Vol. 123, No. 1, 1984 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
August 30, 1984 Pages 404-409

AMINO TERMINAL FRAGMENTS OF HUMAN PROGASTRIN FROM GASTRINOMA

Joseph R. Reeve, Jr.*, John H. Walsh*,
Ronald K. Tompkins**, David Hawke*** and John E. Shively***

*Center for Ulcer Research and Education,
VA Wadsworth Center, Los Angeles, California 90073
**Dept. of Surgery, UCLA Medical School, Los Angeles, California 90024
***Division of Immunology, City of Hope, Duarte, California

Received July 27, 1984

SUMMARY: Two peptides which copurified from a human gastrinoma were found to correspond to the amino acid sequence deduced for the amino terminal portion of human and procine progastrin. The sequence of peptide A is Ser-Trp-Lys-Pro-Arg-Ser-Gln-Gln-Pro-Asp-Ala-Pro-Leu-Gly-Thr-Gly-Ala-Asn-Arg-Asp-Leu-Glu-Leu which is identical to an amino terminal portion of human progastrin. The sequence of peptide B is identical to that of peptide A except it is missing the first five amino acids. If peptide A corresponds to the amino terminus of progastrin, the signal peptidase cleaves at an Ala-Ser bond.

Gastrin molecules of varying chain lengths have been isolated from human gastrin-secreting tumors (1). These include biologically active forms comprised of 34, 17, and 14 amino acids, all apparently fragments of the same precursor. A cDNA copy of mRNA coding for porcine antral preprogastrin has been cloned and the nucleotide sequence was determined (2).

More recently, a single DNA sequence corresponding to the porcine gene was obtained from a human tumor (3). The amino acid sequence for 34 amino acid gastrin was located near the carboxyl end of preprogastrin, and the 58 additional amino acids coded from the amino terminus of gastrin-34 were presumed to consist of the signal peptide and other connecting peptides. The larger forms of gastrin-like immunoreactivity, previously known as Component I (4) and as "big-big gastrin"(5), presumably consist of gastrin-34 attached to one or more connecting peptides.

0006-291X/84 $1.50

Although the presence of these amino terminal connecting peptides has been deduced from the porcine and human genes, the sites of peptide cleavage have not been demonstrated directly in mammalian tissue extracts.

An 0.5g fragment of a gastrinoma was obtained at operation from a peripancreatic lymph node removed from a patient with the Zollinger-Ellison syndrome (6) and was quickly frozen. Aqueous extracts of this tissue were prepared[1] and a portion was applied directly to a reverse phase C18 high performance liquid chromatography column.[2] Monitoring of the eluate at 220 nm and at 280 nm for detection of total peptide and of tryptophan and tyrosine-containing peptides, respectively, revealed one predominant peak along with several minor peaks (Fig.1). Isocratic HPLC of this peak showed that it was apparently homogenous. The original tumor extract contained 40 pmol carboxyl terminal gastrin immunoreactivity per mg tissue, but this immunoreactive gastrin eluted considerably later than the major absorbance peak on gradient HPLC.

An aliquot of the purified peak fraction was subjected to microsequence analysis (7). Identifiable amino acid PTH derivatives were detected in 23 cycles of the Edman reaction. Two different derivatives were obtained in each of the first 20 cycles except for cycles 1 and 4 (Table 1). Arranging the amino

---

[1] The 0.5 g frozen tissue specimen was minced with a scalpel and quickly immersed in 5 ml boiling 0.1 M ammonium bicarbonate and boiled for 5 min. The extract was cooled, centrifuged, the supernatant saved, and the pellet reextracted in 5 ml 2% aqueous trifluoroacetic acid at 4 for 6 h. After centrifugation, the two supernatants were combined.

[2] Aliquots of 1 ml of combined supernatant were applied to the Vydac C18 reverse phase HPLC column equilibrated with 0.1% trifluoracetic acid (Solvant A). The column was rinsed with the same solution for 10 min., then eluted with linear gradient from 0 to 100% buffer B (50% acetonitrile containing 0.1% trifluroacetic acid).

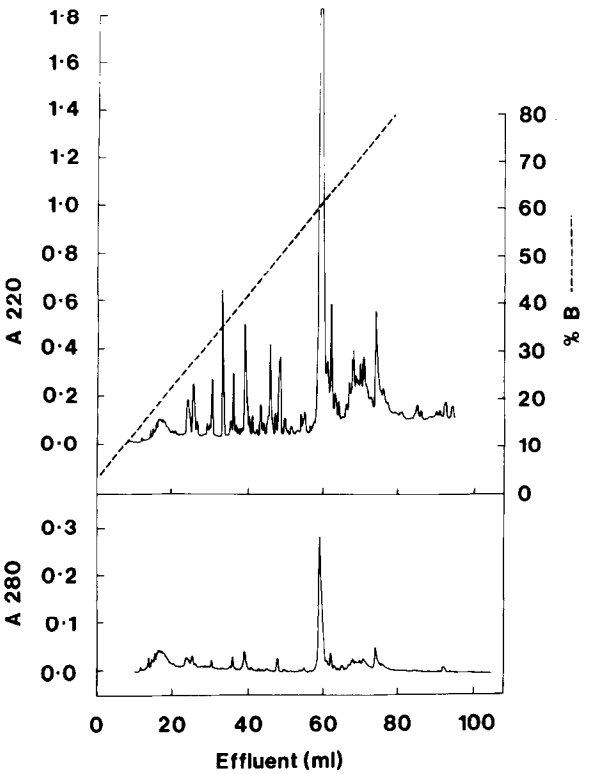

Figure 1: Elution profile of an aqueous extract of a peripancreatic lymph node tumor removed from a patient with the Zollinger-Ellison syndrome. One ml (corresponding to 0.1g of tumor) of extract was loaded directly onto a Vydac C18 reverse phase high pressure liquid chromatography column. The eluate was monitored at 220 nm and 280 nm.

acids according to yield at each step, and calling the more abundant residue a member of peptide A and the other a member of peptide B, resulted in two peptides with the same sequence except peptide B contained five less amino acids at its amino terminus. In order to achieve the alignment shown in Table 1, it was necessary to change the assignment based on yield at five steps of the Edman degradation (cycles 6, 8, 9, 12 and 18). In addition to serine, alanine was identified in cycle one. The significance of this observation is not known. The entire sequences for peptides A and B correspond exactly to the predicted human gene product. The two peptide sequences determined from a combination of the results shown in Table 1 and

TABLE 1

Microsequence Analysis of Human Tumor Progastrin[1]

Cycle	Peptide A (Primary Sequence)[2] pmol		Peptide B (Secondary Sequence)[2] pmol	
1	Ser	(425)	(Ser)[3]	
2	Trp	(394)	Gln	(249)
3	Lys	(725)	Gln[4]	(253)
4	Pro	(312)	(Pro)[4]	
5	Arg	(149)	Asp	(108)
6	Ser	(100)	Ala	(270)
7	Gln	(203)	Pro	(107)
8	Gln	(141)	Leu	(157)
9	Pro5	(116)	Gly	(127)
10	Asp	(81)	Thr	(80)
11	Ala	(235)	Gly	(111)
12	Pro	(72)	Ala	(255)
13	Leu	(196)	Asn	(77)
14	Gly	(96)	Arg	(69)
15	Thr	(50)	Asp	(40)
16	Gly	(87)	Leu	(70)
17	Ala	(145)	Glu	(65)
18	Asn	(39)	Leu	(40)
19	Arg	(44)	Pro	(31)
20	Asp	(28)	(Trp)	(25)
21	Leu	(90)		
22	Glu	(36)		
23	Leu	(60)		

1 Approximately 5 micrograms (2 nmols) of sample were applied to a spinning cup microsequencer. The yield of peptide A was 36% and peptide B 11%. It should be noted that Ser gives rise to multiple PTH amino acid derivatives in Edman chemistry; quantitive yields for only one of the derivatives is given here.

2 The secondary sequence is for the most part a subset (from 6 on) of the primary sequence. Yields in pmols are shown in parentheses. Assignments to primary or secondary sequence are based on relative yields assuming a relative ratio of 2:1 and by analogies between the two sequences.

3 225 pmol of PTH-Ala was also observed, but Ser has been assigned due to the identity seen with the rest of peptide A. The sum of yield of PTH-Ser for peptide A and B is shown.

4 Only the sum of the yield of PTH-Pro for peptide A and B is shown.

5 PTH-Gln was also observed, but is probably a carry-over from the previous two cycles.

maximal homology between chains and with the human gastrin gene are shown in Figure 2.

The amino terminal fragments of human progastrin were present in extremely high concentration in this tumor. We calculated from the absorbance at 280 nm and the amino acid

	5							10							15							20							25
Human gene	S	W	K	P	R	S	Q	Q	P	D	A	P	L	G	T	G	A	N	R	D	L	E	L	P	W	L			
Peptide A	S	W	K	P	R	S	Q	Q	P	D	A	P	L	G	T	G	A	N	R	D	L	E	L						
Peptide B						S	Q	Q	P	D	A	P	L	G	T	G	A	N	R	D	L	E	L	P	W				
Porcine gene	S	W	K	P	G	F	Q	L	Q	D	A	S	S	G	P	G	A	N	R	G	K	E	P	H	E	L			

Figure 2: Sequence of the peptides isolated from the gastrinoma tumor compared with similar regions of human and porcine progastrin as determined cDNA techniques (2,3). The underlined residues of the porcine gene indicates the differences between hog and human progastrin.

analysis that each milligram of tissue contained 1000 pmol progastrin fragment, a concentration 25 times higher than immunoreactive gastrin components in the same tumor. The gastrin-like immunoreactive components were present at 5-10 times higher concentration than in extracts of normal human gastric antrum (8).

The presence of a relatively pure population of gastrin-producing cells in the gastrinoma compared with a minor fraction of gastrin cells in gastric antrum favored the identification of this peptide fragment. Further studies are needed to determine whether or not the storage of amino terminal fragments of progastrin is a tumor-specific phenomenon, but specific identification of these fragments in normal tissues probably will require radioimmunoassay with specific antibodies.

Characterization of these peptides indicates a previously undemonstrated product of the recently characterized human gastrin gene. It also indicates possible cleavage points at which the leader sequence of human preprogastrin are separated from progastrin.

Analysis of a tumor extract by a single step of high pressure liquid chromatography followed by microsequence analysis has produced new information about a gastrin gene product. The results of this peptide analysis and of the cDNA analysis performed on a different gastrin secreting tumor are compatable with a single gene and a single messenger RNA. Recent studies of

cDNA sequences and peptide products of the enkephalin precursors and products and of the calcitonin gene products have shown the importance of direct analysis of naturally occurring peptides in interpretation of gene structure determinations (9-13). Furthermore, the amino terminal portion of gastrin could have independent biological activity as was found recently for a similar portion of the calcitonin precursor (14). This pair of peptides shows how preprogastrin is processed in tumor tissue which may reflect events occuring in normal tissue. The results indicate the cleavage site on preprogastrin for signal peptidase, data that cannot be obtained through cDNA studies.

REFERENCES

1. Gregory, R.A., (1979) Bioorganic Chemistry 8, 497.
2. Yoo, O.J., Powell, C.T., Agarwal, K.L., (1982) Proc. Natl. Acad. Sci. USA 79, 1049.
3. Rehfeld, J.F., (1983) Proc. Natl. Acad. Sci. USA 70 May, 2866-2869.
4. Rehfeld, J.F. and Stadil, F. (1973) Gut 14, 369.
5. Yalow, R.S. and Berson, S.A. ((1972) Biochem. Biophys. Res. Comm. 48, 391.
6. Jensen, R.T., Gardner, J.D., Raufman, J.P., Pandol, S.J., Doppman, J.L. and Collen, M.J. (1983) Ann. Int. Med. 98, 59.
7. Shively, J.E. (1981) Meth. Enzymol. 79, 31.
8. Malmstrom, J. Stadil, F. and Rehfeld, J.F. (1976) Gastroenterology 70, 697-703.
9. Gubler, U., Seeburg, P., Hoffman, B.J., Gage, L.P. and Udenfriend, S. (1982) Nature 295, 206-208.
10. Noda, M. Teranishi, Y., Takahashi, H., Toyosato, M., Notake, M., Nakanishi, S. and Numa S. (1982) Nature, 297, 431-434.
11. Kakidani, H. Furutani, Y., Takahasi, H., Noda, M., Morimoto, Y., Hirose, T., Asai, M., Inayama, S. Nakanishi, S. and Numa, S. (1982) Nature 298, 245-248.
12. Amara, S.G., Jonas, V., Rosenfeld, M.G., Ong, E.S. and Evans, R.M. (1982) Nature 298, 2240-244.
13. Jacobs, J.W., Goodman, R.H., Chin, W.W., Dee, P.C., Habener, J.F. ((1981) Science 213, 457-459.
14. MacIntyre, I. Hilyard, C.J., Murphy, P.K., Reynolds, J.J., Gaines, Das R.E. and Craig, R.K. (1982) Nature 300, 460-462.