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In vitro biosynthesis of fish islet preprosomatostatin: Evidence of processing and segregation of a high molecular weight precursor

(mRNA from islets of Langerhans/wheat germ system/canine microsomal membranes/antibody competition/tryptic maps)

DENNIS SHIELDS

Department of Anatomy, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, New York 10461

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ABSTRACT **Evidence is presented for a precursor to somatostatin that is 10–12 times larger than the authentic secreted hormone. mRNA from angler fish (*Lophius americanus*) islets of Langerhans was translated in the wheat germ cell-free system and the products were identified by immunoprecipitation with specific antibodies to somatostatin followed by sodium dodecyl sulfate gel electrophoresis. One 18,000-dalton polypeptide was specifically immunoprecipitable. Competition experiments showed that authentic somatostatin competed with the 18,000-dalton molecule for antibody binding. When dog pancreas microsomal membranes were present during translation, an additional polypeptide of 16,000 daltons was also immunoprecipitable. Comparison of their tryptic peptides demonstrated that the 16,000-dalton polypeptide was derived from the 18,000-dalton one. Tryptic peptide analysis of somatostatin and the 18,000-dalton precursor demonstrated that the 18,000-dalton polypeptide contains the authentic somatostatin amino acid sequence and suggests that it is located at the carboxyl terminus of the precursor molecule and is preceded by a basic amino acid.**

Somatostatin (somatotropin release inhibiting factor) is a tetradecapeptide that originally was isolated from hypothalamic tissue (1, 2) and has now been detected in various organs (3). Several groups have detected higher molecular weight forms of the molecule in the pancreas and hypothalamus (4–8) and recently, a precursor–product relationship between some of the higher molecular weight immunoreactive forms and secreted somatostatin was demonstrated (6, 8). These findings suggest that the putative precursors undergo several posttranslational cleavages to yield distinct biosynthetic intermediates and the authentic secreted molecule. Many proteins destined for secretion are synthesized as nascent precursors possessing a "signal peptide" or its functional equivalent (9, 10). It is also apparent that several polypeptide hormones are synthesized via higher molecular weight precursor forms (11–14) which undergo posttranslational cleavage. In the case of bovine corticotropin–β-lipotropin, for example, the precursor molecule contains several peptide hormones within its sequence (15).

Consequently, it was of interest to investigate the primary translation product of somatostatin mRNA to compare its size to that of precursor forms found in tissue extracts (4–8). Previous studies (16, 17) using fish islets of Langerhans as a source of mRNA established that preproinsulin was the major translation product; however, at least three other polypeptides with molecular weights up to 18,000 were noted (16). Evidence is presented for an 18,000-dalton precursor (preprosomatostatin) to somatostatin in which the authentic somatostatin sequence (1600 daltons) is located at the carboxyl terminus of the molecule.

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MATERIALS

Rabbit antiserum directed against synthetic somatostatin (S 203), specific for residues 5–8 and 11, was a gift of W. Vale (Salk Institute). Somatostatin was purchased from Peninsula Laboratories (San Carlos, CA) or was a gift of P. Davies or J. Ehrlichman. Bovine proinsulin was a gift of R. E. Chance (Eli Lilly). Tosylphenylalanyl chloromethyl ketone-treated trypsin was from Worthington. ^{3}H and ^{14}C-labeled amino acid mixture, [^{3}H]phenylalanine, and [^{35}S]methionine were purchased at the highest available specific activity from Amersham/Searle. [^{35}S]Cysteine ($\approx$450 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels) was from New England Nuclear, Boston, MA.

METHODS

Most of the methods used for these experiments have been described, including isolation of mRNA from angler fish islets and its translation in the wheat germ system (16), preparation of microsomal membranes from dog pancreas (18), antibody precipitation (19), and $NaDodSO_4$ gel electrophoresis using gradients of 10–15% polyacrylamide (9, 16).

Peptide Mapping. Preprosomatostatin was excised from the dried gel, rehydrated in 0.1 M NH_4HCO_3, and digested with 50 μg of trypsin (20). Then, 20 μg of unlabeled somatostatin tryptic peptides (see below) was added to the sample and the mixture was applied to a 2.5-ml column of Sephadex G-10. The column was eluted with 0.1 M NH_4HCO_3 and sufficient volume was collected to give qualitative recovery of all the unlabeled somatostatin tryptic peptides. The eluate was freeze-dried and dissolved in water prior to analysis on thin-layer cellulose plates. Radioactive tryptic peptides containing approximately 5–10 nmol of unlabeled somatostatin tryptic peptides were applied to 20 × 20 cm cellulose plates. The peptides were subjected to electrophoresis at 55 V/cm in pyridine/acetic acid/water, 100:4:896 (vol/vol), at pH 6.4, followed by chromatography with pyridine/1-butanol/acetic acid/water, 40:60:12:48 (vol/vol). Unlabeled peptides were located by spraying the plate with Fluram; the radioactive spots were localized by fluorography (21).

Electroeluted samples were combined, 1 mg of bovine serum albumin was added, and the samples were precipitated with 2 vol of acetone (16). The precipitates were dissolved in 500 μl of 1% NH_4HCO_3 and digested with 50 μg of trypsin for 4 hr at 37°C. After digestion the samples were freeze-dried, dissolved in 80% acetic acid, and applied to a heated (56°C) water-jacketed column (0.9 × 23 cm) of type P Chromobead ion-exchange resin (Technicon Chemicals S.A., Belgium 7900) equilibrated with 0.05 M pyridine/acetic acid at pH 3.13. The peptides were eluted with a gradient of pyridine/acetic acid as described (22).

Reduction, Alkylation, and Trypsin Digestion of Unlabeled Somatostatin. Five hundred micrograms of somatostatin

(≈300 nmol) dissolved in 100 μl of 0.1 M ammonium acetate (pH 7.0) was treated with 15 μl of 2-mercaptoethanol (approximately 210 μmol) and incubated for 4 hr at room temperature. Then, 400 μmol of iodoacetamide was added and the sample was incubated for 1 hr in the dark. Excess iodoacetamide was removed by passage over a column of Sephadex G-10. The reduced and alkylated somatostatin was lyophilized and dissolved in 50 μl of 0.1 M NH_4HCO_3. Five micrograms of trypsin was added, and the sample was digested at 30°C for 90 min. This digestion procedure was repeated, and digestion was terminated by lyophilizing the sample.

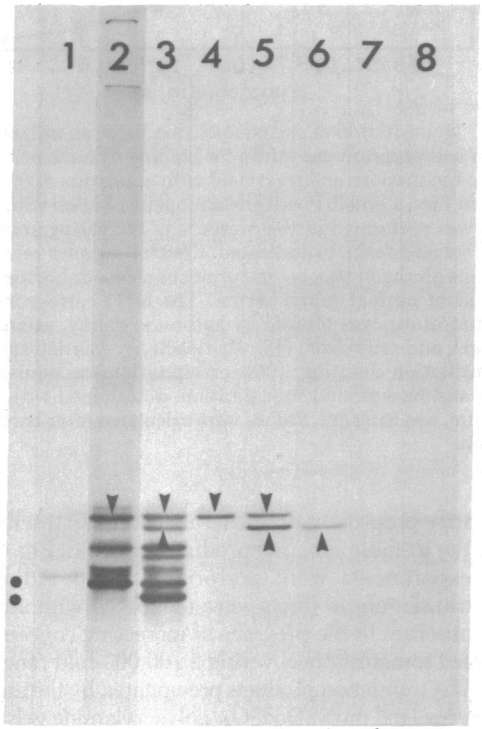

FIG. 1. Autoradiograph of the dried gel after $NaDodSO_4$/polyacrylamide gel electrophoresis. mRNA (1 A_{260} unit/ml) from angler fish islets of Langerhans was translated in the wheat germ system containing 250 μCi of [^{35}S]methionine per ml in the absence (lanes 1, 2, 4, and 8) or presence of dog pancreas microsomal membranes at 5 A_{260} units/ml (lanes 3 and 5–7). Incorporation into acid-precipitable radioactivity was 105,000 and 53,000 cpm/μl in the absence and presence of microsomal membranes, respectively. After incubation at 27°C for 60 min, 10-μl aliquots of translation products were prepared for electrophoresis directly (lanes 2 and 3) and the remainder was subjected to the following posttranslational assays. Immunoreactive translation products (lanes 4 and 5): 50-μl aliquots of the incubation mixture were adjusted to 1% $NaDodSO_4$, incubated in a boiling water bath for 2 min, and cooled to room temperature; then 4 vol of solution A (19) and 2 μl of antisomatostatin antiserum S203 were added; after incubation for 12 hr at 4°C the immunoprecipitates were processed as described (19). Lane 6: immunoreactive translation products as in lane 5 except that a 50-μl aliquot was adjusted to 8 mM $CaCl_2$ and then incubated with a mixture of 250 μg each of trypsin and chymotrypsin per ml for 1 hr at 4°C prior to immunoprecipitation; digestion was terminated by addition of 1000 units of Trasylol and boiling for 2 min in 1% $NaDodSO_4$. Lane 7: as in lane 6 except that proteolysis was performed in the presence of 1% Triton X-100. Lane 1: total translation products synthesized in the absence of microsomal membranes, followed by posttranslational incubation with trypsin and chymotrypsin as in lane 5. Lane 8: as in lane 4 except that the translation products were treated with normal rabbit serum. Downward-pointing arrowheads, putative preprosomatostatin; upward-pointing arrowheads, prosomatostatin; dots to the left of lane 1, migration of *in vitro*-synthesized preproinsulin and proinsulin, respectively.

RESULTS

Previous results demonstrated that angler fish islet mRNA actively supports protein synthesis in the wheat germ cell-free system (16). Analysis of the translation products by $NaDodSO_4$ gel electrophoresis and subsequent autoradiography revealed four major polypeptides ranging in molecular weight from 11,000 to about 18,000 (Fig. 1, lane 2). The most prominent translation product, which had the lowest molecular weight, was preproinsulin (16). Because it is known that fish islets synthesize both glucagon and somatostatin (4), the possibility existed that one (or more) of the bands might correspond to precursor forms of these hormones. To test this hypothesis, translation products were treated with the specific anti-somatostatin serum S203 and analyzed on the same slab gel (Fig. 1). Synthesis in the absence of microsomal membranes followed by treatment with S203 (lane 4) resulted in the precipitation of only one polypeptide which had an apparent molecular weight of 18,000–19,000 (Fig. 1, lane 4; Fig. 2); no translation products were precipitated by the control serum (lane 8). This band was tentatively identified as the primary translation product of somatostatin mRNA and hence would be a precursor to somatostatin that is approximately 12 times larger than the secreted molecule. It was therefore designated preprosomatostatin.

When mammalian (dog pancreas) microsomal membranes were present during translation, at least three additional polypeptides were evident (Fig. 1, lane 3). Treatment of the products synthesized in the presence of microsomal membranes with S203 serum resulted in precipitation of two polypeptides (lane 5). One of these bands exactly comigrated with preprosomatostatin (downward pointing arrow); the other had an apparent molecular weight of about 16,000 (Fig. 2). Concomitant with the appearance of the 16,000-dalton polypeptide, there was a slight reduction in the intensity of the putative

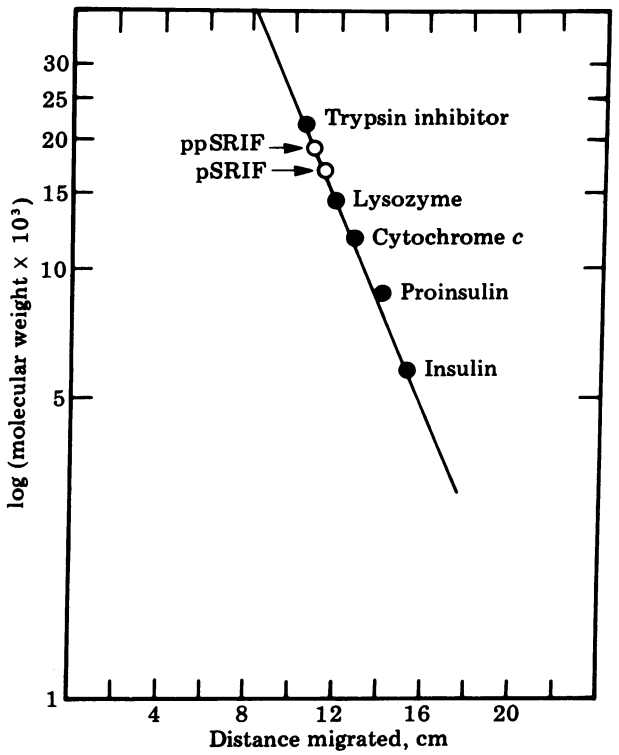

FIG. 2. Calibration of the gel shown in Fig. 1. Soybean trypsin inhibitor, egg white lysozyme, horse cytochrome *c*, bovine proinsulin, and bovine insulin (representing 21.5, 14.3, 11.7, 8.7, and 5.7 kilodaltons, respectively) were applied to the gel shown in Fig. 1 and their relative mobilities were used to determine the molecular weights of preprosomatostatin (ppSRIF) and prosomatostatin (pSRIF).

preprosomatostatin, results which suggest a precursor–product relationship between these two polypeptides. In addition, the 16,000-dalton polypeptide was segregated into the microsomal membrane vesicle because it was resistant to posttranslational proteolysis—in contrast, preprosomatostatin was completely degraded (lane 6). By analogy to previous results (9, 16) this finding suggested that nascent preprosomatostatin (18,000 daltons) was processed, possibly by removal of a signal peptide, to a pro form of somatostatin, prosomatostatin.

That the 16,000-dalton prosomatostatin was segregated into the microsomal vesicles was demonstrated when posttranslational proteolysis was performed in the presence of the detergent Triton X-100 and the resulting products were incubated with anti-somatostatin antibodies (lane 7)—in this case, resistance to proteolysis was completely abolished. As expected, those polypeptides synthesized in the absence of microsomal membranes were entirely degraded when subjected to posttranslational proteolysis (lane 1). Addition of microsomal membranes posttranslationally had no effect on either the polypeptides synthesized in the absence of membranes or those synthesized when membranes were present during translation (data not shown).

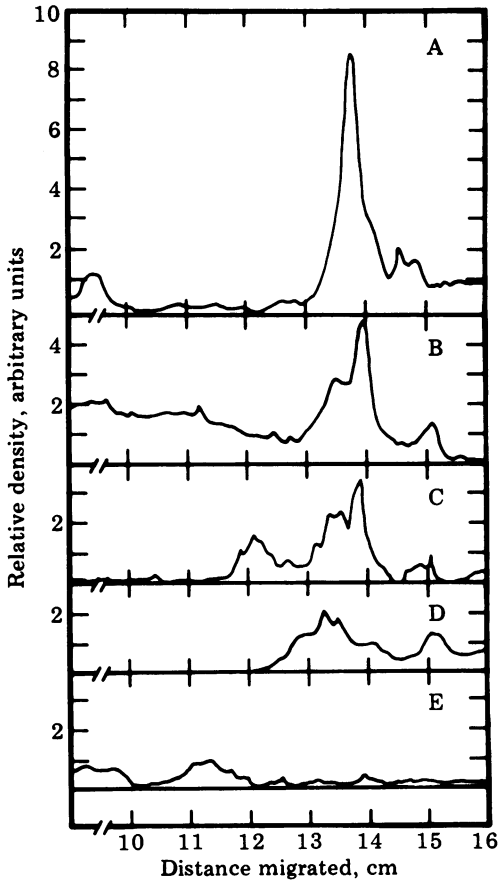

FIG. 3. Immunological characterization of the cell-free translation products containing somatostatin-like material: competition between unlabeled somatostatin and putative preprosomatostatin for binding by antisomatostatin serum. Angler fish islet mRNA was translated in 500-μl incubation mixtures containing 200 μCi of [^{35}S]methionine. After incubation, the mixture was adjusted to 1% NaDodSO$_4$ and treated as outlined in Fig. 1. The sample was divided into equal 200-μl aliquots to which was added 0.1 ng (*B*), 1 ng (*C*), 10 ng (*D*), or 1 μg (*E*) of unlabeled somatostatin; control samples (*A*) received no unlabeled antigen. Each sample was then incubated with 3 μl of antiserum S203 as outlined in Fig. 1 and the immunoprecipitates were processed for gel electrophoresis and autoradiography. The autoradiographs were analyzed by using a Joyce–Loebl double-beam densitometer. The densitometric tracings show the region of the gel in which preprosomatostatin was resolved.

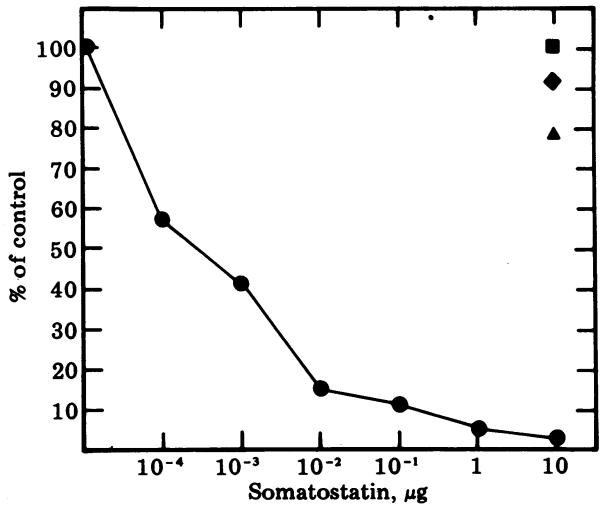

FIG. 4. Quantitation of the competition between unlabeled somatostatin and preprosomatostatin for binding by antisomatostatin antibodies: Incubations and treatment with antiserum S203 were as described in Figs. 1 and 3. Posttranslational incubation with antiserum S203 was performed in the presence of increasing amounts of unlabeled somatostatin, as indicated. Control samples received 10 μg of porcine glucagon (■), bovine proinsulin (♦), or bovine insulin (▲), or 3 μl of normal rabbit serum. The band corresponding to preprosomatostatin was located by autoradiography, excised from the dried gel, and solubilized (18). Radioactivity was determined by liquid scintillation counting; 100% precipitation was equivalent to 5600 cpm and background precipitation, determined with normal rabbit serum, was 215 cpm. Values were calculated after background subtraction.

To identify preprosomatostatin further and to test the specificity of the immunoreactive products shown in Fig. 1, competition experiments were performed. Total radiolabeled cell-free translation products were incubated with antisomatostatin antiserum in the presence of increasing concentrations of unlabeled somatostatin covering a 100,000-fold range (Figs. 3 and 4). The translation products precipitated by the antiserum were then resolved on NaDodSO$_4$/polyacrylamide gels and the autoradiographs were analyzed by densitometry (Fig. 3). The data show that with increasing concentration of unlabeled somatostatin there was a concomitant reduction in the amount of radioactive immunoprecipitable preprosomatostatin. Quantitative analysis of the immunoprecipitates by liquid scintillation counting after solubilization from the gels (Fig. 4) showed that almost 100% of the cell-free synthesized preprosomatostatin could be replaced by synthetic somatostatin. In contrast, glucagon and proinsulin had no effect on antibody precipitation even at the highest concentration of competitor tested (10 μg). Insulin (10 μg) appeared to act as a competitor but only to a minor extent and this can be explained on the basis of contaminants present in crude insulin preparations but not in proinsulin. It should be noted that about 20% of the total preprosomatostatin radioactivity was immunoprecipitable by antiserum S203 under the conditions used for these experiments.

The proposed precursor–product relationship between putative preprosomatostatin and prosomatostatin (Fig. 1) was confirmed when these polypeptides were synthesized in the cell-free system containing either ^{3}H-labeled or ^{14}C-labeled amino acids (Fig. 5 *Upper*). If prosomatostatin (16,000 daltons) were derived from the 18,000-dalton precursor by proteolytic processing—for example, by removal of a nascent signal peptide—it would be expected these two molecules would share many common peptides. The data of Fig. 5 demonstrate that indeed this is the case. With the exception of one peptide,

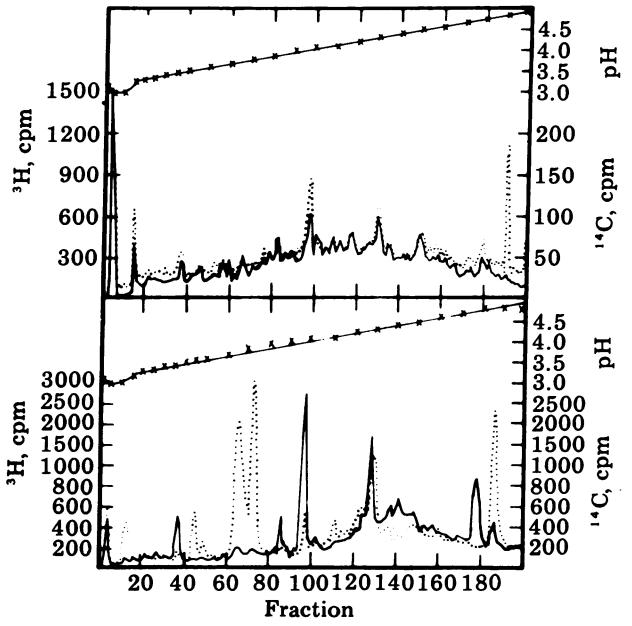

FIG. 5. Comparison of radiolabeled tryptic peptides derived from the cell-free synthesis of putative preprosomatostatin (18,000 daltons), prosomatostatin (16,000 daltons), and proinsulin. Analysis was by chromatography on a 15-ml column of type P Chromobead ion-exchange resin (22). The cell-free translation products synthesized in the absence or presence of dog pancreas microsomal membranes at 8 A_{260} units/ml in incubations containing ^{14}C-labeled amino acid mix (35 μCi/ml) or ^{3}H-labeled amino acid mix (300 μCi/ml) were analyzed by NaDodSO$_4$/polyacrylamide gel electrophoresis. The desired radioactive bands (see Fig. 1) were located by fluorography and excised from the dried gel. After electroelution, the ^{14}C- and ^{3}H-labeled polypeptides were combined, as indicated, and subjected to digestion with trypsin. (*Upper*) Comparison of ^{14}C-labeled preprosomatostatin (—) and ^{3}H-labeled prosomatostatin (·····) tryptic peptides. Input radioactivity was 18,000 cpm and 87,000 cpm, respectively. (*Lower*) Comparison of ^{3}H-labeled preprosomatostatin (—) and ^{14}C-labeled proinsulin (·····) tryptic peptides. Input radioactivity was 150,000 cpm and 126,000 cpm, respectively.

eluting from the column at about pH 4.8 (fractions 188–191), the tryptic peptides of these two molecules were similar. For comparison, the tryptic peptides of preprosomatostatin and angler fish proinsulin (synthesized *in vitro*) are shown in Fig. 5 *Lower*. In this case there are significant differences in the elution profiles of the tryptic peptide fragments.

To establish that the 18,000-dalton putative preprosomatostatin contained the amino acid sequence of authentic somatostatin (molecular weight, approximately 1600), the tryptic peptides of these two molecules were compared directly. The amino acid sequence of somatostatin is identical in mammals and angler fish (2, 6), and it has been shown that three peptides are generated by trypsin treatment of authentic somatostatin (2, 23). Therefore, it can be postulated that, regardless of the location of the authentic somatostatin sequence within the 18,000-dalton precursor, its middle tryptic peptide, T-2, would be present in tryptic peptides derived from the *in vitro* synthesized precursor. This might not necessarily be true for tryptic peptides T-1 and T-3, which could have NH_2-terminal or COOH-terminal extensions, respectively (Fig. 6). Preprosomatostatin synthesized in the cell-free system containing [^{35}S]cysteine and [^{3}H]phenylalanine was subjected to trypsin digestion. The radioactive tryptic fragments were combined with peptide fragments derived from trypsin treatment of unlabeled reduced and alkylated somatostatin and analyzed on thin-layer cellulose plates. As predicted from the amino sequence of somatostatin, three tryptic peptides were evident (Fig. 6). Comparison of the radioactive spots with the unlabeled tryptic fragments revealed several differences between the cell-free products and somatostatin. Most significantly, however, all three tryptic peptides obtained from authentic somatostatin were exactly coincident with radioactive spots—these data demonstrate that the 18,000-dalton precursor does contain the somatostatin amino acid sequence.

DISCUSSION

The results presented here demonstrate that the primary translation product of islet somatostatin mRNA is approximately 18,000 daltons, or about 10–12 times larger than the secreted molecule. Upon translation in the presence of microsomal membranes the nascent 18,000-dalton precursor was proteolytically cleaved to about 16,000 daltons and the proso-

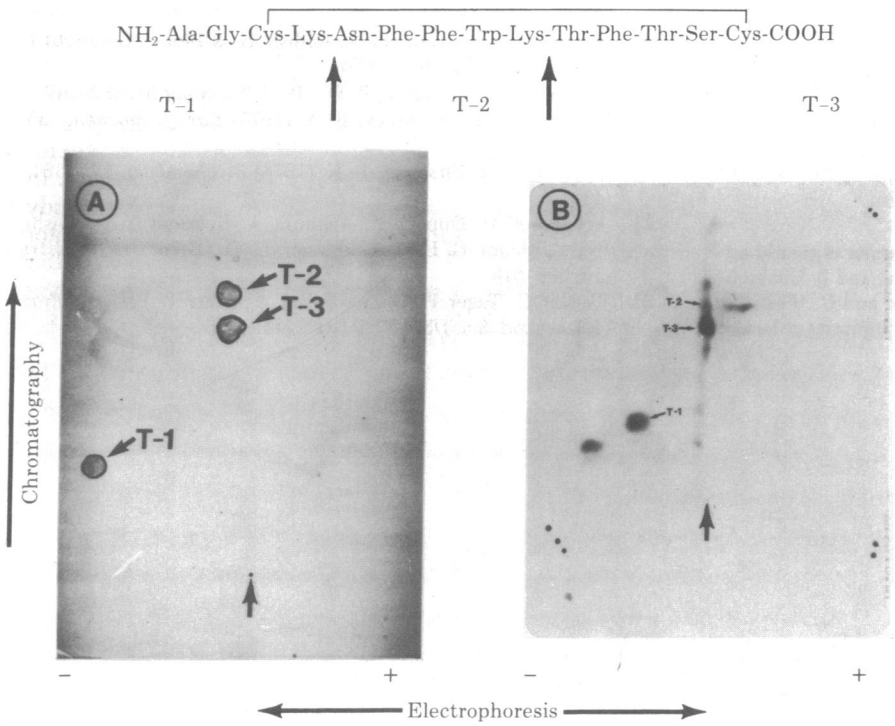

FIG. 6. Two-dimensional tryptic map of *in vitro* synthesized radiolabeled preprosomatostatin and synthetic somatostatin. Islet mRNA was translated in the wheat germ system containing 350 μCi of [^{35}S]cysteine and 250 μCi of [^{3}H]phenylalanine per ml. The translation products were resolved by NaDodSO$_4$ gel electrophoresis, and preprosomatostatin was located by autoradiography. The gel slices containing preprosomatostatin were digested with 50 μg of trypsin. Radioactive tryptic peptides were combined with approximately 10 nmol of unlabeled tryptic peptides obtained from reduced and alkylated somatostatin. The combined tryptic peptides were separated on thin-layer cellulose plates, first by electrophoresis at pH 6.4 and then by chromatography in the second dimension. Approximately 32,000 cpm of ^{35}S radioactivity and 38,000 cpm of ^{3}H were applied. (*A*) Fluram-stained map. (*B*) Fluorograph from a similar peptide map. The origin is designated by an arrow. Tentative identity of the tryptic peptides was assigned on the basis of ^{35}S:^{3}H ratio. The sequence of ovine and porcine somatostatins (2, 23) is shown.

matostatin was segregated into the heterologous membrane vesicles. These results can be explained by assuming that translation in the absence of microsomal membranes resulted in synthesis of preprosomatostatin possessing a signal peptide of 20–30 amino acids—i.e., 2000–3000 daltons larger than prosomatostatin. Thus, given slight discrepancies in procedures for molecular weight determination, the data are entirely consistent with recent reports of a somatostatin precursor of about 15,000 daltons found in whole tissue extracts (6, 8). Although highly suggestive, the above data do not prove that preprosomatostatin has an NH_2-terminal signal peptide; sequencing determinations are needed. It could be argued that the 18,000-dalton preprosomatostatin is not the primary translation product of somatostatin mRNA but resulted from cleavage of an even larger molecule by a protease(s) present in the wheat germ system, but this is unlikely.

Tryptic peptide analysis of the 18,000-dalton precursor conclusively demonstrated that preprosomatostatin contains the authentic somatostatin sequence. It also revealed that the NH_2-terminal tryptic peptide of unlabeled somatostatin comigrated exactly with a radioactive peptide from preprosomatostatin. Determination of the radioactivity in this spot showed that it corresponded to peptide T-1 (data not shown). This suggests that the authentic somatostatin sequence, within the 18,000-dalton precursor, is preceded by a basic amino acid (lysine or arginine). In addition, all three unlabeled somatostatin tryptic peptides comigrated with radioactive spots derived from the 18,000-dalton precursor, suggesting that the authentic somatostatin may be located at the extreme COOH terminus of the precursor molecule. Were this not the case, then the unlabeled COOH-terminal peptide (T-3) would not comigrate with a radioactive counterpart. It is noteworthy that trypsin digestion led to excision of somatostatin peptides from the precursor; this is consistent with the data from *in vivo* labeled angler fish islet prosomatostatin (6) but disagrees with data on the hypothalamic 15,000-dalton prosomatostatin. In this case (8) trypsin was unable to cleave somatostatin from the precursor molecule—only a protease activity that copurified with the precursor could effect such conversion. The possibility exists, therefore, that islet and hypothalamic somatostatins share a common authentic sequence but could be derived from different precursor molecules. This could be tested by comparison of the tryptic peptide maps of preprosomatostatin obtained from these tissues. Finally, as has been recognized for several different peptide hormones (12, 14, 15), there is a significant difference in the size of preprosomatostatin and authentic secreted somatostatin (about 10–12 fold), a finding that is consistent with the possibility that the 18,000-dalton molecule could be a precursor to one or more other polypeptides in addition to somatostatin.

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Note Added in Proof. Patzelt *et al.* (24) recently reported a 12,500-dalton prosomatostatin, from rat islets, in which the authentic sequence was located at the COOH terminus.

1. Brazeau, P., Vale, W., Burgus, R., Ling, N., Butcher, M., Rivier, J. & Guillemin, R. (1972) *Science* **179,** 71–79.
2. Burgus, R., Ling, N., Butcher, M. & Guillemin, R. (1973) *Proc. Natl. Acad. Sci. USA* **70,** 684–688.
3. Arimura, A., Sato, H., Dupont, A., Nishi, N. & Schally, A. V. (1975) *Science* **189,** 1007–1009.
4. Noe, B. D., Fletcher, D. J., Bauer, G. E., Weir, C. C. & Patel, Y. (1978) *Endocrinology* **102,** 1675–1685.
5. Conlon, J. M., Zyznar, E., Vale, W. & Unger, R. H. (1978) *FEBS Lett.* **94,** 327–330.
6. Noe, B. D., Fletcher, D. J. & Spiess, J. (1979) *Diabetes* **28,** 724–730.
7. Zingg, H. H. & Patel, Y. (1979) *Biochem. Biophys. Res. Commun.* **90,** 466–472.
8. Lauber, M., Cannier, M. & Cohen, P. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 6004–6008.
9. Blobel, G. & Dobberstein, B. (1975) *J. Cell Biol.* **67,** 834–851.
10. Lingappa, V. R., Lingappa, J. & Blobel, G. (1979) *Nature (London)* **281,** 117–121.
11. Steiner, D. F., Kemmler, W., Tager, H. S. & Peterson, J. D. (1974) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **33,** 2105–2115.
12. Mains, R. E., Eipper, B. A. & Ling, N. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 3014–3018.
13. Patzelt, C., Tager, H. S., Carroll, R. J. & Steiner, D. F. (1979) *Nature (London)* **282,** 260–266.
14. Brownstein, M. J., Russell, J. T. & Gainer, H. (1980) *Science* **207,** 373–378.
15. Nakanishi, S., Inoué, A., Kita, T., Nakamura, M., Chang, A. C. Y., Cohen, S. N. & Numa, S. (1979) *Nature (London)* **278,** 423–427.
16. Shields, D. & Blobel, G. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 2059–2063.
17. Permutt, M. A., Biesbroeck, R. C., Boime, I., Szczesna, E. & McWilliams, D. (1976) in *CIBA Symposium no. 41: Polypeptide Hormones*, eds. Porter, R. & Fitzsimmons, D. W. (Elsevier, New York), pp. 97–116.
18. Shields, D. & Blobel, G. (1978) *J. Biol. Chem.* **253,** 3753–3756.
19. Lingappa, V. R., Katz, F. N., Lodish, H. & Blobel, G. (1978) *J. Biol. Chem.* **253,** 8667–8670.
20. Lamb, R. A. & Choppin, P. W. (1977) *Virology* **81,** 382–397.
21. Bonner, W. M. & Laskey, R. A. (1975) *Eur. J. Biochem.* **56,** 335–341.
22. Francus, T. & Birshtein, B. K. (1978) *Biochemistry* **17,** 4324–4331.
23. Schally, A. V., Dupont, A., Arimura, A., Redding, T. W., Nishi, N., Linthiam, G. L. & Schlesinger, D. H. (1976) *Biochemistry* **15,** 509–514.
24. Patzelt, C., Tager, H. S., Carroll, R. J. & Steiner, D. F. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 2410–2414.