Communications to the Editor

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SOLUTION SYNTHESIS OF A TETRATETRACONTAPEPTIDE AMIDE WITH GROWTH HORMONE RELEASING ACTIVITY[1)]

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The tetratetracontapeptide amide corresponding to the entire amino acid sequence of growth hormone releasing factor (GRF) was synthesized by assembling eight peptide fragments in solution followed by deprotection with 1M trifluoromethanesulfonic acid-thioanisole in TFA.

KEYWORDS ——— solution synthesis of growth hormone releasing factor (GRF); somatocrinin; 1M trifluoromethanesulfonic acid-thioanisole in TFA as a deprotecting reagent; base-catalyzed ring-closure of Asp(OBzl)

Recently, two peptides, a larger form (1-44) and a smaller form (1-40), with high intrinsic growth hormone releasing activity were isolated from human pancreatic islet tumor and sequenced.[2,3)] These peptides, termed GRF (or somatocrinin[4)]), were synthesized by solid phase technique, together with a number of shorter chain peptides.[2,3)]

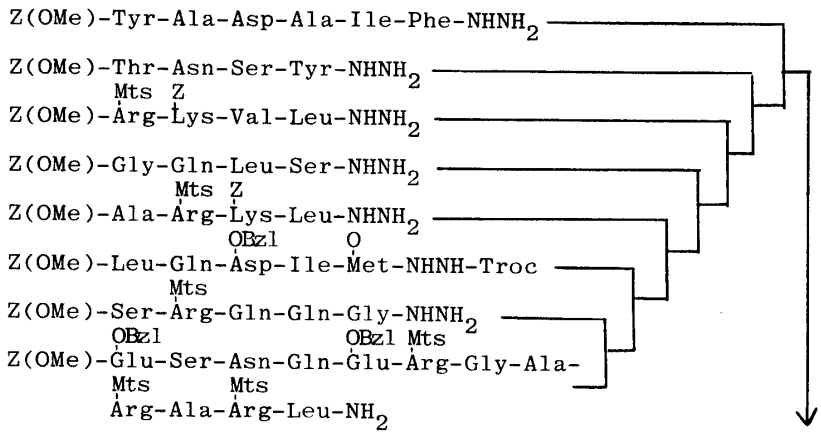
We wish to record the conventional solution synthesis of a tetratetracontapeptide amide corresponding to the entire amino acid sequence of a larger form of GRF.

In a combination of the TFA labile Z(OMe) group for N^{α}-protection, amino acid derivatives bearing protecting groups removable by TFMSA/TFA[5)] were employed, *i.e.*, Lys(Z), Arg(Mts),[6)] Asp(OBzl) and Glu(OBzl). Of these, the Bzl group of the Asp residue at position 3 was purposely removed by hydrogenolysis in order to avoid base-catalyzed ring-closure of Asp(OBzl)[7)] at the stage of fragment synthesis. As building blocks, 8 peptide fragments were selected to construct the entire peptide backbone of GRF (Fig.). Every fragment was synthesized by the known amide-forming reactions. Of these, peptide hydrazides containing the Asp(OBzl) or the Glu(OBzl) group were synthesized with the aid of substituted hydrazine, Troc-$NHNH_2$,[8)] the protecting group of which can be removed by Zn in acetic acid.

The C-terminal dodecapeptide amide was synthesized by the azide condensation of Z(OMe)-Glu(OBzl)-Arg(Mts)-Gly-$NHNH_2$ and H-Ala-Arg(Mts)-Ala-Arg(Mts)-Leu-NH_2, followed by stepwise addition of respective amino acids *via* the corresponding Np esters[9)] or the azide. Then, the rest of the fragments were successively assembled by the azide procedure.[10)] Throughout this synthesis, Leu was taken as a diagnostic amino acid in acid hydrolysis. By comparison of the recovery of Leu with those of newly incorporated amino acids, satisfactory incorporation of each fragment was ascertained.

The protected form of GRF 1-44 was treated with 1M TFMSA/TFA in the presence of thioanisole[11)]

Fig. Synthetic Scheme of GRF



H-Tyr-Ala-Asp-Ala-Ile-Phe-Thr-Asn-Ser-Tyr-Arg-Lys-Val-Leu-Gly-
Gln-Leu-Ser-Ala-Arg-Lys-Leu-Leu-Gln-Asp-Ile-Met-Ser-Arg-Gln-
Gln-Gly-Glu-Ser-Asn-Gln-Glu-Arg-Gly-Ala-Arg-Ala-Arg-Leu-NH_2

and *m*-cresol[12] in an ice-bath for 60 min, and this treatment was repeated twice more to ensure complete deprotection. The deprotected peptide was converted to the corresponding acetate by Amberlite CG-4B (acetate form), then treated with dil. ammonia (pH 8.0) to reverse possible N→O shift. The Met(O) residue was reduced by incubation with dithiothreitol. (37°C, 24 h).

The crude product thus obtained was then purified by gel-filtration on Sephadex G-25, followed by ion-exchange chromatography on CM-Trisacryl® M using gradient elution with 0.25M NH_4HCO_3 (pH 8.2) buffer. The final purification was performed by reverse-phase HPLC on TSK 410KG column (2.15 x 30 cm) using isocratic elution with 41 % acetonitrile in 0.5 % heptafluorobutyric acid to obtain a homogeneous product, $[\alpha]_D^{20}$ - 60.0° (c=0.2 in 0.2N AcOH), which exhibited a single band in disk isoelectrophoresis (pH 9-11) and a sharp single spot on TLC (Rf 0.30 in BuOH-AcOH-Pyridine-H_2O =4:1:1:2, Rf 0.36 in BuOH-AcOH-Pyridine-H_2O=30:6:20:24). Its purity was further confirmed by 6N HCl-hydrolysis [Asp 4.10 (4), Thr 1.01 (1), Ser 3.66 (4), Glu 7.18 (7), Gly 3.05 (3), Ala 5.19 (5), Val 1.01 (1), Met 0.88 (1), Ile 1.96 (2), Leu 5.00 (5), Tyr 1.97 (2), Phe 1.04 (1), Lys 2.02 (2), Arg 5.83 (6), recovery of Leu 85.0 %] and leucine amino peptidase digestion [Asp 2.07 (2), Thr 0.94 (1), Ser 3.99 (4), Glu 2.20 (2), Gly 3.06 (3), Ala 5.27 (5), Val 1.13 (1), Met 0.85 (1), Ile 2.02 (2), Leu 5.00 (5), Tyr 2.00 (2), Phe 1.10 (1), Lys 2.07 (2), Arg 6.03 (6), Asn (2) and Gln (5) were not determined, recovery of Leu 77.5 %].

When tested in the *in vivo* assay according to Guillemin et al.,[2] our synthetic peptide (0.1-0.5 μg) significantly stimulated secretion of immunoreactive growth hormone in rats.

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REFERENCES AND NOTES

1) Amino acids, peptides and their derivatives are of the L-configuration. The following abbreviations were used; Z=benzyloxycarbonyl, Z(OMe)=*p*-methoxybenzyloxycarbonyl, Mts=mesitylene-2-sulfonyl, DCC=dicyclohexylcarbodiimide, Bzl=benzyl, Troc=trichloroethyloxycarbonyl, Np=*p*-nitrophenyl, TFA=trifluoroacetic acid, TFMSA=trifluoromethanesulfonic acid.

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