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The amino-acid sequences of sculpin islet somatostatin-28 and peptide YY

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Two pancreatic peptides, somatostatin-28 and peptide YY, have been isolated from the Brockmann bodies of the teleost fish *Cottus scorpius* (daddy sculpin). Following purification by reverse-phase HPLC, each peptide was sequenced completely through to the carboxyl-terminus by gas-phase Edman degradation. Somatostatin-28 was the major form of somatostatin detected and is similar to the gene II product from anglerfish. Peptide YY (36 amino acids) more closely resembles porcine neuropeptide YY and intestinal peptide YY than it does the pancreatic polypeptides.

Amino acid sequencing; Sculpin; Somatostatin-28; Peptide YY; (Pancreatic islet)

1. INTRODUCTION

Protein sequence analysis of pancreatic hormones from different species not only provides the basis for understanding structure-activity relationships but also, in conjunction with cDNA sequence data, may show the way in which the precursor (prohormone) molecules are processed. The islet organs (Brockmann bodies) from certain of the teleost fish are a particularly rich source of the pancreatic hormones – insulin, glucagon, somatostatin and pancreatic polypeptide [1,2]. In these species (e.g., catfish, anglerfish, salmon), it is apparent that somatostatin can exist in several forms [3–7], evidently derived from two distinct genes (I and II) and also containing various N-terminal extensions to the 14 amino acid 'parent' peptide, S-14. The S-14 I form is identical to that found in higher order species [8] whereas the larger forms (S-22, S-25, S-28) derived from gene II are probably terminal products of prosomatostatin processing, and appear to have modified biological activities compared to S-14 I [7].

Pancreatic polypeptide has been identified in a wide variety of vertebrate species, and isolated and characterised from mammals, birds, and reptiles [9–11]. It is a 36 amino acid peptide with an amidated C-terminus, containing (thus far) 9 conserved residues. Similar peptides have been characterised from porcine brain (NPY) and intestine (PYY) [12,13] while a non-amidated 37 amino acid form was recently found in anglerfish Brockmann bodies [14]. The crystal structure of avian PP has been determined, and suggests that a common three-dimensional structure could accommodate the quite extensive sequence variation seen in this family of peptides [15].

We recently isolated a PP-like peptide along with the other pancreatic hormones, from the islet organs of the teleost fish, *Cottus scorpius* (daddy sculpin) [16]. The complete amino acid sequence of insulin was determined as well as a partial sequence

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Abbreviations: HPLC, high performance liquid chromatography; PTH, phenylthiohydantoin; PP, pancreatic polypeptide; NPY, neuropeptide YY; PYY, intestinal peptide YY; aPY, anglerfish pancreatic peptide YG; sPY, sculpin pancreatic peptide YY

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for the PP-like molecule. This paper reports the complete amino acid sequence of this molecule and also that of somatostatin.

2. MATERIALS AND METHODS

The pancreatic hormones were extracted from Brockmann bodies of daddy sculpin, freshly caught at the Kristineberg Marine Biology Station (Fiskebackskil, Sweden) as described [16]. Gel filtration yielded an absorbance peak of M_r about 4000–6000 which was further fractionated by reverse-phase HPLC (Waters μBondapak alkylphenyl) in 50 mM ammonium acetate/acetonitrile [16]. Individual peaks were re-chromatographed on a Waters-Millipore liquid chromatograph using a Brownlee Aquapore RP-300 column (30 × 4.6 mm) and a 0.1% trifluoroacetic acid/acetonitrile gradient (fig.1). Absorbance was monitored at both 214 and 280 nm. Appropriate fractions were reduced in volume under a stream of nitrogen before being submitted for sequence analysis.

Amino acid sequence analysis was carried out on a model 470A gas phase sequencer with an on-line model 120A PTH analyser (Applied Biosystems, Foster City, CA). For the PP-related material two separate experiments were performed. The first used 60 pmol peptide and yielded sequence information up to cycle 21. The second, using 700 pmol, employed a customised programme for Pro and Ser/Thr at the appropriate sites known from the first run and enabled the complete sequence of 36 residues to be determined. In the case of somatostatin, 1100 pmol of unreduced peptide was sequenced to completion.

C-terminal analysis on the PP-like molecule was attempted using carboxypeptidase Y (EC 3.4.16.1) under conditions established as being successful for other peptides possessing unmodified carboxytermini. The enzyme/substrate ratio used was 1:25 (w/w), twice the concentration of enzyme used in these control experiments. Amino acid analysis was carried out using the Waters-Millipore system described in [16].

3. RESULTS AND DISCUSSION

Previously we had isolated, by gel filtration and reverse-phase HPLC, four distinct fractions from sculpin islet tissue, three of which were identified as glucagon, insulin and PP, respectively [16]. The fourth fraction, which eluted between insulin and the PP-like peptide on HPLC, has now been identified by sequencing as somatostatin-28. The PP-like peptide has also been sequenced and by analogy with other peptides in this family [14,17] is referred to as sculpin pancreatic peptide YY, or sPY, to denote the N-terminal and C-terminal tyrosine residues.

The final purification of the peptides was achieved by an additional reverse-phase HPLC step as shown in fig.1. The yield of somatostatin-28 was approx. 0.12 mg/g tissue, whereas that for sPY was 0.16 mg/g tissue. The

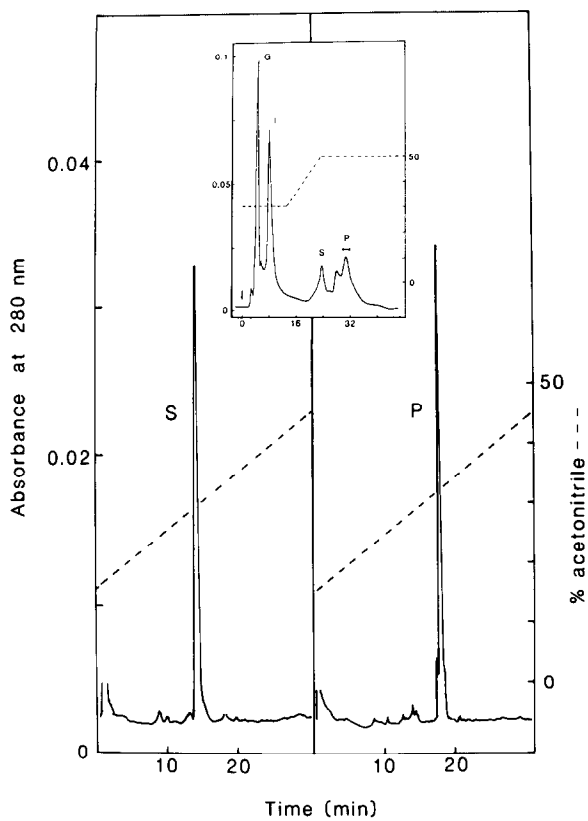

Fig.1. Final purification by reverse-phase HPLC of the somatostatin and PP-like peptide from the islet organs of the daddy sculpin. The peptides were eluted using a 30 min gradient, from 15 to 45% acetonitrile in 0.1% trifluoroacetic acid, at a flow rate of 1 ml/min. The inset shows prior separation of sculpin pancreatic peptides by HPLC as described in [16]. Peaks G, I, S and P refer to fractions containing glucagon, insulin, somatostatin and PP-like peptide, respectively.

unambiguous identification at each cycle of the sequence analyses testified to the homogeneity of the peptides, and could be continued through to the C-terminus. Yields at each cycle are shown in fig.2. Assignment of half-cystine to positions 17 and 28 in somatostatin-28 was made on the basis of (i) lack of a detectable PTH derivative because of the disulphide link between these positions, (ii) the presence of two cysteic acid residues from amino acid analysis of a performic acid treated sample, and (iii) analogy with all known somatostatin molecules. Amino acid analysis confirmed the sequence assignments made for the two peptides.

The PTH derivative of terminal residue 36 of sPY co-eluted with PTH-tyrosine. However it is known that all members of the PP family except anglerfish PY possess an amidated C-terminus at position 36 due to the presence of an obligatory glycine residue at position 37 in the unprocessed peptide [18]. The anglerfish peptide retains Gly-37 [14]. As there was insufficient material to confirm the presence of an amidated tyrosine by the usual dansyl method [19] and because PTH-tyrosinamide and PTH-tyrosine standards co-elute under the conditions used for sequence analysis, support was provided by the carboxypeptidase Y experiment which showed no release of tyrosine over 24 h under conditions established as being favourable.

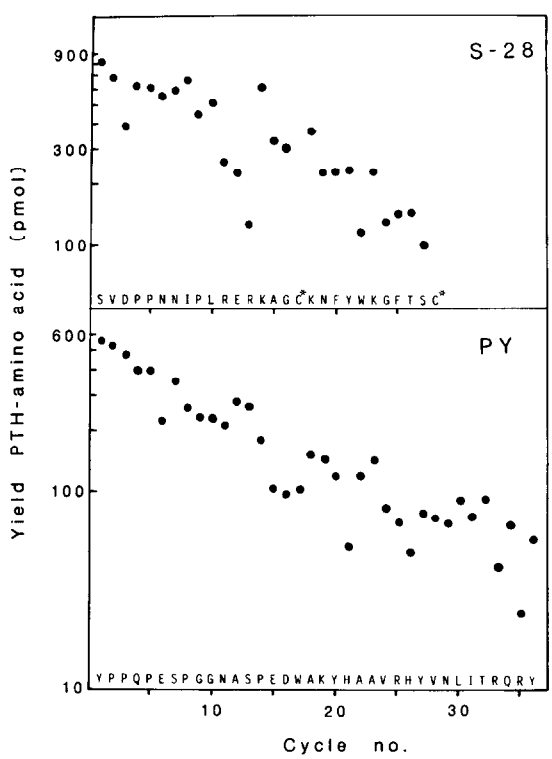

Fig.2. Sequence information for sculpin somatostatin (S-28) and pancreatic peptide YY (sPY). No yield is reported for Cys-17 and Cys-28 of somatostatin as the disulphide bridge between them remained intact during sequencing.

3.1. *Somatostatin*

The somatostatin characterised in this study is an S-28 type, similar to that found in anglerfish islet tissue (designated S-28 II), in which the C-terminal tetradecapeptide shows two sequence changes (Tyr-21 and Gly-24) from standard mammalian S-14 I. It is possible that other forms of somatostatin may also be present, as found in Coho salmon [7] where S-14 I, S-14 II and S-25 II have all been identified. The latter is the major form detected in salmon islet tissue and is clearly homologous to the sculpin S-28 and anglerfish S-28 II sequences, particularly when the tripeptide deletion is adjusted for (table 1). The isolation and characterisation of sculpin S-28 lends support to the notion that gene II prosomatostatin is processed differently from that of gene I in the endocrine pancreas of teleost fish, i.e., more directed towards production of the extended forms of somatostatin.

Overall, considerable sequence variation is seen in the N-terminal sequences of the S-28 forms of somatostatin but this does not necessarily preclude a defined structural role for this section, such as the β-turn thought to be necessary for correct proteolytic processing at the N-terminus [20]. The presence of Pro-4 and Pro-5 in sculpin S-28 is compatible with this view; they may also compensate for the substitution of Pro to Leu at position 10. Alternatively, the biological role of S-28 itself may depend crucially on this early sequence.

3.2. *Pancreatic peptide YY (sPY)*

This peptide, clearly related to the so-called pancreatic polypeptides, is known to exist only in the pyloric and not the splenic Brockmann body of the daddy sculpin [21]. It turns out to be, not surprisingly, similar to aPY from anglerfish except for the C-terminus which is incompletely processed in the

Table 1

Comparison of the amino acid sequences of the extended forms of somatostatin (upper panel) and of the pancreatic polypeptide family (lower panel)

	5	10	15	20	25
Sculpin S-28	S V D P P N N I P L R E R K A G C K N F Y W K G F T S C				
Anglerfish S-28 II	- - - S T - - L - P - - - - - - - - - - - - - - - - - -				
Salmon S-25	S V D - L - P -				
Anglerfish S-28 I	A A S G G P L L A P - - - - - - - - - - - F - - T - - - -				
Porcine S-28	- A N S N P A M A P - - - - - - - - - - - F - - T - - - -				
Catfish S-22 II	D N T V T S K P L N - M - Y F - - S R - A -				

	5	10	15	20	25	30	35
Sculpin sPY	Y P P Q P E S P G G N A S P E D W A K Y H A A V R H Y V N L I T R Q R Y-NH_2						
Anglerfish aPY	- - - K - - T - - S - - - - - - - - - S - Q - - - - - - - - - - - - - - - - G						
Porcine NPY	- - S K - D N - - E D - P A - - L - R - Y S - L - - - I - - - - - - - - -						
Porcine PYY	- - A K - - A - - E D - - - - E L S R - Y - S L - - - L - - V - - - - -						
Alligator PP	T - L - - K Y - - D G - P V - - L I Q F Y D D L Q Q - L - V V - - P - F-NH_2						
Chicken PP	G - S - - T Y - - D D - P V - - L I R F Y D N L Q Q - L - V V - - H - -						
Human PP	A - L E - V Y - - D - - T - - Q M - Q - A - D L R R - I - M L - - P - -						

Abbreviations and references are given in the text. Dashes indicate identical residues to the corresponding sculpin peptides. In somatostatin residues 17 and 28 are disulphide-linked. With the exception of anglerfish aPY, the other members of the pancreatic polypeptide family all possess an amidated C-terminus at residue 36

latter [14]. It appears from the residues conserved in the sequence that the basic folding pattern of turkey PP, as determined crystallographically [22], can be maintained for sPY as has been shown for NPY and PPY [15]. The dimerising surface shows an interesting substitution at position 21, where His replaces Tyr, but the hydrophobic interactions are preserved with Pro-14, Trp-17, Val-24 and Val-28 replacing Val, Leu, Leu and Leu, respectively.

There are 14 changes in sequence from both porcine NPY (brain) and porcine PPY (intestine) but considerably more differences exist with respect to the pancreatic polypeptides (see table 1). The question of whether sPY, and aPY, are new members of the PP family remains open until specific biological roles for all of these peptides can be clearly established.

A fuller understanding of specific prohormone processing pathways will be greatly aided by crystallographic investigations of the prohormones in addition to characterisation of the proteolytic enzymes involved.

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