Communications to the Editor

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SYNTHESIS OF A 42 RESIDUE PEPTIDE CORRESPONDING TO THE ENTIRE AMINO ACID SEQUENCE OF HUMAN GIP[1)]

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Human GIP (gastric inhibitory polypeptide or glucose-dependent insulinotropic polypeptide) was synthesized in a conventional solution method by assembling eight peptide fragments followed by deprotection with 1 M trifluoromethanesulfonic acid-thioanisole in trifluoroacetic acid. New amino acid derivatives, Trp(Mts), Asp(OChp), and Glu(OChp) [Mts=mesitylenesulfonyl, Chp=cycloheptyl] were employed to suppress various side reactions. Synthetic GIP exhibited a significant increase of immunoreactive insulin level in dog plasma under basal ground infusion of glucose.

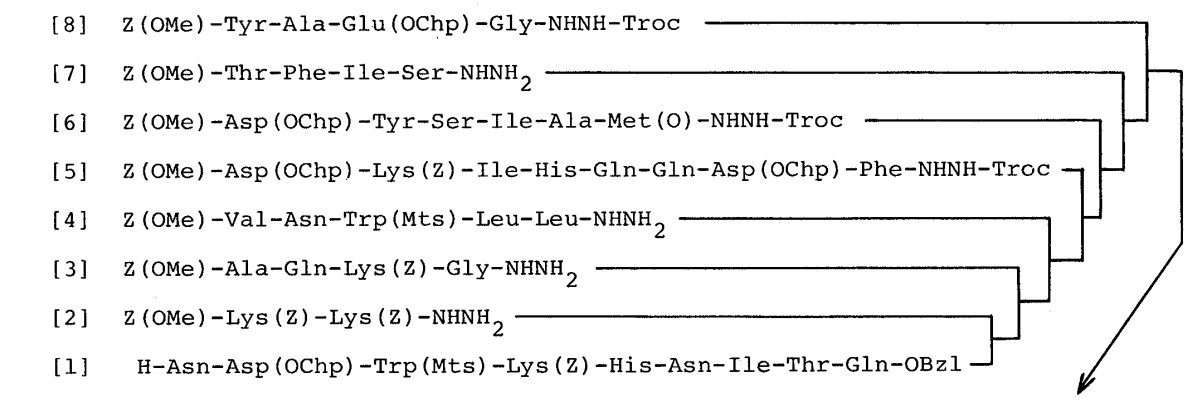
KEYWORDS —— human GIP solution synthesis; β-cycloheptylaspartate; γ-cycloheptylglutamate; N^{in}-mesitylenesulfonyltryptophan; thioanisole-mediated deprotection; trifluoromethanesulfonic acid deprotection; Asp succinimide formation; insulinotropic activity; gastric inhibition

At present, the structures of GIP (abbreviation of gastric inhibitory polypeptide or glucose dependent insulinotropic polypeptide) from three species have been elucidated; i.e., porcine GIP (revised) by Jörnvall et al.,[2)] bovine GIP by Carlquist et al.,[3)] and human GIP by Moody et al.[4)] Here we report the first solution phase synthesis of human GIP, consisting of 42 amino acid residues.

Compared with our previous synthesis of porcine GIP[5)] (Brown's 1971 formula[6)]), several improvements have been made in the present synthesis (Fig.). The thioanisole-mediated deprotecting procedure[7)] was employed. Besides Lys(Z) and Gln-OBzl (C-terminus), three new amino acid derivatives bearing protecting groups removable by 1 M TFMSA-thioanisole in TFA[8)] were employed, i.e., Trp(Mts),[9)] Asp(OChp),[10)] and Glu(OChp).[11)] Trp(Mts) suppresses indole-alkylation during N^{α}-deprotection by TFA. Asp(OChp) was found to suppress significantly base-catalyzed succinimide formation.[12)] Glu(OChp) was employed for the first time to suppress base-catalyzed pyrrolidone formation.[13)]

Eight fragments were selected as building blocks to construct the entire peptide backbone of human GIP. Of these, two fragments, [2] and [7], are known fragments used in our previous synthesis of porcine GIP.[5)] Each fragment was synthesized by the known

Fig. Synthetic Scheme for Human GIP



H-Tyr-Ala-Glu-Gly-Thr-Phe-Ile-Ser-Asp-Tyr-Ser-Ile-Ala-Met-Asp-Lys-Ile-His-Gln-Gln-Asp-Phe-Val-Asn-Trp-Leu-Leu-Ala-Gln-Lys-Gly-Lys-Lys-Asn-Asp-Trp-Lys-His-Asn-Ile-Thr-Gln-OH

amide-forming reactions. Three fragments, [5], [6], and [8], containing the Asp(OChp) or the Glu(OChp) residue were specially synthesized using substituted hydrazine, Troc-$NHNH_2$,[14] the protecting group of which can be removed by Zn[15] or Cd[16] in acetic acid or Zn-NH_4Cl in methanol.[17]

The eight fragments thus obtained were assembled successively by the Honzl and Rudinger's azide procedure[18] according to the scheme (Fig.). The condensations from [1] to [4] proceeded satisfactory as usual. However, the subsequent azide condensations of fragments [5] to [8] were performed at a lower temperature (-18°C) than usual (4°C) in order to minimize the Curtius rearrangement.[19] Each protected product was purified either by precipitation from DMF or DMSO with appropriate solvents, such as methanol or AcOEt, or by gel-filtration on Sephadex LH-60 using DMF as an eluant.

Throughout this synthesis, Thr was used as a diagnostic amino acid. After each condensation, each intermediate was subjected to acid hydrolysis and the recovery of Thr was compared with those of newly added amino acids to assure satisfactory incorporation. The homogeneity of each product was further ascertained by elemental analysis and thin layer chromatography (TLC).

In the final step, the protected GIP thus obtained was treated with 1 M TFMSA-thioanisole in TFA in the presence of m-cresol and ethanedithiol (0°C for 180 min) to remove all protecting groups employed, except Met(O). Met(O) is known to be partially reduced under this thioanisole-mediated condition. The deprotected peptide was treated with dil. ammonia (pH 8.0, 0°C, 30 min) to reverse any possible N→O shift[20] and then incubated with β-mercaptoethanol (37°C, 20 h) to ensure the complete reduction of Met(O). The reduced product, after gel-filtration on Sephadex G-50, was purified by ion-exchange chromatography on CM-cellulose using gradient elution with 0.2 M $AcONH_4$ buffer (pH 6.4). After being desalted by repeated lyophilization, the product was finally purified by HPLC on Nucleosil 5C18 (10 x 250 mm) using gradient elution with acetonitrile (27 to 37% in 1.5 h) in 0.1% TFA at a flow rate of 1.5 ml per min (retention time 64.2 min).

The purified peptide (yield 10% from protected GIP), $[\alpha]_D^{15}$ -39.7° in 1 N AcOH, exhibited a sharp single spot on TLC (Rf 0.26, n-BuOH-pyridine-AcOH-H_2O=4:1:1:2, Rf 0.11, n-BuOH-pyridine-AcOEt-H_2O=1:1:1:1) and a single band in disk isoelectrofocusing

(Pharmalyte pH 3-10). Its purity was further confirmed by 6 N HCl hydrolysis [Asp 6.86(7), Thr 2.00(2), Ser 1.97(2), Glu 5.24(5), Gly 2.08(2), Ala 3.21(3), Val 0.98(1), Met 0.84(1), Ile 3.90(4), Leu 2.18(2), Tyr 1.96(2), Phe 2.07(2), Lys 5.18(5), His 1.91 (2), recovery of Thr 89%] and leucine aminopeptidase digestion [Asp 3.76(4), Thr 1.72 (2), Ser 1.57(2), Glu 0.94(1), Gly 2.00(2), Ala 3.39(3), Val 1.19(1), Met 0.76(1), Ile 3.88(4), Leu 2.20(2), Tyr 1.79(2), Phe 2.03(2), Trp 1.91(2), Lys 5.17(5), His 2.01 (2), recovery of Gly 78%, Gln and Asn were not determined].

Our synthetic GIP (20μg/kg) exhibited a rapid and significant increase of immunoreactive insulin in both peripheral and portal blood in dogs under background infusion of glucose (8μg/h), but no significant inhibition (dose 4-64 μg/kg) against gastric acid secretion stimulated by pentagastrin (1.5 μg/kg) was observed in rats.

REFERENCES AND NOTES

1) Amino acids, peptides and their derivatives are of the L-configuration. The following abbreviations are used: Z=benzyloxycarbonyl, Z(OMe)=p-methoxybenzyloxycarbonyl, Mts=mesitylenesulfonyl, Bzl=benzyl, Troc=2,2,2-trichloroethyloxycarbonyl, Chp=cycloheptyl, TFA=trifluoroacetic acid, TFMSA=trifluoromethanesulfonic acid, DMF=dimethylformamide, DMSO=dimethylsulfoxide.
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