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THE AMINO TERMINAL SEQUENCES OF BOVINE AND HUMAN CHROMOGRANIN A AND SECRETORY PROTEIN I ARE IDENTICAL

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The amino terminal sequences of bovine and human adrenal medullary chromogranin A have been determined. Their sequences are identical and also identical to the published sequence of secretory protein I from the parathyroid gland. This data indicates that the previously published sequence of chromogranin A is incorrect at residues 2 and 19. These data confirm earlier observations of a substantial similarity between secretory protein I and chromogranin A and, in fact, strongly suggest that they are identical.

Chromogranin A is a protein costored and coreleased with catecholamines from storage granules in the adrenal medulla (1,2), while secretory protein I (parathyroid secretory protein) is a protein costored and coreleesed with parathyroid hormone from storage granules in the parathyroid gland (3,4).

A recent publication demonstrated a strong similarity between secretory protein I and chromogranin A (5). The data included similar amino acid composition, physical properties, and immunological cross reactivity. The amino terminal sequences were shown to differ in two positions, residues 2 and 19 (4,5,6).

During the course of our studies on peptides in the adrenal medulla we isolated two peptides whose sequences corresponded exactly to the amino terminal sequences of secretory protein I but not to that of chromogranin A (7). To determine if these peptides did in fact originate from chromogranin A we have sequenced the amino terminal 25 residues of both human and bovine chromogranin A. Immunohistochemistry (8-11) and radioimmunoassays (12,13)

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have demonstrated the presence of chromogranin A immunoreactivity in virtually all neural and polypeptide hormone producing endocrine tissues in rat (10), cow (8,12,13) and man (9,11).

Experimental Procedures

Chromogranin A was purified from catecholamine storage vesicles of human pheochromocytoma and from bovine adrenal medulla using previously described methods (1). The lyophilized protein was dissolved in 100% trifluoroacetic acid and placed at 60°C for one hour to provide as high a yield as possible during sequencing. Each protein (1nmole) was sequenced as previously described (7) on an Applied Biosystems 470A Protein Sequencer using the NVAC program. The phenylthiohydantoin (PTH) amino acids were identified by HPLC on a Supelco C_{18} column with a Bakerbond C_4 guard column. Buffer A was 66mM trifluoroacetic acid /4mM acetic acid brought to pH 5.8 with NaOH. Buffer B was 25% 33mM trifluoracetic acid brought to pH 3.6 with NaOH, 10% acetonitrile, and 65% methanol. The gradient was 28%B at 0 min. with linear steps to 30%B at 13 min., 50%B at 17 min., 70%B at 37 min. and 100%B at 38 min. The HPLC system consisted of a Spectra Physics 8700 HPLC equipped with the dynamic mixer, a Waters 710B WISP, and a Kratos 757 UV detector (at 269nm). Complete separation of all PTH amino acids was achieved with this system.

Results and Discussion

The sequencing data showed a yield of approximately 65% for residue 1 from both chromogranins. The sequences of the amino terminal 20 residues of both proteins are shown in Fig. 1. Also shown is the published sequence of bovine secretory protein I (4,5) and the previously determined sequence of bovine chromogranin A (5,6). As can be seen, the sequences for human and bovine chromogranin A are identical and conflict with the published sequence of bovine chromogranin A at positions 2 and 19 where Arg has been shown instead of Pro and Val, respectively. Species differences cannot be postulated, since prior sequence data on both secretory protein I (4,5) and chromogranin A (5,6) were both obtained from the bovine molecules (4-6).

BA-16	LEU-PRO-VAL-ASN-SER-PRO-MET-ASN-LYS-GLY-ASP-THR-GLU-VAL-MET-LYS
BOVINE CHROM A	LEU-PRO-VAL-ASN-SER-PRO-MET-ASN-LYS-GLY-ASP-THR-GLU-VAL-MET-LYS-(CYS)-ILE-VAL-GLU
HUMAN CHROM A	LEU-PRO-VAL-ASN-SER-PRO-MET-ASN-LYS-GLY-ASP-THR-GLU-VAL-MET-LYS-(CYS)-ILE-VAL-GLU
SP-1	LEU-PRO-VAL-ASN-SER-PRO-MET-ASN-LYS-GLY-ASP-THR-GLU-VAL-MET-LYS- XXX -ILE-VAL-GLU
"CHROM A"	LEU-ARG-VAL-ASN-SER-PRO-MET-ASN-LYS-GLY-ASP-THR-GLU-VAL-MET-LYS- CYS -ILE-ARG-GLU

Figure 1. Amino Terminal Sequences of Chromogranin A From Human And Bovine.

The amino terminal sequences of human and bovine Chromogranin A are shown along with the sequence of the peptide we previously isolated, BA-16 (7), the sequence of secretory protein 1, SP-1 (4) and the previously published sequence of Chromogranin A, "Chrom A", (5). The (Cys) residues were not observed but are inferred from the lack of any other amino acid observed at that residue.

Furthermore, the human and bovine proteins are identical in the amino terminal region (Figure 1), consistent with their other previously described similarities (1). In addition, the substitutions, of Arg for Pro and for Val are non conservative. It therefore appears that the previous published sequence of chromogranin A (6) is incorrect at these two positions. When the sequences of the chromogranins and secretory protein I are compared, it can be seen that they are also identical.

Thus the data of Cohn, et al (5) showing substantial similarities between chromogranin A and secretory protein I are completely understandable. The proteins appear to be identical at least at the amino terminus. This sequence data also suggests that chromogranin A (secretory protein I) may be found in an identical form in all normal polypeptide hormone producing tissues (5, 8-13). Thus, it appears that there are not two similar families of secreted proteins from these tissues but a single family with identical amino terminal sequences.

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