be the most sensitive to structural and conformational changes in the region of the heterodetic joint and the residue in position 2. This part of the CD spectrum is formed by superposition of a pair of oppositely signed amide n-$\pi^*$ bands and an aromatic $B_{1u}$ band of tyrosine chromophore. CD spectra (in neutral buffer solution) of compounds in which the interaction tyrosine - $S^6$ takes place differ from the spectra of the other compounds by a distinctly higher intensity of the positive $B_{1u}$ aromatic band and lower intensity of the positive n-$\pi^*$ band below 220 nm. The latter group of compounds also includes all D-Tyr$^2$ analogs. The typical spectral difference between carba-1 and carba-6 oxytocin is smoothed out in corresponding D-Tyr$^2$ analogs. On the other hand, the change of the tyrosine configuration differentiates greatly the formerly similar spectra of deaminooxytocin (I) and its carba-1 analog (II). This effect might be explained by the higher flexibility of the heterodetic joint in the carba analog. A comparison of CD spectra of carba-analogs including those with a nonaromatic amino acid in position 2 reveals that the conformational difference between the two types of compounds may include partial changes connected with the interaction itself, with the replacement of a sulfur atom with a methylene group and with the change of configuration in position 2. It appears that the change of configuration of the amino acid in position 2 leads to some enhancement of the molecular rigidity. This effect seems to be especially large in the /D-Phe(4-Et)$^2$/deamino-carba-6-oxytocin.

**Fluorescence:** We have also measured the quantum yield of tyrosine aromatic fluorescence in neutral water solution. The tyrosine fluorescence is effectively quenched by the disulfide group (as shown for oxytocin in previous studies (4)) even with analogs where tyrosine is in the D-configuration or where the $Cys^1$ residue has been replaced by the $Pen^1$ residue. The thioether group in carba-analogs also quenches the tyrosine fluorescence but far less than does the disulfide group. We have found only a small and insignificant difference in the quantum yield of fluorescence for carba-1 and carba-6 analogs of deamino-oxytocin. The fluorescence study proves the existence of contacts between the side chain of tyrosine and the heterodetic joint of the ring moiety. The flexibility of the molecules and the effectiveness of the quenching mechanism are too high in aqueous solution, so that the method is not able to distinguish between cases of different accessibility of the quencher for the aromatic group.

On the other hand, preliminary results of fluorescence measurement in dimethylsulfoxide solution have shown a significant difference in quenching between carba-1 and carba-6 oxytocin. Both analogs IV and III almost do not influence the fluorescence. These results are in agreement with the $^1H$ NMR data that show a fixed conformation with sulfur-aromatic interaction in DMSO solution.

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