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Similarity of secretory protein I from parathyroid gland to chromogranin A from adrenal medulla

(secretion/parathormone/epinephrine/chromaffin granule)

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ABSTRACT **We have compared the amino acid and carbohydrate compositions, partial amino acid sequences, immunological crossreactivity, and physical properties of secretory protein I of the parathyroid gland and chromogranin A of adrenal gland. This comparison indicates that these proteins are similar molecules. Because secretory protein I is present in secretory granules containing parathormone and is cosecreted with the hormone, and because chromogranin A is contained within chromaffin granules and, likewise, is secreted with the catecholamines, the present observations raise the possibility that this class of protein plays a general role in hormone secretion or storage mechanisms.**

Two separate and independently pursued lines of investigation have revealed that the parathyroid and adrenal medulla each contain a glycoprotein that is secreted *in vitro* together with the hormone of each gland (1, 2). In the parathyroid, secretory protein I (SP-I) is cosecreted with parathormone; in the adrenal medulla, chromogranin A is secreted with the catecholamines. At this time, the role of neither protein has been elucidated, although there has been speculation that both proteins play some part in hormone storage or secretion. The present report compares the chemical, physical, and immunological properties of SP-I and chromogranin A. We conclude that these are closely related molecules.

EXPERIMENTAL PROCEDURES

Preparation of Chromaffin Granules. Fresh adrenal glands were separated into cortex and medulla portions by surgical means. All of the operations were performed in the cold room (≈5°C). The medullary tissue was homogenized in 10 volumes of 0.3 M sucrose solution. To 0.5-ml homogenates, trichloroacetic acid was added to a final concentration of 25%. The precipitate was collected by centrifugation and dissolved in 1 ml of 1 M NaOH. Portions of these solutions were neutralized with equal volumes of 1 M HCl and diluted for assay. The chromaffin granules were prepared from the medullary homogenate by a differential centrifugation method described by Bartlett and Smith (3).

Radioimmunoassay for SP-1 in Chromaffin Granule Extracts. The chromaffin granule pellet was resuspended in 8 ml of 0.3 M sucrose solution. To 1 ml of suspension, trichloroacetic acid was added to a final concentration of 25%, followed by the treatment described above for the precipitates from tissue homogenates. Anti-SP-I antiserum and conditions for assay were described by Cohn *et al.* (4).

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Table 1. Amino acid composition of bovine SP-I and chromogranin A

	Moles per 100 residues	
	SP-I*	Chromogranin A†
Asx	7.1	8.0
Glx	23.2	22.5
Ser	7.6	7.7
Thr	2.1	2.6
Cys	0.5	0.2
Met	1.6	1.4
Pro	8.7	9.2
Gly	7.9	8.1
Ala	8.9	8.5
Val	3.5	3.9
Leu	7.4	7.3
Ile	1.0	1.4
Phe	1.5	1.7
Tyr	1.0	1.0
Trp	1.1	1.4‡
Lys	8.7	8.4
His	1.6	1.9
Arg	6.6	6.0

* SP-I data are taken from Cohn *et al.* (9).
† Chromogranin A data were calculated from Winkler (1); each datum is the average of 5 sources reported in table 4 of ref. 1.
‡ From ref. 10.

Immunodiffusion. Experiments were performed in 1% agarose in 0.1 M Tris·HCl, pH 8.0/0.15 M NaCl/0.02% sodium azide. Incubation was at room temperature for 48 hr.

Immunoreplica—NaDodSO$_4$/Polyacrylamide Gel Electrophoresis. NaDodSO$_4$/polyacrylamide electrophoresis was carried out with the buffer system of Laemmli (5) and an acrylamide gradient from 10% to 15%. Anti-chromogranin A antiserum was prepared against chromogranin A that had been prepared as described by Fischer-Colbrie (6); immunization of rabbits was by the method of Schneider *et al.* (7), but with intradermal injections. The immunoreplicas were obtained by the method of Burnette (8). In short, the proteins after electrophoresis were transferred to a nitrocellulose sheet that was treated with the antiserum at a dilution of 1:100 for 24 hr. After several washes, the sheet was treated with ^{125}I-labeled protein A (0.2 μCi/ml; 1 Ci = 3.7 × 10^{10} becquerels) for 1 hr. Finally, the sheets were exposed on x-ray film (Cronex 4) at −70°C with intensifying screens. A preimmune serum gave no significant staining of the immunoreplica.

Purification of SP-I. Bovine parathyroid SP-I was prepared by the method of Cohn *et al.* (9).

Abbreviation: SP-I, secretory protein I from parathyroid gland.

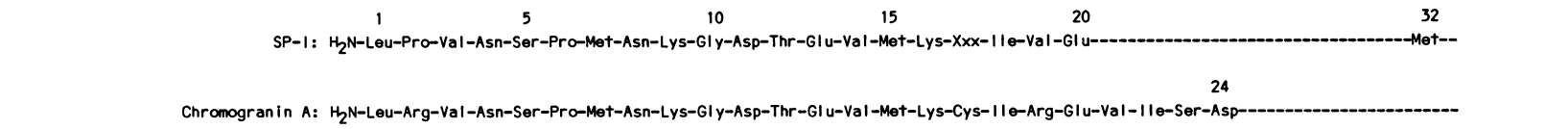

FIG. 1. Partial amino acid sequence of SP-I and chromogranin A. The data for SP-I are from ref. 9 and for chromogranin A are from ref. 10.

RESULTS

Purified bovine cellular SP-I consists of at least two glycoproteins of nearly similar size (72,000 and 70,000 daltons) that have the same amino acid compositions and partial amino acid sequences (9). The differences in size might be accounted for by carbohydrate composition. SP-I contains about 30% acidic amino acid residues and has a pH of 4.5. The amino acid composition (Table 1) or partial sequence (Fig. 1) bear no obvious relationship to parathormone or its precursors (9). SP-I appears to exist within secretory granules of the parathyroid that also contain parathormone (11). Its rate of synthesis is at least equal to that of parathormone, and its appearance in incubation medium (in studies of the incubation of parathyroid slices or cells with radioactive amino acids) occurs about 30 min after synthesis—kinetics equivalent to that for parathormone itself (12, 13). Also, like parathormone, the secretion of SP-I is strongly suppressed by Ca^{2+} and Mg^{2+} (4, 12, 14). The multiple species of SP-I in the cell and the addition of carbohydrate prior to or during secretion has led to speculation that SP-I is directly involved in the process of exocytosis (15).

In adrenal medulla, the catecholamine-containing chromaffin granules contain a family of acidic proteins, the so-called chromogranins (16), and as minor components, the enzyme dopamine β-hydroxylase, enkephalins, and enkephalin precursors (17). These proteins are secreted together with the catecholamines and in the same proportions as they exist in the intact chromaffin granules (7, 18–20). Newly synthesized chromogranins are incorporated into the granules and require about 30 min before they become released from the cell upon stimulation by carbachol (21). Chromogranin A, the major component of the soluble proteins, comprises more than 40% of these proteins (1). The amino acid composition of the total soluble proteins is similar to that of chromogranin A (22), and an immunological crossreaction between the acidic chromogranins has been reported (23). Therefore, it has been suggested (21) that the smaller chromogranins are breakdown products of chromogranin A because of the presence of endogenous proteases. The molecular mass of chromogranin A is in the range of 70,000–80,000 daltons (24–26). Chromogranin A is a glycoprotein with 5.4% carbohydrate (6, 27, 28).

Tables 1 and 2 provide comparative data on amino acid composition, physical properties, and carbohydrate content for SP-I and chromogranin A. The similarities are striking. Considering the fact that these data have been obtained in several different laboratories, the apparent small differences might be attributable to methodological variation. Fig. 1 lists the published partial amino acid sequences for the two proteins. Of the first 20 residues, 18 are identical. At position 2, SP-I contains Pro compared to Arg for chromogranin A; at position 19 a Val compared to Arg. These results show that the two molecules are closely homologous. We herein provide additional evidence based on immunological data that these proteins are similar.

Fig. 2 shows a radioimmunoassay of homogenates from adrenal medulla and chromaffin granules. Each sample displaced ^{125}I-labeled SP-I in a fashion similar to the SP-I standard. On a wet tissue basis, the medulla contained 1.9 mg of SP-I equivalent per g. On a protein basis, the medulla contained 29 μg of SP-I equivalent per mg, and the chromaffin granules contained the very high concentration of 229 μg of SP-I equivalent per mg. (Parathyroid gland yields about 0.8 mg/g or 10 μg/mg, respectively.) Fig. 3 shows the result of an immunodiffusion experiment with a soluble lysate of chromaffin granules in which the chromogranins represent the majority of protein. The antiserum generated against SP-I recognized a protein in the chromaffin granule lysate that reacted identically as SP-I. Additionally, a lysate that had been depleted of dopamine β-hydroxylase (29) by lentil lectin-Sepharose or concanavalin A-Sepharose affinity chromatography also gave a reaction identical to that of SP-I.

Fig. 4 compares the electrophoretic and immunological properties of SP-I, chromogranin A, and a soluble lysate of chromaffin granules. Purified SP-I and chromogranin A moved to the same position in $NaDodSO_4$ gels consistent with having

Table 2. Comparison of SP-I and chromogranins*

Properties	SP-I	Chromogranins
Molecular species		
Major components	2	(Chromogranin A)
Minor components	—	Several
Molecular mass of major component(s)	70 and 72 kDal	70–80 kDal
pI	4.5	Acidic
Carbohydrate, μmol/100 mg of protein		
Mannose	1.1	0.8
Galactose	6.5	6.3
Fucose	0.6	0.6
Glucosamine	0.5	1.1
Galactosamine	1.7	5.4
Sialic Acid	ND	8.3

* Data collected from refs. 2 and 9 for SP-I and from ref. 1 for the chromogranins. Carbohydrate data for SP-I is unpublished data and for chromogranin A is taken from refs. 28 and 29. ND, not determined; kDal, kilodaltons.

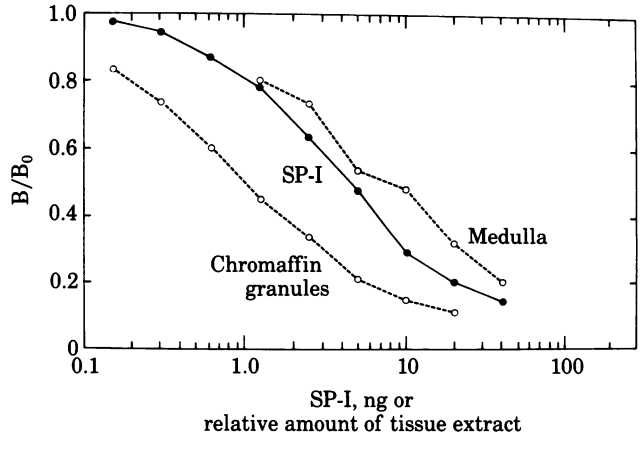

FIG. 2. Immunological reactivity of extracts from adrenal medulla and chromaffin granules to anti-SP-I antiserum. The lowest dilution factor (highest amount) from the neutralized samples was 1:400 for medulla and 1:1,000 for chromaffin granule.

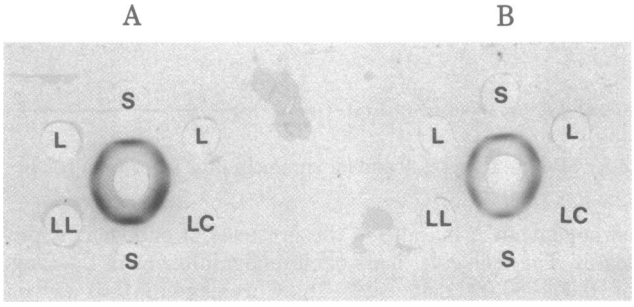

FIG. 3. Agarose immunodiffusion for SP-I and chromaffin granule lysates (*A* and *B*). Center wells, rabbit anti-bovine SP-I; S, purified SP-I; L, chromaffin granule lysate; LL, chromaffin granule lysate that was passed through lentil lectin-Sepharose; LC, chromaffin granule lysate that was passed through concanavalin A-Sepharose. The gel was stained with Coomassie blue. *A* and *B* are identical except that *B* contained one-half the amounts of samples and antiserum as in *A*.

identical molecular weights (Fig. 4, lanes 2 and 3). In both preparations, faster moving components (breakdown products) were present. As shown by the immunoreplica technique, antiserum against chromogranin A reacted with both purified components (Fig. 4, lanes 5 and 6) and with the acidic chromogranins in the soluble lysate (lane 4), which have been shown to be related immunologically to chromogranin A. Practically identical results were obtained with the antiserum raised against SP-I (Fig. 4, lanes 7–9). Of critical importance is that, in each case, each antiserum recognized nearly the same molecular species in the purified SP-I and chromogranin A preparations (lanes 5 versus 6 and lanes 8 versus 9). It should be pointed out that there were

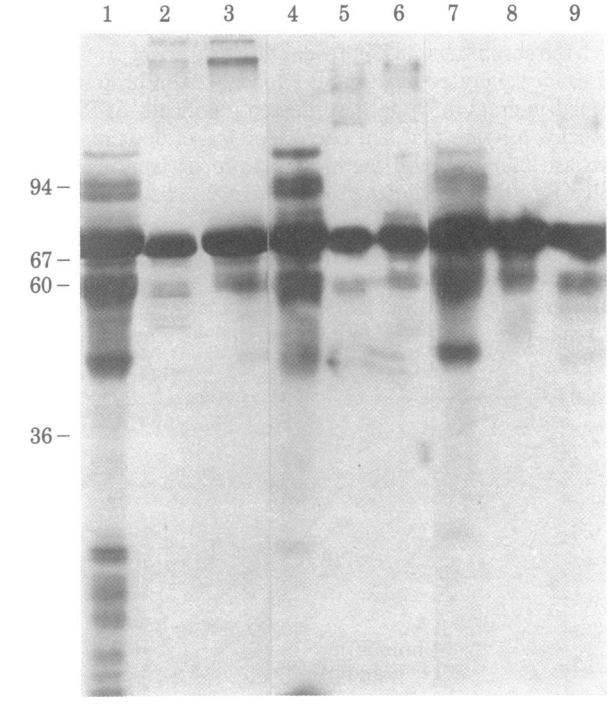

FIG. 4. Slab-gel electrophoresis and immunoreplicas of SP-I and chromogranin A stained with Coomassie blue. Daltons are shown × 10^{-3}. Lanes: 1, total soluble lysate of bovine chromaffin granules; 2, SP-I; 3, chromogranin A; 4–6, results of an immunoreplica experiment with antiserum developed against chromogranin A (lane 4, total soluble lysate of bovine chromaffin granules; lane 5, SP-I; lane 6, chromogranin A); 7–9, results of an immunoreplica experiment with antiserum developed against SP-I (lane 7, total soluble lysate of chromaffin granules; lane 8, chromogranin *A*; lane 9, SP-I).

differences in the molecular species recognized by the antiserum developed to chromogranin A compared to that developed to SP-I. Thus, some high molecular mass species were detected by the former but not by the latter (lanes 5 and 6 versus lanes 8 and 9). These differences are not surprising because the two antisera tested were raised against different protein preparations in different animals. Hence, they would be expected to have somewhat different specificities and would detect related proteins in the chromogranin A and SP-I preparations to different degrees.

CONCLUSIONS

These and previous results presented in Tables 1 and 2 and Fig. 1 make it clear that SP-I and chromogranin A are similar proteins. This study provides evidence that chromogranin A-like proteins occur outside adrenergic tissues, or stated conversely, that SP-I-like proteins exist outside the parathyroid gland. The presence of chromogranin A in the noradrenergic vesicles of sympathetic neurones (30, 31) and its secretion from this tissue (10, 32) had been established earlier. Because both proteins are present in the secretory granules of their respective tissue (1, 11) and are cosecreted with epinephrine and norepinephrine from the adrenals and with parathormone from the parathyroid, the present results showing their close similarity seem more than fortuitous and raise the obvious possibility that they play a general role in the storage or secretory processes of certain glands. It is now necessary to establish if the SP-I/chromogranin A-like proteins exist in other secretory tissues.

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