The Primary Structure of Human Chromogranin A and Pancreastatin*

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A full-length clone encoding human chromogranin A has been isolated from a λgt10 cDNA library of a human pheochromocytoma. The nucleotide sequence reveals that human chromogranin A is a 439-residue protein preceded by an 18-residue signal peptide. Comparison of the protein sequence of human chromogranin A with that of bovine chromogranin A shows high conservation of the NH₂-terminal and COOH-terminal domains as well as the potential dibasic cleavage sites, whereas the middle portion shows remarkable sequence variation (36%). This part of human chromogranin A contains a sequence homologous to porcine pancreastatin at residues 250–301. The sequence variation in this part of human chromogranin A compared to porcine pancreastatin is 32% and thus of the same magnitude as that between human and bovine chromogranin A. Therefore, the difference between porcine pancreastatin and the corresponding portions of bovine or human chromogranin A can be explained by species variation, suggesting that pancreastatin is derived from chromogranin A itself rather than a protein that is only similar to chromogranin A. Moreover, the pancreastatin sequence contained in human chromogranin A is flanked by sites for proteolytic processing. Together, these observations suggest that human chromogranin A may be the precursor for a human pancreastatin molecule and possibly for other, as yet unidentified, biologically active peptides.

Chromogranin A is a member of the secretogranin/chromogranin class of proteins that occur in secretory granules of a wide variety of endocrine cells and neurons (see Ref. 1 and references therein). Proposals as to the function(s) of these proteins have included a role: (i) in the packaging of peptide hormone and neuropeptide precursors; (ii) in the processing of peptide hormone and neuropeptide precursors; (iii) in the organization of the granule matrix, including calcium binding; (iv) as regulatory proteins after secretion; and (v) as precursors of peptide hormones and neuropeptides (see Refs. 1–8 and references therein). It remains to be established which of these proposed functions are relevant in vivo.

A major step toward the elucidation of the function of the secretogranins/chromogranins was the realization that the sequence of bovine chromogranin A, which was recently determined by cDNA cloning (4, 5), contains a portion which is homologous to porcine pancreastatin (9, 10), a peptide reported to inhibit glucose-induced insulin secretion (11). The sequence identity between porcine pancreastatin and bovine chromogranin A of about 70% can be interpreted in two ways. First, in line with a possible precursor function of chromogranin A which had been deduced previously from the presence of potential dibasic cleavage sites in the bovine chromogranin A sequence (4, 5), pancreastatin may be derived from chromogranin A itself, the 30% sequence variation being due to species differences. Second, pancreastatin may not be derived from chromogranin A but from a related molecule. One approach to resolve this issue is to determine the degree of sequence variation of chromogranin A in various mammalian species.

In humans, pancreastatin may be important for the physiological homeostasis of blood insulin levels as well as pathological aberrations such as diabetes mellitus. It is therefore of obvious interest to search for a human pancreastatin and its precursor. Moreover, determination of the human pancreastatin sequence would allow, by means of comparison with the porcine molecule, the identification of conserved parts within pancreastatin that are likely to be crucial for its biological activity. In the present study, we report the complete sequence of human chromogranin A and show that it contains a pancreastatin sequence flanked by sites for proteolytic processing.

EXPERIMENTAL PROCEDURES

Screening of cDNA Libraries—A size-selected λgt10 cDNA library made from mRNA of a human pheochromocytoma (12) was kindly donated by Drs. A. Lamouroux and J. Mallet (CNRS, Gif-sur-Yvette). Screening was performed using techniques described previously (4, 12, 13).

Sequencing of Human Chromogranin A cDNA—The 1.9-kb EcoRI full-length cDNA insert from clone hCgA/42 was subcloned into pBR322, mapped with several restriction enzymes, and sequenced using previously described methods (4, 12).

Northern Blot Analysis—Poly(A)* RNA was isolated and analyzed from rat PC12 cells and various human and bovine tissues as described previously (4, 12). Transfer of mRNA onto GenScreen nitrocellulose filters (Du Pont-New England Nuclear) was performed overnight as described in Maniatis et al. (14) but using 25 mM NaPO₄, pH 7.2, as transfer buffer. After transfer, the mRNA was cross-linked to the filter with UV light (254 nm) for 4 min. Prehybridization was done for 2 h at 65 °C in 0.5 M NaPO₄, pH 7.2, 7% SDS, 1 mM EDTA. Hybridization was performed with the full-length nick-translated 1.9-kb cDNA insert from hCgA/42 (specific activity 8 × 10⁶ cpm/µg) for 16 h with 500,000 cpm/ml in 0.5 M NaPO₄, pH 7.2, 1% SDS, 1 mM EDTA. Washings were done in 40 mM NaPO₄, pH 7.2, 1% SDS, at 0°C.

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† The abbreviations used are: kb, kilobase; SDS, sodium dodecyl sulfate.
65 °C for 2 h with several buffer changes.

**Southern Blot Analysis**—10 μg of genomic human DNA from two individuals was isolated by standard methods and digested for 16 h at 37 °C with 20 units of restriction enzyme and electrophoresed on a 0.7% Tris/acetate/EDTA agarose gel (14). Transfer of DNA onto nitrocellulose filters was performed as described (14). Prehybridization was done overnight in 4 x SSC, 45% (v/v) deionized formamide, 0.1 M NaPO4, pH 6.8, 5 x Denhardt's solution, 0.1% SDS, 100 μg/ml sonicated herring sperm DNA. Hybridization was performed in the presence of 106 cpm/ml nick-translated 1.9-kb cDNA insert from hCgA/42 in 4 x SSC, 45% (v/v) deionized formamide, 1 x Denhardt's solution, 0.1% SDS, 50 mM NaPO4, pH 6.5, 10% dextran sulfate (Pharmacia Biotechnology, Inc., Mt. 3,500,000) at 42 °C for 24 h. Washings were done for 2 h at 65 °C in 1 x SSC, 0.1% SDS with several buffer changes.

**RESULTS AND DISCUSSION**

**Determination and Analysis of the Human Chromogranin A Sequence**—cDNA clones encoding human chromogranin A were isolated from a size-selected λgt19 cDNA library of a human pheochromocytoma (2 x 106 recombinants) by hybridization with the 1.7-kb fragment of Scal/BstH11-digested bovine chromogranin A cDNA (4), which yielded several thousand positive plaques. Thirty plaques were picked and purified. Six clones were analyzed for the length of the insert, and the longest insert (hCgA/42) was sequenced and turned out to be full-length.

The mRNA corresponding to hCgA/42 (see Fig. 1) is 1864 nucleotides long (not including the poly(A) tail) and contains 82 untranslated nucleotides at the 5' end, a 1371-nucleotide coding region, and 411 untranslated nucleotides at the 3' end which include the polyadenylation signal. The nucleotide sequence in the coding region of the human chromogranin A mRNA is 83% identical to that of the bovine mRNA (4). The open reading frame of the human chromogranin A mRNA codes for a 457-residue protein of 50,688 Da. The first 18 amino acids form a typical signal peptide, responsible for the presence of hCgA/42 in adults.

**Fig. 1.** Complete nucleotide and deduced amino acid sequence of human chromogranin A. Nucleotides are numbered at the right and amino acids above the sequence. **Negative numbers** indicate the signal peptide and arrowheads the polyadenylation signal. The known NH2-terminal protein sequence of human chromogranin A codes for a full-length protein. The mRNA corresponding to hCgA/42 (see Fig. 1) is 1864 nucleotides long, contains 82 untranslated nucleotides at the 5' end, a 1371-nucleotide coding region, and 411 untranslated nucleotides at the 3' end which include the polyadenylation signal. The nucleotide sequence in the coding region of the human chromogranin A mRNA is 83% identical to that of the bovine mRNA (4). The open reading frame of the human chromogranin A mRNA codes for a 457-residue protein of 50,688 Da. The first 18 amino acids form a typical signal peptide, responsible for the presence of hCgA/42 in adults.
Asp sequence at residues 43-45 and the disulfide-bonded structure is formed in the human sequence (Fig. 2). The first 91 residues following the signal peptide and the last 84 residues of human chromogranin A are identical to the human sequence are identical to the human sequence and are indicated by dashed lines. The human sequence indicate potential dibasic cleavage sites. The amino- and carboxyl-terminal domains of bovine and human chromogranin A are much more conserved than the middle portion (12). In contrast, human chromogranin A between residues 92 and 355 differs by 36% from the bovine proteins. This observation confirms and extends our earlier finding, obtained by sequence comparison of bovine chromogranin A and human secretogranin I (chromogranin B), that the terminal domains of the chromogranin/secretogranin molecules are much more conserved than the middle portion (12). In contrast to bovine chromogranin A, human chromogranin A contains a potential N-glycosylation site at Asn⁹⁷. It remains to be shown whether this site is used in vivo.

Relationship of Pancreastatin to Chromogranin A—Another feature that is conserved in human and bovine chromogranin A are the potential dibasic cleavage sites. All eight sites of the bovine protein are found in the corresponding positions in the human sequence (Fig. 2). This finding supports the previous suggestion that the presence of potential sites for proteolytic processing may point to a precursor function of chromogranin A. Furthermore, in comparison with bovine chromogranin A, the human molecule contains two additional potential N-glycosylation sites, of which the Arg-Lys pair at position 248-249 is particularly interesting. This dibasic site precedes the beginning of a portion of the human chromogranin A sequence that is homologous to porcine pancreastatin. Another observation is the potential N-glycosylation site in human chromogranin A. The box marks the Arg-Gly-Asp sequence. Asterisks above the human sequence indicate potential dibasic cleavage sites. The solid triangle marks the potential N-glycosylation site in human chromogranin A. The bar indicates human and porcine pancreastatin.

2 U. M. Benedum, P. Rosa, and W. B. Huttner, unpublished data.
It is of interest to note that the sequence variation between porcine pancreastatin and human chromogranin A residues 250-301 is of the same magnitude (32%) as that between the middle portions of bovine and human chromogranin A. It is therefore likely that the previously noted sequence variation between pancreastatin and chromogranin A (9, 10) is due to species variation rather than pancreastatin being derived from a protein that is only similar, but not identical, to chromogranin A. This in turn suggests that chromogranin A itself is the precursor of pancreastatin.

How, then, would pancreastatin be liberated from human chromogranin A? In addition to the potential dibasic cleavage site at the NH₃ terminus of pancreastatin, its COOH terminus is followed by a glycine and a lysine residue at positions 302 and 303, respectively (see Fig. 2). Lys₃₀³ is surrounded by amino acids with high turn-inducing potential which favors proteolytic processing at this monobasic site (18). We therefore conclude that a pancreastatin molecule may be proteolytically liberated from human chromogranin A at Arg²⁴⁸- Lys₂⁴⁹ and Lys₃₀³. In view of the fact that porcine pancreastatin contains an amidated glycine at the COOH terminus (11), it is to be expected that a human pancreastatin molecule, after removal of Lys₃₀³, undergoes COOH-terminal amidation at Gly₃₀₁ using the amide group of Gly₃₀₂. It remains to be shown whether such a processing of human chromogranin A occurs in vivo.

The biological activity of porcine pancreastatin resides in the COOH-terminal part comprising 17 residues (11) which corresponds to residues 284-301 in human chromogranin A (see Fig. 2). Since porcine pancreastatin exhibits biological activity on rat pancreatic tissue and thus seems to act across species (11), we have searched for a conserved sequence in this part of pancreastatin. The only stretch of more than 2 amino acid residues that is identical in this part of human, bovine, and porcine pancreastatin consists of four glutamic acid residues. In addition, the COOH-terminal heptapeptides of human and porcine pancreastatin are identical.

The Human Chromogranin A Gene and mRNA—Southern blot analysis of human genomic DNA was performed using the 1.9-kb EcoRI insert of the full-length hCgA/42 cDNA clone (Fig. 3). Single major bands of 31 and 4 kb were observed after digestion with EcoRV and EcoRI/BamHI, respectively, suggesting a single gene for chromogranin A in the haploid human genome. The presence of two hybridizing bands of 10 and 7 kb in the BglII digestion suggests the presence of intron sequences because there is no BglII restriction site in the chromogranin A cDNA sequence. The present Southern blot data differ from those of Murray et al. (19) who used a partial human cDNA clone as probe.

Northern blot analysis of poly(A)+ mRNA from rat PC12 cells, a human pheochromocytoma, and bovine adrenal medulla reveals a major hybridizing mRNA of 2.1 kb (Fig. 4). In addition, a second hybridizing mRNA of 1.6 kb is seen in human and rat pheochromocytoma. The significance of this second mRNA remains to be investigated. Chromogranin A mRNA is not detected in human liver (data not shown), as
expected from previous data on the tissue distribution of this protein (20).

Perspectives—First, the delineation of the conserved parts of chromogranin A facilitates future studies on the still poorly understood function of this protein. Second, the evidence suggesting that chromogranin A itself is the pancreastatin precursor should stimulate further work on the proteolytic processing of this protein. The present human chromogranin A sequence provides the necessary information to investigate whether, in addition to processing to pancreastatin, any of the other potential sites for proteolytic processing in human chromogranin A are used physiologically to give rise to biologically active peptides. Third, knowledge of the human pancreastatin sequence may prove to be relevant for clinical research. Finally, the isolation and sequencing of a full-length cDNA clone coding for human chromogranin A opens new avenues for studying the chromogranin A gene and its mRNA in man. Studies on the organization and precise localization of the human chromogranin A gene and on chromogranin A mRNA expression in various human tissues in normal and pathological conditions is some of the research now possible.

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