ISOLATION AND AMINO ACID SEQUENCE OF BOVINE SECRETIN

Mats CARLQUIST, Hans JÖRNYALL and Viktor MUTT

Departments of Biochemistry II and Chemistry I, Karolinska Institutet, S 104 01 Stockholm 60, Sweden

Received 9 March 1981

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% thioglycol. 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 X 20 cm) which had been equilibrated with 0.02 M ammonium bicarbonate (at pH 6.6 by bubbling with CO₂). 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 l-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 C₁₈ column (7.8 X 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
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 preycled polypeprene and a 0.1 M quadrupole 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 \(\mu\)g 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.
Table 1

Data for the tryptic peptides of bovine secretin

<table>
<thead>
<tr>
<th>Peptide</th>
<th>T₁</th>
<th>T₂</th>
<th>T₃</th>
<th>T₄</th>
<th>T₅</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recovery (%)</td>
<td>87</td>
<td>93</td>
<td>85</td>
<td>95</td>
<td>89</td>
</tr>
</tbody>
</table>

Composition

<table>
<thead>
<tr>
<th></th>
<th>1.0 (1)</th>
<th>-</th>
<th>0.6 (1)</th>
<th>-</th>
<th>-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asx</td>
<td>1.9 (2)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Thr</td>
<td>2.8 (3)</td>
<td>-</td>
<td>0.9 (1)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ser</td>
<td>1.1 (1)</td>
<td>-</td>
<td>-</td>
<td>1.0 (1)</td>
<td>1.0 (1)</td>
</tr>
<tr>
<td>Glx</td>
<td>1.1 (1)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.0 (1)</td>
</tr>
<tr>
<td>Gly</td>
<td>-</td>
<td>-</td>
<td>1.2 (1)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ala</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.9 (1)</td>
</tr>
<tr>
<td>Val</td>
<td>1.0 (1)</td>
<td>1.0 (1)</td>
<td>-</td>
<td>1.0 (1)</td>
<td>3.0 (3)</td>
</tr>
<tr>
<td>Leu</td>
<td>1.0 (1)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Phe</td>
<td>1.0 (1)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>His</td>
<td>1.0 (1)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Arg</td>
<td>1.0 (1)</td>
<td>1.0 (1)</td>
<td>1.2 (1)</td>
<td>1.0 (1)</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>2</td>
<td>4</td>
<td>3</td>
<td>6</td>
</tr>
</tbody>
</table>

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 T₁ → T₅, 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 Asp₁₅ is 96% and on Leu₁₀₂₂ is 82%.
The sequencer analysis established the sequence of 16 residues, fixed the relative order of all tryptic fragments (T₁–T₅, 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-NH₂ 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 T₁, T₃ and T₅.

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.

Acknowledgements

This investigation was supported by grants from the Swedish Medical Research Council (projects 13X-1010, 13X-3532) the Swedish Cancer Society (project 820), the Knut and Alice Wallenberg's Foundation and Karolinska Institutet.

References