Purification and Structural Characterization of Progastrin-derived Peptides from a Human Gastrinoma*

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Several peptides derived from the gastrin-predicted preprohormone sequence were isolated from a human gastrinoma by gel permeation, anion exchange, and reverse phase chromatography. The peptides were identified and characterized structurally by a combination of radioimmunoassays, mass spectral analysis, and microsequence analysis. The largest peptide, progastrin-(1–35) (cryptogastrin), extends from the putative processing site for the signal peptide to the double basic residues adjacent to the amino terminus of gastrin 34. A shorter form of this peptide, progastrin-(6–35) (cryptogastrin-(6–35)), was also isolated in smaller amounts. In addition, sulfated and nonsulfated gastrin 17 amides (progastrin-(55–71)) and the glycinextended nonsulfated gastrin 17 (progastrin-(55–72)) were identified by radioimmunoassay, and their structures were confirmed by mass spectral analysis. Isolation of cryptogastrin indicates that the signal peptide of human preprogastrin contains 21 amino acid residues, and progastrin, therefore, contains 80 amino acids. There is minimal processing of the cryptic peptide preceding the sequence of gastrin 34. An amidated gastrin form larger than gastrin 34 could contain 71 amino acids. No evidence was obtained for processing that would produce gastrins containing more than 34 but less than 71 amino acid residues.

Gastrin is an important hormone that regulates gastric acid secretion and gastric mucosal growth (1, 2). Hypersecretion of gastrin from gastrin-producing tumors (gastrinomas) leads to hyperplasia of the gastric mucosa and hypersecretion of acid associated with severe peptic ulcer disease, a clinical condition known as Zollinger-Ellison syndrome (3).

Gastrinomas have been the source for purification of several molecular variants of gastrin. Gregory and coworkers isolated and characterized nonsulfated and sulfated heptadecapeptide gastrins (4), "big" gastrins (5), and minigastrins (6). The latter forms of gastrin were found to contain 34 and 14 amino acid residues (7). These peptides all are carboxyl-terminal amidated at a phenylalanine residue and have similar biological activities as stimulants of acid secretion, although the smaller forms are cleared from the circulation more rapidly than the larger forms (8, 9).

Elucidation of the cDNA structure of human gastrin has been done from mRNA extracted from gastrinoma (10) and from normal human stomach (11–13). Based on the nucleotide sequence, the structure of a 101-amino acid human preprogastrin could be deduced. This preprogastrin was predicted to contain a leader sequence of approximately 20 residues and two flanking peptides at the amino-terminal and carboxy-terminal ends of gastrin 34. The carboxy-terminal region also contains a typical amidation region consisting of glycine followed by 2 arginine residues (14). Preliminary characterization of the carboxyl-terminal progastrin peptides has been carried out by use of antibodies specific for the carboxy-terminal flanking peptide (15) and for glycine-extended gastrin (16). Other region-specific antisera have been used to identify differences in posttranslational progastrin processing between gastrinoma and normal human gastric antral tissue (17).

Very little structural analysis has been done to fully characterize gastrin gene products in gastrinoma other than the amidated forms known to be biologically active. We have produced evidence that the site for signal peptide cleavage of preprogastrin is between residues 21 and 22 of preprogastrin (18). This processing site has been confirmed by another group (19). In order to understand fully the complex processing of progastrin so that activities of various peptide products can be studied, it is essential to isolate and characterize each of the products of posttranslational processing in vivo. The present study describes a comprehensive approach to such analyses using a combination of chromatographic separation steps combined with radioimmunoassay, mass spectrometry, and microsequence analysis.

MATERIALS AND METHODS

Extraction of Gastrin and Amino-Terminal Flanking Peptides from Gastrinoma Tissue—A 4.4-g portion of a human gastrinoma, obtained at operation and stored at −70 °C, was broken into small pieces, added to 20 ml of boiling water, and boiled for 15 min. After cooling on ice, ammonium bicarbonate was added to a final concentration of 0.1 M. The tissue was then homogenized with a polytron homogenizer and extracted by mild stirring at 4 °C for 2 h. The extracted tissue was then centrifuged at 31,000 × g for 1 h. The supernatant was then loaded onto two Sep-Pak C18-columns (Millipore Corp.), eluted sequentially, which were washed with 20 ml of 0.1 M ammonium bicarbonate.

The concentrated tumor extract was purified by chromatography on a Vydac pH-stable C4 column in 0.1 M ammonium bicarbonate, using a gradient of 0–50% acetonitrile. The column eluant was monitored by absorbance at 214 nm. Two-milliliter fractions were col-

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lected and assayed for gastrin and glycine-extended gastrin immunoreactivity using region-specific radioimmunoassays (20, 21). Further purification involved rechromatography of the fractions on the same column, using shallower gradients for elution.

**Extraction of Glycine-extended Gastrin from Gastrinoma Tissue—** In order to determine the molecular ion species present for the gastrin samples, the gastrinoma extracts were dissolved in 2-5 μl of dimethyl sulfoxide. Approximately 2 μl of the solution was added to approximately 1 μl of 10% ethanolamine in glycerol (negative ion FAB spectra) or 1 μl of dithiothreitol-dithioerythritol (5:1) (positive ion FAB spectra) on a 1.5 x 6-mm stainless steel sample stage. Excess solvent was evaporated in the vacuum lat. of the direct probe inlet.

**RESULTS**

**Gastrin and Amino-Terminal Progastrin Peptides—** Fig. 1A shows the elution profile from the pH-stable C₈ column for the gastrinoma extract. Two peaks of gastrin immunoreactivity were observed. Based on mass spectral analysis (Table I) of the purified samples, the earlier eluting peak was identified as sulfated gastrin 17 and the later eluting peak as nonsulfated gastrin 17. Two major absorbance peaks with no associated gastrin immunoreactivity were present in the fractions adjacent to those containing gastrin 17 and sulfated gastrin 17. The predominant peptide in each of these fractions was purified to apparent homogeneity by rechromatography on the C₈ column using the same ammonium bicarbonate, acetate system (Fig. 1, B and C). Half of each of these peptides was subjected to microsequence analysis. The amino-terminal sequences obtained are given in Table II. In order to determine their mass, 25% of the sample was injected into a JEOL HX-100HF double-focusing mass spectrometer operating at 5 kV acceleration voltage and a nominal resolution of 3000 unless otherwise noted. Sample ionization was by means of a 6-KeV Xe atom beam. Mass spectra were obtained in two different modes of operation. For broad mass range survey scans, repetitive scans of mass-assigned data were collected directly using a JEOL DA6000 1 The abbreviation used is: FAB, fast atom bombardment.

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the peptide samples were analyzed by mass spectrometry. The smaller peptide gave a protonated molecular ion at m/z 3250, and the larger peptide gave a molecular ion at m/z 3904 mass (Table I, Fig. 2). These molecular weights correspond within experimental error to those of peptides extending up to the double arginine processing site just before the amino terminus of gastrin 34 (Table I, Fig. 3). Both peptides are derived from the amino-terminal region of progastrin that precedes the gastrin 34 sequence. Progastrin-(6–35) (cryptogastrin-(6–35)) could be derived from progastrin-(1–35) (cryptogastrin) by proteolytic cleavage at an arginine residue. Amino acid analysis of both peptides confirms their identity (Table III).

**Glycine-extended Gastrin**—On Sephadex G-50, the major immunoreactive peak of glycine-extended gastrin eluted slightly before the major immunoreactive peak of amidated gastrin, but there was considerable overlap. Total glycine-extended gastrin immunoreactivity represented approximately 20% of the gastrin immunoreactivity eluted from the G-50 column. The glycine-extended gastrin immunoreactivity was further purified by chromatography on a fast protein liquid chromatography Mono Q column. Final purification was achieved on a Vydac pH-stable C8 column, as described under “Materials and Methods.”

Approximately 550 pmol of the glycine-extended gastrin immunoreactivity was desalted as described under “Materials and Methods” and analyzed by FAB mass spectrometry. Upon analysis of half of the sample (at low resolution), two molecular ions were obtained. The average mass of the first was

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**TABLE I**

| Peptide              | Molecular ion form | Monoisotopic mass | Calculated | Observed |
|----------------------|--------------------|-------------------|------------|----------|
| Gastrin 17           | (M – H)            | 2095.8            | 2096.0     |
| Sulfated gastrin 17  | (M – H)            | 2175.8            | 2176.0     |
| Cryptogastrin        | (M + H)*           | 2450.37           | 2450.4     |
| Cryptogastrin-(6–35) | (M + H)*           | 3249.6            | 3249.6     |
| Glycine-extended gastrin 17 | (M + H)* | 2155.3*          | 2155.1*    |

*Average mass.

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**TABLE II**

| Cycle | Residue | Picomoles | Cycle | Residue | Picomoles |
|-------|---------|-----------|-------|---------|-----------|
| 1     | Ser     | 65        | 1     | Ser     | 62        |
| 2     | Gin     | 166       | 2     | Trp     | 65        |
| 3     | Gin     | 125       | 3     | Lys     | 171       |
| 4     | Pro     | 104       | 4     | Pro     | 41        |
| 5     | Asp     | 123       | 5     | Arg     | 37        |
| 6     | Ala     | 35        | 6     | Ser     | 35        |
| 7     | Glu     | 64        | 7     | Asp     | 41        |
| 8     | Leu     | 41        | 8     | Glu     | 42        |
| 9     | Gly     | 49        | 9     | Pro     | 26        |
| 10    | Thr     | 38        | 10    | Asp     | 32        |
| 11    | Gin     | 43        | 11    | Ala     | 25        |
| 12    | Ala     | 46        | 12    | Pro     | 20        |
| 13    | Asn     | 53        | 13    | Leu     | 6         |
| 14    | Arg     | 26        | 14    | Gly     | (14)*     |
| 15    | Asp     | 32        | 15    | Thr     | 3         |
| 16    | Gin     | 29        | 16    | Gin     | 14        |
| 17    | Gin     | 28        | 17    | Ala     | 11        |
| 18    | Leu     | 15        | 18    | Asn     | 11        |
| 19    | Pro     | 31        | 19    | Arg     | 7         |
| 20    | Gin     | 3         | 20    | Gin     | 14        |
| 21    | Leu     | 5         | 21    | Leu     | 9         |
| 22    | Gin     | 7         | 22    | Gin     | 7         |
| 23    | Gin     | 16        | 23    | Gin     | 16        |
| 24    | Gin     | 22        | 24    | Gin     | 22        |
| 25    | Gly     | 14        | 25    | Gly     | 14        |

* Tube number 14 was accidentally lost. The value for Gly is based on carry-over Gly from cycle 15.

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**TABLE III**

| Amino acid | Cryptogastrin-(6–35) | | Cryptogastrin | |
|------------|----------------------|------------------|--|------------------|
|            | Residues/mol | Expected | Residues/mol | Expected |
| AsP        | 3.2          | 3        | 2.9          | 3        |
| Thr         | 1.2          | 1        | 1.5          | 1        |
| Ser         | 2.9          | 2        | 2.8          | 3        |
| Glx         | 4.9          | 6        | 6.3          | 6        |
| Pro         | 2.8          | 4        | 3.6          | 5        |
| Gly         | 4.1          | 3        | 3.5          | 3        |
| Ala         | 3.3          | 3        | 3.0          | 3        |
| Val         | 0.4          | 0        | 0.7          | 0        |
| Met         | 0.4          | 0        | 0.2          | 0        |
| Ile         | 0            | 0        | 0.5          | 0        |
| Leu         | 2.5          | 4        | 4.3          | 4        |
| Tyr         | 0.4          | 0        | 0.3          | 0        |
| Phe         | 0.4          | 0        | 0.4          | 0        |
| His         | 1.6          | 2        | 1.3          | 2        |
| Trp         | 0.5          | 1        | 0.9          | 2        |
| Lys         | 0.9          | 1        | 1.6          | 1        |
| Arg         | 1.0          | 1        | 1.9          | 2        |
2155 u, in agreement with the value calculated for glycine-extended gastrin 17. A second, more predominant molecular ion gave an average mass of 2171 u, corresponding to the molecular mass for glycine-extended gastrin with an oxidized methionine.

**DISCUSSION**

The structural characterization of gastrin offers many challenges. Its blocked amino terminus makes direct sequence analysis impossible, and its heterogeneity with respect to size, sulfation, and oxidation of methionine makes immunoreactivity studies difficult to interpret. We have used a combination of radiomunoassay and FAB mass spectrometry to fully characterize several different forms of gastrin present in a particular human gastrinoma. Sulfated and unsulfated gastrin 17 were detected in this tissue, as well as several progastrin metabolites without known biological activities.

We report here a structural confirmation of the existence of a glycine extended precursor of gastrin 17. Previous studies (16) have demonstrated a substance with the expected immunochemical and chromatographic properties of glycine-extended gastrin 17. Only immunoreactivity was used as the basis for identification (14), and no chemical replicates have been available for direct comparison.

Glycine-extended gastrin is stored in normal gastric cells and is released into the circulation in proportions similar to those of stored forms (24, 25). In cultured rat gastric cells, amidated and glycine-extended gastrin are stored in approximately equal amounts and released in response to secretagogues in similar proportions (26). The relative amounts of glycine-extended gastrins are increased to variable extents in human disease associated with hypergastrinemia, including gastrinoma (27, 28). The biological activity of glycine-extended gastrins appears to be much lower than that of amidated gastrins, although some activity has been detected on basal or stimulated gastric acid secretion (30).

We have also fully characterized by microsequencing and mass spectrometry the amino-terminal peptide of progastrin. This peptide corresponds to the peptide sequence between the signal peptide and the amino terminus of cryptagastrin that was not resolved by the use of an ammonium bicarbonate buffer system permitting microsequence analysis of the two peptides separately. Further characterization by mass spectrometry confirmed the molecular masses of these two peptides. The carboxyl terminus of both peptides is His35 of progastrin, which probably results from cleavage after Arg26-Arg27, followed by two steps of a carboxypeptidase B-like enzyme. This processing system resembles the enzymatic steps needed to process the carboxyl-terminal regions of progastrin to form gastrin 34 and gastrin 17.

The shorter amino-terminal progastrin peptide lacked the five amino acids present at the amino terminus of crypt gastrin and appeared to arise from crypt gastrin by cleavage at a single basic residue. The existence of the same two progastrin peptides from two different human gastrinomas suggests that the processing resulting in these peptides may be found in normal tissue. Development of specific radiomunoassay for these peptides will enable investigators to confirm this presumption.

The progastrin gene has been proposed to have arisen from gene duplication due to a homology between regions 29–54 and 62–87 of preprogastrin (10). The sequence Pro-Trp-Leu-Glu is found in both the progastrin peptides described here (amino acids 24–27) and gastrin 17 (amino acids 57–60) (Fig. 3). This conservation of structure may have physiological significance.

Now that the amino-terminal flanking peptides of progastrin are known, further studies can be directed at determining whether or not specific receptors exist for synthetic duplicates of crypt gastrin or of its shorter fragment. In addition, immunoassays can now be developed to localize and quantitate these peptides in normal tissues, in an attempt to gain insight into the normal biosynthetic processing of progastrin.

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