A Gastric Inhibitory Polypeptide II: The Complete Amino Acid Sequence

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Porcine gastric inhibitory polypeptide is a 43 amino acid residue polypeptide with the amino acid sequence Tyr–Ala–Glu–Gly–Thr–Phe–Ile–Ser–Asp–Tyr–Ser–Ile–Ala–Met–Asp–Lys–Ile–Arg–Gln–Gln–Asp–Phe–Val–Asn–Trp–Leu–Leu–Ala–Gln–Gln–Lys–Gly–Lys–Lys–Ser–Asp–Trp–Lys–His–Asn–Ile–Thr–Gln. Fifteen of the first 26 amino acids occur in the same position as they do in porcine glucagon, and nine of the first 26 in the same position as in porcine secretin. The calculated molecular weight of the polypeptide is 5105.

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Le polypeptide inhibant la sécrétion gastrique du porc est un polypeptide contenant 43 résidus d'acides aminés disposés dans l'order suivant: Tyr–Ala–Glu–Gly–Thr–Phe–Ile–Ser–Asp–Tyr–Ser–Ile–Ala–Met–Asp–Lys–Ile–Arg–Gln–Gln–Asp–Phe–Val–Asn–Trp–Leu–Leu–Ala–Gln–Gln–Lys–Gly–Lys–Lys–Ser–Asp–Trp–Lys–His–Asn–Ile–Thr–Gln. Quinze des 26 premiers acides aminés occupent la même position que dans le glucagon de porc et neuf des 26 premiers, la même position que dans la sécrétine de porc. Le poids moléculaire calculé de ce polypeptide est de 5105.

Introduction

Brown (2) reported that "gastric inhibitory polypeptide" (3) could be degraded by trypsin into eight peptides, of which five were produced as a result of major tryptic cleavages at the lysyl bonds. The alignment of the tryptic peptides in the molecule and the partial sequence of the polypeptide were given as:

NH_2–Tyr–(Ala$_2$,Asx,Glx,Gly,Ile$_2$;Phe, Ser$_2$,Thr,Tyr)–Met–Asp–Lys–Ile–Arg–Gln–(Ala,Asx$_2$,Glx$_{2-3}$,Leu$_2$,Phe,Val,Trp)–Lys–Gly–Lys–Lys–Ser–Asp–Trp–Lys–His–(Asn,Gln,Ile,Thr)–COOH

This report deals with the elucidation of the sequences of tryptic peptides Tr-2, Tr-5, Tr-3b, Tr-7, and Tr-3a, *i.e.* those produced as a result of major tryptic cleavages and hence the sequence of the amino acids in the polypeptide. Confirmation of the sequence has been achieved by degradation of the whole molecule, cyanogen bromide fragments, and tryptic peptides with chymotrypsin and thermolysin.

Materials and Methods

Trypsin (TPCK) and chymotrypsin (CDS) were obtained from Worthington and thermolysin (three times recrystallized) from Calbiochem, and made up to a concentration of 2.0 mg/ml in 1% aqueous NH_4HCO_3. The solutions were stored at −20 °C until immediately prior to use. Carboxymethylcellulose (CM 11) was a product of Whatman Co., England, and DEAE-Sephadex and other Sephadex products were obtained from Pharmacia, Sweden. Ammonia in solution and acetic acid were Aristar quality from BDH England. Polyamide plates were obtained from the Cheng Chin Trading Co., Ltd., Taipai, Taiwan.

Gastric Inhibitory Polypeptide

The purification of the polypeptide and the preparation and purification of the tryptic peptides and the cyanogen bromide fragments have been described previously (2, 3).

Amino Acid Analyses

Peptides were dried, taken up in 6 *M* HCl, and hydrolyzed *in vacuo* at 110 °C for 16 h. Quantitative amino acid analyses were performed on a Biochrom BC 200 amino acid analyzer modified to quantitate 5–10 nmoles.

Tryptophan was detected where relevant using Ehrlich's reagent after spotting onto filter paper.

Dansyl-Edman Degradation

The amino acid sequences and the N-terminal residues in pure peptides were determined by the dansyl-Edman method (DNS-Edman), essentially as described by Gray (6) and Gray and Hartley (7). The dansyl-amino acids were identified using polyamide layers (13) with the development of Bruton and Hartley (4) as quoted by Hartley (8), in which the polyamide sheets are cut to a size of 5 cm^2. The solvent systems employed were solvents 1 and 2 (13), solvent 3 (5), and solvent 4 (1.0 *M* ammonia – ethanol, 1:9 v/v) for the separation of εDNS-lysine, αDNS-histidine, and DNS-arginine.

Amide Determination

Amides were located by the determination of the mobility of the peptide relative to aspartic acid at pH 6.5.

Offord (10) supplies data from which the charge on the peptide can be calculated, following the calculation of the molecular weight from the amino acid composition. In peptides containing lysine which had been subjected to the Edman procedure, the lysine exists as PTC lysine which is no longer charged. In peptides containing more than one Asx or Glx, the mobilities were also determined between the Edman steps by paper electrophoresis at pH 6.5 of samples of 15–25 nmoles withdrawn before and after the Edman cycle removing the Asx or Glx.

Enzyme and Chemical Degradations

Tryptic and chymotryptic digestions were performed in 1% ammonium bicarbonate solutions with a 0.2% polypeptide solution and an enzyme/polypeptide ratio of 1:50 (w/w). The reactions were allowed to proceed for 6 h at 22 °C with addition of fresh enzyme every 2 h.

Thermolysin digestion was performed as above except that the enzyme/polypeptide ratio was 1:20 (w/w) and the reaction proceeded for 2 h only, at 22 °C.

Cleavage with cyanogen bromide (CNBr) and the isolation of the CNBr fragments was performed for 6 h in 70% formic acid as previously described (2).

Separation of Peptides

The isolation and purification of peptides produced by tryptic digestion and cyanogen bromide cleavage have been described (2). Chymotryptic peptides (Cht-peptides) and thermolysin peptides (Th-peptides) of the polypeptide, cyanogen bromide fragments, and tryptic peptides were isolated by high-voltage electrophoresis, in cooled tanks (11), at pH 2.1 or pH 6.5 and 4 kV, and by chromatography in the system of Waley and Watson (12). The peptides were eluted from paper with 1% acetic acid or 0.05 *M* ammonia solution after staining of guide strips with a cadmium ninhydrin reagent (9) or with reagents for tyrosine, tryptophan, and arginine (1).

Results

The complete amino acid sequence of porcine "gastric inhibitory polypeptide" has been established from work on the tryptic peptides, cyanogen bromide cleaved fragments, and chymotryptic and thermolysin peptides. The results presented below are summarized in three tables for clarity in the presentation of the data.

Amino Acid Sequence of Peptide Tr-2

The peptide was purified by the methods previously described (2). The N-terminal amino acid was confirmed as tyrosine and the original amino acid composition of the peptide was confirmed by amino acid analyses on 30 nmoles of material. The presence of a tyrosine, other than the N-terminal residue, and a phenylalanine prompted degradation of the peptide with chymotrypsin. Degradation of 1.0 mg (Ω 0.5 μmole) with chymotrypsin was performed by the method described. High voltage electrophoresis of 20 nmoles of the degradation mixture for 45 min, 4 kV and pH 6.5, revealed the presence of three major ninhydrin-positive peptides, 1 neutral and 2 acidic. The remainder of the degradation mixture was applied to paper, and after electrophoresis, as described, guide strips were cut and stained with ninhydrin. The peptides were located and eluted from paper using 1% acetic acid.

The peptides were labelled Tr-2–Cht-1, -2, and -3. Peptide Tr-2–Cht-1 was nearest the anode. Each peptide (10 nmoles) was hydrolyzed as described for subsequent amino acid analyses, and 2.0 nmoles were taken for N-terminal amino acid determinations by the dansyl technique. Approximately 100 nmoles of each of the peptides were subjected to the DNS-Edman procedure and the sequences were elucidated. This information, when taken together with the information obtained from cleavage of Tr-2 with cyanogen bromide (2), allowed the structure of Tr-2 to be clarified. Confirmation of the sequence of Tr-2 has also been obtained by Edman degradation of CN-1 (Table 1).

Amino Acid Sequence of Tr-5

This peptide had been shown earlier to be isoleucylarginine (2).

Amino Acid Sequence of Tr-3b

The peptide was isolated from paper and not by the method described by Brown (2). Two milligrams of polypeptide were digested with trypsin as described and then subjected to high voltage electrophoresis at pH 6.5 and 4 kV for 45 min. The neutral peptides Tr-3a and Tr-3b were eluted from paper after staining guide strips with ninhydrin. Tr-3b was separated from Tr-3a by chromatography in the system of Waley and Watson (12). The peptide was obtained in low yield, approximately 30% recovery. Fifty nanomoles were subjected to DNS-Edman degradation and the sequence of the peptide was elucidated (Table 2). The position of amides and confirmatory sequences were determined on chymotryptic fragments of the whole molecule and the C-terminal CNBr fragment (CN-2).

The peptide Cht-3a was isolated from a chymotryptic digestion mixture of the polypeptide by paper chromatography (12), followed by high voltage paper electrophoresis at pH 6.5. The peptide was a tryptophan-containing peptide and Ehrlich's reagent was used to detect the

TABLE 1. Amino acid sequence of tryptic peptide Tr-2, the N-terminal peptide

Peptide	Sequence of peptides
Sequence	H_2N–Tyr–Ala–Glu–Gly–Thr–Phe–Ile–Ser–Asp–Tyr–Ser–Ile–Ala–Met–Asp–Lys
CN-1	Tyr–Ala–Glu–Gly–Thr–Phe–Ile–Ser–Asp–Tyr–Ser–Ile–Ala–Met[a]
Tr-2-Cht-2	Tyr–Ala–Glu–Gly–Thr–Phe
Tr-2-Cht-1	Ile–Ser–Asp–Tyr
Tr-2-Cht-3	Ser–Ile–Ala–Met–Asp–Lys
Tr-2-CN-2	Asp–Lys

[a]Identified in CN-1 as homoserine.
NOTE: All peptides were sequenced by the dansyl-Edman procedure. The electrophoretic mobilities of the chymotryptic (Cht) peptides, at pH 6.5, were used to demonstrate the absence of amides in Tr-2.

TABLE 2. Amino acid sequence of tryptic peptide Tr-3b

Peptide	Sequence of peptides
Sequence	Gln–Gln–Asp–Phe–Val–Asn–Trp–Leu–Leu–Ala–Gln–Gln–Lys
CN-2-Cht-2	Asp–Lys–Ile–Arg–Gln–Gln–Asp–Phe
Cht-3a	Val–Asn–Trp
Cht-WW4	Leu–Leu–Ala–Gln–Gln
Tr-3b	Gln–Gln–Asp–Phe–Val–Asn–Trp–Leu–Leu–Ala–Gln–Gln
CN-2	Asp–Lys–Ile–Arg–Gln–Gln–Asp–Phe–Val–Asn–Trp–Leu–Leu–Ala–Gln–Gln–Lys

peptide at each purification stage. After elution from paper, 10 nmoles of peptide were hydrolyzed for subsequent amino acid analysis. The composition of peptide Cht-3a was found to be Asx_1, Val_1, and Trp_1, the latter identified by Ehrlich's reagent. The sequence Val–Asx–Trp was arrived at after two stages of the DNS-Edman procedure. The presence of asparagine was determined by the neutral behavior of the peptide following high voltage electrophoresis at pH 6.5 (10).

The peptide Cht-WW4 was isolated from a chymotryptic digestion mixture of the polypeptide by paper chromatography (12). Amino acid analyses after acid hydrolysis revealed the composition Ala_1, Glx_2, Leu_2. The sequence

Leu–Leu–Ala–Gln–Gln

was elucidated by the DNS-Edman technique, and the presence of the amides was determined by the neutral behavior of the peptide following high voltage electrophoresis at pH 6.5.

The peptide CN-2–Cht-2 was obtained after chymotryptic digestion of the fragment CN-2. The peptide was isolated by high voltage electrophoresis at pH 2.1, 4 kV, and 45 min. After staining guide strips, CN-2–Cht-2 was eluted from the paper and rerun at pH 6.5, 4 kV, and 45 min. The peptide behaved as a neutral peptide at pH 6.5. Two nanomoles were taken for N-terminal amino acid determination using the dansyl technique and 150 nmoles were subjected to the DNS-Edman procedure. The sequence

$$\underrightarrow{\text{Asp}}\text{–}\underrightarrow{\text{Lys}}\text{–}\underrightarrow{\text{Ile}}\text{–}\underrightarrow{\text{Arg}}\text{–}\underrightarrow{\text{Gln}}\text{–}\underrightarrow{\text{Gln}}\text{–}\underrightarrow{\text{Asp}}\text{–}\underrightarrow{\text{Phe}}$$

was determined after seven stages of the Edman procedure. Twenty-five nanomoles were taken at every Glx or Asx N-terminus and subjected to high voltage electrophoresis at pH 6.5 to determine the position of the amides. The sequence of tryptic peptide Tr-3b is summarized in Table 2.

Amino Acid Sequence of Peptide Tr-7

This peptide had been shown earlier to be Gly–Lys–Lys (2).

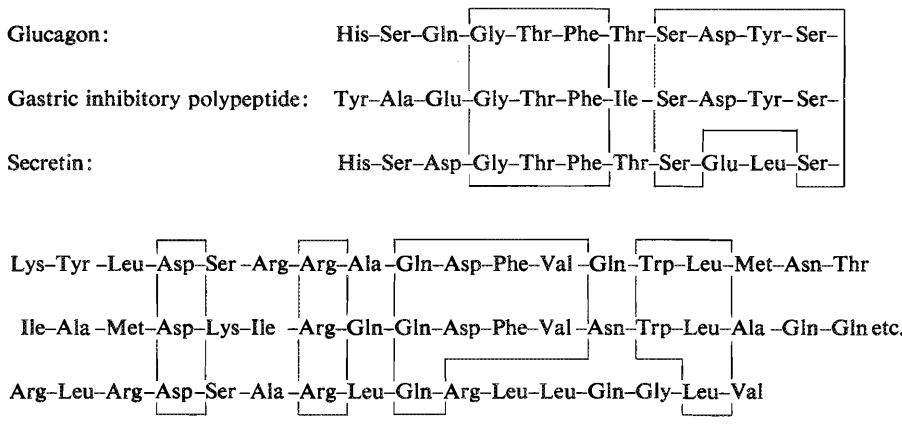
Amino Acid Sequence of Peptide Tr-3a

The peptide was prepared by the method described by Brown (2) and by elution from paper following high voltage electrophoresis at pH 6.5, 4 kV, for 45 min, and paper chromatography (12). In the latter isolation procedure, the peptide was prepared from 2.0 mg of polypeptide. One hundred nanomoles of the peptide were digested with thermolysin and three peptides were obtained which were separated by paper chromatography (12). After elution with 1% acetic acid, 2 nmoles were taken for N-terminal amino acid determination, 10 nmoles for amino acid analyses, and approximately 80 nmoles of each peptide for sequence determination by the DNS-Edman

TABLE 3. Amino acid sequence of tryptic peptide Tr-3a, the C-terminal peptide

Peptide	Sequence of peptides
Sequence	Ser–Asp–Trp–Lys–His–Asn–Ile–Thr–Gln–COOH
Tr-3a	Ser–Asp–Trp–Lys–His–Asn–Ile–Thr–Gln
Tr-3a–Th-1	Ser–Asp–Trp–Lys
Tr-3a–Th-2	His–Asn
Tr-3a–Th-3	Ile–Thr–Gln
Tr-3c	His–Asn–Ile–Thr–Gln
CN-2–Cht-3	Lys–His–Asn–Ile–Thr–Gln

TABLE 4. Similarities in structure of "gastric inhibitory polypeptide" with porcine glucagon and porcine secretin



technique. The position of the amides was determined by the method of Offord (10), and where Glu/Gln or Asp/Asn occurred C-terminally as in Tr-3a–Th-2 and Tr-3a–Th-3, they were confirmed by identification of the dansyl-amino acid, before and after acid hydrolysis. In an earlier study, Brown (2) had described the existence of a tryptic peptide Tr-3c, produced as a result of a minor tryptic cleavage. This peptide was isolated in pure form in this study and sequenced using the DNS-Edman procedure. Additional evidence for the sequence of this peptide was obtained from the sequence of peptide CN-2–Cht-3. This peptide was obtained from a chymotryptic digestion mixture of CN-2. These results and the sequence of Tr-3a are presented in Table 3.

Discussion

The sequence of the N-terminal tryptic peptide Tr-2 is shown in Table 1, and is deduced from the information obtained from the sequences of the chymotryptic fragments of the peptide and from the cleavage of the whole molecule and peptide Tr-2 with cyanogen bromide. It was earlier reported (2) that Tr-2 was followed by Tr-5 in the alignment of the tryptic peptides. The sequence of the amino acids in tryptic peptide Tr-3b caused considerable difficulties because of the low yields obtainable and the presence of a large number of amides. It did prove possible to sequence the peptide by performing 12 stages of the DNS-Edman procedure but the presence of amides and the confirmation of the sequence had to be obtained by studies on fragments produced by chymotryptic digestion of the polypeptide itself or the C-terminal cyanogen bromide fragment of the polypeptide. The peptide Tr-7 had been sequenced earlier (2). After cleavage with thermolysin, the sequence of Tr-3a, the C-terminal major tryptic peptide, was performed without difficulty. The full amino acid sequence of the polypeptide is given in Fig. 1. It can be seen that in these results and those of the earlier

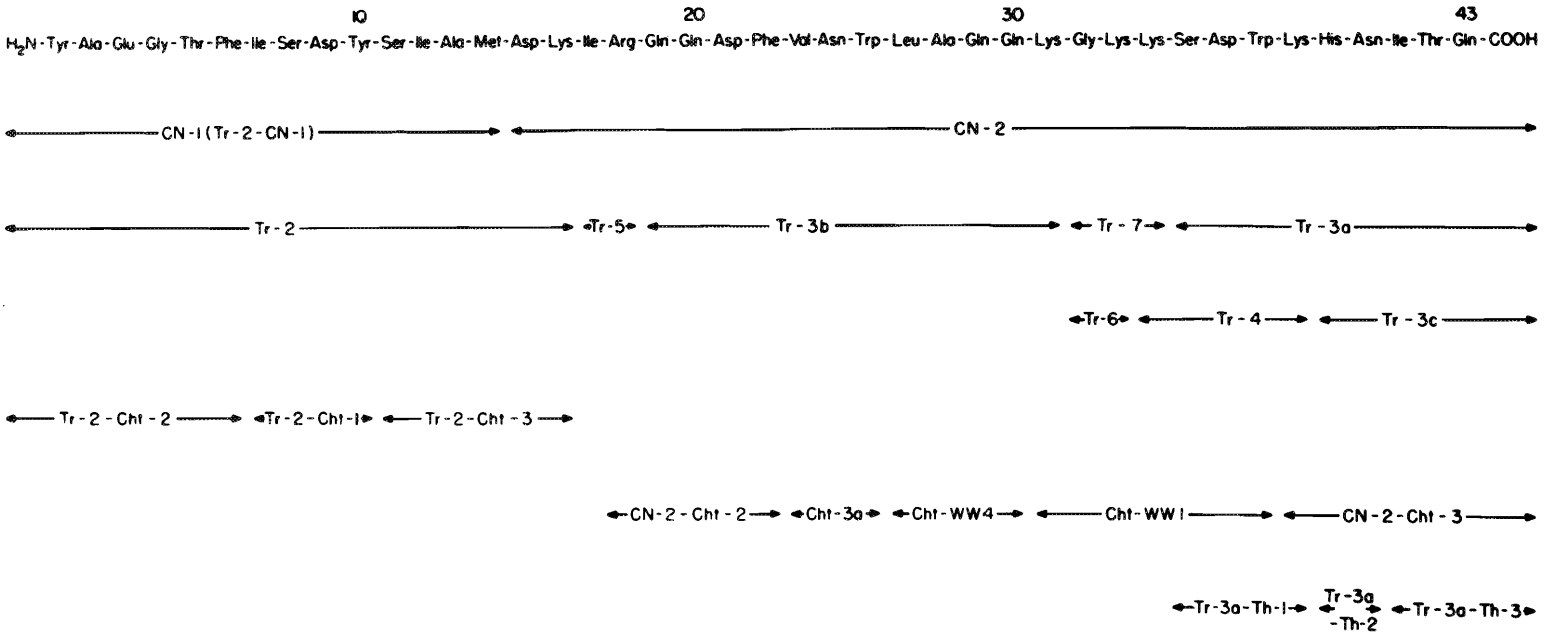


FIG. 1. The amino acid sequence of "gastric inhibitory polypeptide". All peptides except CN-2 were sequenced completely by the DNS-Edman technique.

paper (2) most regions of the molecule have been analyzed for composition and sequence in at least two different ways and the various tryptic peptides have now been purified and analyzed more than once and in different ways.

Of interest are the similarities in structure of "gastric inhibitory polypeptide" with porcine glucagon. In the N-terminal part of the molecule 15 of the first 26 amino acids of "gastric inhibitory polypeptide" occur in the same position as they do in porcine glucagon (Table 4). There are also similarities with the gastrointestinal hormone, porcine secretin; nine of the first 26 amino acids of the "inhibitory polypeptide" occur in the same position as secretin. Whereas to date we have not been able to demonstrate any similar physiological actions between glucagon and "gastric inhibitory polypeptide", both secretin and the polypeptide will inhibit gastrin-stimulated H^+ secretion in dogs.

The molecular weight of the polypeptide, calculated from the amino acid composition, is 5105.

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