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NH_2-Terminal Dodecapeptide of Porcine Big Gastrin: Revised Sequence and Confirmation of Structure by Immunochemical Analysis

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A revised sequence for the NH_2-terminal dodecapeptide of porcine big gastrin is described which differs from that originally reported in the inversion of His^7 and Pro^9 for Pro^7 and His^9. The immunochemical properties of a range of synthetic peptide fragments and analogs of the original and revised sequences of porcine big gastrin were examined with an antiserum raised to the natural porcine peptide. The pattern of immunoreactivity of these peptides indicates that the antiserum has specificity for the 4–9 region of big gastrin. The dodecapeptide with the revised sequence had full immunoreactive potency relative to natural porcine big gastrin, whereas the dodecapeptide with the original sequence had about 1000-fold lower immunoreactivity. It is proposed that the synthetic peptide with the revised, but not the original, sequence is compatible with the structure of big gastrin.

INTRODUCTION

Big gastrin has been isolated from hog antral mucosa as two peptides of 34 residues (pG34) differing only in the presence or absence of sulfation of the tyrosine in the COOH-terminal region of the molecule. Similar peptides (hG34) have also been isolated from human gastrinoma tissue (*1*). The structures of porcine and human G34 were originally determined by Harris (see Ref. (*2*); Fig. 1). The unsulfated forms of these molecules have been synthesized by Kenner and co-workers (*3*, *4*), and hG34 has also been synthesized by Wünsch *et al.* (*5*). During immunochemical studies of the natural and synthetic big gastrins it was found that synthetic pG34 had about 1000-fold lower immunochemical potency relative to natural pG34 with an antiserum raised to the natural peptide and known to be specific for the NH_2-terminal part of the molecule, while synthetic hG34 had about 100 000 times lower immunoreactivity compared with the natural peptide. In contrast, with antisera specific for the COOH-terminal portion of G34 the synthetic and natural big gastrins had equal immunoreactivity (*6*). It was suggested that these differences could be accounted for by an error in the sequence of the NH_2-terminal portion of G34. In keeping with this suggestion we now report a revised sequence for the NH_2-terminal region of pG34 that differs from the original sequence in the substitution of Pro for His at position 7 and His for Pro at position 9 (Fig. 1). The synthetic NH_2-terminal dodecapeptide of PG34 with the revised sequence has full immunoreactivity compared with natural pG34.

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METHODS

Natural peptides. pG34 was isolated from hog antral mucosa (*1*). The sulfated form of pG34 was used for immunization and for sequencing studies. The unsulfated form of pG34 was used for radiolabeling in radioimmunoassays and as a radioimmunoassay standard.

Synthetic peptides. The following peptides were synthesized in the laboratory of Professor G. W. Kenner and were kindly made available for this study: Peptides with sequences corresponding to 1–6, 1–12, 1–19, and 1–34 of the previously described structure of hG34 were synthesized according to published methods (*3*); the synthesis of peptides corresponding to the 1–6 and 1–12 sequences of the original and the revised structure is described in the paper following this (*4*); in addition dodecapeptides containing Pro^4, His^7, and Pro^9, and Leu^4, His^7, and Ser^9, were also studied.

Sequence studies. Harris established that the NH_2-terminal residue of pG34 was blocked as pyroglutamic acid (see Ref. (*2*)) and in the present study this residue was enzymically removed by incubation of 5 nmol natural pG34 with 0.5 mg calf liver pyroglutamate amino peptidase (Boehringer–Mannheim) in 1.5 ml 0.05 *M* ammonium bicarbonate (37°C, 2 hr). The reaction mixture was briefly boiled and centrifuged, and the supernatant fractionated on Sephadex G50 superfine (1 × 100 cm) in 0.05 *M* triethylamine carbonate, pH 7·5, at 4°C. The product corresponding to 2–34 pG34, was identified by radioimmunoassay using COOH-terminal specific antisera and was lyophilized. The amino acid sequence analysis and the determination of the PTH amino acids were carried out as already described (*7*).

Immunochemical studies. The immunochemical properties of natural and synthetic fragments of pG34 were studied in a radioimmunoassay system that employed ^{125}I-labeled natural pG34 and an antiserum previously shown to be specific for the NH_2-terminal part of pG34 (*8*). A population of COOH-terminal specific antibodies that was present in the original antiserum was removed by affinity immunoadsorption to G17 conjugated to Sepharose (*8*). Unsulfated pG34 was labeled with ^{125}I by the chloramine-T method and purified on DE-cellulose. Intact natural pG34 and its NH_2-terminal tryptic peptide (NT pG34) were prepared as previously described (*8*), and were routinely used as radioimmunoassay standards. The immunochemical potencies of synthetic peptides in this system were expressed as the molar ratio of concentrations of test peptide and natural pG34 needed to produce 50% inhibition of binding of ^{125}I-pG34 to antiserum.

RESULTS

Sequence

The revised amino acid sequence of the NH_2-terminal dodecapeptide of pG34 shown in Fig. 1 was unambiguously determined on the spinning cup sequenator. This sequence agrees with that originally described in all but two positions: In the revised sequence there is a substitution of Pro for His at position 7, and of His for Pro at position 9.

	1	2	3	4	5	6	7	8	9	10	11	12
ORIGINAL SEQUENCE, pG34:	GLP-	LEU-	GLY-	LEU-	GLN-	GLY-	HIS-	PRO-	PRO-	LEU-	VAL-	ALA-
REVISED SEQUENCE, pG34:	GLP-	LEU-	GLY-	LEU-	GLN-	GLY-	PRO-	PRO-	HIS-	LEU-	VAL-	ALA-
ORIGINAL SEQUENCE, hG34:	GLP-	LEU-	GLY-	PRO-	GLN-	GLY-	HIS-	PRO-	SER-	LEU-	VAL-	ALA

FIG. 1. Amino acid sequences of the NH_2-terminal dodecapeptides of hG34 and pG34 proposed by Harris (original sequences) and of pG34 determined in the present study (revised sequence).

Immunochemical Studies

Synthetic peptides corresponding to the original and revised sequences of pG34, together with various fragments and analogs, were studied in a radioimmunoassay system employing antisera raised to pG34. The specificity of the assay for the NH_2-terminal region of pG34 was established by showing that the NH_2-terminal tryptic peptide of G34 had full activity compared with natural intact pG34, whereas the COOH-terminal tryptic peptide was inactive in this system (*8*). Synthetic fragments of pG34 corresponding to the 1–12 and 1–19 sequences reported originally were 500 to 1000 times less potent than natural pG34 (Fig. 2 and Table 1). Moreover, synthetic fragments of hG34 corresponding to the originally described 1–12 and 1–19 sequences were still less active, having over 100-fold lower immunoreactivity than their porcine counterparts (Fig. 2 and Table 1). The NH_2-terminal hexapeptides of pG34 and hG34 were markedly less potent than the dodecapeptides (Table 1), but extension of both porcine and human dodecapeptides to give the NH_2-terminal nonadecapeptides produced little further increase in immunoreactivity (Fig. 2). When Pro was substituted for Leu at position 4 in the original porcine dodecapeptide, there was a marked decrease in activity (Table 1); similarly when Ser was substituted for Pro at position 9 in the

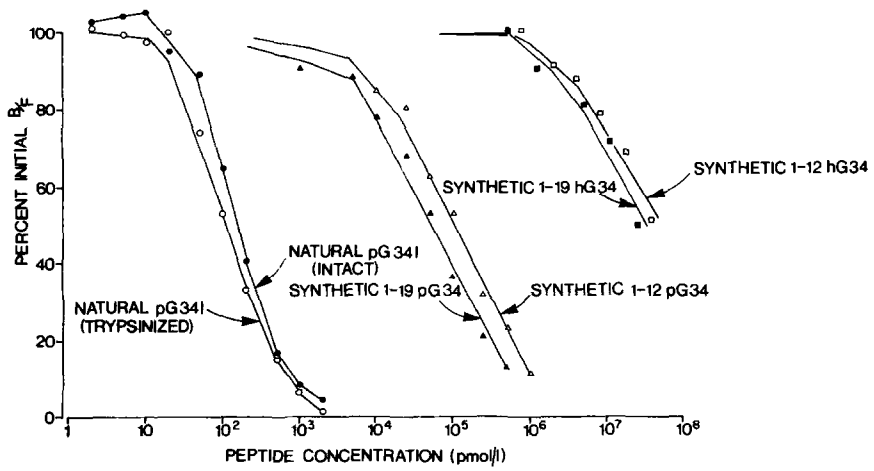

FIG. 2. Inhibition of binding of ^{125}I-pG34 to antiserum by graded concentrations of natural and synthetic fragments of pG34 and hG34 corresponding to the original sequences. Ordinate: ratio of antibody bound (*B*) to free (*F*) ^{125}I-pG34 expressed as a percentage of the initial *B*/*F* ratio in the absence of unlabeled peptide. Abscissa: concentration of peptide. The incubation and separation conditions were as previously described (*8*).

TABLE 1

IMMUNOCHEMICAL POTENCIES OF SYNTHETIC FRAGMENTS OF PORCINE AND HUMAN BIG GASTRIN

Peptide	Immunochemical potency[a]
Natural pG34	1.00
Synthetic 1–6 pG34	0.000 021
Synthetic 1–12 pG34 (Pro^7, His^9)	0.73
Synthetic 1–12 pG34 (His^7, Pro^9)	0.001 5
Synthetic 1–19 pG34 (His^7, Pro^9)	0.003 4
Synthetic 1–6 hG34	0.000 002
Synthetic 1–12 hG34	0.000 009
Synthetic 1–19 hG34	0.000 009
Synthetic 1–12 G34 (Pro^4, His^7, Pro^9)	0.000 012
Synthetic 1–12 G34 (Leu^4, His^7, Ser^9)	0.000 34

[a] Molar potency ratios based on a comparison of the concentration of natural pG34 (=1.00) and synthetic peptide needed to inhibit binding of ^{125}I-pG34 to antiserum by 50%.

original porcine dodecapeptide, there was again a significant decrease in immunoreactivity (Table 1). Taken as a whole these results indicate that positions 4 and 9 in pG34 contribute to the antigenic determinant for the antibody used in this study. In keeping with this conclusion the synthetic peptide corresponding to the revised sequence of pG34 was found to have full immunoreactivity relative to natural pG34 and was about 1000 times more active than the dodecapeptide with the original sequence (Table 1 and Fig. 3). The dilution curves for inhibition of binding by graded concentrations of natural and synthetic peptides were parallel (Fig. 3). The assay system used in the present study is therefore able to distinguish natural pG34 from synthetic peptides with the original sequence, but the synthetic dodecapeptide with the revised sequence is indistinguishable from natural pG34.

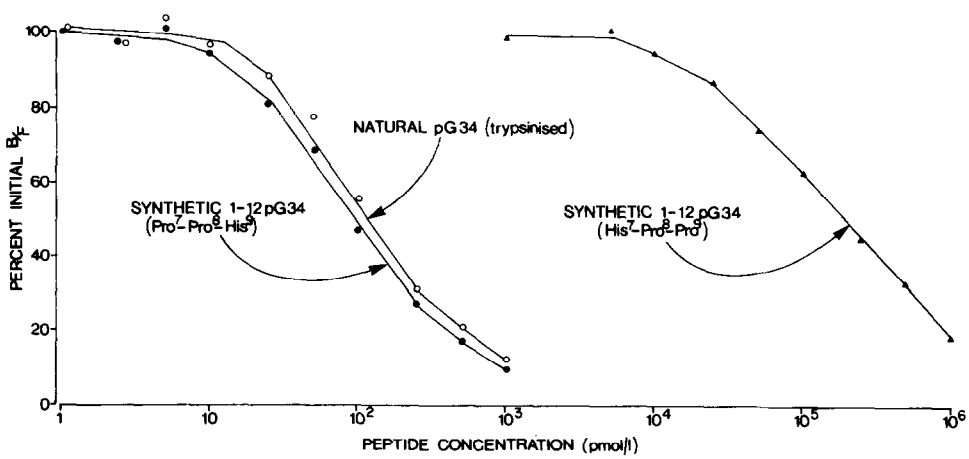

FIG. 3. Inhibition of binding of ^{125}I-pG34 to antiserum by graded concentrations of natural pG34 and NH_2-terminal dodecapeptides corresponding to the two proposed sequences of pG34.

DISCUSSION

We have shown that the synthetic NH_2-terminal dodecapeptide of pG34 with the revised sequence Pro^7–Pro^8–His^9– has virtually full immunoreactivity compared with natural pG34, whereas the dodecapeptide with the sequence His^7–Pro^8–Pro^9– has about 1000-fold lower immunoreactivity. These results therefore allow us to distinguish between two proposed sequences for the NH_2-terminal region of pG34 and are compatible with the revised sequence reported here as being the correct one. Independent evidence in favor of the revised sequence has recently been obtained by Agarwal and co-workers (B. M. Noyes, M. Mevarech, R. N. Stein, and K. L. Agarwal, personal communication), who have established that the nucleotide base sequence coding for the 4–16 portion of pG34 unambiguously confirms the revised structure reported here.

Fragments of hG34 with the original sequence were about 100 times less active than their porcine counterparts. Since the human and porcine dodecapeptides differed in only two residues (human, Pro^4, Ser^9; porcine, Leu^4, Pro^9), one or both of these positions was presumably part of the antigenic determinant for this antiserum. In support of this idea it was found that dodecapeptides with Pro^4 and Pro^9 (i.e., porcine sequence at position 9 and human at position 4) and with Leu^4 and Ser^9 (i.e., human at position 9 and porcine at position 4) both had reduced activity compared with the original porcine dodecapeptide. It seemed likely then that both position 4 and position 9 contributed to the antigenic determinant and consequently that an error in sequence might occur in the 4–9 regions. This suggestion is consistent with the revised sequence described in the present study. The new sequence of pG34 is identical to that originally proposed with the exception of a simple inversion of His^7 and Pro^9 to give Pro^7 and His^9. By analogy with the porcine peptide it is probable that the sequence of hG34 is wrong in the 7–9 region, and further studies on the structure of this peptide are in progress.

The present study illustrates the value of immunochemical methods in establishing the structure of macromolecules and serves to emphasize the capacity of antisera to detect relative minor differences in sequence. In this work we were able to use the immunochemical approach first to detect a possible error in sequence, then to localize the likely region of error, and finally to choose between synthetic peptides with two alternative sequences. Because the biological activity of G34 is determined by the COOH-terminal portion of the peptide chain, the intact synthetic pG34 with the original sequence had full biological activity on acid secretion. The immunochemical probes have therefore allowed us to study the properties of the big gastrins independently of their biological activity. The approach used in the present series of experiments is obviously applicable to structural studies of other macromolecules and should be added to other test systems in the comparison of properties of natural and synthetic proteins and polypeptides.

REFERENCES

1. R. A. GREGORY AND H. J. TRACY, *Lancet* **1**, 797 (1972).
2. R. A. GREGORY AND H. J. TRACY, "Gastrointestinal Hormones," p. 13 (J. C. Thompson, Ed.). Univ. of Texas Press, Austin, 1975.

3. A. M. CHOUDHURY, G. W. KENNER, S. MOORE, R. RAMAGE, P. M. RICHARDS, W. D. THORPE, L. MORODER, G. WENDLEBERGER, AND E. WÜNSCH, "Peptides," p. 257 (A. Loffet, Ed.). Univ. of Brussels Press, 1976.
4. A. M. CHOUDHURY, K. Y. CHU, G. W. KENNER, S. MOORE, K. L. RAMACHANDRAN, AND R. RAMAGE, *Bioorg. Chem.*, **8**, 471 (1979).
5. E. WÜNSCH, G. WENDELBERGER, A. HALLET, E. JAEGER, S. KNOF, L. MORODER, R. SCHARF, I. SCHMIDT, P. THAMM, AND L. WILSCHOWITZ, *Z. Naturforsch. C* **32**, 495 (1977).
6. G. J. DOCKRAY, R. A. GREGORY, AND G. W. KENNER, *Gastroenterology* **75**, 556 (1978).
7. M. HUNKAPILLER AND L. HOOD, *Biochemistry* **11**, 21 (1978).
8. G. J. DOCKRAY, C. VAILLANT, AND C. R. HOPKINS, *Nature (London)* **273**, 770 (1978).