Communications to the Editor

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SYNTHESIS OF A 27-RESIDUE PEPTIDE CORRESPONDING TO THE ENTIRE AMINO ACID SEQUENCE OF HUMAN GASTRIN-RELEASING PEPTIDE (hGRP)[1)]

Haruaki Yajima,*,[a] Suzumitsu Kuno,[a] Kenichi Akaji,[a] Osamu Ikemura,[a] Motoyuki Moriga,[b] Mitsuru Aono,[b] Kazuhiko Mizuta,[b] and Atsushi Takagi[b]

Faculty of Pharmaceutical Sciences, Kyoto University,[a] and The First Department of Internal Medicine, Faculty of Medicine, Kyoto University,[b] Sakyo-ku, Kyoto 606, Japan

Human GRP (gastrin-releasing peptide) was synthesized in a conventional solution method by assembling six peptide fragments followed by deprotection with 1 M trifluoromethanesulfonic acid-thioanisole in trifluoroacetic acid. Before the deprotection, the two Met sulfoxides employed were reduced by phenylthiotrimethylsilane. The synthetic peptide raised the immunoreactive gastrin level in rat plasma like synthetic porcine GRP.

KEYWORDS —— human gastrin-releasing peptide synthesis; N^{in}-mesitylenesulfonyltryptophan; N^{G}-mesitylenesulfonylarginine; Met(O) reduction; phenylthiotrimethylsilane; thioanisole-mediated deprotection; trifluoromethanesulfonic acid deprotection; immunoreactive gastrin release

We wish to report the first solution synthesis of a 27-residue peptide corresponding to the entire amino acid sequence of human gastrin-releasing peptide (hGRP), the structure of which was elucidated by cDNA sequence analysis of its precursor.[2)] This peptide possesses a characteristic C-terminal dipeptide amide unit, Leu-Met-NH_2, similar to tachkinins[3)] and bombesin.[4)]

Unlike our previous syntheses of porcine[5)] and chicken GRPs,[6)] our newly introduced Trp-derivative, Trp(Mts),[7)] was employed to suppress the indole-alkylation observable during TFA-deprotection of the Z(OMe) group. Besides Trp(Mts), amino acid derivatives bearing protecting groups removable by 1 M TFMSA-thioanisole in TFA[8)] were employed, i.e., Arg(Mts)[9)] and Lys(Z). The Met(O)[10)] employed was reduced by a newly found reducing reagent, phenylthiotrimethylsilane.[11)]

The C-terminal 7-residue peptide amide (1) and five peptide hydrazides (2-6) were selected as building blocks to construct the entire peptide backbone of hGRP (Fig.). Of these, the corresponding Boc-derivatives of fragment (3) and (5) were used in our previous GRP syntheses[5,6)] and fragment (1) is an intermediate of our neuromedine C synthesis.[12)] These fragments were prepared by the known amide-forming reactions. Necessary fragments thus obtained were assembled successively by the azide procedure[13)] to minimize possible racemization. The reactions were performed in DMF or a DMF-DMSO mixture and the amount of the acyl component was increased from 1.2 to 2.2 equivalent according to the chain elongation. Protected intermediates were purified by precipitation from DMF or DMSO with appropriate solvents or by gel-filtration on a

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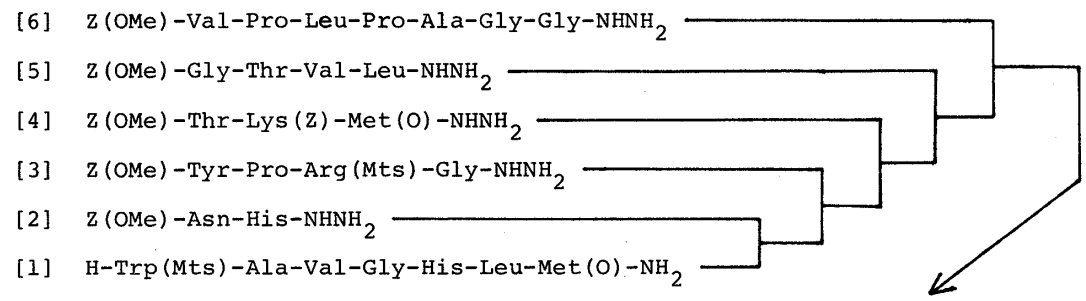
Fig. Synthetic Route to Human Gastrin-Releasing Peptide



H-Val-Pro-Leu-Pro-Ala-Gly-Gly-Gly-Thr-Val-Leu-Thr-Lys-Met-Tyr-Pro-Arg-Gly-Asn-His-Trp-Ala-Val-Gly-His-Leu-Met-NH_2

Sephadex LH-60 using DMF as an eluant. Throughout the synthesis, Leu was selected as a diagnostic amino acid in the amino acid analysis. By comparison of the recovery of Leu with those of newly added amino acids, satisfactory condensation of each fragment was ascertained.

The protected hGRP thus obtained was treated with phenylthiotrimethylsilane (60 eq) at 40°C for 30 min to reduce two residues of Met(O), then it was exposed to 1 M TFMSA-thioanisole/TFA in the presence of m-cresol and ethanedithiol in an ice-bath for 3 h to remove all protecting groups. The deprotected peptide was converted to the corresponding acetate by Amberlite CG-4B (acetate form) and treated with dil. ammonia (pH 8.0) for 30 min to reverse possible N→O shift[14] at the Thr residue. This treatment seems effective in hydrolyzing the O-trimethylsilyl bond at the Thr residue possibly formed during the above reduction of Met(O), as examined using Z(OMe)-Thr-OH. For purification of Met-containing peptides, prolonged incubation with thiol compounds is usually necessary to ensure the complete reduction of the Met(O) employed. However, in the present synthesis, this incubation step was eliminated, since complete reduction of the Met(O) residues was achieved with phenylthiotrimethylsilane before deprotection as stated above. The crude deprotected peptide was then purified by gel-filtration on Sephadex G-25, followed by HPLC on Cosmosil 5C18 (10 x 250 mm) using gradient elution of CH_3CN (28-38%) in 0.1% TFA.

Purity of synthetic hGRP ($[\alpha]_D^{20}$ -99.9° in 1 N AcOH) was ascertained by TLC (Rf 0.44, n-BuOH-AcOH-pyridine-H_2O=4:1:1:2; Rf 0.05, n-BuOH-AcOH-AcOEt-H_2O=1:1:1:1), analytical HPLC on Cosmosil 5C18 (retention time, 13.0 min, under the above elution conditions), isoelectrofocusing on 7.5% polyacrylamide (Pharmalyte, pH 3-10, single band, mobility 6.4 cm from the origin to the cathode, after running at 200 V for 5 h), and amino acid analysis, after 6 N HCl hydrolysis (Asp 0.98, Thr 1.89, Pro 2.97, Gly 4.97, Ala 2.18, Val 2.66, Met 1.91, Leu 3.00, Tyr 0.99, Lys 1.01, His 1.92, Arg 0.96, recovery of Leu 85%, Trp recovery in papain + leucine aminopeptidase digestion 1.04).

Upon i.v. injection into rats, the synthetic human GRP raised plasma immunoreactive gastrin level equivalent to that of synthetic porcine and chicken GRP. Its pancreatic secretion effect was judged to be higher than that of porcine and chicken GRP when tested in rats.

REFERENCES AND NOTES

1) Amino acids, peptides and their derivatives are of the L-configuration. The follow-

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ing abbreviations are used: Z=benzyloxycarbonyl, Z(OMe)=p-methoxybenzyloxycarbonyl, Mts=mesitylenesulfonyl, Boc=tert-butoxycarbonyl, TFA=trifluoroacetic acid, TFMSA=trifluoromethanesulfonic acid, DMF=dimethylformamide, DMSO=dimethylsulfoxide.

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