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Isolation and characterization of the intact gastrin precursor from a gastrinoma

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Antibodies to the extreme C-terminal region of human progastrin have been used to monitor the isolation of high-M_r immunoreactive material in a gastrinoma extract. Microsequence analysis of the product revealed amino acid residues in the first 18 positions corresponding to those predicted from the cDNA sequence for preprogastrin starting at position 22; the sequence and immunochemical data together allow the identification of this material as intact progastrin. Implications for gastrin biosynthesis are discussed.

Progastrin; Hormone synthesis; Amino acid sequence

1. INTRODUCTION

It is now known from cDNA sequencing that the human gastric acid-stimulating hormone gastrin is initially synthesized as a precursor of 101 residues [1–3]. The precursor contains a single copy of the 34-residue peptide, big gastrin (G34), which is the largest chemically characterized form of the hormone. In antral gastrin cells there is evidence that tryptic-like cleavage of the precursor liberates flanking peptides at the C- and N-terminus of G34, and cleaves G34 to give G17, which is the major biologically active form of the hormone, and an N-terminal fragment (NT G34) [4–6]. It seems, however, that different biosynthetic processing pathways occur in other gastrin-producing tissues, e.g. human duodenum and gastrinomas [6]. To study the mechanisms of post-translational modification in more detail we have recently developed antibodies to a range of peptides likely to be derived from the precursor. One of these is specific for the extreme C-terminal region of the intact precursor and in some gastrinomas this antibody reveals abundant quantities of material with apparently high M_r [6]. We report here the isolation of this material together with evidence that it corresponds to intact progastrin and that it also occurs in antral mucosa.

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2. MATERIALS AND METHODS

2.1. *Isolation of progastrin*

The starting material for the present isolation was a large liver metastasis obtained at surgery from a gastrinoma patient. Fresh tissue (21 g) was quickly added to boiling water (210 ml), heated for 10 min to inactivate proteases, and then homogenised. The extract was centrifuged twice (3000 × *g*, 15 min, 4°C, followed by 13000 × *g*, 30 min), and to the supernatant was added chloroform (2 vols) and methanol (1 vol.); the extract was then centrifuged and the aqueous layer lyophilised. Approx. 200 mg of the resulting powder (corresponding to about 10 g tissue) was dissolved in distilled water, centrifuged (50000 × *g*, 30 min) and fractionated by gel filtration on

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Sephadex G-50 (2.5 × 100 cm) in 0.05 M ammonium bicarbonate. Tubes containing immunoreactive material of apparently high M_r were reduced in volume and an aliquot corresponding to approx. 10% of the total was purified by HPLC on a μBondapak C_{18} cartridge in a Waters Z-module using an Altex system and gradient elution with acetonitrile and 0.1% aqueous trifluoroacetic acid (TFA). A final purification was made on a Vydac 5 μm C_{18} (5 × 250 mm) column using a similar system. For analytical studies, extracts of five other gastrinomas resected at surgery, and of antral mucosa (n = 5) resected from patients undergoing antrectomy for duodenal ulcer, were prepared by boiling in water, and fractionated by gel filtration and HPLC on Techsil 5 μm C_{18} (5 × 250 mm) using conditions similar to those described above. The radioimmunoassay (RIA) used to monitor purification has been detailed in [6]. It employe an antibody raised to synthetic Tyr-Ala-Glu-Asp-Glu-Asn, which corresponds to (Tyr) progastrin 97–101 (the predicted extreme C-terminal pentapeptide of the precursor); the same peptide was used for radiolabelling and as standard.

2.2. *Structural studies*

Microsequence analysis was carried out using an Applied Biosystems gas-phase sequencer (470 A) and identification of phenylthiohydantoin (PTH) derivatives was made by HPLC. Possible post-translational modifications of the precursor in the form of sulphation and phosphorylation were examined by: (i) incubation with arylsulphatase (200 or 400 μg) in sodium acetate for 2 h at 37°C [7], followed by trypsinization, HPLC separation (C_{18} Techsil 5 μm) and identification of G17-containing tryptic peptides by N-terminal specific RIA [8]; (ii) phosphate determination by the malachite green method [9].

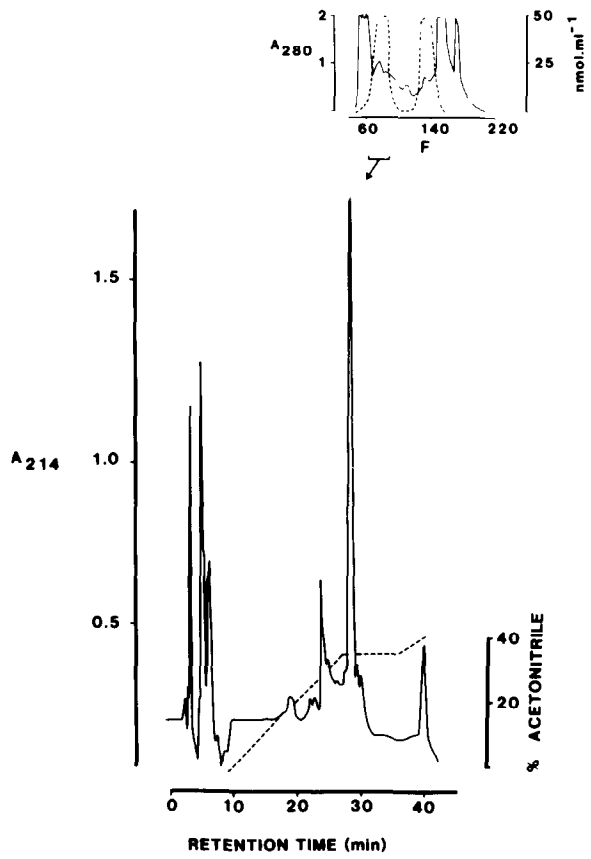

Fig.1. Reversed-phase separation on HPLC of a gastrinoma extract. The major peak (retention time 28 min) reacts with antibodies to the C-terminus of progastrin. The gastrinoma extract was fractionated first on Sephadex G-50 (2.5 × 100 cm) in 0.05 M ammonium bicarbonate when two peaks of immunoreactivity were identified (see inset; ——, A_{280}; – – –, immunoreactivity, nmol·ml^{-1}, abscissa: fraction number, volume 5.5 ml). The earlier eluting peak in the Sephadex eluate was applied to μBondapak C_{18} cartridge in a Waters Z module and eluted with an acetonitrile gradient (– – –) from 0.1% aqueous TFA. Further separation on Vydac C_{18} gave a homogeneous peptide for sequencing.

3. RESULTS AND DISCUSSION

In RIA, antibodies specific for the extreme C-terminus of progastrin revealed two major peaks of immunoreactivity when the gastrinoma extract was fractionated on Sephadex G-50 (inset, fig.1). The earlier eluting material emerged as a single major peak when it was further purified by HPLC (fig.1). A second HPLC step on Vydac C_{18} gave a homogeneous peptide suitable for amino acid analysis. Recovery was >75% at each step. RIA indicated that 9.5 nmol of the final product was obtained, and this agrees well with an estimate of 11.0 nmol determined by A_{280}. The latter estimate is based on the calculated molar extinction coefficient for a peptide with three tryptophan and one tyrosine residue (as predicted from the cDNA se-

	20	22	25	30	35	40
Human progastrin predicted from cDNA	Glu - Ala - Ser - Trp - Lys - Pro - Arg - Ser - Gln - Gln - Pro - Asp - Ala - Pro - Leu - Gly - Thr - Gly - Ala - Asn - Arg -					
Gastrinoma Peptide		Ser - Trp - Lys - Pro - Arg - Ser - Gln - Gln - Pro - Asp - Ala - Pro - Leu - Gly - Thr - Gly - Ala - Asn -				

Gly

Fig.2. Amino acid sequence of the gastrinoma peptide (see fig.1) determined by gas-phase microsequence analysis. PTH amino acids were identified for 18 cycles; at cycle 15 both Gly and Thr were observed. Shown above is the amino acid sequence of progastrin predicted from cDNA sequencing [1–3] starting at position 20.

quence). This yield is equivalent to an original tissue concentration of about 10 nmol/g which represents an overall recovery of 25% from the initial boiling water extract. The final solution was reduced to about 150 μl with a stream of nitrogen and 40 μl was taken for sequencing. Gas-phase microsequence analysis produced identifiable PTH amino acids for 18 cycles. The amino acid sequence obtained was identical to that predicted from the cDNA sequence of human preprogastrin starting at residue 22 (fig.2). There was a single ambiguity at cycle 15 when both Gly and Thr were observed. The latter is predicted, and the former may represent carry over from the previous cycle. The C-terminal extent of the peptide can be deduced from the fact that it reacts with antibodies specific for the predicted sequence of the extreme C-terminus of the precursor. Assuming the signal peptide corresponds to residues 1–21 of preprogastrin, the sequence and immunochemical data therefore allow us to identify the present peptide as corresponding to intact progastrin. The second major peak in Sephadex gel filtration eluates (fig.1) probably corresponds to the C-terminal fragment of the precursor produced when it is cleaved to yield G34 and G17; on the HPLC system shown in fig.1 this material was not retained and was eluted with 0.1% TFA.

The material isolated from tumours also occurs in normal antrum. HPLC of the appropriate fractions of Sephadex G-50 columns of antral mucosa revealed a major peak in the same position as the gastrinoma progastrin. In three of five antral samples examined there was also a second peak with a lower retention time (25 min, fig.3) that accounted for 1–30% of total immunoreactivity in the sample. The identity of this material is presently unknown. It was absent from the gastrinoma used for the present isolation work, but was found in 2 of 5 other gastrinomas (20 and 50% of total).

Peptides derived from preprogastrin are known to be modified by sulphation (G17) or phosphorylation (extreme C-terminal flanking peptide); it was of interest therefore to see if the gastrinoma material was so modified. Digestion with arylsulphatase did not change the elution position of either the intact progastrin, or the N-terminal G17-immunoreactive fragment generated by trypsinization, suggesting that the tyrosine residue in the G17 region of the precursor is not sulphated. However, microphosphate analysis in-

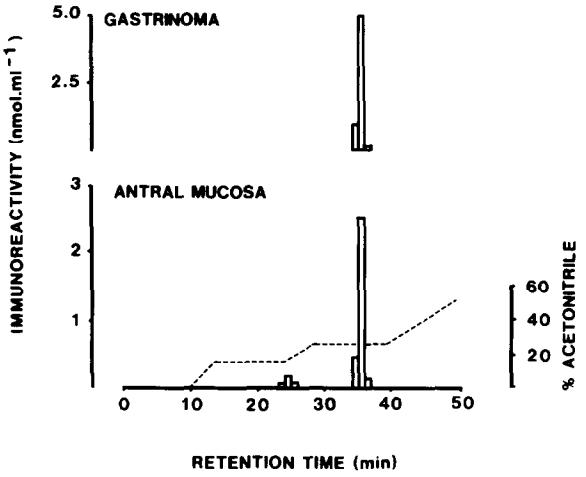

Fig.3. Separation on a Techsil C_{18} column of the immunoreactive material in an antral extract and in the extract of gastrinoma used for isolation studies (figs 1,2). In both cases the material was fractionated first on Sephadex G-50 and the early eluting peak reacting with C-terminal specific progastrin antibodies was taken for reversed-phase HPLC using a gradient of 0.1% aqueous TFA and acetonitrile (– – –). Note that the major peaks coincide (36 min), and the presence of a minor peak (retention time 25 min) in the antral extract.

dicated that in 5.1 nmol progastrin there was 1.8 nmol phosphate, so that about 30% of the precursor would appear to be phosphorylated. The C-terminal flanking peptide of porcine progastrin has recently been shown to occur in both phosphorylated and unphosphorylated forms [10] and the homologous Ser in the C-terminal region of human progastrin is therefore a plausible candidate for the site of phosphorylation.

A variety of intermediates and products of progastrin processing have now been identified; these include, in addition to G17 and G34, Gly-extended G17 and G34, NT G34 and the C- and N-terminal flanking peptides [4,5,11–13]. However, the precise sequence of events involved in the production of these forms remains in doubt. Since they differ in both distribution and biological properties the factors regulating their production are of physiological importance. The capacity to identify the intact gastrin precursor now makes it possible to study post-translational processing mechanisms in detail. Moreover, the present method of isolation should make it possible to obtain sufficient material to raise progastrin-specific antibodies.

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