ISOLATION AND AMINO ACID SEQUENCE OF BOVINE SECRETIN

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1. Introduction

Secretin stimulates secretion of pancreatic juice [1,2] and was originally isolated from porcine upper intestine [3,4]. This peptide, or a secretin-like one, has also been found in the brain [5] but the physiological role in this location is yet unknown. Secretin [4] is chemically and evolutionarily related to glucagon [6] to the vasoactive intestinal polypeptide (VIP) [7] to further peptides in the same group [8] and probably to a still larger group of active peptides [9].

Some peptide structures are highly conserved whereas others are more variable. Glucagon isolated from several species [10], is identical in all mammals investigated. This also applies to known mammalian VIP structures [11]. For secretin, only one mammalian structure is this far established [4]. Surprisingly however, when the primary structure of chicken secretin was determined [12], it was found to be about as similar to porcine secretin as to glucagon. We have therefore isolated bovine secretin and determined its amino acid sequence, making this a second mammalian secretin for which the primary structure is known. Functionally, this allows judgements of the variability in secretin. Parenthetically, high-pressure liquid chromatography (HPLC) was found to give exceptionally high yields of tryptic peptides.

2. Experimental

2.1. *Isolation of bovine secretin*

The starting material and the isolation procedure for bovine intestinal secretin were the same as for bovine VIP [11] until the second treatment with alginic acid and the following precipitation with NaCl. From ~100 kg intestines, 27 g (wet wt) peptide concentrate was obtained.

This material was chromatographed on Sephadex G-25 fine (Pharmacia) in 0.2 M acetic acid. The fractions active by secretin tests in cat [13], were precipitated with NaCl, dissolved in water, adjusted to pH 4.0 with 0.1 M NaOH, and reprecipitated with NaCl.

The precipitate (11 g) was extracted with methanol [14] (50 ml/g) containing 0.5% thiodiglycol. The suspension was filtered, neutralized with 0.1 M NaOH and refiltered. The second filtrate was adjusted to pH 2.7 with 0.2 M HCl and peptides were precipitated with 3 vol. ether and rechromatographed on Sephadex G-25 fine in 0.2 M acetic acid. The fractions containing secretin-activity were lyophilized (310 mg) and further chromatographed on a CM-cellulose (Whatman CM 22) column (3.5 × 20 cm) which had been equilibrated with 0.02 M ammonium bicarbonate (at pH 6.6 by bubbling with CO_2). Elution was performed by a stepwise increase of pH and ammonium bicarbonate from pH 6.6 (0.02 M), pH 7.3 (0.02 M), pH 8.0 (0.02 M), pH 8.0 (0.06 M) and finally pH 8.0 (0.10 M). The secretin fractions at pH 8.0 (0.02 M) were lyophilized (28 mg) partitioned between 1-butanol–0.1 M ammonium bicarbonate [12] and yielded 6 mg.

Final purification was done on a Waters Associates liquid chromatograph equipped with a μ-Bondapak C_{18} column (7.8 × 300 mm), a U6K-injector, two M 6000A pumps, a 660 solvent programmer and a 450 detector. The solvent system consisted of 0.005 M ammonium acetate, 0.1% acetic acid in 50% ethanol and the flow rate was 1.0 ml/min.

2.2. *Analytical methods*

Bovine secretin (50 μg) was digested with trypsin (2 μg) in 0.1 M ammonium bicarbonate (50 μl) at 37°C for 4 h. The tryptic fragments were separated by HPLC as described in the legend to fig.2. Hydrolysis of peptides was performed in evacuated tubes at 110°C for 24 h with 6 M HCl containing 0.5% phenol, and

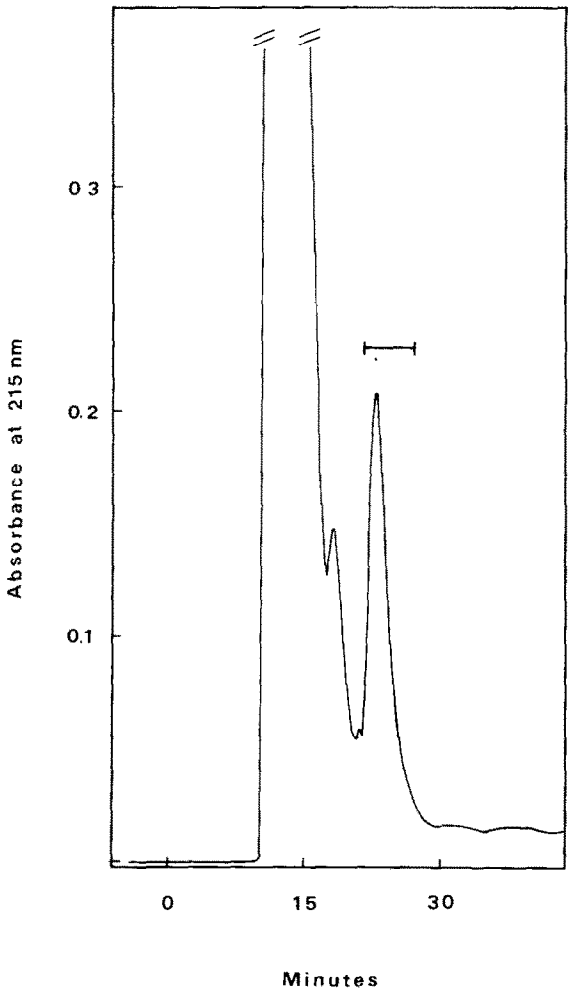

Fig.1. HPLC of 1.2 mg secretin-active material after the partition step. Column: 7.8 X 300 mm μ-Bondapak C_{18}. Solvent: 0.005 M ammonium acetate; 0.1% acetic acid in 50% ethanol. Flow rate 1.0 ml/min. The secretin fraction is indicated by a bar.

the amino acid compositions were determined on a Beckman 121M instrument.

N-Terminal amino acids were analyzed with the dansyl-Edman method [15,16], and the amidated C-terminal residue was determined as a dansyl derivate [17]. Step-wise Edman degradation of the intact peptide was carried out in a Beckman 890 C liquid-phase sequencer using precycled polybrene and a 0.1 M quadrol peptide program [18]. PTH-amino acids were analyzed by HPLC [19] and by thin-layer chromatography (TLC) [20].

3. Results and discussion

3.1. *Isolation of bovine secretin*

The isolation procedure for bovine secretin involved 8 steps as in section 2. After the final steps of reverse-phase HPLC a homogeneous material was obtained (fig.1) in a yield of 220 μg pure peptide from 100 kg intestines. This material was used for the determination of the amino acid sequence of bovine secretin.

3.2. *Structural analysis of bovine secretin*

The tryptic digest was separated by HPLC into 5 fragments, T_1–T_5 (fig.2). The amino acid compositions and N-terminal residues of the fragments are given in table 1. Yields obtained were noticeably high (table 1) in comparison with other purifications on paper of similar peptides [11,12]. The N-terminal structure of intact secretin (50 nmol) was determined by liquid-phase sequencer-assisted degradations. The complete sequence is shown in fig.3.

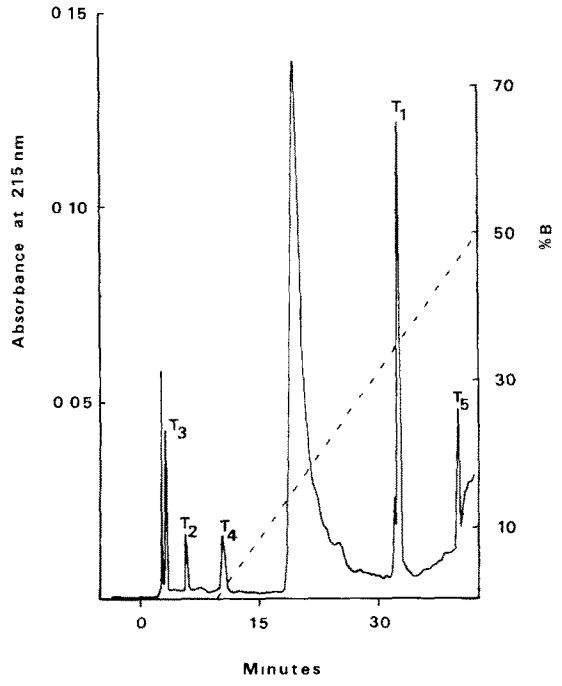

Fig.2. Separation of tryptic fragments from secretin (20 μg) by HPLC on μ-Bondapak C_{18} (3.9 X 300 mm) with a gradient (shown by dashed line) of solvent B (90% ethanol and 10% of solvent A) in solvent A (0.005 M ammonium acetate, 0.1% acetic acid and 1% ethanol). Flow rate 2.0 ml/min. The large peak eluted between 15 and 30 min is due mainly to acetic acid. The tryptic fragments are indicated by T_1–T_5.

Table 1
Data for the tryptic peptides of bovine secretin

Peptide	T_1	T_2	T_3	T_4	T_5
Recovery (%)	87	93	85	95	89
Composition					
Asx	1.0 (1)	-	0.6 (1)	-	-
Thr	1.9 (2)	-	-	-	-
Ser	2.8 (3)	-	0.9 (1)	-	-
Glx	1.1 (1)	-	-	1.0 (1)	1.0 (1)
Gly	1.1 (1)	-	-	-	1.0 (1)
Ala	-	-	1.2 (1)	-	-
Val	-	-	-	-	0.9 (1)
Leu	1.0 (1)	1.0 (1)	-	1.0 (1)	3.0 (3)
Phe	1.0 (1)	-	-	-	-
His	1.0 (1)	-	-	-	-
Arg	1.0 (1)	1.0 (1)	1.2 (1)	1.0 (1)	-
Total	12	2	4	3	6
N-terminus	His	Leu	Asp	Leu	Leu

Values are molar ratios without correction for destruction, incomplete hydrolysis or impurity; Cys, Ile, Lys, Met, Pro, Trp and Tyr are absent from all peptides

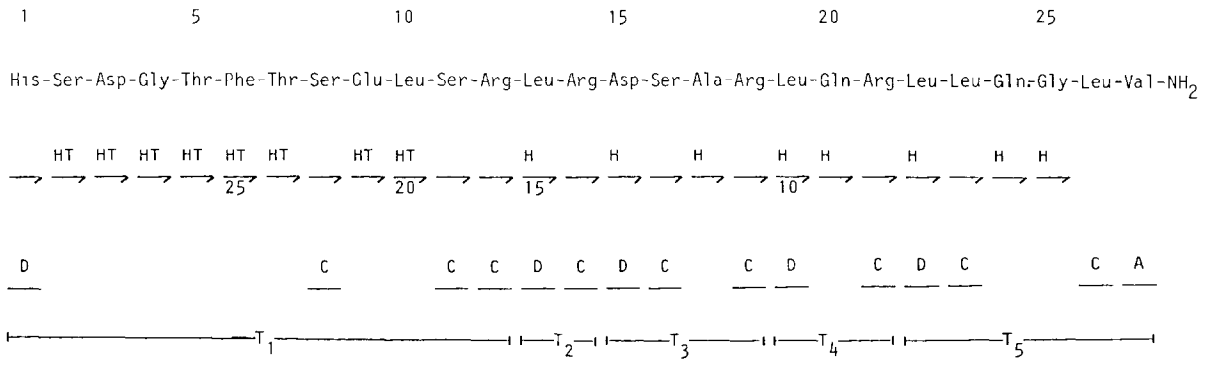

Fig.3. The amino acid sequence of bovine secretin. Sequencer degradation is shown by (—→; values are nmol recovered). The tryptic fragments are indicated by T_1–T_5, and residue identifications by H (HPLC), T (TLC), D (dansyl method), C (composition from a tryptic fragment) or A (analysis of amidated C-terminal residue). Repetitive yield calculated on Asp_{3-15} is 96% and on Lue_{10-22} is 82%.

The sequencer analysis established the sequence of 16 residues, fixed the relative order of all tryptic fragments (T_1–T_5, table 1) and showed their C-terminal residues to be at positions 12,14,18 and 21. These arginine residues, although incompletely identified in the sequencer degradations, are further established from the total compositions (table 1). The amidated C-terminus of secretin (position 27) was Val-NH_2 as identified after dansylation [17]. The N-terminal residues of the tryptic fragments confirm the identities of positions 1,13,15,19 and 22. Finally the serine residues at positions 8,11,16 and the two leucine residues at positions 23 and 26 not reached by the sequencer degradation, are given from the total compositions of fragments T_1, T_3 and T_5.

The whole structure determination was limited by lack of material (200 μg in total, for both digestion and direct sequencer analysis) but is anyway fully sufficient, especially since results from sequencer degradations, enzymatic specificities and total compositions agree.

Three secretin structures have now been determined, those from pig, chicken and cow. Bovine and porcine types of glucagon [10] and VIP [11] have also been determined. The results show that there is no species variation between pig and cow for any of these peptides. This, however, does not exclude some variation between distantly related species, as has been shown for avian and mammalian glucagons [21], VIPs [22] and secretins [12]. However, the conservation within studied mammals for glucagon, VIP and now secretin, is significant in relation to more variable hormone structures like ACTH (corticotropin), calcitonin, gastrin, PTH (parathyroid hormone) and insulin [8,23].

These results therefore show that secretin, like glucagon and VIP, is structurally conserved, suggesting that these molecules are under strong functional restrictions.

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