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Observation of a conformational effect in peptide molecule by reversed-phase high-performance liquid chromatography

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ABSTRACT

RP-HPLC of analogues of oxytocin containing a reduced peptide bond was studied using various columns and buffers. Anomalous behavior of one analogue served as a basis for the discussion of possible conformational consequences of this substitution.

INTRODUCTION

Reversed-phase liquid chromatography has been used for conformational studies in peptide chemistry by several workers [1–7], because the interaction of a peptide with the rigid hydrophobic surface may provide information about the flexibility of the peptide chain or about the accessibility of various hydrophobic residues in the chain, depending on its conformation. Extensive studies in this field have been performed by Hodges' and Houghten's groups [1–6], who synthesized several model peptides containing the same amino acid residues and showed that their retention depends on their most probable conformation. Another paper [7] speculated about the similar conformation of a number of oxytocin analogues based on the fact that their retentions followed the same dependence as those of similary modified benzene derivatives.

Recently we have synthesized a range of oxytocin analogues with one peptide bond at a time replaced with a CH_2NH bond [8]. We found that in almost all instances the biological activity of these analogues was substantially decreased if not completely eliminated. The two exceptions were the analogues with CH_2NH replacement between Cys and Tyr in positions 1 and 2 and between Pro and Leu in positions 7 and 8. In the former instance the analogue was a fairly potent inhibitor and in the latter it was an active agonist. We were interested in establishing whether this activity change is reflected by a change in retention on a reversed-phase material.

EXPERIMENTAL

The analogues used in this study were the same as those synthesized earlier [8]. Isocratic high-performance liquid chromatography (HPLC) was carried out on an SP-8800 instrument equipped with an SP-8450 detector and SP-4290 integrator (all from Spectra Physics, Santa Clara, CA, USA). We used columns of Vydac C_{18} (250 × 4 mm I.D., 5 μm) (Separations Group, Hesperia, CA, USA) and Separon SI C_{18} (250 × 4 mm I.D., 5 μm) (Tessek, Prague, Czech Republic). The mobile phases used were mixtures of methanol with either 0.05% trifluoroacetic acid in water or 0.1 *M* ammonium acetate (pH 7.5). Chromatography was per-

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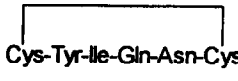
formed at room temperature with a flow-rate of 1 ml/min.

RESULTS AND DISCUSSION

The retention times of the oxytocin analogues containing the reduced peptide bond (**II–IX**) were compared with those of oxytocin (**I**) using two different columns with reversed phases and buffers of acidic and neutral pH. As can be seen from Table I, reduction of reptide bond resulted in the expected decrease in the retention times with an acidic mobile phase, and therefore in increased hydrophilicity, in all but one instance. The most pronounced decrease was observed for **IV** and **VIII**, where the reduced bond is situated between Ile and Asn or at the carboxyterminus of the molecule. This behavior can be explained by the relatively high exposure of these bonds to the exterior of the molecule (thus increasing its hydrophilicity), as they are claimed to connect (in the native molecule) the corner residues of putative β-turns. A relatively small decrease in retention time was observed for **IX**, with the reduced bond between Leu and C-terminal Gly. However, **II** showed an increased retention in the comparison with the parent molecule oxytocin.



Oxytocin

The overall hydrophobicities calculated from the partial hydrophobicities of all the structural elements in the molecules of **II–IX** are apparently identical (the secondary amino group is completely ionized) and therefore the higher retention of **II** in an acidic mobile phase can be explained only by the better interaction of this compound with the stationary phase. Inaccessibility of the generated amino group might explain the smaller than expected decrease in retention time, but it cannot explain its increase.

Retention times in a neutral mobile phase may not reflect precisely the hydrophobicity of the analogues, as under these conditions amino groups might be ionized to different extents, depending on the character of the amino acid residue. Therefore, we did not attempt to draw any conclusions from the behavior of the analogues in a neutral mobile phase. The com-

TABLE I

RETENTION CHARACTERISTICS OF OXYTOCIN ANALOGUES

No.	Analogue	k'			
		Vydac C_{18}		Separon SI C_{18}	
		pH 2[a]	pH 7.5[b]	pH 2[c]	pH 7.5[d]
I	Oxytocin (OXT)	9.00	6.74	7.56	5.77
II	[$^{1}\Psi^{2}$-CH_2NH]OXT	13.25	7.89	10.62	6.13
III	[$^{2}\Psi^{3}$-CH_2NH]OXT	2.37	11.86	2.46	9.93
IV	[$^{3}\Psi^{4}$-CH_2NH]OXT	1.94	6.67	2.05	5.91
V	[$^{4}\Psi^{5}$-CH_2NH]OXT	4.50	6.54	4.15	5.69
VI	[$^{5}\Psi^{6}$-CH_2NH]OXT	3.87	6.40	4.07	5.32
VII	[$^{6}\Psi^{7}$-CH_2N]OXT	2.37	4.86	2.53	5.12
VIII	[$^{7}\Psi^{8}$-CH_2NH]OXT	2.06	9.03	2.36	7.08
IX	[$^{8}\Psi^{9}$-CH_2NH]OXT	6.75	8.96	5.24	6.86

[a] 27.5% MeOH.
[b] 38% MeOH.
[c] 45% MeOH.
[d] 57% MeOH.

parison of two mobile phases might be further complicated by their different ionic strengths and the use of different counter ions [3].

A peptide bond imposes certain restrictions on the conformation of the peptide chain, which is released when it is replaced with the CH_2NH group. We have shown recently [9] that the steric fixation of the aromatic side-chain in position 2 in oxytocin had a dramatic effect on its biological activity. It is well known (for a review, see, *e.g.*, ref. 10) that the hydrophobicity of the amino-terminal position of the oxytocin molecule is responsible for the interaction with its uterotonic receptor. A change in configuration of the aromatic amino acid in position 2 led to the design of potent uterotonic inhibitors. Elimination of steric restrictions by peptide group replacement led to a similar result to a change in configuration in this position. Also, in both instances the retention on a reversed phase was substantially increased. (For the behavior of analogues containing D-amino acids in position 2, see refs. 11–13.)

We can speculate that the increased retention of **II** in an acidic mobile phase on a reversed phase reflects a conformational relaxation in the amino-terminal part of the oxytocin molecule, which correlates well with the properties of a uterotonic inhibitor that were observed for this analogue.

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