Cotranslational and Posttranslational Proteolytic Processing of Preprosomatostatin-I in Intact Islet Tissue

Bryan D. Noe,*‖ Phillip C. Andrews,‡ Jack E. Dixon,‡ and Joachim Spiess§

*Department of Anatomy and Cell Biology, Emory University School of Medicine, Atlanta, Georgia 30322;
‡Department of Biochemistry, Purdue University, West Lafayette, Indiana 47907;
§Clayton Foundation Laboratories for Peptide Biology, The Salk Institute, La Jolla, California 92037; and
‖The Marine Biological Laboratory, Woods Hole, Massachusetts 02543

Abstract. Preprosomatostatin-I (PPSS-I) is processed in anglerfish islets to release a 14-residue somatostatin (SS-14). However, very little is known regarding other processing events that affect PPSS-I. This is the first study to identify and quantify the levels of non-somatostatin products generated as a result of processing of this somatostatin precursor in living islet tissue. The products of PPSS-I processing in anglerfish islet tissue were identified in radiolabeling studies using a number of criteria. These criteria included immunoreactivity, specific radiolabeling by selected amino acids, radiolabel sequencing, and chromatographic comparison to isolated, structurally characterized fragments of anglerfish PPSS-I using reverse-phase high performance liquid chromatography. Intact prosomatostatin-I (aPSS-I) was isolated from tissue incubated with [^{3}H]tryptophan and [^{14}C]leucine. Significant ^{14}C radioactivity was observed in the products of 11 of the first 44 sequencer cycles in positions consistent with the generation of a 96-residue prosomatostatin. These results indicate that signal cleavage occurs after the cysteine located 25 residues from the initiator Met of PPSS-I, resulting in a signal peptide 25 amino acids in length.

Nonsomatostatin-containing fragments of the precursor were also found in tissue incubated with a mixture of ^{3}H-amino acids. Only a small quantity of the dodecapeptide representing residues 69–80 in the prohormone was found (10 nmol/g tissue). Two other fragments of aPSS-I, also observed to be present in low abundance, were found to correspond to residues 1–27 (16 nmol/g tissue) and to residues 1–67 (7 nmol/g tissue) of aPSS-I. No evidence for the presence of the fragment corresponding to residues 29–67 was found. However, large quantities of SS-14 were observed (287 nmol/g tissue), indicating that the major site of aPSS-I cleavage is at the basic dipeptide immediately preceding SS-14. Recovery of much lower levels of the non-somatostatin fragments of aPSS-I suggests that prohormone processing at the secondary sites identified in this study occurs at a low rate relative to release of SS-14 from aPSS-I.

S OMATOSTATIN-14 (SS-14)[1] is synthesized as part of a larger precursor, preprosomatostatin. This has been demonstrated by results from experiments using pulse-chase incubations or chemical characterization of products from intact tissue (1, 18, 25, 26, 28, 38, 39). In addition, use of recombinant DNA methodologies has provided the deduced amino acid sequences for prosomatostatins from a number of sources (13–17, 20, 21, 32–35, 40–43). Prosomatostatins or prosomatostatin cleavage products have been identified in pancreatic islets of anglerfish (15, 17, 25, 26, 34, 35, 43), catfish (1, 20, 21, 41), and rats (8, 28); in hypothalamus of mouse (18), rat (4, 6–8, 19, 38), sheep (10, 39), and pigs (30); gut of pigs (29); and from diverse tissues such as a medullary thyroid carcinoma cell line (2, 3, 13, 14, 16) and

a phaeochromocytoma (45). Additionally, both the human (33) and rat (40) genes that code for preprosomatostatins have been characterized. For purposes of comparison, the basic structures of human preprosomatostatin (PPSS) and the two known forms of anglerfish PPSS are depicted in Fig. 1. All mammalian PPSS species that have been characterized share a structure very similar to that of human PPSS.

The preprosomatostatins are cleaved co-translationally to remove the signal peptide and yield prosomatostatin (14, 34, 43). One aspect of somatostatin production that is not completely understood is the nature of the posttranslational processing events leading to cleavage of the prohormone to products. It is known that prosomatostatin processing in mammals is tissue-specific with SS-28 being the predominant product in the gut and SS-14 in the pancreas and brain (27). In addition, a multiplicity of fragments of prosomatostatins of varying size have been isolated from diverse tissue sources (2–10, 27, 29–31, 38, 39, 45). Except for SS-14, SS-

---

1. *Abbreviations used in this paper*: aPSS-I, anglerfish prosomatostatin-I; HPLC, high performance liquid chromatography; PPSS-I, (anglerfish) preprosomatostatin-I; RIA, radioimmunoassay; SS-14, somatostatin-14.

28, and SS-28$_{1-12}$, it is not clear whether any of these fragments of prosomatostatin are metabolic cleavage products. In most cases, it cannot be excluded that these peptides are random degradation products of the somatostatin precursor. To ascertain whether any of these peptides are metabolic cleavage products, it is necessary to perform biosynthetic studies.

Recently, several fragments of prosomatostatin-I (aPSS-I) have been isolated from anglerfish islets and characterized by Andrews and Dixon.[2] The fragments of aPSS-I were initially identified by amino acid analysis of peptides from anglerfish islet extracts that had previously been purified to homogeneity by ion-exchange chromatography and high performance liquid chromatography (HPLC). The primary structure of each peptide was determined by microsequencing or Fast Atom Bombardment mass spectrometry. The availability of these peptides has made it feasible to study their production in intact islet tissue. The purified natural product peptides were used as retention time markers in HPLC runs to identify the peptides in biosynthetic studies. We report here the results of experiments designed to quantitate the amounts of these peptides that are synthesized and stored in islets along with data that confirm the cleavage site for the signal peptide of PPSS-I.

Materials and Methods

Materials

A ^{3}H-L-amino acid mixture containing 15 amino acids ranging in specific activity from 7 to 50 Ci/mmol was purchased from ICN Biochemicals Inc., Irvine, CA. [^{3}H]Tryptophan (8.0 Ci/mmol) and [^{14}C]leucine (340 mCi/mmol) were purchased from New England Nuclear, Boston, MA. Bio Gel P-2 (100–200 mesh) and P-30 (100–200 mesh) were obtained from Bio-Rad Laboratories, Richmond, CA. Monofluor scintillation cocktail was from National Diagnostics, Inc., Somerville, NJ. HPLC grade acetonitrile was purchased from Fisher Scientific Co., Pittsburgh, PA, and sequanal grade trifluoroacetic acid used in the HPLC solvent system was obtained from Pierce Chemical Co., Rockford, IL. The 30-K antiserum to mammalian glucagon was obtained from the laboratory of Dr. R. H. Unger, the University of Texas Southwestern Medical School, Dallas, TX. [^{125}I]Glucagon was purchased from Cambridge Isotope Laboratories, Woburn MA. The R141 antiserum to SS-14 was a gift of Dr. R. P. Elde, University of Minnesota, Minneapolis, MN. [^{125}I]Tyr-SS-14 was purchased from New England Nuclear, Boston, MA.

Incubation of Islet Tissue

Decapsulated islets were prepared and incubated as described previously (23, 25). After a 30-min incubation in buffer alone, islets were incubated 5 h in the presence of either [^{3}H]Trp (100 μCi) plus [^{14}C]Leu (50 μCi), or 500 μCi of the ^{3}H-amino acid mix.

Extraction and Gel Filtration

Labeled tissue was homogenized and extracted in 2 M acetic acid. The crude extracts were desalted by filtration through 2.5 × 17-cm columns of Bio-Gel P-2 in 2 M acetic acid, and the desalted extracts were subjected to gel filtration on 1.6 × 95-cm columns of Bio-Gel P-30, also in 2 M acetic acid. Radioactivity in the column eluates was monitored by taking aliquots from eluate fractions. The remainder of each eluate was separated into three pools containing M_r 1,000–2,500, M_r 2,500–9,000, or M_r 9,000–15,000 peptides, and lyophilized. The concentrated material was solubilized in freshly prepared 3 M acetic acid, and the samples were filtered through 0.22-μm filters before subjecting aliquots to reverse-phase HPLC.

HPLC

The HPLC system consisted of a Perkin-Elmer (P-E) series 3B liquid chro-

matograph linked to a P-E LC-75 spectrophotometer and a P-E Sigma 10 chromatography data station (Perkin-Elmer Corp., Instrument Div., Norwalk, CT). Reverse-phase HPLC was performed using a Vydac C-18 column (0.46 × 25 cm; 300 Å pore size, 5 μm bead size, end-capped with C-2). Elution of peptides was accomplished using a mixture of solutions A and B. Solution A was 60% acetonitrile in 0.1% trifluoroacetic acid. Solution B was 0.1% trifluoroacetic acid. Samples were run at 23°C with a flow rate of 0.8 ml/min and a column pressure of 1,400 psi. Gradient elution was used in all runs. The acetonitrile content of the eluant is given in the figure legend or shown on the figures. Ultraviolet absorbance was monitored at 210 nm. Small amounts of the aPSS-I fragments (0.5–1.0 μg of the purified natural product peptides), used as column eluate markers, were always run on the same day under identical elution conditions to those applied to samples being analyzed.

Radioimmunoassay for Glucagon and Somatostatin

Determination of glucagon-like immunoreactivity in HPLC eluate fractions was achieved by radioimmunoassay (RIA) as described previously (23, 24) using the 30-K antiserum which has been shown to be COOH-terminally directed (11). Determination of SS-14-like immunoreactivity in HPLC eluates was performed by SS-14 RIA as previously described (12). The assay, as used, detects only SS-14-like immunoreactive components in anglerfish islet extracts and not [Tyr^7, Gly^{10}]SS-14 containing peptides (22).

Radiosequencing

Direct micro-sequence analysis was accomplished in a Wittmann-Liebold modified (44) Beckman 890C sequencer. Anglerfish PSS-I was isolated by HPLC. Preparation of the peptide for sequencing included reduction and carboxymethylation (39). Introduction into the cup and sequencer programming were as previously reported (36, 37). The products from each sequencer cycle were monitored by liquid scintillation counting.

Results

Identification and Isolation of aPSS-I

From the predicted amino acid sequence data of Goodman et al. (15, 16) and Hobart et al. (17), it is known that leucine is found only in the 1–80 region of aPSS-I and that tryptophan is found only in SS-14 (or aPSS-I$_{83-96}$; See Fig. 1). Islet tissue (111 mg) was incubated 5 h in the presence of [^{3}H]tryptophan and [^{14}C]leucine. The tissue extract was subjected to gel filtration, and the M_r 9,000–15,000 portion of the eluate was pooled and lyophilized. The peptides recovered were subjected to reverse-phase HPLC as indicated in Materials and Methods and in the legend to Fig. 2. Aliquots of the eluate were taken to determine the distribution of ^{3}H and ^{14}C radioactivity as well as glucagon- and somatostatin-like immunoreactive components. In the resulting chromatogram (Fig. 2), only one of the products monitored was doubly labeled with ^{3}H and ^{14}C and exhibited SS-14-like immunoreactivity with a complete absence of glucagon-like immunoreactivity. This was a component that eluted at a retention time of 51 min. The material eluting at retention time 59 min in Fig. 2 was heterogeneous. As it had been demonstrated previously that the RIA used for SS-14 does not detect [Tyr^7,Gly^{10}]SS-14 or other peptides containing this analogue of SS-14 (22) the material eluting at 51 min was tentatively identified as aPSS-I. The portion of the eluate indicated by the bar on Fig. 2 was pooled, lyophilized, and subjected to reduction and S-carboxymethylation as described previously (39).

Radiosequencing of aPSS-I

The results from radiosequencing of the peptide prepared as indicated in Fig. 2 are shown in Fig. 3. Significant [^{14}C]Leu
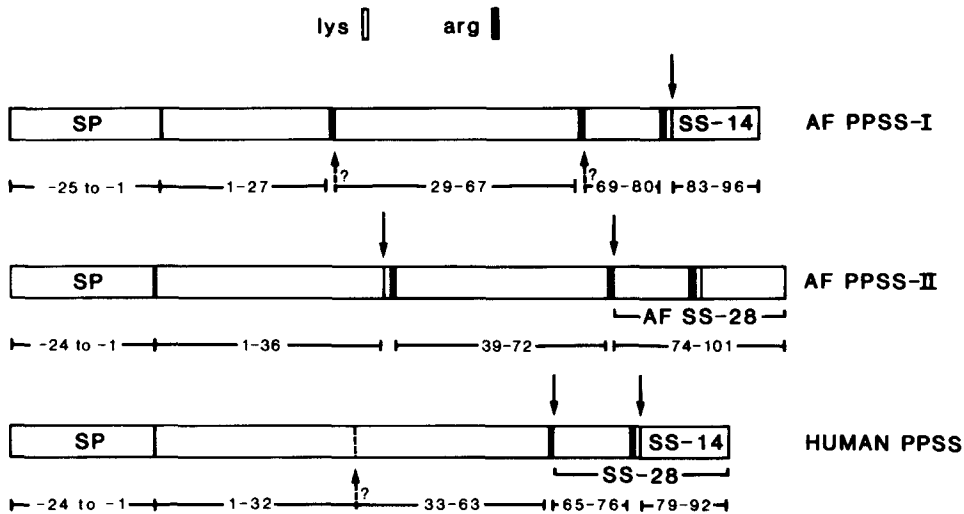
Figure 1. Size and cleavage site comparisons between anglerfish islet and human preprosomatostatins. Numbering of residues assigns the N-terminal amino acid of prosomatostatin as +1; residues of signal peptides (SP) are assigned negative numbers. Known sites of posttranslational endopeptidase and exopeptidase cleavage are indicated by downward pointing solid arrows. Postulated sites of endopeptidase cleavage are indicated by upward pointing dashed arrows. Positions where arginine residues are located are indicated by solid rectangles; lysines are indicated by open rectangles. These diagrams were developed from data in references 15–17, 32, 33, and 43.

radioactivity was observed in Edman cycles 10, 12, 13, 14, 19, 31, 34, 35, 36, 39, and 40. The positions of each of these leucine residues correspond precisely with the positions of 11 leucine residues in the sequence of PPSS-I as determined by Hobart et al. (17) and Goodman et al. (15, 16). Given this distribution pattern of the N-terminal 11 leucine residues over 44 sequencer cycles, the absence of any significant ^{3}H labeling in these cycles, and considering the observation that the peptide exhibits SS-like immunoreactivity (Fig. 2), it is highly probable that the leucine residues found in the radiosequence analysis were part of aPSS-I. As it would be expected that signal sequences would be removed from any

prohormones extracted from the islet tissue, these results then indicate that the N-terminal amino acid of aPSS-I is located nine positions N-terminal to the first leucine residue observed. It follows that the signal cleavage site occurs just before this amino acid. According to the sequence of PPSS-I (15–17), the signal cleavage site would be at a Cys-Ser bond, indicating a signal peptide 25 amino acids in length (Fig. 1).

Examination of Tissue Extracts for Potential Posttranslational Cleavage Products

Andrews and Dixon have isolated and characterized several peptides that are potential metabolic cleavage products of
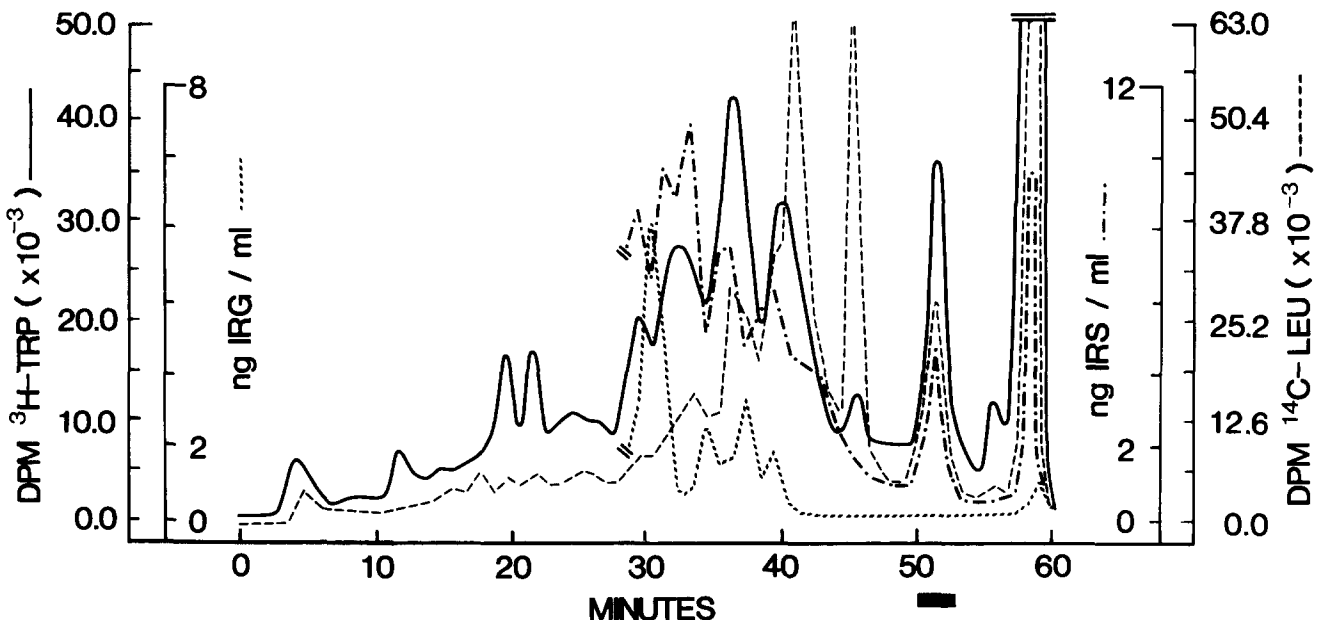

Figure 2. Reverse-phase HPLC of anglerfish islet M_r 9,000–15,000 peptides labeled with [^{3}H]tryptophan and [^{14}C]leucine. See Materials and Methods for column and solvent system. The column was equilibrated at 22% CH$_3$CN. After sample injections, a linear gradient reaching 35% CH$_3$CN in 35 min was instituted. This was followed by a 10-min isocratic elution at 35% CH$_3$CN. Then a 2-min gradient to 60% CH$_3$CN was instituted followed by a 10-min elution at 60% CH$_3$CN. Fractions of 0.4 ml (0.5 min) were collected and aliquots were removed for assay of radioactivity and for the RIAs. The region of the eluate indicated by the bar, containing a peptide doubly labeled with ^{3}H and ^{14}C which exhibited somatostatin-like but not glucagon-like immunoreactivity, was removed and the peptide was subjected to radiosequencing (Fig. 3).
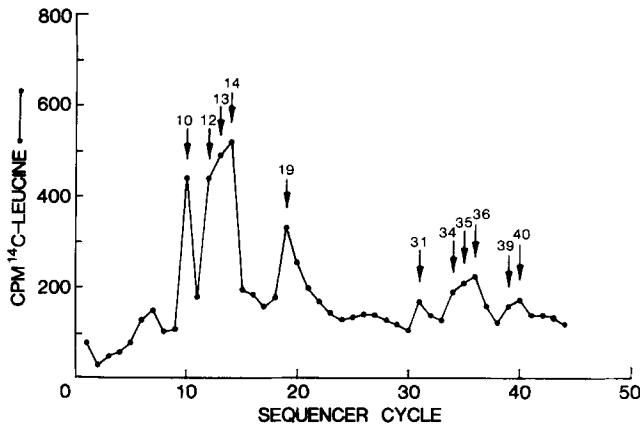
Figure 3. Radiosequencing of a SS-14 containing polypeptide from anglerfish islets. The material (~14 nCi; 24,000 cpm; 0.4-0.6 pmol) recovered as indicated in Fig. 2 was subjected to repetitive Edman degradation in the modified Beckman 890 sequencer. The products of each sequencer cycle were collected and assayed for ^{3}H and ^{14}C radioactivity. No appreciable ^{3}H label was monitored through the first 44 cycles. Significant ^{14}C label was recovered from the cycles indicated by the numbered arrows. The positions of these 11 leucine residues align precisely with the placement of 11 leucines in aPSS-I (references 15-17).

aPSS-I.[2] These are aPSS-I$_{1-27}$, aPSS-I$_{1-67}$, and aPSS-I$_{69-80}$ (Fig. 1). These investigators also prepared aPSS-I$_{29-67}$ by tryptic digestion of the 1-67 peptide. It might be predicted that each of these peptides is a potential cleavage product of aPSS-I because each is flanked by basic amino acids in the precursor (Fig. 1). Moreover, it was found that the C-terminal basic residues had been removed in the 1-27, 1-67, and 69-80 peptides isolated by Andrews and Dixon, suggesting the action of a carboxypeptidase in the final processing of these potential products.

We have performed biosynthetic studies to determine whether, and in what amounts, each of these peptides is produced during the processing of aPSS-I. Extracts of tissue incubated for 5 h in the presence of a ^{3}H-amino acid mixture were subjected to gel filtration and peptides of varying molecular size were then separated by reverse-phase HPLC. The distribution of radioactivity incorporated was compared to the absorption profile of the eluted peptides generated by monitoring at 210 μm. Representative examples of typical elution patterns obtained from M_r 1,000-2,500 and M_r 2,500-9,000 peptide pools are shown in Figs. 4 and 5, respectively. Presentation of the data in this format allows comparison of the relative amount of each peptide synthesized during incubations (A) with amounts of each peptide accumulated and stored in the tissue (B). The data in Fig. 4 demonstrate that the predominant peptide in the M_r 1,000-2,500 pool is SS-14 (B) which is also by far the most heavily labeled peptide in this pool (A). The label appearing in the void volume (RT 3-5 min; Fig. 4 A) can be attributed to unincorporated amino acids that were not removed during the desalting procedure. Of the other products of aPSS-I available for testing, only aPSS-I$_{69-80}$ would be expected in the M_r 1,000-2,500 pool. Under the conditions used, this peptide, which is analogous to SS-28$_{1-12}$ purified from mammalian species, was found to have a retention time of 18.2 min. It is clear from the data in Fig. 4 that, relative to the amounts of SS-14 produced, the quantity of aPSS-I$_{69-80}$
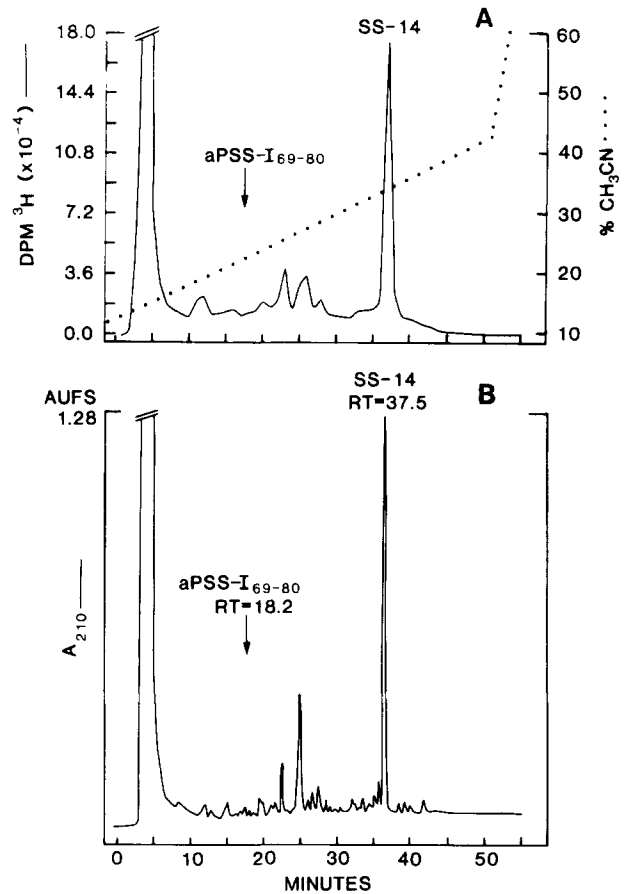

Figure 4. HPLC analysis of M_r 1,000-2,500 peptides labeled with ^{3}H-amino acids. Islet tissue (100 mg) was incubated 5 h with a ^{3}H-amino acid mix. The M_r 1,000-2,500 and M_r 2,500-9,000 peptides were separated by gel filtration and pooled. (A) HPLC chromatogram of one fourth of the M_r 1,000-2,500 peptides. Fractions of 0.8 ml (1.0 min) were collected. (B) Corresponding absorbance pattern obtained at 210 nm. The elution positions of aPSS-I$_{69-80}$ and SS-14 as determined in separate runs are shown by arrows on the chromatograms. AUFS, absorbance units full scale; RT, retention time; ....., CH$_3$CN gradient.

synthesized and stored in the tissue is either very small, or that this fragment is not produced at all.

All of the other potential cleavage products of aPSS-I that were tested in the present study are found in the M_r 2,500-9,000 pool. The chromatograms in Fig. 5 illustrate the relative amounts of each of these peptides recovered and the relative radiolabeling of each. It was found that, when compared with the amounts of SS-14 recovered, the quantities of aPSS-I$_{1-27}$ and aPSS-I$_{1-67}$ were very low. No aPSS-I$_{29-67}$ was detected (Fig. 5).

To quantitate more accurately the relative amounts of each of these potential cleavage products of aPSS-I that are stored in islet tissue, data from the Sigma 10 computing integrator were used. From a series of runs such as those depicted in Figs. 4 and 5, each of the peptides was identified in chromatograms by its retention time, and the peak area from the integrator was used to calculate nanomoles of peptide recovered per gram of islet tissue. The results were very consistent from all samples tested and are tabulated in Table I. These data indicate that SS-14 is by far the predominant cleavage product of aPSS-I, whereas the other peptides are minor
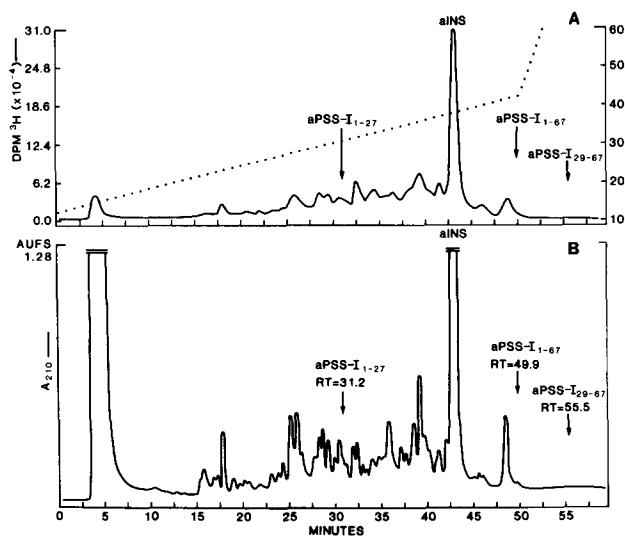
Figure 5. HPLC analysis of M_r 2,500–9,000 peptides labeled with ^{3}H-amino acids. One fourth of the M_r 2,500–9,000 peptides obtained from the tissue sample indicated in Fig. 4 was subjected to HPLC. (A) Distribution of ^{3}H radioactivity in the eluate. (B) Corresponding absorbance pattern observed at 210 nm. To enhance resolution, 0.4-ml (0.5 min) fractions of the HPLC eluates were collected for determination of radioactivity. The elution positions of aPSS-I$_{1-27}$, anglerfish insulin (aIns), aPSS-I$_{1-67}$, and aPSS-I$_{26-67}$ as determined in separate runs are indicated by arrows on the chromatograms. AUFS, absorbance units full scale; RT, retention time; ....., CH$_3$CN gradient.

Table I. Comparison of the Amounts of Prosomatostatin-I-related Peptides Recovered from Islet Tissue Extracts

Peptide	Nmol/g tissue*	Peptide as percent of SS-14
SS-14	287.1 ± 20.1	100.0
aPSS-I$_{69-80}$	10.4 ± 3.5	3.6
aPSS-I$_{29-67}$	ND	0.0
aPSS-I$_{1-67}$	6.9 ± 1.5	2.4
aPSS-I$_{1-27}$	16.0 ± 1.1	5.5

* Data are mean ± SD from five determinations. ND, none detected.

cleavage products of this precursor. None of the "pro-peptide fragments" was found in quantities >5.5% of the amount of SS-14 present.

Discussion

The partial radiosequencing of aPSS-I (Fig. 2) indicates that the position of signal cleavage in PPSS-I lies within the dipeptide Cys-Ser, yielding a signal peptide 25 amino acids in length (Fig. 1). This is the first identification of the amino terminus of aPSS-I extracted from islet tissue. These results confirm data from previous work of Warren and Shields (43) who demonstrated signal cleavage at this site by performing cell-free translation of PPSS-I mRNA in the presence of dog pancreatic microsomes. Our results are also consistent with the fact that two of the peptides isolated and characterized by Andrews and Dixon[2] were aPSS-I$_{1-27}$ and aPSS-I$_{1-67}$, both potential cleavage products of aPSS-I. The fact that these peptides are present in islet extracts suggests that they might be natural cleavage products derived from the N terminus of aPSS-I. Taken together, the results of the present and these other studies establish the signal cleavage site for PPSS-I in anglerfish islets at the position after the 25th residue C-terminal to the initiator Met of PPSS-I.

The examination of a wide variety of tissue extracts from different species for peptides contained within the structure of prosomatostatin-I has led to the identification of a number of peptides in addition to SS-14 and SS-28, which may be cleavage products of this precursor. Prominent among these peptides derived from mammalian tissues are SS-28$_{1-12}$ (human PSS$_{65-76}$ in Fig. 1) (4, 6–9) and a 32-residue peptide, corresponding to human PSS$_{1-32}$ (31) (Fig. 1). A peptide

similar to PSS$_{1-32}$ has been found in rat medullary thyroid carcinoma (3) and can be inferred to be present from work in which prosomatostatin cleavage products in rat brain were characterized (5). Other fragments of mammalian prosomatostatin have been described as well (5, 7, 9). However, just the fact that these peptides can be extracted from somatostatin-producing tissues is not sufficient to demonstrate that they are normal metabolic cleavage products of prosomatostatin. Whether a peptide is an actual processing product cleaved from prosomatostatin or an artifact of nonspecific proteolysis can be determined accurately only by quantitating the amount of each peptide produced in biosynthetic studies and by monitoring the amount of each of these peptides accumulated and stored in somatostatin-producing tissues. After comparing the amounts of the proposed product synthesized and stored with the quantity of SS-14, which is synthesized and stored in the same tissue, estimates can then be made regarding the relative abundance of other PSS cleavage products.

In the present study, we have made this type of comparison for SS-14 and several potential cleavage products of aPSS-I which have been isolated from islet tissue and characterized. The data in Figs. 4 and 5 and Table I indicate that SS-14 is a major cleavage product of aPSS-I. This observation, together with our finding that relatively low levels of aPSS-I$_{1-27}$, aPSS-I$_{1-67}$, and aPSS-I$_{69-80}$ are recovered, suggests that the major site of aPSS-I cleavage occurs at the basic dipeptide, Arg-Lys, which immediately precedes SS-14 in the precursor (Fig. 1). The results also indicate that secondary cleavages occur but appear to be minor, resulting in the production of the small amounts of the nonsomatostatin-containing fragments that were recovered. It is possible that these peptides are not normal metabolic cleavage products of aPSS-I at all. It cannot be excluded that each may be generated by random basic residue-specific endopeptidase activity combined with a carboxypeptidase B-like activity. It should be noted, however, that in the study of Andrews and Dixon[2] the relative proportions of each of these peptides recovered from tissue extracts differed slightly from those reported here. These differences may relate to differential extraction and/or recovery of each of the peptides as a result of the procedures used in each laboratory. It is also possible that the larger peptides are not as readily extracted as the smaller peptides. However, in both laboratories the levels of the "pro-peptide" fragments recovered were found to be significantly lower than the amounts of SS-14 recovered. Andrews and Dixon found that the quantities of aPSS-I$_{1-27}$, aPSS-I$_{1-67}$, and aPSS-I$_{69-80}$ were 2.6, 17.2, and 20.2%, respectively, of the amounts of SS-14 that were monitored in extracts of fresh tissue. This is consistent with the argument that none of the

"pro-peptide fragments" is a major cleavage product of aPSS-I.

In view of the data from both of these studies, a question remains regarding the nature of the major cleavage product(s) of aPSS-I other than SS-14. Considering the observation that aPSS-I_{29-67} was not detected at all in tissue extracts in the present study, one possible cleavage product other than SS-14 is aPSS-I_{29-80} (See Fig. 1). If aPSS-I_{29-80} were a cleavage product, then the other major cleavage product would be aPSS-I_{1-27}. However, since the recovered levels of aPSS-I_{1-27} were significantly lower than those of SS-14 (Figs. 4 and 5 and Table I), this is considered unlikely. Thus, in accordance with the observation that recovery of aPSS-I_{69-80} was also quite low (Fig. 4, Table I), it is proposed that the major cleavage product of aPSS-I other than SS-14 may be aPSS-I_{1-80}. This peptide has not yet been identified in extracts of anglerfish islets. While it is possible that this may be the result of poor recovery of this peptide relative to the other aPPS-I-related products, it is more likely that it reflects lack of an appropriate probe to identify the peptide. An alternative explanation is that all nonsomatostatin-containing portions of aPSS-I are subjected to random degradation or processing at sites other than Arg^{28}, Arg^{68}, and Arg^{81}-Lys^{82}, resulting in either complete breakdown, or in generation of cleavage products different from those identified to date. If this were the case, it could explain the observation of such low levels of the other potential cleavage products relative to SS-14 (Table I).

Although the evidence available indicates that many similarities exist, it is clear that the characteristics of prosomatostatin processing in mammalian somatostatin-producing tissues cannot be completely deduced from the results of the present study. To examine prosomatostatin processing in mammalian tissues rigorously, the methodology used here should be applied. Application of these procedures would establish the relative amounts of the various putative cleavage products of prosomatostatin which are actually produced and stored in the tissue. This information, in turn, could then be used to determine which, if any, of the prosomatostatin fragments found in these tissues might possibly play a biological role. It is possible that varying amounts of specific cleavage products may be produced in the same tissue under differing physiological conditions as a result of differential cleavage. This is a possibility that can readily be tested in the anglerfish islet system and probably in mammalian systems as well.

References

1. Andrews, P. C., and J. E. Dixon. 1981. Isolation and structure of a peptide hormone predicted from a mRNA sequence - a second somatostatin from the catfish pancreas. *J. Biol. Chem.* 256:8267-8270.

2. Aron, D. C., P. C. Andrews, J. E. Dixon, and B. A. Roos. 1984. Identification of cellular prosomatostatin and nonsomatostatin peptides derived from its amino terminus. *Biochem. Biophys. Res. Commun.* 124:450-456.

3. Aron, D. C., and B. A. Roos. 1986. Nonsomatostatin secretory peptide(s) derived from prosomatostatin's amino terminus in a rat medullary thyroid carcinoma cell line. *Endocrinology.* 118:218-222.

4. Bakhit, C., R. Benoit, and F. E. Bloom. 1983. Release of somatostatin-28 (1-12) from rat hypothalamus *in vitro. Nature (Lond.).* 301:524-526.

5. Benoit, R., P. Bohlen, F. Esch, and N. Ling. 1984. Neuropeptides derived from prosomatostatin that do not contain the somatostatin-14 sequence. *Brain Res.* 311:23-29.

6. Benoit, R., P. Bohlen, N. Ling, A. Briskin, F. Esch, P. Brazeau, S.-Y. Ying, and R. Guillemin. 1982. Presence of somatostatin-28(1-12) in hypothalamus and pancreas. *Proc. Natl. Acad. Sci. USA.* 79:917-921.

7. Benoit, R., N. Ling, B. Alford, and R. Guillemin. 1982. Seven peptides derived from pro-somatostatin in rat brain. *Biochem. Biophys. Res. Commun.* 107:944-950.

8. Benoit, R., N. Ling, C. Bakhit, J. D. Morrison, B. Alford, and R. Guillemin. 1982. Somatostatin-28(1-12)-like immunoreactivity in the rat. *Endocrinology.* 111:2149-2151.

9. Conlon, J. M., and D. M. McCarthy. 1984. Fragments of prosomatostatin isolated from a human pancreatic tumour. *Mol. Cell. Endocr.* 38:81-86.

10. Esch, F., P. Bohlen, N. Ling, R. Benoit, P. Brazeau, and R. Guillemin. 1980. Primary structure of ovine hypothalamic somatostatin-28 and somatostatin-25. *Proc. Natl. Acad. Sci. USA.* 77:6827-6831.

11. Faloona, G. R. 1972. The structure-function relationships of pancreatic glucagon. *In* Glucagon, Molecular Physiology, Clinical and Therapeutic Implications. P. J. LeFebvre and R. H. Unger, editors. Pergamon Press, Elmsford, NY. 201-204.

12. Fletcher, D. J., B. D. Noe, G. E. Bauer, and J. P. Quigley. 1980. Characterization of the conversion of a somatostatin precursor to somatostatin by islet secretory granules. *Diabetes.* 29:593-599.

13. Funckes, C. L., C. D. Minth, R. Deschenes, M. Magazin, M. A. Tavianini, M. Sheets, K. Collier, H. L. Weith, D. C. Aron, B. A. Roos, and J. E. Dixon. 1983. Cloning and characterization of a mRNA-encoding rat preprosomatostatin. *J. Biol. Chem.* 258:8781-8787.

14. Goodman, R. H., D. C. Aron, and B. A. Roos. 1983. Rat preprosomatostatin: structure and processing by microsomal membranes. *J. Biol. Chem.* 258:5570-5573.

15. Goodman, R. H., J. W. Jacobs, W. W. Chin, P. K. Lund, P. C. Dee, and J. F. Habener. 1980. Nucleotide sequence of a cloned structural gene coding for a precursor of pancreatic somatostatin. *Proc. Natl. Acad. Sci. USA.* 77:5869-5873.

16. Goodman, R. H., J. W. Jacobs, P. C. Dee, and J. F. Habener. 1982. Somatostatin-28 encoded in a cloned cDNA obtained from a rat medullary thyroid carcinoma. *J. Biol. Chem.* 257:1156-1159.

17. Hobart, P., R. Crawford, L.-P. Shen, R. Pictet, and W. J. Rutter. 1980. Cloning and sequence analysis of cDNAs encoding two distinct somatostatin precursors found in the endocrine pancreas of anglerfish. *Nature (Lond.).* 288:137-141.

18. Lauber, M., M. Camier, and P. Cohen. 1979. Higher molecular weight forms of immunoreactive somatostatin in mouse hypothalamic extracts: evidence of processing *in vitro. Proc. Natl. Acad. Sci. USA.* 76:6004-6008.

19. Lechan, R. M., R. H. Goodman, M. Rosenblatt, S. Reichlin, and J. F. Habener. 1983. Prosomatostatin-specific antigen in rat brain: localization by immunocytochemical staining with an antiserum to a synthetic sequence of preprosomatostatin. *Proc. Natl. Acad. Sci. USA.* 80:2780-2784.

20. Magazin, M., C. D. Minth, C. L. Funckes, R. Deschenes, M. A. Tavianini, and J. E. Dixon. 1982. Sequence of a cDNA encoding pancreatic preprosomatostatin-22. *Proc. Natl. Acad. Sci. USA.* 79:5152-5156.

21. Minth, C. D., W. L. Taylor, M. Magazin, M. A. Tavianini, K. Collier, H. L. Weith, and J. E. Dixon. 1982. The structure of cloned DNA complementary to catfish pancreatic somatostatin-14 messenger RNA. *J. Biol. Chem.* 257:10372-10377.

22. Noe, B. D. 1981. Synthesis of one form of pancreatic islet somatostatin predominates. *J. Biol. Chem.* 256:9397-9400.

23. Noe, B. D., and G. E. Bauer. 1975. Evidence for sequential metabolic cleavage of proglucagon to glucagon in glucagon biosynthesis. *Endocrinology.* 97:868-877.

24. Noe, B. D., G. E. Bauer, M. W. Steffes, D. E. R. Sutherland, J. S. Najarian. 1975. Glucagon biosynthesis in human pancreatic islets: preliminary evidence for a biosynthetic intermediate. *Horm. Metab. Res.* 7:314-322.

25. Noe, B. D., D. J. Fletcher, G. E. Bauer, G. C. Weir, and Y. Patel. 1978. Somatostatin biosynthesis occurs in pancreatic islets. *Endocrinology.* 102:1675-1685.

26. Noe, B. D., D. J. Fletcher, and J. Spiess. 1979. Evidence for the existence of a biosynthetic precursor for somatostatin. *Diabetes.* 28:724-730.

27. Patel, Y. C., T. Wheatley, and C. Ning. 1981. Multiple forms of immunoreactive somatostatin: comparison of distribution in neural and non-neural tissues and portal plasma of the rat. *Endocrinology.* 109:1943-1949.

28. Patzelt, C., H. S. Tager, R. J. Carroll, and D. F. Steiner. 1980. Identification of prosomatostatin in pancreatic islets. *Proc. Natl. Acad. Sci. USA.* 77:2410-2414.

29. Pradayrol, L., H. Jornval, V. Mutt, and A. Ribet. 1980. N-terminally extended somatostatin: the primary structure of somatostatin-28. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 109:55-58.

30. Schally, A. V., W.-Y. Huang, R. C. Chang, A. Arimura, R. W. Redding, R. P. Millar, M. W. Hunkapiller, and L. E. Hood. 1980. Isolation and structure of pro-somatostatin: a putative somatostatin precursor from pig hypothalamus. *Proc. Natl. Acad. Sci. USA.* 77:4489-4493.

31. Schmidt, W. E., V. Mutt, H. Kratzin, M. Carlquist, J. M. Conlon, and

W. Creutzfeldt. 1985. Isolation and characterization of proSS$_{1-32}$, a peptide derived from the N-terminal region of porcine preprosomatostatin. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 192:141-146.

32. Shen, L.-P., R. L. Pictet, and W. J. Rutter. 1982. Human somatostatin I: sequence of the cDNA. *Proc. Natl. Acad. Sci. USA.* 79:4575-4579.

33. Shen, L.-P., and W. J. Rutter. 1984. Sequence of the human somatostatin I gene. *Science (Wash. DC).* 244:168-171.

34. Shields, D. 1980. *In vitro* biosynthesis of fish islet preprosomatostatin: evidence of processing and segregation of a high molecular weight precursor. *Proc. Natl. Acad. Sci. USA.* 77:4074-4078.

35. Shields, D. 1980. In vitro biosynthesis of somatostatin. Evidence for two distinct preprosomatostatin molecules. *J. Biol. Chem.* 255:11625-11628.

36. Spiess, J., J. Rivier, C. Rivier, and W. Vale. 1981. Primary structure of corticotropin-releasing factor from ovine hypothalamus. *Proc. Natl. Acad. Sci. USA.* 78:6517-6521.

37. Spiess, J., J. Rivier, C. Rivier, and W. Vale. 1982. Microsequence analysis of ovine hypothalamic corticotropin releasing factor. Proc. Int. Conf. Methods Protein Sequence Anal., IVth. Humana Press, Clifton, NJ. 131-138.

38. Spiess, J., and W. Vale. 1980. Multiple forms of somatostatin-like activity in rat hypothalamus. *Biochemistry.* 19:2861-2866.

39. Spiess, J., J. Villarreal, and W. Vale. 1981. Isolation and sequence anal-ysis of a somatostatin-like polypeptide from ovine hypothalamus. *Biochemistry.* 20:1982-1988.

40. Tavianini, M. A., T. E. Hayes, M. D. Magazin, C. D. Minth, and J. E. Dixon. 1984. Isolation, characterization and DNA sequence of the rat somatostatin gene. *J. Biol. Chem.* 259:11798-11803.

41. Taylor, W. L., K. J. Collier, R. J. Deschenes, H. L. Weith, and J. E. Dixon. 1981. Sequence analysis of a cDNA coding for a pancreatic precursor to somatostatin. *Proc. Natl. Acad. Sci. USA.* 78:6694-6698.

42. Warren, T. G., and D. Shields. 1982. Cell-free biosynthesis of somatostatin precursors: evidence for multiple forms of preprosomatostatin. *Proc. Natl. Acad. Sci. USA.* 79:3729-3733.

43. Warren, T. G., and D. Shields. 1984. Cell-free biosynthesis of multiple preprosomatostatins: characterization by hybrid selection and amino-terminal sequencing. *Biochemistry.* 23:2684-2690.

44. Wittmann-Liebold, B. 1980. Current advances in sequencing as applied to the structure determination of ribosomal proteins. *In* Polypeptide Hormones. R. F. Beers, Jr. and E. G. Bassett, editors. Raven Press, New York. 87-120.

45. Wu, P., E. Penman, D. H. Coy, and L. H. Rees. 1983. Evidence for direct production of somatostatin-14 from a larger precursor than somatostatin-28 in a phaeochromocytoma. *Regul. Pept.* 5:219-233.