

SYNTHESIS AND PROPERTIES OF ANTIPARALLEL DIMER OF DEAMINO-1-CARBA-OXYTOCIN

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Introduction

Dimer forms of neurohypophyseal hormones were studied already at the very beginning of synthetic activities in this field. Parallel and antiparallel dimers of oxytocin, dimers and trimers of lysine-vasopressin, and dimeric forms of several other analogs were described (1). In all cases significant biological activities were found (0.25 - 4 % activity of the parent hormone), but usually little attention was paid to their further evaluation. The question remains whether the dimeric form can really fulfill the requirements of the receptor and evoke the response or whether the activity found is the result of monomeric form either contaminating the dimer or formed during the biological evaluation due to dimer-monomer equilibrium established by the transsulfidation reaction.

Synthesizing such analog of neurohypophyseal hormones that would model the dimeric form stabilized against transsulfidation reaction and evaluating its biological properties would throw more light into this problem. For this purpose, the antiparallel dimer of deamino-1-carba-oxytocin (dCOT-1) was chosen. Monomeric form of dCOT-1 is known to have at least three times higher uterotonic activity than oxytocin (2).

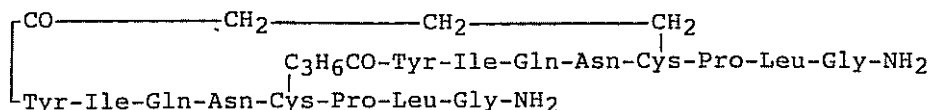
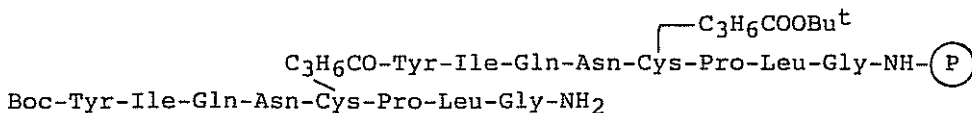
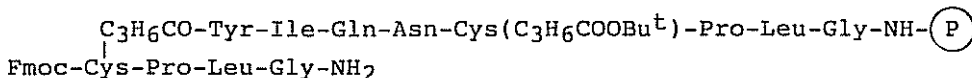
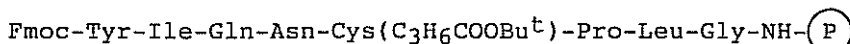
Experimental

The dimer of dCOT-1 was synthesized using two methods:

(i) it was isolated from the reaction mixture after the synthesis and cyclization on the polymer matrix using repeated gel filtration on Bio-Gel P-4 and further purified by reversed-phase HPLC. The cyclization on the polymer affords higher or lower amount of higher-molecular weight products, depending on the solvent which we use for the last synthetic step. Dichloromethane, dimethylformamide or their mixtures gave us comparable results, but the use of trifluoroethanol, as recommended by Schiller (3) provided higher monomer to dimer ratio. The structure of dimer was proven by FAB-MS.

(ii) The independent synthesis was based on the assembling of the octadecapeptide (dimer precursor) on the polymer carrier, its cyclization and cleavage from the resin (Scheme 1). The first three residues were coupled as Boc-amino acids and from the fourth step the synthesis was performed with Fmoc- α -amino-protecting group, since the side chain carboxyl group of modified cysteine residue was protected as tert.butyl ester. After the eighth step the peptide Fmoc-Cys(C₃H₆COOH)-Pro-Leu-Gly-NH₂ was coupled to the growing peptide chain and the synthesis was

Scheme 1



continued stepwise. As the last amino acid Boc-tyrosine was coupled and carboxyl and amino protecting groups were removed by trifluoroacetic acid. Cyclization was performed by the action of dicyclohexylcarbodiimide and hydroxybenzotriazole in trifluoroethanol. All solid phase steps were monitored by bromophenol blue as described previously. Product obtained by this way was found identical with that isolated after cyclization of dCOT-1 monomer.

The product was dissolved in 20% acetic acid (1mg per ml) and tested for its uterotonic activity in the rat uterotonic test in vitro, in the galactogogic test in vivo and in the pressor test.

Results and Discussion

The antiparallel dimer of deamino-1-carba-oxytocin was shown to be completely inactive in the uterotonic test in vitro and in the pressor test. It displayed negligible galactogogic activity (0.03 IU per mg), that amounts to about 0.005% of the activity of monomer. Our results indicate that the intrinsic activity of the dimeric form of neurohypophyseal hormones can be seriously questioned if not ruled out. We suggest that the observed activities were the activities of in situ generated monomeric forms of hormones or analogues due to dimer-monomer equilibrium established by the transsulfidation reaction.

References

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2. Barth T., Krejčí I., Kupková B., Jošt K. 1973. Eur.J. Pharmacol. 24, 183.
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